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# TITLE: Topically Applied therapies for the treatment of skin disease: Past Present and Future

**RUNNING TITLE: Topically applied medicines**

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MB and FG edited the manuscript. All authors wrote or contributed to the writing of the manuscript as follows: MB – I-IV, VI and VII; AW – II, IV and VI, BB - IV; JL and CB -V; RC – I and II ; CE and LA – VI ; FG-1,II and VI; JH - III and VI , MM - I and III, RV – III.



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**ABBREVIATIONS:**

| | |
|---|---|
| AA | Alopecia Areata |
| ACD | Allergic Contact Dermatitis |
| AD | Atopic Dermatitis |
| Aks | Actinic keratoses |
| AMP | Antimicrobial Peptide |
| BCC | Basal Cell Carcinoma |
| BCC | Basal Cell Carcinoma |
| BP | Bullous pemphigoid |
| CIN | Cervical Intraepithelial Neoplasia |
| CM | Cutaneous melanoma |
| DALYS | Disability adjusted life years |
| DEB | Dystrophic epidermolysis bullosa |
| DHH | Desert Hedgehog |
| DLSO | Distal and lateral subungual onychomycosis |
| EB | Epidermolysis Bullosa |
| EBA | Epidermolysis bullosa aquisita |
| EBS | Epidermolysis bullosa simplex |
| EO | Endonyx Subungual Onychomycosis |
| EPP | Erythropoietic Protoporphyria |
| FD | Folliculitis Decalvans |
| HF | Hair follicle |
| Hh | Hedgehog Pathway |
| HPV | Human Papilloma Virus |
| HS | Hidradenitis suppurativa |



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| | |
|---|---|
| ICD | Irritant Contact Dermatitis |
| IHH | Indian Hedgehog |
| JAK | Janus kinase inhibitor |
| JEB | Junctional epidermolysis bullosa |
| KC | Keratinocyte carcinoma |
| LI | Lamellar ichthyosis |
| LP | Lichen Planus |
| MBCC | Morpheaform BCC |
| MBCC | Morpheaform Basal Cell Carcinoma |
| mBCCs | Locally advanced or rare metastatic BCCs |
| MC | Molluscum contagiosum |
| NBCC | Nodular Basal Cell Carcinoma |
| NBCC | Nodular Basal Cell Carcinoma |
| NBCCS | Nevoid Basal Cell Carcinoma Syndrome |
| NSAID | Non-Steroidal Anti-Inflammatory Drugs |
| NSV | Nonsegmental Vitiligo |
| OTC | Over-The-Counter products |
| PAR-2 | Protease-Activated Receptor-2 |
| PC | Pachyonychia congenita |
| PSO | Proximal Subungual Onychomycosis |
| PUVA | Psoralen and Ultraviolet A |
| ROS | Reactive Oxygen Species |
| SCC | Squamous Cell Carcinoma |
| SD | Seborrheic dermatitis |
| SHH | Sonic Hedgehog |
| SV | Segmental Vitiligo |
| SWO | Superficial White Onychomycosis |



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| | |
|---|---|
| TCI | Topical calcineurin inhibitors |
| TCS | Topical corticosteroid |
| TODO | Total dystrophic onychomycosis |
| XLP | X linked Protoporphyria |
| XP | Xeroderma pigmentosum |
| cNFs | Cutaneous Neurofibromas |
| 5-FU | 5-Fluorouracil |

**ABSTRACT**

The purpose of this review is to summarize essential biological, pharmaceutical and clinical aspects in the field of topically applied medicines that may help scientists when trying to develop new topical medicines. After a brief history of topical drug delivery, a review of the structure and function of the skin, routes of drug absorption and their limitations is then provided. The most prevalent diseases and current topical treatment approaches are then detailed, the organization of which reflects the key disease categories of autoimmune and inflammatory, microbial infections, skin cancers and genetic skin diseases. The complexity of topical product development through to large scale manufacture along with recommended risk mitigation approaches is then highlighted. As such topical treatments are applied externally patient preferences along with the challenges they invoke are then described and finally the future of this field of drug delivery is discussed with the emphasis on areas that are more likely to yield significant improvements over the topical medicines in current use or would expand the range of medicines and diseases treatable by this route of administration.

**SIGNIFICANCE**

This review of the key aspects the skin, its associated diseases and current treatments along with the intricacies of topical formulation development should be helpful in making judicious decisions about the development of new or improved topical medicines. These aspects include the choices of



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the active ingredients, formulations, the target patient populations preferences and limitations and the future with regards to new skin diseases and topical medicine approaches.

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## I. INTRODUCTION

The following provides a very brief account of the history of topical therapies and is primarily based on modern interpretations of historical documents such as Sumerian clay tablets, Egyptian hieroglyphs and Greek or Roman papyri. Such antiquities can be notoriously difficult to interpret and so are indicative of ancient practices rather than unassailable evidence. However, it is safe to assume that skin medicaments have been ubiquitous throughout human history and have been developed by civilisations throughout the world.

The history of topical treatments dates to the first human records (Figure 1). Perhaps the earliest evidence (~3,000 BCE) is from well preserved bodies and associated paraphernalia in China, Europe and South America that are indicative of tattooing, bloodletting or acupuncture using crude instruments fashioned from shards of stone, bone and wood (Dimitrov et al., 2017) It is tempting to speculate that the presence of non-pigmented plant material on such archaeological specimens is suggestive of their use for cutaneous or transdermal drug delivery. At around the same time, opioid salves were likely used by the Egyptians and Sumerians (Finch & Drummond, 2015).

An ever-present threat to our ancient ancestors would have been bacterial infection of wounds. There is evidence, reviewed by (Whitaker et al., 2007), that the Aborigines, Myanmar, and Mayan civilisations practiced larval therapy (maggots) to treat such injuries. Indeed, the prevention and treatment of infected wounds is a recurring theme in the historical records and there is speculation that ointments and poultices containing cannabis were recognised for their anti-microbial effect as early as ~2,000 BCE (Río et al., 2018; Russo, 2007) Aloe vera (first described in Sumerian tablets around 2100 BCE (Anon, 2016) also has a long history of use for treating infected wounds (Sánchez-Machado et al., 2017). Incidentally, the Sumerians are also thought to be amongst the first to develop soap (~3,000 BCE), with subsequent methods outlined by the Babylonians (~2,000 BCE) and Phoenicians (~600 BCE) (Routh et al., 1996). Other treatments for skin infections dating from ~1700 BCE include honey, mouldy bread (possibly due to the presence of penicillium) and onions (which contains various bactericidal compounds such as alliin and allicin), with castor oil and



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willow extract as anti-inflammatories (Hartmann, 2016) and menthol (extracted from the plant genus Mentha) for analgesia (Finch & Drummond, 2015).

A fascinating historical aspect is the combined use of photoreactive substances and sunlight, which predates modern phototherapy for dermatological conditions by over 3,500 years. Such treatments are described in Indian (1,400 BCE) and Chinese (950 BCE) texts, where plants containing psoralens were administered to patients with vitiligo and leukoderma prior to exposure to sunlight (Fitzpatrick & Pathak, 1959; Roelandts, 2002). At around the same time (~1500 BCE), cosmetic skin peels were being used for the treatment of wrinkles and contained, amongst other ingredients, ox gall, bastard sponge, raisins, mustard, barley, Euphorbia, sea salt, bran, pumice, burnt sepia shells and ostrich eggshell flour (Ursin et al., 2018).

Whilst its first use dates to the bronze age, tar produced from pine and birch trees has likely been used in the Scandinavian region since ~1,000 BCE as a treatment for various dermatological conditions such as dermatitis, psoriasis, chronic lichen simplex, sunburn and other pruritic conditions (Al-Asmari et al., 2014; Barnes & Greive, 2017).

The classical antiquity period (700 BCE – 150 CE) includes multiple dynasties and empires (e.g., Assyrian, Carthaginian, Chinese, Egyptian, Greek, Persian, Phoenician, Roman, etc.) and, correspondingly, a wealth of dermatological and cosmetic formulations, with ingredients ranging from pharmaceutically based plant extracts to the rather surreal (

Table 1). Of particular note from this era is the development of an opium patch, known as Olympic Victor's Dark Ointment; OVDO (Finch & Drummond, 2015; Harrison et al., 2012). The formulation comprised zinc carbonate, gum Arabic, antimony, saffron, myrrh, mastic gum, zinc oxide, frankincense, Aloe vera, beaten egg and raw opium (~10% w/w morphine). The rheological properties of the formulation enabled good application and adhesion to the skin and recent work has indicated that the transdermal delivery rates of morphine from OVDO are broadly comparable with modern day transdermal patches.



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Of historical interest is the misconception that Cleopatra VII bathed in soured donkey's milk as a skin peel to improve complexion. The scientific rationale is that soured milk will contain elevated concentrations of alpha-hydroxy acids such as lactic acid, which cause superficial removal of the epidermis (Fischer et al., 2010). Whilst Cleopatra (69 – 30 BCE) may not have 'immersed' herself in this particular practice, there is evidence that the second wife of the Roman emperor Nero (Poppaea Sabina, 30 – 65 CE) bathed in the milk of five hundred donkeys (Ursin et al., 2018).

Advice on skin care was provided by the Roman poet Ovid (Publius Ovidius Naso, 43 BCE – 17 CE) in the third book of his trilogy "Ars Amatoria". However, interpretation of this text, which suggest the use of crocodile dung to lighten the skin, is a matter of some debate (Hendry, 1995). In sweet contrast, liquorice mixed with honey was described in the Roman texts of Dioscorides and Plinius the Elder as a topical treatment for warts and ulcers (Fiore et al., 2005).

Unani medicine, a traditional medicine established in South Asia (~1100 CE) espoused a range of herbal remedies for skin conditions, including seeds of the Abrus precatorius (anti-suppurative, wound healing), ammonium chloride (anti-bacterial), camphor (cooling, anti-inflammatory) and black nightshade (anti-inflammatory, anti-bacterial) and have been suggested for the modern treatment of acne vulgaris (Ansari, 2019).

The history of topical medicaments may have many oddities, but some of the traditional dermatological therapies listed above contain pharmaceutically active ingredients which have been adopted into dermatological formulations (Table 2). A prime example is the humble fig (Ficus genus) which is known to contain a range of bioactive compounds (Badgujar et al., 2014; Cheng et al., 2020) that have been investigated for dermatological conditions such as warts (Bohlooli et al., 2007), seborrhoea (Ditthawutthikul et al., 2021), acne vulgaris(Pasalar et al., 2022), malignant melanoma (Conforti et al., 2012) alopecia (H.-J. Lee et al., 2019), eczema (Abbasi et al., 2017), wound healing (Trombetta et al., 2006) and cutaneous infection (Khémiri et al., 2019).

Our modern day understanding of the benefits of topical dermal delivery has been well established and include:

- Direct access for localised delivery to the pathological site.



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- The avoidance of first pass metabolism and other variables associated with the GI tract such as pH, gastric emptying time and enzymatic degradation.
- Reduction of adverse side-effects associated with systemic toxicity.
- Non-invasive administration, with consequent improved patient compliance.

Correspondingly there have been several important breakthroughs over the last seventy years that involve the direct application of medicines to the skin, including:

- Bacitracin (cyclic polypeptide antibiotics) for topical treatment of acute and chronic skin infections (1948).
- Introduction of topical steroid (hydrocortisone) (1951).
- Treatment of acne vulgaris with tretinoin (1971).
- Bactroban (mupiricin) for treatment of infected skin lesions (1987).
- Approval of Dovonex (calcipotriol/ calcipotriene active form of Vitamin B) for the treatment of psoriasis (1991).
- Use of Differin (0.3% adapalene) gel as a lower potency retinoid to reduce irritation in treatment of acne vulgaris (1996).
- Luxiq (betamethasone Valeriate) approved as the first foam formulation for topical application (1999).
- Approval of DUAC (a combination of 1.2% clindamycin phosphate and 5% benzoyl peroxide) gel for the topical treatment of acne vulgaris (2000).
- The introduction of tacrolimus (Protopic®) ointment, a non-steroid topical agent for use in atopic dermatitis (2000), with subsequent approval a year later for a second agent, pimecrolimus (Elidel®) cream for the same indication.
- Evoclin (clindamycin phosphate foam) approved for treatment of acne vulgaris (2004).



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- Approval of Aldara (Imiquimod) for Actinic keratoses (AKs) and then basal cell carcinoma (BCC) (2004).
- Lamisil once (terbinafine) as the first 'once only' treatment for Athlete's Foot (2005).
- EUCRISA™ (crisaborole), a novel non-steroidal topical ointment for mild to moderate Atopic Dermatitis (2016).
- Opzelura™ (ruxolitinib) cream (2021) for the short-term and non-continuous chronic treatment of mild to moderate atopic dermatitis (AD). Later approved for repigmentation in nonsegmental vitiligo (2002).
- Approval of VTAMA® (tapinarof) cream, 1%, an aryl hydrocarbon receptor agonist, indicated for the topical treatment of plaque psoriasis in adults (2022). VTAMA® cream is the first and currently the only FDA-approved steroid-free topical medication in its class.
- Vyjuvek, a herpes-simplex virus type 1 (HSV-1) vector-based gene therapy, approved for the treatment of wounds in patients 6 months of age and older with dystrophic epidermolysis bullosa (DEB) with mutation(s) in the collagen type VII alpha 1 chain (COL7A1) gene (2023).

Although the number of medicines for application to the skin remains relatively small compared to the more conventional oral or IV routes, market research predicts future growth in the topical drug market due to the growing elderly population and age-related dermatological conditions. The body also starts to encounter several other problems as the population ages, such as delayed wound healing, greater sensitivity to ultraviolet (UV) radiation, increased susceptibility to infections, and diminished subcutaneous fat. It is estimated that this market was worth ca. $15-50 billion in 2021, with a predicted compound annual growth rate of 4 – 9 % to 2030.

Regardless of market size and predicted growth, skin disorders represent a significant portion of total global diseases, affecting hundreds of millions of people worldwide with up to 70% of people in developing countries suffering skin disease at one time and 20% of all children having some



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form of dermatitis (eczema). In the developed world, 15% to 30% of outpatient medical care in health systems of developed countries, incorporating a wide arsenal of diagnostic, therapeutic, and aesthetic resources. In fact, skin conditions are now recognized as the fourth leading cause of nonfatal disease burden worldwide in disability-adjusted life years (DALYs) (Karimkhani et al., 2017), with females tending to have a higher burden of disease than males.

The purpose of this review is designed to provide an overview of the structure and function of the skin and diseases that affect it, along with current and future topical treatments and future and critical processes required to move new treatments from the petri dish to the patient.

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## II. STRUCTURE AND FUNCTION OF THE SKIN

### *A. Introduction*

The skin is a large, complex organ covering the entire body surface to protect against physical, chemical and biological challenges and plays key roles in moisture retention, thermoregulation, metabolic and immunologic functions, and sensory perception. In essence, skin comprises three main layers: hypodermis, dermis and epidermis. The hypodermis (subcutaneous layer) with the dermis, form the major part the skin and comprise adipose and connective tissue elements, respectively (Figure 2) and are well vascularised. The overlying, avascular epidermis consists of primarily of keratinocytes and is delineated into four layers, viz., the stratum basale; stratum spinosum; stratum granulosum and stratum corneum (Marieb & Hoehn, 2018). A further layer, the stratum lucidum, is seen in thickened skin regions, such as the soles of the feet and palms of the hand, but is difficult to detect microscopically (Corcuff et al., 1993; Delgado-Charro & Guy, 2002). Importantly, a further superficial layer, that is often overlooked for topical drug delivery, is the 'acid mantle'. The acid mantle is a complex mixture of sebum and residual skin and environmental debris and is the first point of contact for a topically applied xenobiotic which could act as potential barrier for topical drug delivery (Chilcott, 2008). Additionally, proteolytic products from filaggrin (which act as a natural moisturizing factor) are present within the stratum corneum and may form part of this mixture (Shetage et al., 2014). Filaggrin is a protein made in the viable epidermis which is subsequently hydrolysed to produce a complex mixture of hygroscopic free amino acids, amino acid derivatives and salts which together constitute the natural moisturising factor (NMF) which is mainly responsible for maintaining the water content of the stratum corneum and contributes to skin barrier function (Arezki et al., 2017).

The skin's role as a chemical barrier is bi-directional, controlling the loss of water, electrolytes and other constituents from the milieu interior whilst concomitantly reducing the entry of harmful or unwanted xenobiotics from the environment (Barry, 2001; Walters & Roberts, 2002). Microbiologically, it prevents the penetration of microorganisms (bacteria and viruses) and also protects against ultraviolet radiation through the action of melanocytes in the basal layer (Walters



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& Roberts, 2002). Changes in these features have been reported to promote the ingress of foreign bodies, such as allergens, and may contribute to the development of inflammatory skin conditions (Cork et al., 2009).

The excellent barrier properties of the skin are one of the major limitations of both topical and transdermal drug delivery, further confounded by the inherent inter- and intra-variability of skin permeability (Southwell et al., 1984) and skin sensitivity upon application of certain drugs/excipients (Carmichael, 1994). In addition, some areas of the body such as the load bearing soles of the feet and the palms of the skin have a different structure (typically thicker stratum corneum and presence of an additional layer, the stratum lucidum) in comparison with other areas, such as the lips, where the stratum corneum is thin. An understanding of the structure and function of healthy skin must first be understood to appreciate the skin barrier properties and how this is affected by diseases and the action of topical treatments. However, it is also important to know that these properties are not uniform and can vary significantly due to body site, gender, age, race and disease (Brown & Williams, 2019).

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### *B. Healthy skin structure and function*

#### i. *The subcutaneous fat layer*

The subcutaneous tissue, also called the hypodermis, is the deepest (innermost) layer of the skin beneath the dermis (Walters and Roberts, 2002) and can be several millimetres thick. g Fat microlobules and fibrous collagen are its main constituents, but it also houses the blood vessels, lymphatics and nerves. Its functions are to protect against physical trauma to underlying tissues, store energy, insulate the skin and provide a connection to underlying structures such as muscle and bone while allowing movement over them (Jepps et al., 2013). The thickness of this layer depends on sex, age, endocrine levels and nutritional status (Kanitakis, 2002). In addition, if an API reaches this layer, it is typically considered to have already entered into the systemic circulation, unless it is protein bound. As such, subcutaneous injections have been extensively utilised as a delivery route to circumvent low oral bioavailability and when oral dosing is not well tolerated and, in some cases, to modify or extend the release characteristics in efforts to prolong systemic exposure (McLennan et al., 2005).

#### ii. *The dermis*

Overlying the hypodermis, the dermis is typically 3 – 5 mm thick and contains fibroblasts along with macrophages, mast cells and a large population of resident T-cells. The dermis is composed of two heterogenous layers; lower (reticular) and upper (papillary) of the dermis. The reticular layer is the deep layer of the dermis that supports the blood vessels, lymphatics, nerves, hair follicles, sebaceous glands, and the sweat glands. The reticular layer is composed of connective tissue, predominantly collagen fibres for mechanical strength, and elastic fibres for flexibility that are embedded in an amorphous gel-like matrix that contains water, glycoproteins and glycosaminoglycans (e.g. hyaluronic acid and dermatan sulphate) with smaller amounts of heparan sulphate and chondroitin sulphate. The papillary layer is named after the dermal papillae that interconnect the dermis with the ridges of the epidermis and overlays the reticular layer. The papillae is made up of more loosely arranged collagen and functions increasing the surface area



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between the dermis and epidermis and contain terminal networks of capillaries which increase the exchange of nutrients, waste products and oxygen between the two layers. This rich blood supply also promotes systemic delivery of drugs applied to the skin by removing permeants rapidly from the dermal-epidermal boundary. The interdigitation of the dermal papillae and epidermal rete ridges provides added mechanical strength.

The vasculature in the dermis helps to regulate body temperature whilst supporting the absorption of oxygen and nutrients to the tissue and removing toxins and waste products. The rich blood flow allows for efficient removal of compounds from the dermis to maintain "sink conditions", providing the driving force for diffusion. The lymphatic system extends to the epidermal/dermal junction to facilitate waste removal, immunological responses to microbial assault and the removal of permeated molecules from the dermis. (Cross & Roberts, 2011) showed that whilst dermal blood flow affected the clearance of relatively small solutes, such as lidocaine, lymphatic flow was a significant determinant for the clearance of larger molecules such as interferon.

Three main appendages found on the surface of human skin originate in the dermis and are detailed below:

*Pilosebaceous unit*

The pilosebaceous unit is composed of the hair follicle, the hair shaft, the adjacent arrector pili muscle and the sebaceous gland. As the unit pass through the stratum corneum and epidermis, the hair follicle offers a potential route for local (e.g. acne, folliculitis, etc), topical and transdermal delivery and has been considerably investigated.

The hair shaft consists of two to three layers; the cortex or the hair's protective layer, the cortex providing the bulk of the hair, and sometimes the medulla (thicker hairs). The outer root sheath is a keratinised layer that is continuous with the epidermis and is thus considered the most likely contributor for drug diffusion. The infundibulum is the outer a part of the hair follicle extending all the way down to the sebaceous duct where the hair shaft isn't always in close contact with the pores and skin and can move quite freely. The thickness of the stratum corneum decreases deeper



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inside the infundibulum, resulting in a potential for increased drug diffusion due to the reduced barrier. There is some evidence to suggest that hair may provide a reservoir for lipophilic chemicals that may be available for subsequent dermal absorption.

Each hair follicle is associated with one or more sebaceous glands comprised of sebocytes that are responsible for producing sebum which is a mixture of lipids including cholesterol, squalene, triglycerides and free fatty acids (Patzelt & Lademann, 2013). The primary function of the sebum is to lubricate the hair and skin to prevent from drying out and the sebum also functions as an antimicrobial due to its low pH (ca. 5.5). The highest density of sebaceous glands is on the face and scalp, while the palmoplantar regions are completely devoid of sebaceous glands.

*Eccrine (sweat) glands.*

The eccrine glands are coiled glands found in the majority of the body's surface at a density of 100-200 cm/cm$^2$. These coiled glands are typically 500 – 700 µm in diameter primarily located in the dermis or hypodermis with a duct that empties on the surface of the skin. These glands function to produce a slightly acidic solution or sweat to help with thermoregulation through evaporation. These glands are innervated through the sympathetic nervous system in response to increases in temperature, exercise, and glands located in the palms and soles of the feet in response to emotional stress.

*Apocrine glands*

The apocrine glands are only located in certain areas of the skin found primarily in the axillae, ear canal, nipples, eyelids, and anogenital regions. The apocrine glands are similar in size to an eccrine gland but secrete through much larger ducts into the upper regions of the hair follicle. The apocrine glands secret a "milk" protein which is a mixture of protein, water, salts, and carbohydrate waste. These secretions provide the odour of "sweat" by acting as the nutrients for skin bacteria and bacterial decomposition.



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These appendages offer a potential route of drug delivery by offering a route for compounds to bypass the protective barrier of the stratum corneum to deeper layers of the skin. These "shunt routes" may also be responsible for any drug permeation shortly after application, since permeation through the bulk stratum corneum typically has a time lag. The shunt route may also be important for large polar molecules. However, for most permeants, the available fractional area offered by these shunt routes is relatively small and so the main pathway for molecules to traverse the tissue remains across the bulk of the skin surface. The mechanisms by which molecules traverse human skin, including the influence of shunt route transport, are discussed later.

*Basement Membrane (dermal-epidermal junction)*

The basement membrane separates the dermis from the epidermis serving as the dermal-epidermal interface or junction. The basement membrane consists of collagen, laminins, nidogens, and perleclan. Electron microscopy has shown that the basal lamina can be divided into two layers the lamina densa and the lamina lucida. The basement membrane acts as a support for keratinocytes while also functioning as a mechanical barrier for cell trafficking for cells migrating from the dermis. The dermal-epidermal membrane also has a regulatory role in cell proliferation and a barrier to the diffusion of large molecules from the epidermis to the dermis.

iii. *The epidermal layer*

The principal barrier properties of the skin have been shown to lie predominantly in the outer epidermal layer, initially postulated by Homelle (1853) and Duriau (1856) who found that the skin was not completely impermeable. The epidermal layer can be considered as several histologically distinct layers as depicted in Figure 3: The stratum basale (also called the stratum germinativum), stratum spinosum, stratum granulosum, stratum lucidium (where present) and the outermost stratum corneum (Wickett & Visscher, 2006.)



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*The stratum basale (stratum germinativum or, more commonly, basal layer)*

The stratum basale is a single cell layer of columnar or cuboidal cells attached via hemidesomsomes to the basement membrane using intergrins on the basal surface to adhere the cells to the extracellular matrix. The stratum basale is connected to the stratum spinosum by desmosomes. The keratinocytes of this layer are metabolically active as they contain the typical organelles such as mitochondria and ribosomes. As such, this is the only layer that undergo cell division or proliferation, where a daughter cell remains while the other cell migrates upwards through the epidermis towards the skin surface. The continuous replication of the columnar keratinocytes in this layer is a result of the pluripotency, and so this layer has been used as a source of non-embryonic stem cells for regenerative medicinal purposes (Dinella et al., 2014). At this anatomical level, the extensive keratin network is made up primarily of keratins K 5 and K 14 which prevent water loss and the adsorption of foreign substances (Morganti et al., 2001).

The stratum basale contains omelanocytes which synthesise melanin in two forms eumelanin and phaeomelanin. The more common eumelanin is brown/black while the less common is phaeomelanin which is red or yellow where melanocytes contain a mixture of the two in differing ratios from person to person. The pigment granules to pass from the melanocytes to the keratinocytes through dendritic connections to adjacent, and this allows. On facial skin, there may be up to one melanocyte for every five basal layer keratinocytes, but on less exposed surfaces, such as the trunk, this ratio may be only one melanocyte to twenty keratinocytes. Melanocyte presence appears to be inducible with chronic exposure to light increasing the relative proportion of the pigment forming cells within the basal layer. As they absorb UV radiation and are free-radical scavengers, they provide an energy sink. The number of melanocytes between darker and lighter skin types are similar however the melanocytes in darker skin types are more active and efficient.

Langerhans cells, which derive from bone marrow, are also found within the stratum basale (and in the overlying stratum spinosum as well as the papillary dermis) and are recognised as the major



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antigen-presenting (dendritic) cells. Antigens readily bind to the cell surfaces and, although Langerhans cells are not themselves efficiently phagocytic, they can present the antigens to lymphocytes at local lymph nodes. Clearly, when compared to other membranes of the body, the skin is exposed to many potential antigens and hence the Langerhans cells play an important role in conditions such as allergic contact dermatitis.

One other specialised cell type is found within the basal layer is the Merkel cell, which are associated with nerve endings, found on the dermal side of the basement membrane, and are found in greatest numbers around the touch sensitive sites of the body, such as the lips and fingertips.

*The stratum spinosum (spinous layer / prickle cell layer)*

The stratum spinosum is on top of the basal layer, and together these two layers are termed the Malpighian layer. This spinous layer consists of 2-6 rows of keratinocytes that change morphology from columnar to polygonal cells. Here, the keratinocytes begin to differentiate and synthesise keratins that aggregate to form tonofilaments. Desmosomes connecting the cell membranes of adjacent keratinocytes are formed from condensations of the tonofilaments, which help to maintain a distance of approximately 20 nm between adjacent cells.

The cells become “spinous” due to the more prominent desmosomes on the cell surface which gives this layer its name (Delgado-Charro & Guy, 2002). Once cells reach the spinosum, their capacity to divide is lost and the synthesis of the keratins K5 and K14 (Section 1.2.3.1) is replaced with the accumulation of keratins K1 and K10 to form filaments. Cornified envelope precursors begin to appear in the cells in the form of lamellar granules and the enzyme transglutaminase and the proteins loricrin and involucrin are also formed, which help to form the insoluble cornified envelope in the later stages of differentiation (Morganti et al., 2001).

*The stratum granulosum (granular layer)*

As the keratinocytes pass from the stratum spinosum to the stratum granulosum, they continue to differentiate, synthesise keratin and start to flatten. Only 1-3 cell layers thick, the stratum granulosum is characterised by the presence of dense, irregular amorphous deposits known as



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keratohyalin granules - that contain profilaggrin, loricrin, cysteine-rich progein and keratin. Profilaggrin facilitates the bundling of keratin that cause the corneocytes to flatten. Loricrin is an insoluble (hydrophobic) protein which undergoes polymerisation (via cross-linking) to provide mechanical strength. Membrane-coating granules (lamellar bodies) are also synthesised, probably in the endoplasmic reticulum and Golgi apparatus, and contain the lamellar subunits arranged in parallel stacks as precursors for the intercellular lipid lamellae seen in the stratum corneum. As the cells approach the upper layer of the stratum granulosum., the lamellar bodies are extruded from them into the intercellular spaces. At this point lysing enzymes are released which start to degrade viable cell components such nuclei and organelles. Subsequently the keratinocytes become flattened and compacted in the form of non-viable corneocytes.

The stratum granulosum keratinocytes are classified by the two types of granules that are formed within the cells; body rich protein keratohyaline granules and lamellar granules containing lipids (Wickett & Visscher, 2006). The protein granules contain profilaggrin and loricrin. These proteins are rendered insoluble by the transglutaminase cross-linking occurring at this stage. The granules start to migrate to the outside of the keratinocytes to exit the surface of the cell, which also allows the cells to lie parallel to the surface of the skin. More lamellar bodies which are actively synthesising these lipids also concentrate in the upper part of the granular cells (Elias, 1991). These lamellar bodies contain multi-layered stacks of flattened lipid vesicles which contain the lipid precursors for the intercellular lipid matrix of free fatty acids, glycerol and ceramides (Menon et al., 2012) found in the SC. The vesicles are extruded from the cells as they approach the upper region of the granule layer. Though only a few cells thick, the complex biotransformations occurring in the stratum granulosum and their regulation are essential in the formation of a fully functioning stratum corneum barrier (Wickett & Visscher, 2006).

*The stratum lucidum*

The stratum lucidum, sometimes known as the transition zone (Corcuff et al., 1993; Delgado-Charro & Guy, 2002), is a thin and translucent layer (hence the name) three to five cells thick.



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Here, the cell nucleus disintegrates and there is an increase in keratinisation of the cells; the cells contain eleidin, derived from keratohyalin which is then converted to keratin in the upper stratum lucidum and stratum corneum. Concomitantly, the cells undergo further morphological changes and flatten, potentially entering apoptosis. Eventually, the membrane coating granules fuse with the cell membrane to release their contents (Delgado-Charro & Guy, 2002). Occasionally, droplets of an oily substance may be seen in this cell layer, probably arising from exocytosis of the lamellar bodies. The stratum lucidum is most clearly observed in relatively thick skin specimens, such as from the load-bearing areas of the body (soles of feet and palms). Indeed, some researchers question whether this layer is functionally distinct from the other epidermal layers, or if it is an artefact of tissue preparation (Williams, 2003b). Many researchers tend to view the stratum lucidum as the lower portion of the stratum corneum and hence combine these two layers together.

*Stratum Corneum*

Extensive research was conducted in the first half of the 20$^{th}$ century to further isolate the skin's barrier function to a sub-layer of the epidermis. In 1944, following partial and full removal of the outermost skin layer by sandpapering and correlating this change with an increase in transepidermal water loss (TEWL) the stratum corneum finally emerged as the principal contributor to epidermal barrier function (Winsor, 1944).

The stratum corneum, the end-product of epidermal differentiation, is a 10-20 μm thick heterogeneous layer with a highly complex composition (Bouwstra & Gooris, 2010) and is fundamental to the skins defence. The exact structure and composition of the stratum corneum has been widely discussed with many studies aiming to elucidate its structure, characterise the lipid composition and correlate these features with a healthy barrier performance. The upper layer of the stratum corneum is known as the stratum dysjunctum, and normally consists of ~3-5 cell layers that are actively undergoing desquamation (sloughing). The lower layer of the stratum corneum, the stratum compactum, is thicker and more densely packed and has a higher density of corneodesmosomes as serine, cysteine and aspartic proteases are excreted into the extracellular



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spaces to cleave desmosomes for desquamation. Typically, it takes 14 days for a daughter cell from the stratum basale to differentiate into a stratum corneum cell, and the stratum corneum cells are typically retained for a further 14 days prior to shedding.

The 'bricks and mortar' model presented by Michaels et al. (1975) characterised the structure of the stratum corneum as an arrangement of anucleated, keratin rich corneocytes (analogous to bricks) separated by a heterogeneous lipid-enriched extracellular domain (mortar) (Figure 4). Serre et al., (1991) later added to this model, suggesting the presence of specialised desmosomes (corneodesmosomes) which are incorporated into the corneocyte envelope, promoting cohesion of the stratum corneum by binding together adjacent corneocytes.

As previously stated, the corneocytes of the stratum corneum arise from differentiation of keratinocytes at the stratum granulosum level and TEWL can, in part, be influenced by the constituents of these cells, which help to control water flux and maintain adequate hydration of the stratum corneum (Rawlings & Harding, 2004). Filaggrin, a filament aggregating protein, plays a vital role in corneocyte production as it encourages the aggregation of keratin intermediate filaments, promoting the collapse of corneocytes into flat cells for organised packing (Wickett & Visscher, 2006). The subsequent organised 'brick' like packing of corneocytes provides a tortuous pathway for molecules, increasing the diffusional path length and promoting the stratum corneum's defensive properties (Rawlings & Harding, 2004). Following the production of corneocytes, filaggrin is degraded into amino acids which are later involved in the formation of natural moisturising factors (NMFs) (Dale et al., 1997). NMF (a mixture of amino acids, amino acid derivatives and salts, along with the intercellular lipid matrix) contributes to the water retaining functions of the stratum corneum which is vital to maintaining stratum corneum elasticity and flexibility (Jokura et al., 1995). Filaggrin is particularly important when considering the altered barrier function in diseased skin as there a clear association between filaggrin mutations (resulting in loss of function) and atopic dermatitis (Harding & Rawlings, 2005; Kezic et al., 2008; Seguchi et al., 1996). In particular, a loss of function filaggrin mutation has been associated with a reduction in NMFs, corneocyte size and elevated TEWL (Cork et al., 2003).



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Furthermore Nemoto-Hasebe et al., (2009) reported a correlation between filaggrin related atopic dermatitis and the severity of the condition, postulating that these mutations may play a crucial role in the pathogenesis of atopic dermatitis. Similarly, Menon et al. (2012) reported that the disruption of the fillagrin gene expression, leads to a defective corneocyte envelope and loss of water retention, such as those seen in ichthyosis vulgaris as well as atopic dermatitis.

The intercellular lipid matrix of the stratum corneum is comprised predominantly of three main lipid classes: cholesterol, ceramides and free fatty acids (FFAs) (Jungersted et al., 2010). Cholesterol provides support and stabilisation of the stratum corneum lipids, the synthesis of which involves the enzyme 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase). It has been shown that an up regulation of HMG-CoA reductase occurs in the skin following an acute barrier disruption consequently affecting the cholesterol levels in the stratum corneum and stabilisation of this layer (Harris et al., 1997).

Formation of lipid bilayers within the intercellular matrix is thought to be partially attributed to the presence of FFAs within the stratum corneum and this lipid class is the primary source of ionisable head groups and has a role to play in stratum corneum structure and surface pH (Jungersted et al., 2008; Wickett & Visscher, 2006). The role of FFAs in healthy skin and their effects on barrier function when absent were assessed by Mao-Qiang et al., (1993) who found that, after barrier disruption, inhibition of fatty acid synthesis significantly impaired barrier recovery but topical application of FFAs improved recovery. These findings were supported by Fluhr et al., (2001) who also attributed structural changes in the stratum corneum to low FFA content.

The role of ceramides within the stratum corneum has been of specific interest when determining stratum corneum structure, function and barrier properties due to the great differences in their chemical structure. Bleton et al. (2001) suggested the presence of over a thousand different ceramides structures within the stratum corneum. Until recently, ceramides have been categorised according to nine structural classes following a classification system proposed by (Motta et al., 1993). However, Masukawa et al. (2008) extended this system upon the discovery of a new



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sphingoid base, dihydrosphingosine (dS), resulting in two new structural classes and hypothesised the presence of another ceramide class which was later confirmed by van Smeden et al.(2011).

Ceramides are currently grouped according to 12 subclasseses. Although with continual improvements in the techniques for ceramides characterisation it is expected that this will expand even further. At present, all structures present a sphingoid base coupled to a fatty acid chain. Sphingoid bases can be classed as one of four structures: sphingosine (S), phytosphingosine (P), 6-hydroxyspingosine (H) or (dS). These bases are coupled to one of three fatty acids which vary in chain length, non-hydroxy (N), α-hydroxy (A) or ω-hydroxy (EO), EO fatty acid chains have been found to link to linoleic acid, increasing the chain length and further supporting stratum corneum lipid packing and are of particular importance when attempting to understand the development of atopic dermatitis. Macheleidt et al. (2002) compared the percentage weight of EO ceramides in the epidermis of healthy and atopic dermatitis subjects, finding a change from 52 % in healthy individuals to 10 % in atopic dermatitis sufferers, highlighting a link between EO ceramides presence and diseased skin.

The physical properties of ceramides, such as the long hydrophobic carbon chains, are also thought to aid in the formation of lamellae structures within the stratum corneum by binding adjacent corneocytes, helping to prevent TEWL and increasing the mechanical strength (Masukawa et al., 2008; Wertz et al., 1985). It is thought that as well as performing this role within the lipid matrix, ceramides such as EOS attach, to proteins of the corneal envelope through the ω hydroxy fatty acid group, providing support by anchoring corneocytes into the extracellular lipid matrix (Raith et al., 2004), a function referred to as a ‘molecular rivet’.

The formation of a healthy stratum corneum layer relies primarily on the delicate balance of lipids of the intercellular matrix and successful differentiation of cells to produce a defensive layer analogous to the aforementioned ‘bricks and mortar’ model. It is the complex nature of this heterogeneous, selectively permeable, differentiated layer that allows the stratum corneum to perform so remarkably as a multifaceted barrier (Fig. 4 and Fig. 5).



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In addition, stratum corneum barrier integrity is dependent on the water content of the tissue which acts as a plasticiser, maintains pliability and hence prevents the stratum corneum from cracking. The outermost portion of the stratum corneum has ~15% (w/w) water whereas the innermost layers typically contains twice this amount; both lower than the underlying viable dermis (70% by weight) and hence a water gradient exists through the skin. Water within the stratum corneum also mediates the activity of some hydrolytic enzymes (e.g., proteases) since environmental humidity affects the activities of enzymes involved in the desquamation process. Additionally, keratinocyte water activity regulates enzymes involved in the generation of NMF.

Overlying the outer surface of the stratum corneum is an “acid mantle”, a thin film comprising sebum, corneocyte debris and sweat components. Sebum, and specifically triglycerides, wax esters and squalene, provides the major components of the acid mantle and, given the variability in follicular density across skin sites, then clearly the composition of the acid mantle varies with anatomical region. The mantle essentially provides an acidic surface to the stratum corneum, in the region of pH 5 in contrast to the viable epidermis pH of ~7.4. The acid mantle thus provides a non-specific defence mechanism against the ingress of pathogenic organisms. If such organisms adapt to this slightly acid surface pH, such pathogens are poorly adapted to survive within the more alkaline environment of the viable epidermis.

*Skin microbiome and epidermal systems.*

The skin is home to millions of bacterial, fungi and viruses that comprise the skin microbiome. Similar to those in our gut, skin microorganisms have essential roles in the protection against invading pathogens, the education of our immune system and the breakdown of natural products as reviewed by Byrd et al. (2018) and Eisenstein, (2020). The skin microbiome is seeded at birth with the first microorganisms helping to train the immune system to tolerate commensal organisms (which have a neutral or beneficial impact on their host) while remaining alert to pathogens. These microbial communities continue to grow and diversify until puberty, when hormonal and developmental changes determine the final composition that is carried throughout adulthood. Recently evidence of extensive communication between bacteria, skin cells and



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immune cells has been discovered, which are believed to help to reinforce and repair the barrier formed by the skin, bolster the body's defences against infection and temper excess inflammation. As such some skin diseases are associated with an altered microbial state, reversion of which can provide prevention or treatment strategies (see later). In addition, such organisms can influence the delivery of some topically applied drugs.

As previously outlined, human skin contains many enzymes such as stratum corneum tryptic and chemotryptic proteases that are required for specific purposes such as desquamation. In addition, the epidermis contains many drug metabolising enzymes and histochemical and immunohistochemical methodologies suggest that the majority of these are localised in the viable epidermis, sebaceous glands and hair follicles. A common misconception is that the skin is an "inert" tissue however most phase 1 (e.g. oxidation, reduction, hydrolysis) and phase 2 (e.g. methylation, glucuronidation) reactions can occur within the skin, though these tend to be at <10% of the specific activities found in the liver. Interestingly, esterases tend to have relatively high activities within skin and, considering that there is a large skin surface area, metabolism of some drugs can be significant in some cases/ This can be exploited in the delivery of prodrugs such as steroid esters with the increased lipophilicity afforded by an aliphatic chain improving drug uptake into the tissue where esterases can liberate the free drug within the skin. Micro-organisms present on the skin surface, such as *Staphylococcus epidermidis* may also metabolise topically applied drugs.



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## III. DISORDERS OF THE SKIN AND CURRENT TREATMENTS

The skin is the largest organ in the body and thus it is perhaps not surprising that in excess of 3,000 acute and chronic skin diseases have been described that affect individuals of all ages and social conditions. Some of them, such as skin cancer, may be life-threatening. However, even when this is not the case, they may pose a heavy burden on patients in terms of quality-of-life impairment and costs, resulting in the fourth leading cause of nonfatal burden expressed as years lost due to disability. As a result, skin disorders represent a significant portion of the global total of diseases, affecting millions of people worldwide, with up to 70% of people living in developing countries suffering from skin disease at one time. In the developed world, 15% to 30% of outpatient medical care in health systems concerns skin diseases, incorporating a vast arsenal of diagnostic, therapeutic, and aesthetic resources (Buxton & Maorris-Jones, 2009; Ferreira et al., 2021). As a result, skin conditions are an important public health concern and are the most frequent reason for consultation in general practice. However, it is important to note that many patients never seek medical advice and self-treat using over-the-counter medicines. As indicated in Figure 6, the top five skin diseases with the highest market volume in Europe and the US are:

i. Psoriasis
ii. Atopic dermatitis
iii. Acne
iv. Rosacea
v. Tinea unguium

However, in the topical market, although the highest sales volumes relate to the same skin diseases (Figure 7), the ranking is different: ￼

1. Acne
2. Psoriasis
3. Atopic dermatitis
4. Tinea unguium
5. Rosacea



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Skin diseases are a heterogeneous and multifactorial group of conditions, and although an obvious oversimplification, to provide a logical review, the most common diseases have been categorized into the following cause/descriptors: Autoimmune dysregulation and inflammatory dermatoses, infection (bacterial, viral, or fungal, parasites), cancers and orphan/genetic diseases.

## *A. Skin Inflammatory Diseases*

### *i. Psoriasis*

Based on the observations of the British dermatologist Robert Willan, psoriasis was first classified as a separate disease from leprosy by Thomas Bateman in 1836 (Nestle et al., 2009). This chronic inflammatory skin disease has a strong genetic predisposition and an autoimmune component (Armstrong & Read, 2020; Rendon & Schäkel, 2019).

#### *Epidemiology*

Worldwide, approximately 2-4% of the population has psoriasis. However, this number may vary by geographic location, being less common in Asian and some African countries but more prevalent (up to 11%) in Caucasian and Scandinavian populations (Armstrong & Read, 2020; Rendon & Schäkel, 2019). Psoriasis is mostly observed in adult men and women rather than in children. Even though psoriasis can manifest at any age, a bimodal age distribution can be fully identified at 18 to 39 years and from 50 to 69 years old (Armstrong & Read, 2020; Rendon & Schäkel, 2019). The age of psoriasis onset might be related to genetic and environmental factors; for example, the presence of the human leukocyte antigen (HLA)-C*06 allele is commonly observed in an earlier psoriasis onset age (Armstrong & Read, 2020; Rendon & Schäkel, 2019).

#### *Clinical features*

The dermatologic manifestations of psoriasis are varied, as they are highly dependent upon the psoriasis variant. Briefly, the psoriasis types can be summarized as follows:

1) Plaque psoriasis: This type represents approximately 80 to 90 % of all psoriasis



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manifestations. The classical clinical features include sharply demarcated, erythematous plaques covered in silvery scales (Armstrong & Read, 2020; Rendon & Schäkel, 2019). Most patients report that the lesions itch, burn and sting. The common affected areas include the trunk, the extensor surfaces of the limbs and the scalp (Burns et al., 2008; Rendon & Schäkel, 2019).

2) Guttate psoriasis: Characterized by the appearance of small lesions, round or slightly oval, from 2 or 3 mm to 1 cm in diameter. These are scattered evenly throughout the body. Guttate psoriasis usually affects children or adolescents - and commonly - after acute streptococcal infection (Burns et al., 2008; Rendon & Schäkel, 2019).
3) Inverse psoriasis: This variant, also called flexural psoriasis, involves the groins, vulva, axillae, submammary folds, gluteal cleft, and other body folds. Clinically, due to these regions being warm and moist environment, the psoriatic plaques are not usually scaly but macerated, often bright red and fissured. For this reason, the diagnosis might be challenging. However, the sharp delineation of the lesions enables the distinction from other pathologies, such as intertrigo, candidiasis, contact dermatitis and tinea cruris. Inverse psoriasis is more common in adults than children (Burns et al., 2008; Elston, 2010; Rendon & Schäkel, 2019)
4) Erythrodermic psoriasis: An uncommon psoriasis variant where at least 75% to 80% of the total body surface develops coalescent erythema, scales, or exfoliation. This acute condition is treated as a life-threatening emergency due to extensive electrolyte disturbances and desquamation. Two erythrodermic psoriasis forms can be identified; the first can be regarded as extensive plaque psoriasis, where the chronic lesions gradually evolve into an exfoliative phase involving most of the skin surface. The second form may appear at any time, suddenly and unexpectedly, or after a period of increasing intolerance to local applications, UV therapy and loss of control over the disease (Armstrong & Read, 2020; Burns et al., 2008; Elston, 2010; Rendon & Schäkel, 2019).
5) Pustular psoriasis: Characterized by multiple sterile pustules and erythema. Although a histological feature common to all psoriasis types includes the neutrophilic accumulation in the epidermis, clinically, the term “pustular psoriasis” is reserved for those forms of the



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disease in which pustules appear. Pustular psoriasis can be either localized or generalized. In the first type, the disease is confined to the hands and feet and tends to be chronic. In the generalized forms, the whole body may be involved, md so this condition is potentially life-threatening (Armstrong & Read, 2020; Burns et al., 2008; Elston, 2010; Rendon & Schäkel, 2019)

*Aetiology and pathophysiology*

While one variant typically predominates in a person; different psoriasis types may coexist in a person at any single point in time. Nevertheless, there are key clinical features common to most psoriasis variants, namely the presence of well-demarcated plaques and silvery scales caused by the hyperproliferation of the epidermis. These are accompanied by erythema arising from the underlying inflammatory state (Armstrong & Read, 2020; Rendon & Schäkel, 2019). These rashes tend to be dry and sore and may sometimes result in bleeding (Burns et al., 2008).

A psoriasis diagnosis typically begins with questions about the patient's health, followed by examining the skin, scalp and nails. If extra tests are required, a skin biopsy might be taken for microscopic evaluation (Mayo Clinic, 2022).

The histological analysis of the psoriatic plaque reveals the presence of acanthosis (epidermal hyperplasia) and neovascularization, accompanied by inflammatory infiltrates composed of dermal dendritic cells, macrophages, T cells and neutrophils (Armstrong & Read, 2020; Rendon & Schäkel, 2019). Taking this information into account, a closer evaluation of the psoriasis physio pathological mechanisms, which are, without doubt, highly complex and yet to be fully elucidated, enables a clear identification of both autoimmune and autoinflammatory components to the disease (Armstrong & Read, 2020; Rendon & Schäkel, 2019).

In the autoimmune component, excessive activation of parts of the adaptive immune system has a central role in the pathogenesis of psoriasis (Rendon & Schäkel, 2019). In psoriasis pathogenesis onset, several cell types, such as plasmacytoid dendritic cells, keratinocytes, natural killer T cells, and macrophages induce cytokine release, which in turn will activate myeloid dendritic cells. For instance, the reactivity to endogenous signals such as DNA-LL37 complexes stimulates



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plasmacytoid dendritic cells to secrete interferon alfa (IFN-α), which in turn will be responsible for the activation of myeloid dendritic cells, ultimately leading myeloid dendritic cells to secrete IL-12 and IL-23 (Armstrong & Read, 2020; Rendon & Schäkel, 2019a. Cytokine IL-12, due to its proinflammatory role, enhances the cytotoxic activity of T lymphocytes by stimulating their differentiation from naive T cells to $T_H1$ cells. On the other hand, IL-23 has a key role in the survival and proliferation of $T_H22$ and $T_H17$, the latter being known to produce Interleukin-17 (IL-17) and IFNγ-producing $TH_1$ cells. Keratinocytes respond to these activated T cells and cytokines by increasing proliferation and producing additional proinflammatory cytokines and chemokines. These keratinocyte-derived effectors act upon dendritic and T cells, perpetuating a chronic inflammatory state (Rahman et al., 2015). Even though other pathways are involved in psoriasis, the IL-23-mediated activation of the $T_H17$ pathway is considered highly relevant (Armstrong & Read, 2020; Rendon & Schäkel, 2019). The inflammatory traits of the disease are interconnected with IL-23 signalling. As the activation of this pathway is mediated intracellularly via Tyk2-Jak2 and STAT3, the transcription of key inflammatory mediators takes place (Armstrong & Read, 2020; Rendon & Schäkel, 2019). These cytokines are responsible for the downstream keratinocyte proliferation, increased expression of angiogenic mediators, endothelial adhesion molecules and infiltration of immune cells into the skin lesions (Armstrong & Read, 2020; Rendon & Schäkel, 2019). Considering this background, psoriasis can be defined as a complex inflammatory disease (Burns et al., 2008). Psoriasis treatment aims to prevent the fast growth of skin cells, treat the skin inflammation and simultaneously remove the current scales from the skin.

*Disease management and therapeutic approaches*

Emollients, like ointments, play a major role in the maintenance therapy of inflammatory skin conditions. These products are designed to treat the symptomatic manifestations of inflammatory skin conditions by creating a skin barrier, reducing TEWL and improving stratum corneum hydration and restoring the integrity of the skin barrier (Loden & Maibach, 1999; Rawlings & Harding, 2004).). This occlusive effect is best achieved using large molecular weight hydrophobic ingredients such as paraffins. and may also contain hygroscopic excipients, such as urea and



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glycerol. These ingredients display water-binding capabilities to varying extents (Lodén, 2003). Collectively, these mechanisms help to relieve dermatological manifestations such as dryness, stretching and itching, and at the same time also help to restore the defective skin barrier, by improving skin flexibility. The general guidance related to emollient application states that these products should be frequently applied and that a generous amount of product should be used per week (250-600 g per week) (Ersser et al., 2012). Within this scope it is also important that the patient chooses products with acceptable sensorial characteristics, as these are extremely important to treatment compliance. An additional consideration is that paraffin-based formulations may represent a fire risk when impregnated into clothing and so patients should be advised accordingly.Drug treatment options ultimately depend on the disease severity, comorbidities, and access to health care. For mild cases, topical formulations (with or without corticosteroids) are the preferred treatments and are available in a wide range of dosage forms (oils, ointments, creams, lotions, gels, foams, sprays and shampoos). Topical corticosteroids (TCS) are widely used in psoriasis management. . The introduction of hydrocortisone in the market was in the early 1950s, however since that date there several new topical corticosteroids have been introduced in the market. These ultimately led to an expansion of the therapeutic indications of TCSs (Charman et al., 2000). The majority of the currently available TCS are synthetic derivatives of hydrocortisone, in which the main modification focuses the basic corticosteroid structure. This chemical modification improves the potency, specificity and duration of action. Two widely used TCSs are mometasone furoate (potent UK classification) and clobetasol propionate (very potent UK classification). For these compounds, the specificity of the molecule was optimised through the addition of a double bond at carbon one and substitution at carbon sixteen (Wiedersberg et al., 2008). In addition, it was found that the halogenation at carbon nine, induced an increase in the lipophilicity of the molecule, which improved skin permeation, as well as extended the action duration (Ponec et al., 1981). Nevertheless, by increasing the molecular potency of the TCSs, there is also a higher chance for both local and systemic side effects. These may include skin atrophy and adrenal suppression and are more common when the TCS are applied for prolonged periods of time and in large areas of skin (Del Rosso & Friedlander, 2005). Current clinical guidelines



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suggest that TCSs prescription by the healthcare professionals should be made with full appreciation for the potency and associated side effect profiles. Clinical guidance for the use of TCS in practice often supports limiting their use, with the British National Formulary recommending that TCSs should be 'spread thinly on the skin but in sufficient quantities to cover the affected areas. The most common therapeutic regimen concerns the application of the product twice daily (Williams, 2007).

However, whilst such suggestions are typically made, clinical recommendations issued by the NHS Health Technology Assessment programme following a systematic review of the available body of clinical efficacy data support a reduced frequency of application of TCS to once daily.

In the UK, TCSs are ranked according to a 4-point potency scale: mild, moderate, potent and very potent based on molecular potency. In contrast, the United States classification system, based on the vasoconstriction assay, accounts for the effect of the vehicle on the potency of TCS; for example, mometasone furoate 0.1 % w/w cream is classified as a mildly potent TCS, whereas the equivalent strength ointment is ranked as a potent TCS. The latter classification system, accounting for the nature of the vehicle, highlights the importance of considering formulation effects and the molecular potency of a drug when determining the potency class (Afifi et al., 2017)The use of the vasoconstrictor assay to determine the extent of skin blanching and, hence, potency as a measure of pharmacodynamic effect was first described by (McKenzie A. W. (1962). The formulation effects on the extent of skin blanching induced by TCS have since been well documented. Compared to commercial TCS formulations, McKenzie (1962) found ointments to exhibit superior in vivo blanching responses compared to the equivalent cream or lotion formulations. This was further confirmed in a study documented by Poulsen & Rorsman (1980)where corticosteroid in an ointment formulation were found to be of a higher potency classification, as determined by the skin blanching assay, than the equivalent cream formulation.Stoughton (1987), later used the skin blanching assay to determine the bioequivalence of generic and trade TCSs, finding certain TCS formulations to produce inequivalent vasoconstrictive effects despite containing equal concentrations of the active drug. The



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vasoconstrictor assay has since been adopted as a means of determining the potency and bioequivalence of TCSs on the market in the USA (FDA, 1995).

There are currently 15 different proprietary TCS drugs available for UK prescribing, effective in the treatment of atopic dermatitis, seborrheic dermatitis and psoriasis (British National Formulary, 2019); these can be subdivided by formulation type and drug concentration. Of these 15 drugs, mometasone furoate, betamethasone dipropionate and clobetasol propionate are classified as potent and very potent TCSs, respectively. As such, the risk of adverse effects, both local and systemic, should be considered before prescribing (National Institute for Health and Care Excellence, 2018). It should also be noted that TCS are often combined with emollient treatment, and care needs to be given when considering the order of application and time between using the two products as the regime may not only improve the effectiveness of the TCS but also potentially reduce it (Beebeejaun et al., 2023).

Other topical therapies for psoriasis include vitamin D analogues, retinoids, calcineurin inhibitors, salicylic acid, coal tar, and anthralin and combinations thereof, such as Daivobet® (calcipotriol/betamethasone), which is currently considered the top-selling topical drug. However, this is likely to change with the recent approval of the aryl hydrocarbon receptor agonist VTAMA® (Tapinarof), the first and only FDA-approved steroid-free topical medication in its class.

In moderate to severe cases, phototherapy is usually selected, either alone or in combination with systemic immunosuppressive drugs. If both topical and phototherapies do not appear to work, or if the case is more severe, other oral or injected medications might be favoured. Oral medication can include retinoid pills, methotrexate, and cyclosporine for severe psoriasis, whilst injected medications usually concern a biologic drug or rarely a corticosteroid injection (Mayo Clinic, 2022).

Biological therapies targeting key inflammatory mediators are nowadays major treatments employed in psoriasis. The most relevant biological drugs in psoriasis management are (i) Stelara® (ustecinumab), a monoclonal antibody for (IL)-12/23; (ii) Humira® (adalimumab) human monoclonal IgG1 antibody; (iii) Skyrizi® (risankizumab), humanized immunoglobulin G1 (IgG1)



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monoclonal antibody selective for the protein interleukin (IL)-23, and; (iv) Cozentyx® (secukinumab), human monoclonal IL-17A antibody. In addition, phosphodiesterase 4 (PDE4) and JAK inhibitors, such as apremilast (Otezla®) and Xeljanz® (tofacitinib), respectively, have also gained popularity (Mayo Clinic, 2022).

*i. Atopic dermatitis*

Atopic dermatitis (AD) is a highly prevalent, chronic and relapsing inflammatory skin disease whose incidence in the past decades has been increasing, especially in developed countries (Bieber, 2022; Ferreira et al., 2021; Torres et al., 2019). AD, also known as atopic eczema, belongs to the atopic disorders spectrum, together with food allergy, allergic asthma and allergic rhinoconjunctivitis. These disorders share a common feature: the presence of Immunoglobulin E (IgE)-related allergic reactions to environmental allergens (Bieber, 2022). Even though there is a pronounced heterogeneity in the clinical manifestations, the most common features of AD include sensitive and dry skin, together with localized or disseminated eczematous lesions, which are responsible for a severe itching sensation (Bieber, 2022). These symptoms may lead to skin trauma and sleep disturbances, leading to considerable morbidity and quality of life impairment.

*Epidemiology*

The WHO Global Burden of Diseases Initiative's data reports that at least 230 million people worldwide suffer from AD, being the most common inflammatory skin disorder in the developed world (Torres et al., 2019). Even though it is more common in children (10-20%), recent evidence shows that AD is becoming increasingly prevalent in adults in developed countries, especially in the United States (US), Europe, and Japan. The maximum prevalence may reach 30% in some populations (Kapur et al., 2018; Torres et al., 2019). Several large-scale studies account for numerous genetic, social and/or environmental factors as the main drivers for the registered 2 to 3-fold AD increase in the past decades in industrialized countries (Kapur et al., 2018; Torres et al., 2019).



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Approximately 45% of AD cases appear in the first six months of life, 60% during the first year, and 85% before five years of age. AD is often followed by other atopic diseases, such as allergic rhinitis, asthma and food allergy, in the so-called 'atopic march'. This concept addresses a particular sequence of atopic diseases that precede the development of other allergic disorders later in life (Torres et al., 2019). Evidence suggests that up to 50% of children who develop AD before two years old will develop asthma during subsequent years. However, luckily, the course of AD may also present a relapsing-remitting pattern, with up to 70% of children going into clinical remission before adolescence (Kapur et al., 2018; Torres et al., 2019).

*Aetiology and pathophysiology*

The pathophysiological mechanisms that evolved in AD are not entirely understood; however, there has been a growing understanding that the atopic states and the symptoms of AD involve abnormalities not only of immune regulation but also derive from defects in skin barrier function as well as environmental and infectious agents.

The epidermal barrier in individuals with AD may be impaired due to loss of the filaggrin gene expression (see section II.B.iii). Furthermore, several reports point out that deficiency in ceramides and antimicrobial peptides such as cathelicidins are also common in AD — the compromised skin barrier results in high rates of TEWL. The inability of the skin to retain moisture also impairs its ability to protect against other irritants, allergens, and environmental factors (Burns et al., 2008).

This barrier dysfunction and an inherent hyper-reactive epidermal state activate innate and adaptive immune systems. The initial acute stage of AD is characterised by a predominantly $T_H2$-mediated inflammatory state, where the IL-4, IL-5, IL-13, IL-31, and the thymic stromal lymphopoietin (TSLP) cytokine. The release of these pro-inflammatory cytokines induces the production of immunoglobulin E (IgE) and stops the expression of loricrin and involucrin, leading to pruritus. Simultaneously, it down-regulates antimicrobial peptides, causing increased susceptibility to bacterial infections, especially by *Staphylococcus aureus* (Bieber, 2022; Torres et al., 2019). Human papillomavirus-induced warts, fungal infections, viruses (such as HSV1 and 2,



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vaccinia, coxsackie A and the pox virus of molluscum contagiosum) are also frequent pathogens in AD and may cause severe complications (Burns et al., 2008). Afterwards, as the condition becomes chronic, the disease becomes a $T_H1$-dominated inflammation where IFNγ, IL-12 and TGFß are often upregulated (Torres et al., 2019).

*Clinical features*

AD manifestations depend upon the clinical phenotype, partly conditioned by age, ethnicity and disease severity (Torres et al., 2019). The main clinical features of the disease include:

- Itching;
- Macular erythema;
- Papules or papulovesicles;
- Eczematous areas with crusting;
- Lichenification and excoriation;
- Dryness of the skin;
- Secondary infection.

AD lesions can occur on any body part. A chronic or relapsing disease course is characteristic. Flare-ups of eczematous, oozing or weeping pruritic lesions over dry skin are highly characteristic of AD acute stages. On the other hand, red or brownish patches of dry, cracked or scaly skin with lichenification and pruriginous nodules are often observed in chronic lesions (Torres et al., 2019).

As there is no specific test or pathognomonic laboratory biomarker for AD, the diagnostic is mainly supported by the presence of the characteristic clinical features, along with the existence of pruritus, disease evolution and a personal and/or family history of atopy. Several sets of criteria support disease identification; the most used are the Hanifin and Rajka criteria (Torres et al., 2019).



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*Disease management and therapeutic approaches*

As previously described, AD exhibits a broad spectrum in its clinical phenotype, derived from the complex and multidimensional interactions between all the involved mechanisms. All these pathways represent potential fields of preventive and therapeutic interventions (Bieber, 2022).

The primary goal of therapy in AD is to reduce pruritus and, at the same time, to control the disease to capacitate patients to have a fully functional life at home, work and school. Hence, typically, AD treatment includes more than one topical product. Furthermore, it is also extremely important to correctly identify the relevant external disease triggers to avoid them. These can involve (i) mechanical irritants, such as wool, irritant fabrics and fibres; (ii) chemicals, like acids, bleaches, solvents, and surfactants used in cosmetic and hygiene products; (iii) biological irritants, like allergens and microbes, and finally (iv) air pollutants, like tobacco smoke, volatile organic compounds, and traffic exhaust (Hoare, 2000).

The treatment package for AD usually comprises an emollient for skin hydration, which forms the basis of continuous management, a topical corticosteroid (TCS) to manage inflammatory flare-ups, and occasionally a topical antibiotic in cases of clinically infected skin (Hoare, 2000).

Hydrating and lubricating the skin is the backbone of therapy for patients with mild-to-moderate AD. Using moisturizers with non-aqueous emollients, occlusive agents, and humectants daily reduces AD signs and symptoms, thereby reducing the need for topical corticosteroids (Torres et al., 2019).

Topical corticosteroids (TCS) and calcineurin inhibitors are the predominant treatments for AD inflammation. Both pharmacotherapeutic classes are indicated for flare management and/or proactive therapy for long-term control (Burns et al., 2008; Torres et al., 2019). TCS represent the first line of treatment, reducing disease recurrence when used intermittently in patients with established disease. The selection of the TCS to be used largely depends upon the skin lesions location, extent, acute or chronic state, and the patient's age and disease severity (Bieber, 2022; Torres et al., 2019). Less potent topical corticosteroids should be employed on the lesions of the eyelids, face, axillae, groins, and inner thighs. Children who are less than one year old should also



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use less potent TCS due to the possibility of systemic absorption (Bieber, 2022; Burns et al., 2008; Torres et al., 2019). High-potency TCS should be employed in older patients with lichenified and chronic prurigo-like lesions and palm lesions. The top-selling AD Topical drug in North America and Europe is Eucrisa® (crisaborole), a topical phosphodiesterase inhibitor. This drug inhibits the intracellular enzyme cAMP-specific 3′,5′-cyclic phosphodiesterase 4 (PDE4), being another alternative to topical corticosteroids for individuals with mild-to-moderate AD or in anatomically sensitive sites (Kapur et al., 2018). Then, the 2nd most sold topical product is Protopic® (tacrolimus). This drug and pimecrolimus belong to the topical calcineurin inhibitors (TCIs) class. TCI are approved for both short-term and chronic intermittent use in children from age two and adults. These drugs are usually employed in sensitive skin areas, such as the face and flexural skin (Torres et al., 2019). The pharmacological mechanisms of TCI regard the inhibition of cutaneous T cell activation and proliferation; additionally, this class may also exert epidermal barrier repair actions. Their reduced efficacy sometimes constrains their clinical use, and some side effects include a burning or pruritus sensation, commonly observed during the first week of use (especially with tacrolimus), and high cost (Torres et al., 2019). The sales figures of Opzelura (ruxolitinib), a topical JAK inhibitor launched in 2022, were unavailable to the authors at the time of writing but will likely disrupt the current market.

As previously mentioned, the skin of patients with AD is often heavily colonized with S. aureus, even at uninvolved sites. Therefore, short-term topical and/or oral antibiotic therapy is often recommended. Appropriate systemic antibiotics such as first- or second-generation cephalosporins or anti-staphylococcal penicillins are frequently employed in widespread secondary infections (Kapur et al., 2018).

In the cases where AD is not responsive to topical treatment, short-term phototherapy can also be employed as an adjuvant, preferably resorting to narrow-band ultraviolet B (NB-UVB/UVB 311 nm) and medium-dose ultraviolet A1 (UVA1) light (Torres et al., 2019).

When topical therapies and phototherapy fail or become unacceptable or impractical, systemic therapy should be employed (Torres et al., 2019). Systemic non-biologic therapies usually regard nonspecific immunosuppressants, such as cyclosporine, azathioprine, methotrexate and



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mycophenolate mofetil. These are established and widely available options for severe refractory cases.

Several biological agents targeting the immune pathways stated on AD can also be employed. A relevant example regards dupilumab injection (Dupixent®), which has been showing promising results in treating moderate-to-severe AD not responsive to topical therapies, or in cases when topical drugs are not advisable.

*ii. Contact dermatitis*

Contact dermatitis is a highly prevalent inflammatory skin disease affecting an estimated 15% of the adult population over a lifetime (Johansen et al., 2022). Two primary forms of contact dermatitis can be identified: (i) Allergic Contact Dermatitis (ACD) and (ii) Irritant Contact Dermatitis (ICD). The first type is caused by repeated and direct skin exposures to contact allergens, whilst ICD, which is the most common type (covering up to 80% of patients), is caused by skin exposure to irritants (Johansen et al., 2022). In about two-thirds of contact dermatitis cases, the clinical presentation of the disease is in the hands, followed by the face and foot (Johansen et al., 2022). Due to its high prevalence, contact dermatitis is a global problem, affecting all age groups and being a significant factor in quality of life and ability to work impairment.

*Epidemiology*

Approximately 4 to 7% of dermatology consultations in secondary care regard contact dermatitis. A study by Nguyen and colleagues reports that contact dermatitis is the fifth most prevalent dermatological disease in American patients and that the associated costs with this condition are in the top eight, accounting for more than 1.5 billion US dollars in total direct medical costs per year (Nguyen & Yiannias, 2019). This skin disease is more common in young females and is thought to



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be linked to exposure to substances, specifically nickel, within certain jewellery and cosmetics (Adler & DeLeo, 2021).

ACD and ICD epidemiology depend highly on domestic and occupational exposure trends, technological developments, and regulations (Johansen et al., 2022). Therefore, it is pretty challenging for qualified dermatologic work groups to be continuously updated on the trends and new sources of the disease, especially ACD. Nevertheless, surveillance networks have successfully identified contact allergens related to significant morbidity. Furthermore, mechanisms are now in place to closely monitor the beneficial effects deriving from the restructured regulation on consumer products (Johansen et al., 2022).

*Aetiology and pathophysiology*

Advances in immunology have enabled a better understanding of the complexity and heterogeneity underlying contact dermatitis. As previously mentioned, ICD is caused by xenobiotics' proinflammatory and noxious effects, commonly present in soaps, detergents, and solvents. On the contrary, ACD is a type-IV or delayed-type hypersensitivity reaction caused by low molecular weight (LMW) reactive chemicals (<1000 Da, also called haptens) (Brites et al., 2022). Generally, haptens display lipophilic residues and electrophilic moieties, making them suitable agents to cross through the stratum corneum and establish covalent bonds with the nucleophilic residues of endogenous cutaneous proteins. Then, the hapten–protein complexes lead to the activation of the innate immune system and the efficient priming of T cells, which mediate cutaneous inflammation (Brites et al., 2022). Haptens may include naturally occurring substances, such as urushiol that is found in the resin of poison ivy. They can also be synthetic components like dyes, fragrances, drugs, or metal ions, for instance, nickel $Ni^{2+}$ and chromium $Cr^{3+}$ (Brites et al., 2022). A key ACD feature relies on the fact that once the individual is sensitized, they will always develop ACD after contact with the allergen. Therapeutically, the development of preventive strategies would be highly beneficial (Brites et al., 2022).

Even though ICD is caused by xenobiotics exposure, both the genetic predisposition of the patient and the skin barrier integrity are key parameters affecting the aetiology and pathophysiology of



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ICD. (Johansen et al., 2022). In this sense, atopic dermatitis has a significant interplay with ICD (Johansen et al., 2022).

*Clinical features*

Pruritus is the hallmark of contact dermatitis. Furthermore, the affected skin area will appear leathery, darker, swollen and might have a burning sensation. Bumps and blisters are also frequent (Burns et al., 2008; Johansen et al., 2022). The diagnosis of contact dermatitis begins with a discussion of the symptoms and signs; only afterwards is the skin examined. For ACD, a positive result of a patch test to a contact allergen and previous exposure of the patient to that substance are required to strengthen the diagnosis (Johansen et al., 2022).

*Disease management and therapeutic approaches*

ACD and ICD treatment significantly rely on patient education concerning avoiding irritants, allergens, and other triggers. This process should also be accompanied by restoring the skin barrier using emollients and anti-inflammatory topical drugs. However, systemic agents and sometimes phototherapy may also be appropriate (Johansen et al., 2022). When dealing with ACD, where the trigger regards a component related to the patient's professional activity, the use of protective equipment, sick leave, and, ultimately, job change must be evaluated (Johansen et al., 2022). The regular use of hydrating emollients and soap substitutes should be employed. If fissures in the fingers, palms and soles exist, they should be covered with hypoallergenic tape. Alternatively, zinc and salicylic acid paste twice daily may be helpful (Burns et al., 2008).

Besides these measures, contact dermatitis is traditionally treated with topical corticosteroids; however, recalcitrant and disabling cases may require consideration of immunosuppressive therapy such as azathioprine and cyclosporine (Burns et al., 2008; Johansen et al., 2022). Triamcinolone 0.1% is a typical example of a topical steroid applied in contact dermatitis, although methylprednisolone, clobetasol, betamethasone, and others also have a considerable market expression. The therapeutic regimen is highly dependent upon the disease state and anatomical location.



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A potent topical corticosteroid should be used in acute, severe, localized allergic contact dermatitis. On the other hand, in more chronic or widespread contact allergies, the drug's potency may need to be reduced. For long-term use in specific sites (such as face, genitals and flexures), mild topical corticosteroids are indicated. For dermatitis cases on the palms and soles, the longer-term intermittent use of a potent corticosteroid preparation is usually successful (Burns et al., 2008).

*iii. Seborrheic dermatitis*

Seborrheic dermatitis (SD) is a very common chronic and relapsing inflammatory dermatosis. While diagnosing this disease may be challenging, there are typical features that can be pointed out, namely the presence of red, sharply marginated lesions covered with greasy-looking scales with a characteristic distribution pattern. The lesions usually occur in areas rich in sebaceous glands, such as the face, scalp, presternal area. These include corticosteroids, calcineurin inhibitors, and vitamin D analogues (Burns et al., 2008; Elston, 2010; Tao et al., 2021). Dandruff (mild scalp scaling without visible inflammation) appears to be a mild form of SD.

*Epidemiology*

SD is a highly prevalent condition, affecting approximately 50 million US citizens. and this accounts for a total of $300 million annually spent on over-the-counter products (OTC) (Adalsteinsson et al., 2020). Worldwide, it is estimated that SD and dandruff together affect half of the adult population. Even though the disease can manifest at any age, a predominantly bimodal distribution can be designated, with a peak occurring in infancy (2-12 months) and another in early adulthood (Adalsteinsson et al., 2020). Furthermore, SD displays an increased prevalence (30% to 83%) in individuals with HIV or chronic neurological conditions such as Parkinson’s disease (Adalsteinsson et al., 2020).

A wide range of endogenous and exogenous factors have been implicated in the development of the disease. Exogenous factors include the *Malassezia* fungus and other microbiota, stress, poor



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skin and hair care practices, hot, humid weather conditions and certain medications such as antineoplastic agents and epidermal growth factor receptor (EGFR) inhibitors (Adalsteinsson et al., 2020). Endogenously, the disease appears to be more prevalent in males due to their higher androgen levels that enhance sebaceous gland activity (Adalsteinsson et al., 2020)

*Aetiology and pathophysiology*

Despite the high prevalence of SD, its aetiology is not yet fully understood. However, the disease is thought to be a result of the complex interplay between *Malassezia*, keratinocytes and the immune response against an altered lipid composition in the skin (Burns et al., 2008). As described by Adalsteinsson *et al*., the pathogenesis of SD can be divided into five stages:

1. Sebaceous glands secrete lipids onto the skin surface.
2. *Malassezia* colonizes the areas with an increased lipid content.
3. The secretion of lipase by *Malassezia* induces the formation of free fatty acids (FFA) and lipid peroxides, which are then responsible for the activation of the inflammatory response.
4. The release of cytokines, such as IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12 and TNF-α, stimulate keratinocyte proliferation and differentiation.
5. All these mechanisms disrupt the skin barrier, leading to erythema, pruritus and scaling (Adalsteinsson et al., 2020).

*Clinical features*

As previously mentioned, the symptoms and signs of SD include flaking skin on areas such as the scalp and facial hair. The patches of greasy skin covered with these white or yellow flakes can appear on the face, chest, armpits, groin area, or under the breast. Rashes can also suggest signs of seborrheic dermatitis, especially if they are ring-shaped and itchy. The colour of these rashes is dependent upon skin pigmentation. In brown or black-skinned individuals, the lesions tend to look pink, slightly purple, or lighter than the surrounding skin.



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*Disease management and therapeutic approaches*

Seborrheic dermatitis can generally be suppressed by several pharmacological treatments, such as antifungal agents, keratolytics, topical low-potency corticosteroids, and calcineurin inhibitors (Piquero-Casals et al., 2019). However, it should be pointed out that there is no permanent cure, which means that the condition may require regular treatment for several years (Burns et al., 2008a). Taking this into account, despite topical corticosteroids being most useful in an acute phase, their continuous use might lead to complications such as atrophy, erythema, and telangiectasia, especially when applied to the face (Burns et al., 2008). Most authors consider topical antifungals as first-line treatment and agreed that topical corticosteroids and calcineurin inhibitors should only be used for significant symptoms and to manage moderate to severe flare-ups. The top two selling topical drug products in the US and Europe for SD are ketoconazole formulations; the third is a ciclopirox shampoo (Dafnegin).

Acute forms of SD on the face and trunk are usually sensitive to mild steroid ointments, such as hydrocortisone ointment (0.5%), particularly when combined with sulphur (0.5%). Nevertheless, the selection of ketoconazole formulations is highly common due to its effectiveness (Burns et al., 2008). Topical immunosuppressants such as tacrolimus and pimecrolimus are particularly useful for facial seborrheic dermatitis when there is concern about the overuse of topical corticosteroids (Burns et al., 2008). Other active substance ingredients like topical ciclopiroxolamine metronidazole and tacalcitol have been reported to be helpful.

iv. *Radiation dermatitis*

Skin reactions are one of the most common side effects of radiation therapy, with up to 95% of patients being affected (Burns et al., 2008; Hegedus et al., 2017). One of the most common side effects of radiation therapy is radiodermatitis. The radiation responsible for the onset of radiodermatitis regards both superficial and deep X-ray radiation, ranging from 0.01 to 10 nm, shorter than those of ultraviolet rays but typically longer than gamma rays (Hegedus et al., 2017).



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*Clinical features*

Radiation-induced skin changes can be divided into two main groups: (i) acute erythema and desquamation and (ii) chronic atrophy and fibrosis. The severity of these reactions may lead to postponement or treatment limitation. As such, a straightforward prevention and management approach to radiation-induced skin injury is needed (Hegedus et al., 2017). Table 3 summarises the main clinical manifestations of both acute and chronic radiation dermatitis.

*Aetiology and athophysiology*

The adverse cutaneous effects deriving from ionizing radiation occur due to the formation of free radicals and reactive oxygen intermediates in the dividing cells of the basal layer and underlying dermis. As the skin damage is repetitive and accumulates throughout the course of treatment, these aggressions lead to a DNA impairment that ultimately results in changes to the protein, lipid, and carbohydrate skin tissue components (Hegedus et al., 2017). Concomitantly, the barrier function of the epidermis is deeply affected (Hegedus et al., 2017).

As previously described, acute skin changes are the direct outcome of tissue injury and local inflammatory reactions. The transient erythema observed within the first 24 hours after treatment ensues due to the capillary dilation and vascular permeability increase caused by the release of prostaglandins and leukotrienes. Subsequently, the generalized erythema that manifests in the subsequent 2–4 weeks is a direct consequence of the leukocyte infiltration into the underlying tissue which causes epidermal degeneration and dermal oedema (Hegedus et al., 2017). Conversely, chronic skin changes are a direct result of alterations in the vasculature and connective tissue of the dermis and the subcutaneous tissue (Hegedus et al., 2017).

*Disease management and therapeutic approaches*

The prevention and treatment of radiodermatitis are extremely challenging, as studies conducted on various topical agents and wound dressings display conflicting results. Therefore, most applied interventions and recommendations ultimately rely on clinical experience rather than supporting



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data (Hegedus et al., 2017). Nonpharmacological measures include the use of loose-fitting clothing to avoid friction or rubbing in the treatment areas and avoiding extreme heat, cold and sun exposure (Hegedus et al., 2017). In terms of medications, a variety of topical agents are used in radiodermatitis. However, there is limited evidence to support the use of one cream, gel or ointment over another. The choice of topical treatment depends on the characteristics of the lesions, clinical experience, considering that the emollient used must be effective in improving symptoms and be hypoallergenic. Sometimes it is necessary to resort to the use of corticosteroid ointments due to their anti-inflammatory effect.

Predominantly, two main topical products are employed to treat this condition: Biafine® and Linola® Radio-Derm. The first product, Biafine®, consists of an oil-in-water emulsion with trolamine. This product's radioprotective effect or prophylactic benefits have been demonstrated. However, no overall clinical differences were observed when comparing Biafine® to other care ointments (Hegedus et al., 2017; Jensen et al., 2011).

The main active component of the Linola® Radio-Derm cream is linoleic acid. This product provides quick relief from itching and is well tolerated. It was tested in radiodermatitis patients at the University Hospital in Kiel (Linola, 2023).

v. *Rosacea*

Rosacea is a prevalent chronic inflammatory disease with characteristic symptoms that primarily target the face. The most common clinical features include erythema, flushing centrofacial transient telangiectasia, burning/stinging sensation, skin thickening, dry skin and pruritus. It can be accompanied by the presence of papules and pustules (Nowicka et al., 2023; Zhang et al., 2021). Individuals with rosacea commonly go through chronic, less symptomatic periods which are then interrupted by exacerbations that may last from a couple of weeks to months (Nowicka et al., 2023). Based on presenting symptoms, the disease can be classified into four major subtypes: erythematotelangiectatic, papulopustular, phymatous and ocular.



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*Epidemiology*

A study by L. Gether and colleagues showed that in the general population, the prevalence of rosacea is ~5.5%, being more common in younger women although cases presenting with rhinophyma occur more in males (Elston, 2010). Generally, there is an age dependence in rosacea, with 13.5% of people aged 18–25 years developing the disease. This decreases with age, with only 1.0% in people aged 55 years and older displaying the condition (Gether et al., 2018; Nowicka et al., 2023). Furthermore, the disease is more prevalent in fair-skinned individuals of European descendancy (Daou et al., 2021).

*Aetiology and pathophysiology*

The cause of rosacea is still unknown; present theories point out a dysregulation of innate and adaptive immunity, aberrant neurovascular signalling, chronic inflammation, overgrowth of commensal skin organisms, external factors and a combination of all these (Daou et al., 2021). The immune response dysregulation leads to reactive oxygen species (ROS) forming, which appear to have a preponderant role in rosacea's pathophysiology (Daou et al., 2021). Equally, several studies also point out the role of the skin microbiome in developing rosacea. As with most organs, the microbiome is intrinsically connected with the immune system. Organisms such as *Demodex folliculorum*, *Bacillus oleronius*, *Helicobacter pylori*, *Staphylococcus epidermidis* and *Chlamydia pneumoniae* have been studied as potential players in rosacea's pathogenesis due to activation of the innate immune system via the Toll-like receptor 2 (Daou et al., 2021; Nowicka et al., 2023).

Individuals with rosacea present increased levels of cathelicidin, an antimicrobial peptide (AMP), which is expressed by both leukocytes and epithelial cells (Daou et al., 2021). The presence of AMP triggers a series of events ranging from leukocyte chemotaxis, vasodilation, angiogenesis, and extracellular matrix deposition, which ultimately results in the development of a dysbiotic cutaneous state (Daou et al., 2021).



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*Clinical features*

Rosacea is not a life-threatening condition; however, due to its chronic course and the fact that the lesions primarily manifest in the face, it is often accompanied by anxiety and depression, affecting patients' quality of life (Nowicka et al., 2023). Some environmental triggers may contribute to some rosacea symptoms. According to a study from 2022 promoted by the US National Rosacea Society that enrolled 1066 patients, sun exposure, emotional stress and hot water were appointed as the main external factors contributing to rosacea exacerbations. Other triggers included spicy food, alcohol, temperature extremes, emotions, exercise, or cosmetic, skin or hair care products. In this context, patients are encouraged to modify their lifestyles and avoid harmful factors (Nowicka et al., 2023).

As dermatologic manifestations of rosacea are varied, there was an attempt to further classify the disease into four subtypes: Ocular rosacea, Erythematotelangiectatic rosacea, Papulopustular rosacea and Phymatous rosacea. However, current guidelines advocate that a phenotype approach may better address patient’s characteristics, thus providing a more comprehensive choice of treatment (Nowicka et al., 2023). Table 4 describes the classical symptoms of Rosacea according to the disease stage.

*Disease management and therapeutic approaches*

Most rosacea treatments focus on symptom relief instead of addressing the underlying cause of the disease. The stating point of most management treatments concerns an extensive patient education that is highly focused on trigger avoidance. Within this scope, keeping a diary maybe a valuable strategy to identify all stimuli responsible for symptom exacerbation (Daou et al., 2021). Furthermore, patients are advised to avoid irritant cosmetic products and to use daily sunscreen.

The products with higher market expression in rosacea pharmacological management are Soolantra® (ivermectin 10 mg/g cream), Periostat® (doxycycline 20 mg film-coated tablets) and metronidazole.



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In general, an algorithmic approach based on increasing symptom severity and subtype of rosacea is followed. For cases where erythema of the face prevails, topical β-blockers or α2-adrenergic agonists such as brimonidine tartrate can be employed (Daou et al., 2021). Topical antibiotics such as azelaic acid, metronidazole and ivermectin are also commonly employed. For patients who exhibit refractory symptoms to topical therapy, tetracycline compounds (e.g., doxycycline) are advised (Daou et al., 2021).

vi. *Vitiligo*

Vitiligo is a chronic autoimmune disease characterized by the appearance of amelanotic lesions in the skin, which exhibit a macular chalky-white appearance (AL-smadi et al., 2023; Bergqvist & Ezzedine, 2020, 2021). Even though the exact pathophysiology of the disease is not completely elucidated, it is known that genetic and environmental factors, together with metabolic, oxidative stress and cell detachment irregularities may contribute towards vitiligo development. Although this condition does not affect life expectancy, as the lesions may occur in visible areas of the skin, it is often a psychologically devastating disease, leading to patients' disfigurement and social stigma. In this context, it is vital not to address it as a cosmetic condition (Al-smadi et al., 2023; Bergqvist & Ezzedine, 2020, 2021).

*Epidemiology*

Vitiligo has an estimated worldwide prevalence of 0.1 –2 % (Bergqvist & Ezzedine, 2021). This condition can manifest in any sex, ethnic group or skin type. Nevertheless, there appears to be an increased prevalence in certain geographic locations, such as Africa and India (Bergqvist & Ezzedine, 2021). Furthermore, vitiligo usually manifests in young people (Bergqvist & Ezzedine, 2020, 2021).



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*Aetiology and pathophysiology*

Vitiligo is a multifactorial disorder in which melanocyte function is impaired. Due to its autoimmune nature, several mechanisms are held accountable for melanocyte destruction, including genetic, autoimmune responses, oxidative stress, inflammatory mediators and melanocyte detachment mechanisms. Furthermore, the immune system's innate and adaptive arms appear to be involved (Al-smadi et al., 2023; Bergqvist & Ezzedine, 2020, 2021). A brief description of these pathways is addressed below.

**Genetic component**

Familiar clustering is observed in vitiligo with large-scale epidemiological studies recording that around 20% of vitiligo patients have at least one first-degree relative with the disease. Furthermore, vitiligo risk, although not absolute is mainly attributable to genetic factors (80%), with 20% attributable to the environment. (Bergqvist & Ezzedine, 2020).

Several studies of vitiligo genetic architecture have revealed that there are over 50 different genetic loci increasing vitiligo risk (Bergqvist & Ezzedine, 2020). The disease might be considered a complex polygenic disease, in which approximately 70% of the genetic risk arises from common genetic variants with the remainder derived from rare genetic variants (Bergqvist & Ezzedine, 2020).

The main variants are within the transcriptional enhancers located in the class I and II regions of the major histocompatibility complex (MHC) on chromosome 6 (Bergqvist & Ezzedine, 2020). HLA-A polymorphisms represent the most relevant genetic risk of vitiligo, followed by other genes related to antigen presentation and processing (Bergqvist & Ezzedine, 2020). The main genes with a disclosed relationship with vitiligo pathophysiology are:

**TYR gene:** Tyrosinase is an enzyme that catalyzes the rate-limiting steps of melanin biosynthesis. In vitiligo patients, this enzyme encoded by the TYR gene is the most expressed autoantigen (Bergqvist & Ezzedine, 2020).

**NALP1 gene:** The NACHT leucine-rich repeat protein 1 is encoded by the NALP1 gene on chromosome 17p13. This protein acts as a regulator of the innate immune system and has been



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linked with vitiligo-associated multiple autoimmune disease and other autoimmune and autoinflammatory syndromes (Bergqvist & Ezzedine, 2020).

**XBP1P1 gene:** Encodes the X-box binding protein 1. This protein plays a major role in mitigating the unfolded protein response and driving stress-induced inflammation *in vivo* (Bergqvist & Ezzedine, 2020).

**Oxidative stress**

The preponderant role of oxidative stress in vitiligo pathogenesis is thought to be the initial lead event (Bergqvist & Ezzedine, 2020). The increase in reactive oxygen species (e.g., hydrogen peroxide) in the intra-epidermal layer dysregulates mitochondria functions, causing apoptosis and death of melanocytes. Furthermore, vitiligo patients have distinct expression profiles of oxidative markers such as malondialdehyde, selenium, vitamins C and E, glutathione peroxidase, superoxide dismutase, and catalase (Al-smadi et al., 2023). The imbalance between the increased expression of these oxidative stress markers and the decrease of antioxidative components in the skin and blood is thought responsible for the increased sensitivity of melanocytes to external pro-oxidant stimuli, which will, over time will lead to a prosenescent status (Bergqvist & Ezzedine, 2020).

**Innate immunity**

Innate immunity mechanisms are intrinsically linked with oxidative stress and adaptive immunity in this disease. Innate immunity cells are activated due to exosomal excretion by melanocytes. These exosomes will be responsible for the delivery of vitiligo target antigens to nearby dendritic cells, which will in turn induce their differentiation into efficient antigen-presenting cells (Bergqvist & Ezzedine, 2020).

**Adaptive immunity**

Studies with vitiligo patients have disclosed the presence of antibodies to surface and cytoplasmic melanocyte antigens, thus highlighting the role of both humoral and cell-mediated immune responses (Bergqvist & Ezzedine, 2020). These autoantibodies are known to induce melanocyte destruction. Nevertheless, the concentration of these antibodies does not seem to correlate with vitiligo activity. Furthermore, even though the presence of antibodies is systemic, vitiligo lesions



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are well-demarcated. These observations appear to suggest that anti-melanocyte antibodies are not the main driver supporting vitiligo pathogenesis (Bergqvist & Ezzedine, 2020).

The concentration of autoreactive cytotoxic $CD8^+$ T cells in vitiligo patients is higher when compared to healthy individuals (Bergqvist & Ezzedine, 2021). These cells are activated by interferon-γ, being the IFNγ-induced CXC chemokine-ligand 9 (CXCL9), CXCL10 and CXCL11 highly expressed genes in the transcriptional profile of lesional skin in vitiligo patients. The activated $CD8^+$ T cells infiltrate the perilesional skin, target the melanocytes, which will ultimately lead to their destruction (Bergqvist & Ezzedine, 2021).

*Clinical features*

Vitiligo classification was revised in 2011 by an international consensus and the disease is nowadays grouped into two major forms. The first group regards nonsegmental vitiligo (NSV), which represents 80–90 % of all vitiligo cases (Al-smadi et al., 2023) and includes acrofacial, generalized, focal, mucosal, universal, mixed and others vitiligo variants with the first two being most common subtypes. Clinically, generalized vitiligo patients exhibit bilateral, often symmetrical, depigmented macules or patches that display a random distribution over the entire body surface (Al-smadi et al., 2023). Acrofacial vitiligo lesions are located in the distal extremities and/or the face (Al-smadi et al., 2023).

The second type of the disease exclusively refers to segmental vitiligo (SV) the patients of which have white patches of a unilateral distribution. Another characteristic clinical feature concerns hair depigmentation, which impacts the follicular melanocyte reservoir (Al-smadi et al., 2023). This disease has an early onset and is less common than NSV.

*Disease management and therapeutic approaches*

Treatment of vitiligo focuses on reducing oxidative stress and autoimmune inflammation to allow for the repopulation of healthy melanocytes. These include corticosteroids, calcineurin inhibitors, vitamin D analogues and UVA or UVB phototherapy. Emerging treatments include targeted anti-



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inflammatory molecules such as Janus kinase inhibitors (often used for rheumatologic autoimmune disorders) and procedural laser dermabrasion (Manga et al., 2016).

The vitiligo subcommittee of the European Dermatology Forum has reported guidelines for the management and treatment of this disease (Taieb et al., 2013). Four lines of treatments were considered: (i) corticosteroids and calcineurin inhibitors; (ii) phototherapy (NB-UVB and psoralen and UVA [PUVA]), together with systemic steroid treatment; (iii) surgical grafting techniques and finally (iv) depigmenting treatments (Taieb et al., 2013).

In 2021, the topical product with the highest market share was Elidel® VTRS (10 mg/g pimecrolimus cream). Pimecrolimus is a topical ascomycin immunomodulating macrolactam that acts as a topical calcineurin inhibitor. Pimecrolimus affects T cells' activation/maturation, thereby inhibiting cytokine production, such as TNF-α. Furthermore, this API contributes to melanocyte migration and differentiation (Taieb et al., 2013). In 2023, Opzelura® which contains the Janus Kinase (JAK) inhibitor Ruxolitinib gained marketing authorization. This drug is able to suppress the IFN-γ–CXCR3–CXCL9/10 axis required for T Cell recruitment and function (Sheikh et al., 2022).

vii. *Alopecia Areata*

Alopecia areata (AA) is a chronic inflammatory, autoimmune disorder that involves the hair follicle (HF) leading to localized or diffuse hair loss (Burns et al., 2008). This disease exhibits a variable, typically relapsing pattern that is often persistent, especially when the hair loss is extensive (Pratt et al., 2017).

Typically, AA patchy hair loss occurs due to inflammatory cell infiltrates concentrated around the HF (Pratt et al., 2017). The scalp is the most common afflicted area for both men and women, where the disease manifests in well-demarcated regions. Afflictions of the nails may also be observed, including pitting, trachyonychia, and thinning or thickening (Wasserman et al., 2007).



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*Epidemiology*

According to several epidemiological studies performed in Europe, North America and Asia, AA is likely to affect 2% of the general population at some point during their lifetime (Pratt et al., 2017). AA might appear at any age, but the mean onset is between 25 and 36 years old and there appears to be no sexual dichotomy, as the condition equally affects both sexes (Pratt et al., 2017; Strazzulla et al., 2018). However, data retrieved from the Rochester Epidemiology Project indicates that men tend to be diagnosed earlier when compared with women (mean age at diagnosis, 31.5 vs 36.2 years) (Mirzoyev et al., 2014; Strazzulla et al., 2018). In cases when the disease manifests earlier, it typically concerns a more severe AA subtype, such as alopecia universalis (Pratt et al., 2017).

*Aetiology and pathophysiology*

The HF is an immune-privileged site, similar to the anterior chamber of the eyes and the pregnant uterus (Pratt et al., 2017; Strazzulla et al., 2018). Due to its nature, there is local immunoinhibitory signalling in and around the HF that suppresses any putative autoantigens. The breakdown of the HF immune privilege is thought to be a major driver in the development of AA (Pratt et al., 2017; Strazzulla et al., 2018). However, to understand the pathophysiological mechanisms involved in AA, it is crucial to understand the HF growth cycle in which three distinct phases have been identified:

(i) The active growth phase – Anagen: The epithelial cells in the hair bulb undergo active growth and differentiation, which ultimately leads to the formation of the hair fibre with its inner root sheath, as well as the inner layer of the outer root sheath. This phase can be subdivided into six stages (anagen I – VI).

(ii) The apoptosis of epithelial cells – Catagen: This is the end of the extensive epithelial cell division observed during the anagen phase. During catagen, the proximal end of the hair shaft keratinizes to form a club-shaped structure, which eventually sheds, and the lower part of the follicle is involuted by apoptosis (Pratt et al., 2017).

(iii) The resting phase – Telogen. This marks the period between follicular regression and the



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onset of the next anagen phase (Pratt et al., 2017; Strazzulla et al., 2018).

In AA, the HF cycle becomes disrupted, with anagen VI follicles being prematurely precipitated into the telogen phase (Pratt et al., 2017; Strazzulla et al., 2018). Even though the HF can re-enter the anagen phase, when in the anagen III/IV stage, the HF development stops and prematurely goes into the telogen phase. These disrupted cycles will keep occurring until the disease activity declines (Pratt et al., 2017; Strazzulla et al., 2018).

In the HF of AA patients, there are prominent aggregations of CD56+NKG2D+ NK cells, resulting from the increase in major histocompatibility complex (MHC) I and II molecules alongside adhesion molecules. These will ultimately lead to an increased leukocyte trafficking into the dermis. Among the leucocytes, CD56+ NK cells are responsible for expressing NK cell-activating receptors (such as NKG2D and NKG2C). Polymorphism in the ULBP gene cluster, which encodes NKG2D ligands, has been found in patients with increased susceptibility to AA (Pratt et al., 2017; Strazzulla et al., 2018). Furthermore, the reduced expression of receptors for both vasoactive intestinal peptides (VIPR1 and VIPR2) located in the HF has also been observed in the hair bulbs of AA patients. However, the VIP ligand expression was normal in nerve fibres, suggesting that the abnormal processes relate to the VIPR-mediated signalling (Pratt et al., 2017; Strazzulla et al., 2018).

*Clinical features*

The key clinical features of AA predominantly manifest in the scalp, even though other areas of the body may also be affected. Waxing and waning focal alopecia are initially observed, and the skin in the affected areas appears slightly red. A characteristic clinical feature of AA concerns the presence of “exclamation point hairs” in the lesion’s surroundings (Pratt et al., 2017). With the progression of the disease, the lesions may expand, resulting in a wider hair loss, nevertheless, there are cases in which the hair regrows (Strazzulla et al., 2018).

Generally, the patients present no symptoms although tingling, itching, and dysesthesia are sometimes reported before the hair loss (Pratt et al., 2017). The condition is associated with several concurrent diseases such as depression and anxiety, but also with thyroid disease



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(hyperthyroidism, hypothyroidism, goitre and thyroiditis), lupus erythematosus, vitiligo, psoriasis, rheumatoid arthritis and inflammatory bowel disease (Pratt et al., 2017).

Due to the complexity of the clinical features associated with this disease, it is possible to classify it into the following types:

- Patchy AA: Characterized by the presence of one, or multiple separate or conjoined (reticular) patches of hair loss.
- Alopecia totalis: Characterized by total or near-total hair loss on the scalp.
- Alopecia universalis: In this AA type, the total to near-total hair loss occurs on all-haired surfaces of the body.
- Alopecia incognita: Characterized by the diffuse total hair loss with a positive pull test, yellow dots, short, miniaturized regrowing hairs, but with no nail involvement (Pratt et al., 2017; Strazzulla et al., 2018).
- Ophiasis: In this AA type, hair loss occurs in a band-like shape along the circumference of the head, more specifically along the border of the temporal and occipital bones.
- Sisaipho: consists of scalp hair loss sparing the temporal and occipital areas, just the opposite of ophiasis.
- Marie Antoinette syndrome (also called canities subita) is an acute episode of diffuse alopecia with sudden ‘overnight’ greying and preferential loss of pigmented hair (Pratt et al., 2017).

*Disease management and therapeutic approaches*

Some AA patients respond well to current treatments; however, this scenario fails to register when dealing with more severe AA types (alopecia totalis, alopecia universalis or a combination of both) (Pratt et al., 2017).

Topical corticosteroids such as clobetasol administered within a lotion or shampoo can be used for patchy alopecia areata treatment, favouring hair regrowth in mild AA cases. As these treatments are long (at least three months), folliculitis is a frequent complication occurring in these patients. On the other hand, corticosteroids may also be subcutaneously administered in the lesions (Pratt et



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al., 2017). Kenacort® (triamcinolone acetonide injection) is also used in AA and contains a slow-release steroid which is administered by fine-needle injection or by a needleless device into the upper subcutaneous tissue in order to stimulate hair growth at that specific location (Pratt et al., 2017). Several studies reported a decrease in CD3+ T cells, CD8+ T cells, CD11c+ dendritic cells and CD1a+ Langerhans cells around the HF in patients receiving intralesional corticosteroids. Furthermore, the downregulation of genes encoding several interleukins and chemokines (such as IL-12β, CC-chemokine ligand 18 and IL-32) and upregulation of genes encoding several keratins (KRT35, KRT75 and KRT86) was likewise reported (Pratt et al., 2017). Based on these observations, the corticosteroids appear to dampen the inflammation underpinning follicular damage, thus slowing hair loss and optimally allowing regrowth (Pratt et al., 2017).

Topical minoxidil (e.g. Regaine®, Minoxil®, Minoxidil Bail), a potassium-channel facilitator, is also used in AA (and androgenetic alopecia). Such products are also administered with other treatments, such as dithranol cream or oral corticosteroids (Gilhar et al., 2012).

viii. *Folliculitis Decalvans*

Folliculitis decalvans (FD) regards a chronic inflammatory skin disease, being the most common type of neutrophilic scarring alopecia. This disease is responsible for causing painful and recurrent purulent follicular exudation (Matard et al., 2020; Rambhia et al., 2019).

*Aetiology and pathophysiology*

The aetiology of FD is still uncertain; however, it is believed that a staphylococcus infection may be the root cause (Burns et al., 2008). A hypothetical model proposed by Bruno Matadar and colleagues suggests that the origin of FD can be ascribed to an epidermal barrier rupture, caused by opportunistic bacteria, such as *S. aureus* or other bacteria of the transient flora (Matard et al., 2020) prior to colonization by opportunistic bacteria. The greater prevalence of S. aureus on the lesions might be explained by the higher colonizing ability of this bacteria (Matard et al., 2020; Rambhia et al., 2019).



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Histological analysis of the scalp reveals the occurrence of follicular abscesses, with a dense perifollicular polymorphonuclear infiltrate, scattered eosinophils and plasma cells (Burns et al., 2008). Furthermore, foreign-body granulomas caused by follicular disruption can also be observed. This occurrence ultimately leads to scars, followed by a total replacement of the hair follicle with extensive fibrosis (Burns et al., 2008).

*Clinical features*

Round bald spots are the main signs of FD. On rare occasions, hair loss can be seen on other areas of the body including the armpits, face, legs, and arms. The majority of people only show signs once they start to lose hair, others can get an itchy sensation, resembling dandruff. The areas that lose hair can feel painful and tight, causing scaly skin that can ooze and form scabs. FD can cause the hair to grow in tufts, and when the follicle dies, the tufts of hair fall out, leaving scarring and bald spots. The diagnosis of this condition involves a scalp examination. In addition, a swab of the fluid might be taken to check for *Staphylococcus aureus* strands (Cherney & Roland, 2022).

*Disease management and therapeutic approaches*

A cure has yet to be found for FD, but treatments are used to help reduce inflammation and prevent future scarring and hair loss. Duac® (clindamycin phosphate and benzoyl 10 mg/g + 50 mg/g peroxide) gel, Curacne® (isotretinoin) and Zindaclin® 10 mg/g clindamycin gel were the most sold topical products for FD in Europe and North America in 2021. Clindamycin is useful for treating localized areas, whilst isotretinoin has been used to alter the follicular environment, thereby decreasing the colonization of staphylococcus aureus (Burns et al., 2008).

ix. *Lichen Planus*

Lichen Planus (LP) is a group of chronic inflammatory diseases affecting stratified squamous epithelia. It is a T cell-mediated autoimmune disease, in which cytotoxic cells are recruited into the skin, leading to interfacial dermatitis. Over 20 different expressions are described in LP; most can be included in three main subtypes: Cutaneous LP, mucosal LP and appendageal LP. Both



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genetic (certain HLA alleles and SNP loci) and environmental factors (drugs, infections) are associated with LP. The diagnosis is based on the clinical presentation and characteristic histological findings. Although the disease is often self-limiting, an intractable pruritus and painful mucosal erosions can result in significant morbidity. The current first-line treatments are topical and/or systemic corticosteroids; immunosuppressants may be used as corticosteroid-sparing agents. These, however, are often not sufficient to control the disease; Janus kinase inhibitors and biologics (anti-IL-12/23, anti-IL17) have emerged as novel future treatment options in cases of refractory disease (Boch et al., 2021; Elston, 2010; Katta, 2000; Mayo Clinic, 2023a).

*Epidemiology*

Lichen Planus is estimated to manifest in less than 1 percent of the population (Li et al., 2020). Cutaneous lichen planus most frequently develops between the ages of 30 and 60 years, and its skin manifestations affect both men and women equally, although women are more prone to oral expressions, which tend to outnumber LP in most study populations (Katta, 2000). The epidemiological profile of LP is not fully delineated; however, the literature indicates 14 to 250 cases/100,000 person-years. According to a recently published systematic review and meta-analysis, oral LP tends to develop ten years later than cutaneous LP, and the prevalence of this condition is 0.89% of the general population and slightly higher (0.98%) in hospitalized patients (Li et al., 2020). Furthermore, the incidence of this type of LP is higher in women, it predominantly affects more than 40 years old patients, and it is more common in non-Asian countries. However, as pointed out by the authors, there are several limitations to these the above-mentioned review. These include lack of access to some databases, reduced sample size of some studies and the heterogeneity in patients age across studies (Li et al., 2020).

*Clinical Features*

The primary signs and symptoms for this condition are purplish, polygonal, shiny, flat-topped, firm papules and plaques with white streaks (Wicham striae) located commonly on the inner forearm, wrist, ankle, and in some cases, the genitals. These lesions (bumps) can be itchy, painful,



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and have blisters. Lacy white patches can also be observed on the mouth, lips or tongue. Other symptoms include hair loss, change in scalp colour, and nail damage or loss. The mouth is affected in around 50% of all cases, and dentists often make the diagnosis. The most predominant symptom is pruritus, which can be severe and refractory to standard therapies. Several diseases can be associated with LP, such as systemic viral infections, thyroid disease, dyslipidemia, diabetes mellitus, and autoimmune conditions (Li et al., 2020).

A diagnosis for lichen planus can be made by assessing the symptoms and medical history of the patient. A physical examination might be valuable, as well as some tests, such as a biopsy, hepatitis C test, or allergy test. Most skin cases regress within 6 to 9 months. However, oral and genital forms may be more persistent (Li et al., 2020).

*Aetiology and pathophysiology*

Lichen planus arises when the immune system attacks skin cells or mucous membranes. It has been discovered that numerous factors, like hepatitis C infection, flu vaccine, some medications for heart disease, high blood pressure or arthritis, and certain pigments, chemicals, or metals, can trigger it. It is unknown why these factors tend to trigger lichen planus. Pathogenic models talk about an afferent phase, where tolerance to an autoantigen is lost (e.g., in instances where the diversity of local microbiome is low) and autoreactive T cells are activated followed by an efferent phase, where CD8 T cells induce an inflammatory state with proteases and mast cell activation (Boch et al., 2021).

Mast cell degranulation, and T-cell secretion of matrix metalloprotein (MMP-2, MMP-7, MMP-9), as well as chymase and tryptase, may contribute to basement membrane disruption, enabling migration of CD8+ T cells into the epithelium, thus causing the manifestation of the disease (Boch et al., 2021).

Additional autoimmune dermatoses such as alopecia and vitiligo are often represented in patients with lichen planus. Changes in cytokine expression include IL-5, IL-6, IL-8, IL-10, IL-12, IL-17 and IL-22, as well as TNFα, TGFβ, and IFNγ. Chemokines CXCR3, CXCL10, CXCL12, CCR4, and CCL17 are also unregulated (Boch et al., 2021). Evidence suggests a strong Th1-mediated



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inflammatory response of CD8+ T lymphocytes, including elevated type I interferons leading to the recruitment of cytotoxic T cells and keratinocyte damage (Wenzel et al., 2006).

*Disease management and therapeutic approaches*

Once a diagnosis has been reached, treatments can be discussed. The cutaneous lesions of most patients spontaneously disappear within 12–24 months. However, relapses are common and healing may be more challenging in cases where post-inflammatory hyperpigmentation occurs.

Firstly, the possibility of drug-induced LP should always be considered and excluded before initiation of immunosuppressive therapy. Similarly, any LP-associated diseases should be checked in each patient. Some forms of LP lesions can be premalignant, therefore regular follow-up and biopsies should be considered in their management.

The main goal of LP treatment is to resolve the lesions and associated symptoms. Frequently, the first line of treatment for limited LP is potent topical corticosteroids. However, where there is disseminated disease, the use of systemic corticosteroids is common. Besides this drug class, other first-line therapies include systemic retinoids (acitretin/isotretinoin) or cyclosporin (Mayo Clinic, 2023). If the disease remains unresponsive, a second-line therapy should be considered. This often resorts to sulphasalazine and phototherapy. Topical drugs such as calcineurin inhibitors can also be used to reduce the side effects of topical corticosteroids. If the disease remains unresponsive, third-line drugs treatments may include hydroxychloroquine, azathioprine, methotrexate, mycophenolate mofetil, or biologics targeting IL- 12/23 (Husein-El Ahmed et al., 2019). Since LP proinflammatory signalling pathways result from T-cell-dependent immune response, oral JAK inhibitors may represent a future treatment option. Topical antipruritic agents such as menthol, camphor, or polidocanol can be prescribed as an adjuvant to the main treatment. However, the use of systemic antihistamine drugs is more common.



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x. *Pruritis/Itch*

As frequently mentioned throughout this section, pruritus (itching) is one of the most characteristic symptoms of skin disease (Burns et al., 2008). Itch can be defined as a poorly localized, non-adapting, usually unpleasant sensation which provokes an immediate desire to scratch (Burns et al., 2008).

Chronic pruritus is an itch that persists for more than six weeks and is associated with a markedly reduced quality of life comparable with chronic pain, because the patients are often sleep-deprived (Yosipovitch & Bernhard, 2013). Chronic pruritus can be classified according to its extent, being considered generalized (involving all skin) or localized when occurring only in particular areas, such as the scalp, upper back, arms, or groin (Yosipovitch & Bernhard, 2013). Pruritus incidence increases with age and appears to be more prevalent in Asians than in Caucasians (Yosipovitch & Bernhard, 2013).

According to Twycross et al. (2003), the origin of pruritus can be categorized into the following areas:

- Pruritoceptive: caused by dermatological diseases such as scabies, atopic eczema, psoriasis, lichen planus, and urticaria, amongst others.
- Neuropathic: due to lesions on the afferent pathways of the nervous system (e.g., peripheral neuritis, nerve entrapment, brain tumours).
- Neurogenic: resulting from centrally acting mediators which do not damage the central nervous system (e.g., morphine, opioid peptides of cholestasis).
- Psychogenic: induced by delusional parasitosis.

Taking all the above into account, the symptoms and signs of pruritus are highly dependent on the trigger, but commonly, there tends to be erythema, scratch marks, bumps, spots, dry skin, and scaly patches (Elston, 2010).



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*Aetiology and pathophysiology*

The neurophysiological pathways for itch start with the transmission of the itch signal by the small unmyelinated C fibres present in the skin. It is thought that histamine-triggered neurons and nonhistaminergic neurons have an important role in this process. These mediators form a synapse with secondary neurons that then cross over the contralateral spinothalamic tract and then distribute to several areas of the brain responsible for process sensations, such as the evaluative processes, emotions, rewards and memories. Interestingly, these areas overlap with the ones activated by pain (Yosipovitch & Bernhard, 2013).

Patients with chronic pruritus often display peripheral and central neural hypersensitization, meaning that the sensitized fibers are extremely reactive to the harmful stimulus that usually inhibits itch. Factors such as extreme heat, scratching or even a simple touch may be perceived as itch (Yosipovitch & Bernhard, 2013).

*Disease management and therapeutic approaches*

General treatments for pruritus include topical therapy. For mild or localized pruritus, the use of skin emollients is considered to be one of the first lines of treatment due to their ability to hydrate the stratum corneum and by doing so to improve the barrier function of the skin (Benson & Watkinson, 2012). The treatment of pruritus associated with skin xerosis (e.g., winter itch) is one of the case scenarios in which the application of this non-pharmacological measure is highly efficient (Yosipovitch & Bernhard, 2013).

Anaesthetics such as capsaicin may also be useful in managing pruritus due to their desensitizing action on the peripheral nerve fibres. Furthermore, the eutectic mixture of lidocaine and prilocaine 2.5% cream (EMLA™) has been reported to display short-term beneficial effects for neuropathic, facial and anogenital itch (Yosipovitch & Bernhard, 2013).

The use of coolants, such as menthol, may also be useful for mild or localized pruritus management. This molecule relieves pruritus by activating A-delta cold afferents, which transmit a cold sensation due to activating an ion channel (TRPM8) (Yosipovitch & Bernhard, 2013).



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Corticosteroids, due to their anti-inflammatory characteristics, constitute a common treatment line (Yosipovitch & Bernhard, 2013). In more severe cases, oral medication can also be used, including antidepressants from the selective serotonin reuptake inhibitors class (e.g. fluoxetine, sertraline) and tricyclic antidepressants class (e.g., doxepin). Light therapy can also help reduce symptoms in such circumstances (Mayo Clinic, 2023b).

### *B. Skin Infections*

In the next section a brief overview of the main skin infectious diseases is presented. Skin and soft tissue infections include a group of heterogeneous conditions, affecting the epidermis, dermis and subcutaneous tissue. According to the aetiologic agent, four different types of skin infections are considered: bacterial, viral, fungal and parasitic. Aspects such as epidemiological profile, pathophysiology mechanisms, clinical features, and disease management approaches are presented.

#### *i. Acne*

Acne vulgaris is the most common skin disease and is a direct consequence of a blockage occurring in hair follicles and associated sebaceous glands, together known as the pilosebaceous units (Mahto, 2017). This condition primarily manifests in high sebaceous follicle density areas, such as the face, upper chest and back (Mahto, 2017).

*Epidemiology*

Acne is a widespread condition, affecting 80 % of people at some point between 11 and 30 years of age, but it can also manifest in adults (Mahto, 2017). The disease often occurs in males. However, when acne persists into adulthood, 26 % of women are affected, whilst 12 % of men will develop the condition (Zaenglein, 2018a) Worldwide, acne ranks 8$^{th}$ in overall disease



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prevalence, with the highest rates being reported in Western Europe, “high-income” North America and southern Latin America (Zaenglein, 2018).

*Aetiology and pathophysiology*

As previously mentioned, acne is a primary inflammatory disorder involving the pilosebaceous unit (Zaenglein, 2018). The pathogenesis of this disease can be ascribed to four main factors: (i) increased sebum production, (ii) hyperkeratinization of the follicular infundibulum, (iii) inflammation, and finally, (iv) *Cutibacterium acnes* (formerly referred to as *Propionibacterium acnes*) (Zaenglein, 2018). On a side note, as the number and size of sebaceous glands are inherited, there is also a genetic component to the disease (Zaenglein, 2018).

The increased sebum production occurs due to hormonal influence, specifically, to the androgen dihydrotestosterone. During puberty, this hormone is elevated and targets the sebaceous gland, thereby increasing sebum production and excretion (Mahto, 2017). The high sebum concentration and the abnormal follicular hyperkeratinization will lead to the appearance of “sticky” keratinocytes that block the pilosebaceous duct. Then, bacterial colonization by the anaerobic *Cutibacterium acnes* is deemed to occur (Mahto, 2017). Besides this order of events, studies point out that increased numbers of $CD3^+$ and $CD4^+$ T cells and macrophages in uninvolved skin from acne patients are probably related to an underlying inflammatory state (Zaenglein, 2018). The compounding presence of *cutibacterium acnes* triggers a pro-inflammatory state through the activation of toll-like receptors (TLRs), which detect exogenous microorganisms. The resulting signalling cascade includes IL-12 and IL-18, as well as antimicrobial defensins. In addition to TLRs, *cutibacterium acnes* has been shown to induce IL-1α, IL-8, and matrix metalloproteinase as a result of protease-activated receptor-2 (PAR-2) activation. The sebaceous gland of the skin is also a culprit in acne inflammation, producing IL-1α and IL-1ß (Tanghetti, 2013).

*Clinical features*

Acne is characterized by comedones, papules, pustules, nodules, and cysts (Mahto, 2017).

The typical distribution of the lesions occurs in the face, upper back, chest, and shoulders.



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A brief description of the main acne lesion types is as follows:

- **Comedones**: These are the most common acne lesions and can be closed or open. The former comprises small, plugged follicles whose contents are not exposed to the skin surface (whiteheads). On the other hand, open comedones are small follicles with dilated openings onto the skin, which display a black colour due to the oxidation of the debris within the follicle (blackheads) (Mahto, 2017);
- Papules: Small, usually red, raised elevations of the skin.
- Pustules: Are similar to papules but have a purulent center.
- Nodules and cysts are larger painful swellings, usually more than 5 mm in size (Mahto, 2017).

As the face is usually the first thing noticed about a person, the psychological effects of acne can be profound. Therefore, people with the disease are at risk for substantial, negative effects on their quality of life similar to those observed in people with asthma, epilepsy or arthritis (Eichenfield et al., 2021; Zaenglein, 2018b).

*Disease management and therapeutic approaches*

The choice of treatment is based on symptom severity and location of the lesions. However, prior to setting up a treatment plan, it is imperative to advise the patient to include the use of an adequate cleanser as well as moisturizer (Zaenglein, 2018) in their skin treatment regime.

The therapeutic approach to acne may include (i) topical anti-inflammatories, such as retinoids; (ii) antibacterials, including clindamycin and erythromycin, as well as (iii) benzoyl peroxide and salicylic acid that mainly target sebum production. Severe cases of acne may also require systemic antibiotics like doxycycline and minocycline (Oge' et al., 2019).

According to the standard guidelines from the American Academy of Dermatology and the American Academy of Pediatrics, the first-line treatment for acne regards the use of topical agents such as retinoids, benzoyl peroxide, and/or topical antibiotics (Eichenfield et al., 2021). For more severe cases, combinations of topical drugs with systemic agents should be used (Eichenfield et al., 2021). Topical retinoids, such as tretinoin, adapalene and trifarotene, are vitamin A derivatives



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which bind to retinoic acid receptors and retinoid X receptors in the keratinocyte cytoplasm. By doing so, they translocate into the nucleus to initiate transcription changes, namely epidermal cell proliferation stimulation, loosen connections among cells in the stratum corneum, speed up sebum removal from sebaceous ducts, as well as microcomedone clearance stimulation (Eichenfield et al., 2021).

Benzoyl peroxide is a bactericidal agent highly effective at reducing the concentration of *C. acnes* in the sebaceous follicles due to the release of free oxygen radicals (Mahto, 2017).

*ii. Dermatophytosis*

A dermatophytosis is an infection in which the etiologic agent consists of a dermatophyte (Elston, 2010). Dermatophytes are a group of fungi with a high affinity for keratinized structures (Sahoo & Mahajan, 2016). Clinically, dermatophytosis can be classified on the basis of the affected area (see also see Table 5):

- Dermatophytoses of keratinized epidermis include tinea facialis, tinea corporis, tinea cruris, tinea manus and tinea pedis.
- Dermatophytoses of nail apparatus (a form of onychomycosis, also called Tinea unguium).
- Dermatophytoses of hair and hair follicles include dermatophytic folliculitis, Majocchi (trichophytic) granuloma, tinea capitis and tinea barbae (Elston, 2010a).

Dermatophytes can be classified into three genera: (i) *Trichophyton* (which causes infections on skin, hair, and nails), (ii) E*pidermophyton* (which causes infections on skin and nails), and (iii) *Microsporum* (which causes infections on skin and hair) (Sahoo & Mahajan, 2016b). Furthermore, dermatophytosis can be classified according to their native habitat as anthropophilic (humans), zoophilic (animals), or geophilic (soil) (Martinez-rossi et al., 2021a).

Dermatophytosis are the most common fungal infections worldwide, being especially prevalent in tropical and subtropical countries where the environmental temperature and relative humidity are high (Sahoo & Mahajan, 2016b). Nevertheless, other etiological factors contribute towards the rise of dermatophyte infections, such as socioeconomic status, occupation and climate change, as well



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as individual predisposing factors, such as diabetes mellitus, lymphomas, immunocompromised status, or Cushing's syndrome and older age (Sahoo & Mahajan, 2016b).

Dermatophytosis pathogenesis depends upon the interaction between host, agent and environment (Sahoo & Mahajan, 2016b). The secretion of keratinases by dermatophytes destroys keratin and by doing so, it sustains the existence of fungi in keratinized structures. These events lead to the development of an inflammatory response in the skin (Elston, 2010; Martinez-Rossi et al., 2021). In the following sections, the most common types of dermatophytosis are addressed.

*Tinea Corporis*

Tinea corporis, also referred to as ringworm or tinea circinate, is a skin dermatophytosis of the skin other than the hands (tinea manuum), feet (tinea pedis), scalp (tinea capitis), bearded areas (tinea barbae), face (tinea faciei), groin (tinea cruris) and nails (tinea unguium) (Leung et al., 2020).

The etiological agents commonly involved in tinea corporis are *Trichophyton rubrum*, T. *tonsurans*, and *Microsporum canis*. However, T. *rubrum* infections are most prevalent (Leung et al., 2020). Tinea corporis is the most common dermatophytosis worldwide, having an increased incidence in tropical regions (Leung et al., 2020). The disease is commonly registered in post-pubertal children and young adults, and there appears to be no sex predominance (Leung et al., 2020). The most common infection route is by close contact with an infected individual, which, in most cases, regards a household family member (Leung et al., 2020).

Tinea corporis vesiculopustular lesions may be single or in multiple plaques (Burns et al., 2008a). Their appearance is usually circular, red, and demarcated (Willey et al., 2008). However, this clinical pattern is often modified in immunodeficient patients (Burns et al., 2008). The annular appearance of tinea corporis lesions is also observed in other ringworm infections, and it is ascribed to the elimination of the fungus from the centre of the lesion, with the subsequent resolution of the inflammatory host response at that site.

The inflammatory process is highly dependent upon the fungal species, as well as the immunity status of the host (Leung et al., 2020).



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As fungi thrive in moist and warm environments, some non-pharmacological measures are extremely useful in tinea corporis management. These include the use of light and loose-fitting clothing, as well as keeping the skin clean and dry (Leung et al., 2020).

In pharmacological treatment, antifungal products are the standard of care in tinea corporis (Burns et al., 2008; Elston, 2010). Usually, localized or superficial tinea corporis is responsive to topical antifungal therapy applied to the lesion when applied at least 2 cm beyond the lesion once or twice daily for 2–4 weeks (Leung et al., 2020). The most frequent topical antifungal agents include azoles, such as ketoconazole, miconazole, clotrimazole, and allylamines, mainly terbinafine.

*Tinea Cruris*

Tinea cruris is a dermatophytic infection of the groin, and there are many similarities with tinea corporis, which include:

- The pathogenesis;.
- The main etiological agent causing the disease (*T.rubrum*). However, *T. Mentagrophytes* var. *interdigitale* and *E. floccosum* also account for some cases (Burns et al., 2008).
- Greater prevelance in tropical zones, particularly among migrants from temperate countries.
- Similar treatment: Canesten® (clotrimazole), Lotrimin® AF (miconazole) and ketoconazole.

Despite these common features, tinea cruris has some particularities over tinea corporis, including the fact that it has a higher prevalence in men and that the route of infection usually involves the sharing of towels and sports clothing (Burns et al., 2008).

In the early stage of the infection, the lesions are erythematous plaques, curved with sharp margins, extending from the groin down to the thighs (Burns et al., 2008; Elston, 2010). In some cases, the infection might extend to the buttocks. However, the scrotum and penis are rarely involved (Burns et al., 2008).



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*Tinea Pedis*

Tinea pedis is a dermatophytosis of the feet and toes, commonly referred to as Athlete's foot. This condition is highly prevalent worldwide due to its connection with hot, humid climates, sporting activities and hyperhidrosis (Ilkit & Durdu, 2015).

The main etiological agent responsible for tinea pedis is *Trichophyton rubrum*, followed by *Trichophyton interdigitale* and *Epidermophyton floccosum* (Ilkit & Durdu, 2015). Tinea pedis can manifest into four distinct forms: (i) interdigital, (ii) inflammatory (vesicular), (iii) chronic hyperkeratotic (moccasin) and (iv) ulcerative.

**Interdigital tinea pedis** is the most common form of tinea pedis. The main clinical manifestations are interdigital erythema, scaling, maceration and fissuring. In more severe cases, the lesions are accompanied by itching, burning and malodour.

**Inflammatory or vesicular (vesiculobullous) tinea pedis** is clinically characterized by hard, tense vesicles, bulla and pustules on the instep or mid-anterior plantar surface. The lesions usually have a size of 1 to 5 mm and are deeply installed within the epidermis (Ilkit & Durdu, 2015). The vesicles may sometimes group into bullae. In the early stages, their content is clear or lemon-yellow, but this scenario tends to change if superinfection occurs, most often due to *Staphylococcus aureus* or group A *Streptococcus* (Ilkit & Durdu, 2015). In vesicular tinea pedis, the itching, together with burning and pain, may impair the patient's walking (Ilkit & Durdu, 2015).

**Chronic hyperkeratotic tinea pedis** is also referred to as moccasin tinea due to the involvement of the plantar surface of the feet. As deducted by the name, the most common feature of this disease regards chronic plantar erythema, which may range from slight scaling to diffuse hyperkeratosis. After affecting the entire plantar surface, the lesions may also extend to the lateral foot; nevertheless, the dorsal surface of the foot is usually not affected. This scenario may change if the host is immunocompromised. Pruritus can be mild or severe, and fissures can be painful while walking. One or both feet may be involved and the longer the infection lasts, the higher the probability of developing tinea unguium (Ilkit & Durdu, 2015). Due to the likelihood of scratching



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the foot, around 50% of patients will develop the "two feet-one hand'' syndrome, in which one hand becomes infected with dermatophytes (tinea mannum) this syndrome is common, but the cause of the unilateral involvement of the hands is unknown (Ilkit & Durdu, 2015). For this reason, the prevalence of tinea manuum directly relates to the level of tinea pedis in the population (Burns et al., 2008).

**Ulcerative tinea pedis** is typically caused by anthropophilic *T. interdigitale*. Clinically, it is characterized by a rapid spreading of vesiculopustular lesions, ulcers and erosions, which often accompany a secondary bacterial infection. Ulcerative tinea pedis is usually observed in immunocompromised and diabetic patients (Ilkit & Durdu, 2015).

The treatment of tinea pedis involves the application of topical antifungal treatments. These include allylamines, azoles, butenafine, and ciclopiolfine (Sahoo & Mahajan, 2016). The highest-selling drug in tinea pedis is the terbinafine containing Lamisil® range of various dosage forms, including creams, gels, and sprays, while the next Naftin® contains Naftifine and is available in a gel. Both drugs belong to the allylamines antifungal class (Sahoo & Mahajan, 2016).

*Tinea unguium*

Tinea unguium refers to a dermatophytosis of the nail and is also known as Onychomycoses, as dermatophytes are the etiological agents in about 85% of cases. However, nail infections by yeasts or non-dermatophyte moulds (NDM) can also occur (Asz-Sigall et al., 2017).

The etiological agent most commonly involved in Tinea unguium is *Trichophyton rubrum* (71–88 % of the cases), followed by *Trichophyton interdigitale* (9–22 % of cases) (Asz-Sigall et al., 2017). This disease is extremely common worldwide, and its prevalence increases with advancing age with more than 50% of cases occurring in individuals >70 years old (Elston, 2010). The infection route is usually from one person to another, by fomite or direct contact, and as such is highly common among family members (Elston, 2010). As the toenails are more frequently affected than the fingernails, tinea pedis is likely to occur simultaneously in most patients. Epidemiologically, the disease appears to be more frequent in males (Asz-Sigall et al., 2017). As with other tinea infections, the onset of tinea unguium might be related to diabetes mellitus, Down



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syndrome, psoriasis, HIV infection, peripheral vascular impairment, peripheral neuropathies, and podiatric abnormalities. However, the disease can also be consequence of sports activities and traumatic nail disorders (Asz-Sigall et al., 2017).

Depending on the way fungi colonize the nail, there are several possible infection patterns: (i) distal and lateral subungual onychomycosis (DLSO), (ii) superficial white onychomycosis (SWO), (iii) proximal subungual onychomycosis (PSO); (iv) endonyx subungual onychomycosis (EO), and finally (iv) total dystrophic onychomycosis (TDO).

**DLSO** is the most frequent type. A white patch is denoted on the distal or lateral undersurface of the nail and nail bed, usually with subungual hyperkeratosis, discolouration of the nail plate, nail thickening and onycholysis (Asz-Sigall et al., 2017). **SWO** manifestations are usually related to immunosuppression. Usually, a white, chalky plaque is observed on the proximal nail plate. This can become eroded with the loss of the nail plate. SWO can coexist with DLSO (Asz-Sigall et al., 2017; Elston, 2010). In **PSO**, the ventral portion of the nail's proximal plate is affected, with the possibility of total plate involvement. In **EO**, there is fungal penetration through the full thickness of the nail from directly under the skin. The nail bed is not infected. This disease is commonly found in immunocompromised patients. Finally, **TDO** is the most severe form of onychomycosis and is usually a DLSO evolution (Asz-Sigall et al., 2017; Elston, 2010).

Historically, the treatment of onychomycosis has been challenging (Rodgers & Bassler, 2001)

Most tinea unguium patients are first treated with oral antimycotics, such as terbinafine (Noguchi et al., 2018). Even though topical drugs are available and display the highest sales volumes compared to other drugs using distinct administration routes, nails generally offer increased resistance to topical antifungal drugs (Asz-Sigall et al., 2017; Noguchi et al., 2018). However, topical therapy can benefit SWO and mild-to-moderate DLSO cases not involving the lunula region (Asz-Sigall et al., 2017).

The three most commonly used drugs in Europe and the US for the treatment of tinea ungium are Jublia® (10% efinaconazole topical solution), Loceryl™ (50 mg/mL amorolfine nail lacquer) and Dafnegin (8% ciclopirox nail lacquer).



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*iii. Molluscum Contagiosum*

Molluscum contagiosum (MC) is a viral self-limited infectious dermatosis (Meza-Romero et al., 2019). The prevalence of this disease is relatively unknown as most patients do not seek medical care when showing signs. However, it appears to be frequent in the paediatric population, sexually active adults, and immunocompromised individuals (Meza-Romero et al., 2019). Clinically, the disease is characterized by umbilicated pink or skin-coloured papules (Burns et al., 2008; Elston, 2010; Meza-Romero et al., 2019).

*Epidemiology*

As previously mentioned, this infectious dermatosis is caused by the Molluscum contagiosum virus (MCV), which belongs to the Poxvirus family, specifically in the molluscipox genus (Burns et al., 2008). Four different genotypes can be identified: MCV 1, MCV 2, MCV 3 and MCV 4. The first type is more prevalent, accounting for 76–97 of MCV infections, whilst the second type is less frequent, but it manifests more often in adults and HIV patients (Burns et al., 2008a). The remaining two types are extremely rare (Meza-Romero et al., 2019).

The infection is transmitted by direct contact with the infected skin, which can be sexual, non-sexual, or by autoinoculation. Furthermore, the disease can be transmitted by contaminated fomites, like bath sponges or towels (Burns et al., 2008b; Meza-Romero et al., 2019).

*Aetiology and pathophysiology*

The virus enters the basal epidermis, where it replicates in the cytoplasm. This cellular proliferation leads to the appearance of lobulated epidermal growths, which in turn compress the papillae until they appear as fibrous septa between the lobules, which are pear-shaped with the apex upwards (Burns et al., 2008). The cells in the lesion centre are ultimately destroyed, appearing as large hyaline bodies (molluscum bodies), which entail the cytoplasmic masses of the virus (Burns et al., 2008). Even though the inflammatory process in the dermis is not considerable, in more chronic cases, a chronic granulomatous infiltrate can occur (Burns et al., 2008).



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*Clinical features*

The clinical features of this disease consist of shiny, pearly white, hemispherical, umbilicated papule, which may show a small dent or dot on the top. If the lesions are small (less than 1 mm in diameter), they may be identified by means of a hand lens or a dermatoscope. After 6-12 weeks, the lesions may reach a 5-10 mm diameter. Only in rare cases do larger lesions occur (Burns et al., 2008). As previously mentioned, skin inflammation might occur spontaneously after several months or following trauma. In such cases, suppuration, crusting and eventual destruction of the lesion might be observed (Burns et al., 2008). The duration of the non-treated disease is highly variable, although most cases are self-limiting within 6–9 months (Meza-Romero et al., 2019).

*Disease management and therapeutic approaches*

As this is an infection, some non-pharmacological measures are useful for containing the virus. These include: (i) Avoid lesion scratching to prevent virus release from the papules and to protect adjacent skin; (ii) not sharing towels; (iii) avoiding contact sports and communal bathing (Meza-Romero et al., 2019). In circumstances where dry skin or eczema are present, the use of skin emollients may suffice (Meza-Romero et al., 2019).

The main target of the MC pharmacological treatment is to destroy the infected epidermal cells, stimulate an immunological response or act directly against the virus (Meza-Romero et al., 2019). Considering this information, physical methods to remove molluscum contagiosum skin lesions are commonly employed. These include surgical removal by curettage, cryotherapy, carbon dioxide or pulsed dye lasers, and photodynamic therapy (Meza-Romero et al., 2019).

Pharmacologically, there are several topical drugs capable of eliciting an immune response against the MC through the establishment of a mild to moderate inflammatory process. Even though the product strength and application regimen of these drug products must be carefully defined, their use is suitable in MC management in order not to elicit skin damage. These products include cantharidin, trichloroacetic acid, diluted liquefied phenol, salicylic acid, adapalene, nitric oxide cream, potassium hydroxide solution, imiquimod and tretinoin. Topical anaesthetics such as



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EMLA™ cream (2.5% lidocaine and 2.5% prilocaine) are also commonly used in MC as they are applied prior to surgical removal of the skin lesions (Meza-Romero et al., 2019).

*iv. Viral warts*

Viral warts, or verruca or myrmecia (warts that are found on the palmoplantar location), are benign papillomas caused by the human papillomavirus (HPV) (Elston, 2010; Lynch et al., 2014; Soenjoyo et al., 2020). Viral warts can affect mucosal surfaces (genital or oral) and the skin, the latter being the focus of this review. Children have a higher prevalence of viral warts (Soenjoyo et al., 2020). Even though in most cases the lesions spontaneously disappear within 2 years, there are many patients that seek medical treatment due to pain and discomfort caused by viral warts (Lynch et al., 2014b; Soenjoyo et al., 2020).

*Epidemiology*

The prevalence rates for warts varies with age and sex. According to a systematic review by Kwok CS et al. (2012), visible viral warts are uncommon in infancy, common in childhood, and their prevalence rapidly declines from the second decade of life onwards (Kwok et al., 2012).

The infection is likely to be transmitted by contaminated fomites, therefore young people who regularly expose their bare feet in changing rooms and swimming pools, are more likely to develop plantar warts (Kwok et al., 2012). Furthermore, there appears to be a trigger component related with the patient professional activity, i.e. fishmongers, butchers, and other meat handlers (Kwok et al., 2012). Similarly, to other skin diseases, immunosuppressed individuals whether due to drugs or disease, are more prone to get infected by HPV, and thus more likely to develop viral warts lesions (Kwok et al., 2012).

*Aetiology and pathophysiology*

Cutaneous viral warts are caused by the Human papilloma virus. HPV is a small DNA virus that displays a tropism for stratified squamous epithelial cells (Burns et al., 2008). The virus starts by



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infecting the basal layer of the epithelium, then viral replication takes place in fully differentiated keratinocytes, which are located in the upper stratum spinosum and stratum granulosum. This series of events leads to the appearance of warty papules, or plaques, on the skin (Burns et al., 2008).

The appearance and location of verruca is highly dependent on the HPV type (there are more than 150 types), as well as the anatomical site of the infection (Burns et al., 2008; Kwon & Lee, 2017). For instance, HPV-1 replicates in heavily keratinized skin of palms and soles, whilst HPV-16 has a preference for genital areas and HPV-11 replicates in genital and laryngeal epithelium (Burns et al., 2008).

Generally, viral warts are benign, however a small percentage can evolve to dysplasia or neoplasia. Nevertheless, this occurrence is far more common in cervical carcinoma (Burns et al., 2008).

*Clinical features*

The clinical features consist of firm papules (1 to 10 mm diameter) with a hyperkeratotic and clefted surface (Elston, 2010a). Viral skin warts can be classified according to the lesion location, as follows:

- **Verruca Vulgaris** (Common Wart): Palmar lesions disrupt the normal line of the fingerprints.
- **Verruca plantaris** (plantar wart): Affects the plantar foot. These warts are usually solitary.
- **Verruca plana** (flat wart): These warts are sharply defined and display a “flat surface”. They usually occur on the face, beard area, dorsal of hands and shins.
- **Epidermodysplasia Verruciformis**: It is a rare autosomal recessive genodermatosis, with cases linked to chromosome X, that clinically resembles Pityriasis versicolor–like lesions and primarily occurs on the trunk.



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*Disease management and therapeutic approaches*

Viral warts often disappear without any treatment, however depending on location they can be unsightly and painful (Kwok et al., 2012). Similarly, to MC treatment, the main focus of the verruca pharmacological treatment is to physically or chemically ablate the warts, or to stimulate an immune response (Soenjoyo et al., 2020).

A strong peeling medicine, such as salicylic acid, is used to remove the layers off the wart, eventually removing it completely and Actikerrall® (fluoroucil and salicylic acid cutaneous solution 5 +100 mg/g), Verrumal (fluoroucil and salicylic acid cutaneous solution 5 +100 mg/g) and Duofilm® (Salicylic acid 16.7% w/w, Lactic acid 16.7% w/w cutaneous solution) are products with high market expression. However, this type of treatment usually takes a few weeks or months depending on the wart. Therefore, it is important that the patient receives clear instructions on the importance of treatment compliance, as well as information on the irritant potential of these products (Lynch et al., 2014).

Cryotherapy, usually resorting to liquid nitrogen, is often used, but it is less convenient, more painful and also more expensive (Kwok et al., 2012).

*v. Impetigo*

Impetigo is a common cutaneous infection, especially prevalent in children (Pereira, 2014a). Two main forms can be identified: (i) non-bullous impetigo, also referred to as impetigo contagiosa or Tilbury Fox impetigo, and (ii) bullous impetigo. Both types vary in their aetiology. The first, is primarily caused by *Staphylococcus aureus*, *Streptococci*, or by both organisms together, whilst bullous impetigo is mainly triggered by *Staphylococcus aureus* (Burns et al., 2008; Pereira, 2014).

*Epidemiology*

The prevalence rates of non-bullous impetigo are high throughout the world and large outbreaks often occur, frequently in children (Burns et al., 2008; Koning et al., 2012). In fact, impetigo is the third most common skin disorder in children, after dermatitis/eczema and viral warts (Koning et al., 2012).



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On the other hand, non-bullous impetigo is less frequent, except in warmer and more humid areas, where the streptococcal form predominates and is endemic (Koning et al., 2012) Peak incidence occurs between the ages of one and eight years, furthermore the disease often manifests in late summer (Koning et al., 2012).

*Aetiology and pathophysiology*

The colonization process by pathogenic bacteria, such as the one involved in impetigo, starts by the irreversible adherence of the host cell, by means of an adhesin, to the skin. In staphylococcus and streptococci, the main component of the adhesins are teichoic acids. In the skin, the receptor involves an epithelial cell composed of fibronectin. Depending upon the anatomic site, pathogenic potential is different as it is highly dependent upon the receptor's particularities (Darmstadt & Lane, 1994). Taking the above into account, intact stratum corneum provides the primal mechanism for skin defence, because according to the bricks and mortar model, the stratum corneum cells are tightly adherent, thereby providing a strong physical barrier to the ingress of microorganisms (Lane, 2013). Furthermore, due to the stratum corneum turnover every 14 days, there is a reduced potential for pathogenic microflora adherence maintenance. However, increased temperature, humidity, presence of cutaneous disease such as atopic dermatitis and patient age are all factors that contribute to the modification of the skin resident flora. As previously mentioned, bullous impetigo is almost universally caused by *S. aureus* (Pereira, 2014). This agent produces exfoliative toxins which are responsible for hydrolyzing one of the intracellular adhesion molecules, desmoglein-1, present in the desmosomes of keratinocytes located in the stratum granulosum. This will lead to the formation of large blisters (Burns et al., 2008; Pereira, 2014). Due to the inflammatory process, neutrophils migrate through the spongiotic epidermis into the blister cavity, that contains cocci. Occasional acantholytic cells may be seen due to the neutrophils action (Burns et al., 2008).

In impetigo, the most common lesions are reddish sores, often around the nose and mouth, that quickly rupture and can ooze for a few days. A yellow-coloured crust will cover the burst sore. The lesions can be itchy and sore, but are usually mild (Mayo Clinic, 2023a).



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*Disease management and therapeutic approaches*

Impetigo is commonly treated with antibiotics, the selection of which is highly dependent upon the administration ease, the adverse reaction potential, and overall therapeutic effectiveness. Mupirocin available as an ointment or a cream, is the most sold drug product for impetigo treatment in Europe and North America. Mupirocin is the major metabolite of pseudomonas fluorescens fermentation (Darmstadt & Lane, 1994; Pereira, 2014). This molecule reversibly inhibits the bacterial isoleucyl-tRNA synthetase. As it has a low affinity for mammalian isoleucyl-tRNA synthetase, as well as for microorganisms belonging to the natural skin flora, mupirocin is well tolerated (Darmstadt & Lane, 1994). Furthermore, fusidic acid is also commonly used in impetigo. It is highly effective against *S. aureus*, displays a good penetration into the cutaneous surface, as well as significant concentration at the infection site (Pereira, 2014).

*vi. Cellulitis*

Cellulitis is strictly an acute inflammation of the loose connective tissue involving the deep dermis and subcutaneous fat, in which the etiological agent is a bacteria (Burns et al., 2008; Bystritsky, 2021). This condition is often undistinguished from erysipela, an infection disease involving the superficial skin and lymphatics (Burns et al., 2008; Bystritsky, 2021).

Cellulitis can be classified into two groups: purulent or nonpurulent. The first is associated with a pustule, abscess, or purulent drainage, whilst the second does not present these clinical manifestations (Bystritsky, 2021).

*Epidemiology*

Cellulitis is a common infection with the literature reporting that in North America the incidence of cellulitis/ abscess or erysipelas was 27 cases/1000 patients, which is a higher incidence than the one registered for of pneumonia and urinary tract infections combined (Bystritsky, 2021). The disease is more prevalent in middle-aged and older adults, furthermore in temperate climates, cellulitis tends to occur more often in summer (Bystritsky, 2021). This infectious disease may be



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triggered by the presence of wounds or ulcers, tinea pedis, chronic edema/lymphedema, venous insufficiency, excoriating skin disease, as well as obesity (Bystritsky, 2021).

*Aetiology and pathophysiology*

The main etiological agent of cellulitis and erysipelas cases is beta-hemolytic *Streptococci*, nevertheless, in purulent skin and soft tissue infections, the cause is usually *staphylococcus aureus* (Burns et al., 2008; Bystritsky, 2021). For the most implicated beta-hemolytic *streptococci* groups, group A (*Streptococcus pyogenes*) and group G (predominantly *Streptococcus dysgalactiae*) are highly prevalent, followed by groups B and C (Bystritsky, 2021). Infections caused by group B infection are primarily observed in patients under 3 months. However, when occurring in adults (especially after surgery) they usually cause pelvic erysipelas (Burns et al., 2008).

Despite beta-hemolytic *streptococci* being the most common etiological agent, there is a wide range of pathogens implicated in cellulitis and erysipelas, especially if the patient is immunocompromised. For instance, in young children, when the disease manifests in the ocular and buccal regions, the cause is usually *Haemophilus influenzae* type B. Most fortunately, such cases are becoming rarer due to the conjugated vaccine (Bystritsky, 2021).

*Clinical features*

The clinical features of this disease include skin erythema, swelling, tenderness and warmth (Bystritsky, 2021). The disease primarily manifests in the lower extremities, however, as previously reviewed, the infection can take place in any other skin region e.g. orbital cellulitis, vulvar or inguinal cellulitis, abdominal wall cellulitis, among others (Bystritsky, 2021).

Even though distinguishing erysipelas from cellulitis may be a laborious task, sometimes, their clinical features are slightly different, with erysipelas being characterized by a sharp demarcation of the involved skin (Bystritsky, 2021). Furthermore, in erysipelas, blistering is common, and there may be superficial haemorrhages into the blisters, or in intact skin, especially in elderly people (Burns et al., 2008a). In the most severe cases of cellulitis, there can be bullae that progress to dermal necrosis, and uncommonly to fasciitis or myositis. In such cases, lymphangitis and lymphadenopathy are also common (Burns et al., 2008).



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Without effective treatment, the patient might die, as the disease can evolve to fasciitis, myositis, subcutaneous abscesses, septicemia and, in some streptococcal cases, nephritis (Burns et al., 2008).

*Disease management and therapeutic approaches*

As cellulitis is a bacterial infectious disease, the treatment preference is an oral antibiotic such as penicillin VK (penicillin V potassium) and amoxicillin. However, in order to include coverage against methicillin-susceptible *Staphylococcus aureus*, the inclusion of amoxicillin-clavulanate, dicloxacillin, cephalexin and clindamycin is also common (Bystritsky, 2021).

The most sold topical drug products for cellulitis are Betadine (antiseptic topical solution), and Cryoserv® (dimethyl sulfoxide solution).



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### *C. Skin Cancers*

Outdoor activities are becoming more and more popular, consequently, the prolonged skin exposure to sunlight directly results in a significant increase of skin cancers worldwide (Cummins et al., 2006). In fact, skin cancer is the most frequently diagnosed cancer in the United States and worldwide (Wysong, 2023). Nonmelanoma skin cancers, also referred to as keratinocyte carcinomas represent the most common type of skin cancers (Wysong, 2023). Within this group, three main types can be readily identified: actinic keratosis (a premalignant skin disease, AK), basal cell carcinoma (BCC) and cutaneous squamous cell carcinoma (cSCC). Due to their ever-growing relevance an overview of these cancers with regards to their epidemiological profile, pathophysiology, clinical features and disease management approaches is presented.

#### *i. Actinic Keratosis*

Actinic keratosis (AK) is the most frequent premalignant skin disease in the Caucasian population, its main aetiology being exposure to ultraviolet radiation. This disease displays a prevalence of 37.5% among 50 years of age or older Caucasian patients. Due to its high prevalence, AK is one of the most common reasons for patients to visit a dermatologist (Jansen et al., 2019). If this condition is left untreated, it may evolve into basal cell or squamous-cell carcinoma, which are the most common cancers in the USA, as well as in other countries with mostly light-skinned populations (Jansen et al., 2019). However, no specific clinical characteristics distinguish which AK lesions pose as a higher risk and what percentage of the lesions have the ability to progress into a skin carcinoma (Siegel et al., 2017). AK is a major public health concern due to its high prevalence, considerable financial impact, as well as potential for malignant transformation.

##### *Epidemiology*

As previously referred, AK lesions are common among lighted-skinned individuals, and they typically manifest on sun-exposed areas. In Australia, it is estimated that 40-50% of the Caucasian population over 40 years, will develop AKs (Costa et al., 2015). Consequently, Australia also



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displays the highest incidence of Basal Cell Carcinoma worldwide. In the US, the prevalence of this condition varies from 11 to 26%, on the other hand in Europe, this percentage is lower with solely 15% of men and 6% of women, being reported to be affected. Individuals with Fitzpatrick type I or II skin characteristics, such as fair skin, freckles, light-coloured eyes (blue or green) and blonde or red hair are more likely to develop this condition, due to the fact that they are more sensitive to damage from chronic sun exposure. Other risk factors include older age (as this condition is associated with chronic sun damage, the elderly population is favoured. In most cases, the lesions appear almost exclusively in individuals over 45), male gender, cumulative UV exposure, proximity to the equator (AK prevalence is higher in warmer climates), and immunosuppression therapy (Costa et al., 2015).

Individuals with AKs often present 6 to 8 lesions, which usually manifest on the head, bald scalp, face, dorsal forearms and hands. Immunocompromised patients (e.g. with organ transplant and on immunosuppressive therapy) are up to 250 times more likely to develop this condition (Siegel et al., 2017). AKs are diagnosed in up to 10% of dermatology visits, making them one of the most common diagnoses.

*Aetiology and pathophysiology*

Actinic keratoses are the most common lesions. AK may follow 3 different pathways: Regression, persistence or progression to in situ or invasive SCC, a condition with lower potential for metastasize (Costa et al., 2015). However, the risk of an AK's evolve to invasive SCC is not accurately estimated, as the rate may diverge from the 0.1% to 20%. For a patient displaying multiple AK lesions (more than five), the risk of developing invasive cutaneous SCC is between 0.15% to 80%. Histological studies have documented that most of SCC arise from AK lesions, therefore taking into account that it is not possible to predict which AK lesions will advance to this skin cancer, it is important to treat them as soon as possible.

In what concerns the pathophysiology of the disease, the UV radiation damages DNA keratinocyte, thereby reducing the skin immunity (Burns et al., 2008). The mutations involved in the AK pathogenesis, as well as in the ability of the disease to progress into SCC are p16 (INK4a,



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on chromosome 9p21); p14 (ARF); p15 (INK4b) and p53. On a molecular level, UV-A (320-400 nm), is the most abundant source of solar radiation. These rays penetrate the skin more deeply, when compared to UV-B. Molecularly, this depth of penetration induces oxidative damage in the nucleic acids structure, membrane lipids, causing cell proteins damage, alongside with reactive oxygen species (ROS) production. These agents are known to affect the cellular transduction pathways, as well as cell-cell signalling mechanisms, causing an altered proliferation. Thymine (T) — guanine (G) is the signature mutation of UV-A, due to the formation of 8-hydroxyguanine.

In what concerns UV-B radiation (290- 320 nm), it is responsible for the formation of cyclobutane pyrimidine dimers, as well as and 6-4 photoproducts, which are responsible for the cytosine (C) - T and CC -TT mutations, which are also highly characteristic. Furthermore, p53 (tumour suppressor protein) inactivation by UV-B light, is also a key mechanism contributing to the AKs development, as with generates genetically unstable keratinocytes. Mutations in the TP53 gene have been reported in 90% of human cutaneous SCCs. In animal models (mice), the main function of this gene is to protect the skin cancer induction by UV light. In the absence of this gene, other DNA mutations go on to promote the carcinogenesis. Furthermore, the absorbed UV light increases the production of arachidonic acid, corresponding metabolites, pro-inflammatory cytokines, as well as reactive oxygen species which induce lipid peroxidation and cellular destruction. Taking this scenario into account, the main mechanisms that lead to AK development are inflammation, oxidative stress, immunosuppression, impaired apoptosis, mutagenesis, cell growth dis-regulation and proliferation, and tissue remodelling. Due to the potential of AK to regress or progress into SCC, some authors classify this condition as premalignant; however, many publications also suggest that AK can be classified as "cancerous". In fact, Cockerell proposed to use the "keratinocyte intraepidermal neoplasia" (KIN) nomenclature, following the classification used for cervical intraepithelial neoplasia.

Recently, it was documented that cutaneous human papilloma virus (HPV) infection, is associated with AK development. Even thought, the exact cellular mechanism has not been completely elucidated, it is hypothesized that the E6 protein of cutaneous HPV appears to contribute to a



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reduction of Bak protein levels. The E6 protein presents pro-apoptotic effects, being activated as a protective mechanism in keratinocytes exposed to UV light.

The differentiation between AK and early SCC is extremely challenging, due to the resemblance of several cellular and histopathological features, shared in both conditions. However, a common way to distinguish both diseases is the relative depth of dysplastic cells within the skin (Siegel et al., 2017).

In what concerns AK progression into invasive SCC, there are two main pathways: the classical and the differentiated. In the first one, there are atypical keratinocytes present in the lower one-third of the epidermis–keratinocyte intraepidermal neoplasia (KIN I). with the progression of the disease, these cells proceed to fill the lower two-thirds (KIN II), and ultimately the full thickness (KIN III) of the epidermis, before becoming invasive SCC. This mechanism is similar to the progression of cervical intraepithelial neoplasia (CIN), present in the human papilloma virus (HPV). On the other hand, in the 'differentiated pathway', which may be more aggressive and more prevalent, this progression is not observed.

*Clinical features*

The clinical features of AK regard the presence of erythematous or flesh coloured, scaly papules or plaques with a gritty, sandpaper-like texture. Some patients present telangiectasias, within or surrounding the lesion, whilst others develop highly pigmented lesions. The appearance of horny proliferations, referred to as verrucous keratosis may also manifest. AKs range in size from a few millimeters to more than 2 cm in diameter, often coalesce, and as previously mentioned, the lesions are mainly located in sun-exposed areas (face, dorsum of the hands, shoulders, and balding scalp). In severe hyperkeratosis cases, a cutaneous horn which reveals a red and fissured base if removed, may also be identified. The main symptoms of AKs include itch and tenderness, however there are many asymptomatic cases. In accordance with the clinical presentation, we can distinguish three different grades of AKs: grade I) Visible and slightly palpable; grade II) Visible and palpable; grade III) Frankly visible and hyperkeratotic (Costa et al., 2015).



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Histologically, AKs are characterized by atypical keratinocytes, that exhibit a loss of polarity, nuclear pleomorphism and increased mitotic figures in the basal epidermis, which may extend into the entire epidermis. Keratinocytes of the sweat glands and hair follicles are not affected. The stratum corneum exhibits alternating parakeratosis and hyperkeratosis secondary to abnormal keratinocyte development. On the other hand, stratum basal and stratum spinosum keratinocytes present a disordered pleomorphic and anaplastic nuclei. Many AKs show solar elastosis, alongside a mild inflammatory infiltrate of lymphocytes and plasma cells, in the dermis. Despite these common features, AKs vary histologically, and can, accordingly, be divided into six types: hypertrophic, atrophic, bowenoid, acantholytic, lichenoid and proliferative (Siegel et al., 2017).

*Disease management and therapeutic approaches*

Treatment primarily consists of lesion-directed destruction, or field treatment with topical drugs, with the purpose of preventing the progression of the lesions into skin cancer, as well as to improve the appearance and reduce associated symptoms (Siegel et al., 2017). Unfortunately, the recurrence rate after treatment is high, which in many cases leads to recurring treatments. However, studies suggest that the effect of fluorouracil can be extremely long (Siegel et al., 2017). Solitary lesions are often treated with cryotherapy. However, patients with actinic keratosis often present multiple lesions in one continuous area (so-called field change). In these scenarios, the application of field-directed therapies is preferred, not only due to their therapeutic effectiveness, but also due to their prophylactic role on the development of new lesions, and ability to prevent the development of squamous-cell carcinoma. Current guidelines provide no clear recommendations about which treatment approach is preferred. Ultimately, the choice of treatment often depends on the preferences of patients, as well as their treating physicians. Topical diclofenac (Solaraze) is one of the drugs prescribed for this disease. It is believed to exert its effects through the inhibition of cyclooxygenase (COX), especially COX-2. W Without this enzyme, the production of prostaglandin, that suppresses the immune system enabling tumour formation, is reduced and the cascade is disrupted. The treatment regimen regards two applications per day, during a period of 90 days (Costa et al., 2015). Other favoured treatments include



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fluorouracil cream, imiquimod cream, photodynamic therapy (PDT) and metvix® (methyl aminolevulinate hydrochloride 160 mg/g cream); A network meta-analysis, which evaluated AK therapies based on complete clearance rates, ranked topical treatments from highest efficacy to lowest efficacy as follows (Costa et al., 2015):

- 5% 5-FU.
- 0.5% 5-FU.
- Photodynamic therapy (PDT) with aminolaevulinic acid (ALA);
- Imiquimod.
- Methyl aminolaevulinate PDT (MAL-PDT). This product regards the 2nd most sold product in AK management (Metvix®: methyl aminolevulinate hydrochloride).
- Cryotherapy.
- Diclofenac with hyaluronic acid.
- Placebo.

In 2012 a Cochrane review, stated that according to data retrieved from 83 different randomized controlled trials, the use of diclofenac, 5-FU, imiquimod, and ingenol mebutate displayed similar efficacies. Furthermore, it was not possible to document a clear relationship between the different AK treatments, with decreased rates of SCC (Gupta et al., 2012). Additionally, high-dose topical tretinoin has not been effective in AK prevention. As systemic retinoids display a significant side-effect profile, these should be avoided (Gupta et al., 2012).

Topical 5% imiquimod cream (Aldara®), originally indicated for genital and perianal warts and used off-label to treat Bowen's disease, invasive SCC, lentigo maligna, molluscum contagiosum, keloid scars, as well as other diseases, has also been approved for AKs and superficial basal cell carcinoma management. Imiquimod disrupts tumour proliferation by acting as a toll-like receptor-7 agonist that modulates the immune response and stimulates apoptosis. Stockfleth and colleagues showed that 84% of AK patients showed clinical clearance, with solely one 12-week cycle of 5% imiquimod therapy. However, the authors also describe that local irritant reactions are a common side effect (Stockfleth et al., 2008). Furthermore, patient compliance may be also challenging due



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to the long treatment duration (twice weekly for 16 weeks). Systemic effects, include fatigue, flu-like symptoms, headaches, myalgia, and angioedema, however these are rare.

Regulatory approval was granted by Health Canada in December 2009 and by the US FDA in March 2010 to imiquimod 3.75% (Zyclara™) for the treatment of AKs on the face or balding scalp (Lebwohl et al., 2012). Patients applying imiquimod 3.75% achieved a median lesion reduction of 82%, while just over one-third demonstrated complete lesion clearance. This efficacy profile is similar to the one registered with imiquimod 5%, twice weekly for 16 weeks. However, the lower dosage appears to significantly improve patient tolerance to treatment. This regimen was found to be safe, and no serious adverse events were reported. However, erythema was seen in most patients, with up to 25% developing severe erythema. Despite of this side effect, no patients were withdrawn from the study and compliance rates were noted to be higher than 90% (Lebwohl et al., 2012). Even through this product is not in the top 10 most sold products for AK, overall, imiquimod 3.75% is a reasonable alternative to imiquimod 5%, as it demonstrates a comparable efficacy profile, allowing for a much simplified, shorter dosing regimen, and seemingly this therapy presents less severe adverse effects. Due to the appointed reasons, imiquimod 3.75% is approved for the treatment of a larger surface area (up to 200 $cm^2$), whilst the 5% formulation should only be used in an area up to 25 $cm^2$.

*ii. Basal cell carcinoma*

Basal cell carcinoma (BCC) is a skin carcinoma derived from epidermal cells, being the most common skin malignancy worldwide (Dika et al., 2020) Despite BCC being a locally invasive and aggressive cancer, it has a negligible mortality rate due to its slow growing characteristics and reduced tendency to metastasize (Dika et al., 2020; Elston, 2010; Fania et al., 2021; Peris et al., 2019).



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*Epidemiology*

Even though there are a wide range of factors connected with BCC development, the importance of exposure to Ultraviolet light (UV), skin phototype and age, are well documented in the pathogenesis of this cancer. Due to these reasons, it is easy to understand why, epidemiologically, BCC is more prevalent in the Caucasian population. In fact, the incidence of BCC is intrinsically connected with the geographic latitude of the country, as well as the pigment status of the populations. Notwithstanding, the disease appears to have reached a plateau in Australia, which regards the country with the highest incidence of BCC worldwide. Conversely, in other European and Asian countries, BCC incidence continues to grow (Dika et al., 2020).

As previously mentioned, BCC is the most common skin cancer worldwide, accounting for 75% of all skin cancers (Peris et al., 2019). BCC incidence is higher in men, in comparison with women and this cancer is more prevalent in the elderly population (>60 years). Nevertheless, the number of young females (<40 years) affected by BCC is higher than in men in this specific age group. This occurrence maybe ascribed to changes in women clothing, as well as sun exposure behaviours (Peris et al., 2019).

*Aetiology and pathophysiology*

There are several drivers linked with BCC development: exposure to UV light, age, skin phototype, gender, pharmacological and radiation therapy, family history of skin tumours, long-term exposure to arsenic, immunosuppression, as well as some genetic syndromes (Fania et al., 2021).

UV light exposure is one of the main etiological factors, thus explaining why most of the tumours are located on sun exposed areas (Peris et al., 2019). The cumulative exposure to UV radiation (UVA and UVB wavebands) can provoke direct cell damage through the following distinct mechanisms:

- DNA mutations e.g. C:T or CC:TT transitions at dipyrimidine sites (Fania et al., 2021);
- Induction of oxidative stress;



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- Induction of local inflammatory processes;
- Suppression of cutaneous antitumor immunity (Fania et al., 2021).

Furthermore, it should also be mentioned that indoor tanning, as well as a high number of psoralen and ultraviolet A (PUVA) (>100–200) and UVB (>300) treatments are also proved to be risk factors for BCC development (Fania et al., 2021).

Age, is also a significant etiological factor due to its impact on DNA repair capacity, genomic instability, decline of immune system function and chronic inflammation (Fania et al., 2021). These events lead to an accumulation of DNA damage and senescent cells, which together with the chronic inflammation, create a suitable environment for tumour development (Fania et al., 2021).

There are also certain hereditary disorders linked with the development of BCC, such as the Gorlin syndrome, Xeroderma Pigmentosum and the Bazex syndrome (Dika et al., 2020). The pathophysiology mechanisms involved in the appearance of this cancer in the general population, are yet to be fully disclosed, however the importance of the Hh (Hedgehog pathway) genes (PTCH1, SMO, SUFU, TP53) is undeniable (Dika et al., 2020; Peris et al., 2019). A whole exome sequencing analysis of 293 BCC has revealed that 85% of sporadic BCCs display mutations in these genes, therefore, as mentioned by K. Peris and colleagues, an aberrant activation of Hh signalling represents the hallmark of BCC pathogenesis (Peris et al., 2019). There are other tumours in which the Hh pathway is also extremely important, such as medulloblastoma and neuroblastoma (Peris et al., 2019). The Hh pathway plays a critical role in embryogenesis, cell differentiation, as well as cell proliferation mechanisms. The physiological activation of this signalling pathway initiates with the binding of extracellular HH ligands (e.g. sonic hedgehog (SHH), Indian hedgehog (IHH) and desert hedgehog (DHH) in mammals) to PTCH1. This connection will lead to the suppression of PTCH1 and consequently, to the release of SMO. This event will initiate a signalling cascade, responsible for the release and activation of the GLI family of transcription factors (GLI1, GLI2, and GLI3) which are sequestered in the cytoplasm by several proteins, including the Suppressor of Fused (SUFU) (Peris et al., 2019).



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In BCC, somatic mutations in the PTCH1 gene are extremely common (70-75% of BCC), followed by mutations in the SMO gene (10-20%). Finally, a small fraction of BCCs present mutations in a gene homologue of PTCH1 (PTCH2) and SUFU, being these mutations responsible for the cancer development (Peris et al., 2019). However, it should also be referred that the activation of noncanonical Hh pathways (e.g., EGFR, IGF, TGF pathways) may likewise contribute to BCC development (Peris et al., 2019).

Other etiological factor of this disease is the use of potentially photosensitizing drug which can act as co-carcinogens together with UV radiation, thereby increasing the risk of skin cancer. Examples of these drugs regard tetracyclines, thiazide diuretics, NSAIDs and retinoids (Fania et al., 2021).

*Clinical features*

BCC lesions usually manifest on the face and hairy skin, including the upper and lower extremities (Fania et al., 2021). Only in rare cases, the lesions appear in the genital mucosa (Fania et al., 2021).

There are several clinical subtypes of BCC, but the most relevant types are the nodular, the superficial and the morpheaform:

- Nodular BCC (NBCC) is the most prevalent type, accounting for 50-79% of BCC cases. Clinically, it manifests as a sharp border papule or nodule, with a pearly, shiny edge, as well as small arborizing telangiectasias (Fania et al., 2021). NBCC lesions most frequently appear in the cheeks, nasolabial folds, forehead and eyelids (Fania et al., 2021).
- Superficial BCC is the second most prevalent type, being more common in younger patients when compared to other subtypes. The lesions primarily manifest in the trunk and extremities, and are characterized by a sharply circumscribed, scaly, pinkish macule, papule or thin plaque (Fania et al., 2021).
- Morpheaform BCC (MBCC) accounts for 10% of BCC. This subtype is more aggressive than the other forms and may lead to extensive local destruction. Clinically, MBCC is very similar to an indurated plaque of localized scleroderma, as the lesions are ivory-white, shiny, smooth,



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indurated plaques or depressions with poorly defined edges (Fania et al., 2021).

Only 1% of BCC progress to giant BCC, also referred to as ulcus rodens. This clinical subtype concerns an extremely destructive BCC form which is characterized by a deep tissue invasion, that displays a high potential of post-surgical recurrence (Fania et al., 2021).

As with other dermatological diseases, several subtypes of BCC can coexist in a single lesion (Fania et al., 2021). In fact, BCCs are highly polymorphic cancers, being often challenging to classify the lesions into a single subtype (Peris et al., 2019). In these circumstances, which represent 40% of the BCC cases, the cancer is referred to as a mixed tumour (Fania et al., 2021).

Clinically, BCC can also be categorized into low risk and high risk, with this classification being extremely important to define the therapeutic strategy to implement. There are several parameters that increase the risk of BCC:

- Tumours with larger size.
- Tumours with poorly defined lesions.
- Whenever the BCC type if morpheaform or and metatypical.
- The presence of perineural and/or perivascular invasion.
- When the lesions are in high-risk zones: nose, periorificial areas of the head and neck (Fania et al., 2021).

*Disease management and therapeutic approaches*

The main goal of BCC treatment regards the complete removal of the tumour, preferably maintaining both cosmetic and functional aspects. For this reason, surgery is the most common treatment for BCC (Fania et al., 2021). Mohs Micrographic Surgery, or margin control techniques represent the gold standard surgical approaches to BCC, displaying high cure rates (Fania et al., 2021).

Destructive therapies with curettage, electrocautery (electrodesiccation), cryotherapy and laser ablation are fast and cost effective, especially in low-risk non-facial BCC, or for multiple small



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BCCs (Dika et al., 2020; Fania et al., 2021). Other therapeutic approaches concern photo dynamic therapy, as well as interlesional therapy.

In cases where surgery or radiotherapy are not appropriate, systemic Hh pathway inhibitors can be used for locally advanced or rare metastatic BCCs (mBCCs) treatment. The Hh inhibitors regard Vismodegib and Sonidegib are both approved by EMA and FDA. These drugs act as suppressors of SMO, which as previously mentioned displays and important role in BCC pathogenesis (Dika et al., 2020).

Topical treatments are also available; however, their applicability is mainly targeted to superficial BCC or to small BCCs located in low-risk areas. Furthermore, topical treatments are also useful in patients with severe comorbidities, or in cases where the surgery is contra indicated. The main products regard Topical 5-fluorouracil (5-FU) 5% cream, and imiquimod 5% cream (Fania et al., 2021).

Imiquimod is an immune response modifier approved by both EMA and the FDA for small BCC treatment in immunocompetent adults. The therapeutic regimen consists of five daily applications, for a period of 6 weeks (Peris et al., 2019). 5-FU is a pyrimidine analogue which inhibits pyrimidine metabolism and DNA synthesis, thereby functioning as an antineoplastic agent. For BCC treatment, Topical 5-FU 5% cream is used in low-risk lesions on the trunk and lower limbs (Burns et al., 2008a). This product is also approved by EMA and FDA and should be applied twice a day for a period of 2 to 4 weeks (Peris et al., 2019).

xi. *Cutaneous Squamous Cell Carcinoma*

Cutaneous squamous cell carcinoma (cSCC) is a highly prevalent nonmelanoma skin cancer which shares several similarities with BCC. Worldwide cSCC is the second most common skin cancer (following BCC) and the incidence of this disease is dramatically increasing, with cSCC accounting for 20% of all skin cancers (Caudill et al., 2023). This disease is also mainly circumscribed to white populations exposed to sunlight (Caudill et al., 2023). Nevertheless, it should be mentioned that in non-white populations, cSCC regards the most common skin cancer,



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even though the lesions differ in their anatomical location, aetiological factors and prognosis whenever compared to the Caucasian population (Burns et al., 2008a).; Similarly to BCC, in cSCC a malignant proliferation of epidermal keratinocytes with dermal invasion is observed, mainly triggered by DNA damage (Stratigos et al., 2020). The overall prognosis for patients with cSCC is excellent, such as the one observed in BCC. However, in cSCC there is a higher tendency to mestasize, with nodal metastases occurring in 1.9 to 5.2% of cases. The overall mortality of this cancer is 1.5 to 3.4% (Wysong, 2023).

*Epidemiology*

cSCC is the second most common skin cancer, accounting for 1 million of new cases every year (Wysong, 2023a). The incidence of this disease continues to grow as a direct result of population aging, higher levels of sun exposure, use of tanning beds, rising numbers of immunocompromised individuals, but also to an increased focus on skin cancer screening and detection (Wysong, 2023a). Similarly to BCC, the chances of developing cSCC increase with white populations, age, male sex and low latitude (Stratigos et al., 2020).

*Aetiology and pathophysiology*

The main etiological factor of cSCC concerns UVR exposure, as most cSCC present the UV dinucleotic mutation signature: C > T or CC > TT (Stratigos et al., 2020). In this context, people highly exposed to sunlight, tanning beds or patients receiving high doses of photochemotherapy display an increased risk (Elston, 2010; Stratigos et al., 2020; Wysong, 2023). Furthermore, there are other environmental hazards that contribute to the appearance of this cancer: arsenic, itch, tar, crude paraffin oil, fuel oil, creosote, lubricating oil, as well as nitrosoureias exposure (Wysong, 2023). As with BCC, there are phenotypic traits that increase the probability of developing cSCC, such as light skin, red or blonde hair and light-colored eyes (Wysong, 2023). Furthermore, a family history of cSCC also poses as an additional risk. Genetically inherited disorders including xeroderma pigmentosum, epidermolysis bullosa, oculocutaneous albinism, Fanconi anaemia and Muir Torre syndrome, contribute to an earlier age onset (Stratigos et al., 2020).



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Besides the above-mentioned dinucleotide mutations, there are several genes affected by UV light: TP53 and CDKN2A which are involved in cell cycle control, NOTCH1 and NOTCH2, the epigenetic regulators KMT2C, KMT2D, TET2 and mutations of TGFb receptors, leading to their inactivation (Stratigos et al., 2020). Worldwide genomic studies have also revealed the involvement of several polymorphisms, such as in the MC1R, ASIP, TYR, SLC45A2, OCA2, IRF4, BNC2, CADM1, AHR, and SEC16A (Stratigos et al., 2020). A comprehensive overview of these molecular pathophysiological mechanisms can be found in the European interdisciplinary guideline on invasive squamous cell carcinoma of the skin (Stratigos et al., 2020).

The incidence of the disease is also higher in immunocompromised patients, including organ transplants recipients, patients receiving long-term immunosuppressive therapy, as well as patients suffering from chronic inflammation. Furthermore, HPV infection also poses as a risk factor for the development of periungual and anogenital squamous-cell carcinoma (Wysong, 2023). The appearance of cSCC can also provoked by several drugs, such as voriconazole hydrochlorothiazide, BRAF inhibitors, and tumor necrosis factor inhibitors (Wysong, 2023).

*Clinical features*

The main clinical features of this cancer concern scaly, erythematous, or bleeding lesions, most often on sun-exposed areas (Wysong, 2023). However, when cSCC develops in non-white populations, the lesions occur on the palms of the hands, soles of the feet, nails, anogenital regions, as well as in chronic inflammation or scaring body sites (Wysong, 2023).

cSCC can be classified as primary or advanced. The first type is more prevalent, displays a low metastatic potential, and the treatment is simple. Primary cSCC can be further classified as low or high risk, depending on the recurrence probability. On the other hand, advanced Cscc can be classified as locally advanced (lacSCC), or metastatic (mcSCC) including locoregional metastatic or distant metastatic cSCC, respectively (Stratigos et al., 2020) The overall prognosis for patients with cSCC is excellent, with 5-year cure rates of greater than 90% (Stratigos et al., 2020).



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*Disease management and therapeutic approaches*

The gold standard treatment of most cSCC concerns surgical removal (Caudill et al., 2023). The most commonly employed methods are standard excision, Mohs surgery, curettage and electrodesiccation (Caudill et al., 2023). Mohs micrographic surgery or resection with peripheral and deep exhaustive margin assessment is the recommended approach to achieve local control for high-risk and very-high-risk cases (Wysong, 2023). Other surrogate treatment regimens include cryosurgery, photodynamic therapy and radiation therapy (Wysong, 2023).

In patients where neither curative surgery nor radiation therapy are applicable, conventional chemotherapy, immunotherapy, and targeted therapies are also available. For immunotherapy, cemiplimab and pembrolizumab are used in patients with recurrent, locally advanced (stage la) disease, who are not candidates for surgery, as well as for patients with new or recurrent regional disease or distant metastasis. Cetuximab together with radiation therapy, has also proved to be a promising therapeutic strategy for advanced cSCC cases (Wysong, 2023).

xii. *Cutaneous Melanoma (malignant melanoma)*

Cutaneous melanoma (CM) is one of the most aggressive andterminal forms of skin cancer. CM derives from melanocytes, pigment-producing cells, that originate from the neural crest located along the choroidal layer of the eye, mucosal surfaces and meninges in the hair follicles, as well as the basal epidermis (Naik, 2021). This cancer may occur in any tissue that contains these cells, including noncutaneous sites such as the oral mucosa, nasopharynx, paranasal sinuses, tracheobronchial tree, vulva, vagina, anus, urinary tract, central nervous system, and eye. However, most CM appear on the skin surface (Cummins et al., 2006).

Even though solely 3% of skin cancers are diagnosed as CM, this disease alone accounts for 65% of skin cancer deaths. The incidence worldwide, similarly to the previously detailed skin cancers, is on the rise. CM is nowadays one of the most common cancers in young adults (Naik, 2021; Pavri et al., 2016). The timely diagnosis of CM is crucial for a good clinical outcome, due to the high metastatic potential, characteristic of this cancer (Naik, 2021).



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*Epidemiology*

The American Cancer Society in 2023, estimates that in the US approximately 97,610 new melanomas will be diagnosed yearly (about 58,120 in men and 39,490 in women), and that about 7,990 people are expected to die of CM (about 5,420 men and 2,570 women) (R. L. Siegel et al., 2023).

The overall frequency of cutaneous melanoma is increasing over the past few decades, however there are slight differences according to the age group. In adults aged 50 and older, the rates continue to increase in women by about 1% per year (from 2015 to 2019), however this increase appears to have stabilized in men (A. C. Society, 2023).

CM is more prevalent in white people. Overall, the lifetime risk of getting the disease for White people is about 2.6%, 0.1% for Black people, and 0.6% for Hispanic people (A. C. Society, 2023). Age is also an important factor, 65 being the average age of people diagnoses. Nevertheless, it should be pointed out that CM is one of the most common cancers in young adults (A. C. Society, 2023).

*Aetiology and pathophysiology*

As with all skin cancers, the development of CM is dependent on the interaction between the host and the environment (Naik, 2021). The main etiological factors of CM are:

- Ultraviolet radiation exposure: Strong epidemiological evidence connects UV exposure to CM development, being the principal etiological factor of this disease with UV-B being the biggest cause (Cummins et al., 2006);
- Phenotypic characteristics: Individuals with lighter skin and hair tone, display low melanin levels and are at increased risk of melanoma development (Naik, 2021). Furthermore, individuals with a higher amount of moles also have an increased risk (Cummins et al., 2006; Naik, 2021);
- Inherited factors: A positive family history also increases the risk, due to sun exposure habits and/or inherited genetic mutations.
- Genetic features: Mutations on the CDKN2A gene on chromosome are believed to increase



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the risk of melanoma (Naik, 2021). Furthermore, BRAF mutations are found in approximately 50 % of melanomas, especially in younger patients (Pavri et al., 2016).

*Clinical features*

There are five clinical signs of CM, broadly referred in the literature as the "ABCDE rule" (Elston, 2010). These are:

**A** - **A**symmetry in shape.

**B** - **B**order is irregular—the edges are irregularly scalloped, notched and sharply defined.

**C** – The **C**olor is not uniform; The lesions may display all shades of brown, black, gray, red, and white.

**D** – The **D**iameter is usually large (6 mm being a typical guide).

**E** – An **E**levation is almost always present and is irregular. Since most lesions tend to be elevated, more recent guidance has suggested "**E**volution" (i.e. a change in the lesion) as a better indicator of CM.

Even though the clinical presentations may differ, this criterion highlights the key characteristics of suggestive CM skin lesions (Cummins et al., 2006).

Clinically, four main types of CM can be identified: Superficial spreading, nodular, lentigo maligna and acral lentiginous CM. A brief description of the main clinical features of each subtype will be briefly overviewed.

The **superficial spreading subtype** accounts for approximately 70% of melanomas. The lesions usually develop from pre-existent dysplastic nevus. Firstly, a radial growth phase can be identified, however, afterwards, the lesion extends downwards into the dermis. In this stage, the metastatic potential increases. This clinical subtype equally affects men and women, and the lesions are mainly circumscribed to the trunk in men, and to the lower extremities in women. Superficial melanoma may evolve for a period of 1 to 7 years (Cummins et al., 2006; Naik, 2021).



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**Nodular melanomas** represent about 15% to 30% of CM and are more prevalent in men (Cummins et al., 2006; Naik, 2021). This clinical presentation is the most aggressive of all CM subtypes. The lesions are characterized by (i) the absence of a radial growth phase, and therefore the lesions may be smaller; (ii) the presence of a high degree of vertical invasion, thus meaning a worse prognosis; and finally (iii) a variable presentation, that frequently tends to meet fewer ABCDE criteria, thus often impairing the diagnosis (Cummins et al., 2006). The lesions often appear as a smooth, shiny, dome-shaped nodule of uniformly dark black or blue colour and display a rapid growth (Cummins et al., 2006).

**The lentigo maligna CM** is the less commonly diagnosed subtype (about 5% of cases). The progression pattern is slow, and the lesions usually manifest in sun-exposed areas, such as the face, head, as well as the dorsum of hands (Naik, 2021). This subtype is more prevalent in women in their seventh decade of life (Cummins et al., 2006).

The **acral lentiginous** variety is also less common, however this subtype is highly common in darkly pigmented individuals, accounting for approximately 35% to 65% of cases (Cummins et al., 2006; Naik, 2021). The lesions usually manifest in the nailbeds, hands and soles (Cummins et al., 2006; Naik, 2021).

*Disease management and therapeutic approaches*

The vast majority of CM patients are diagnosed in early stages of the disease. In such cases, and similarly to the previously reviewed nonmelanoma skin cancers, excision is the gold standard approach, and often the only necessary treatment (Naik, 2021). For obvious reasons there are no current topical products On the other hand, when the diagnosis occurs at an advanced stage and metastasis are already present, there are several therapeutic approaches that can be considered which specifically focus on immunomodulation and targeted molecular therapy (Pavri et al., 2016).

Immunomodulatory drugs used for CM regard: pembrolizumab, ipilimumab, nivolumab, peginterferon alfa-2b, trametinib, cobimetinib, Interleukin (IL)-2, vemurafenib and dabrafenib. The last two drugs are BRAF inhibitors, a gene which is highly expressed in CM (Pavri et al.,



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2016). Pembrolizumab, ipilimumab, and nivolumab are PD1 and CTLA4 antibodies, whereas cobimetinib and trametinib are MEK inhibitors (Naik, 2021). For obvious reasons there are no current topical products

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*D. Rare skin diseases*

A rare disease (RD) is a health condition with a relatively low prevalence, when compared with common diseases [Gorini et al 2021] and affect up to 6% of the worldwide population (Nguengang Wakap et al., 2020). Due to their low prevalence, the majority of patients with RD experience difficulties in accessing appropriate treatment. In fact, less than one-tenth of patients have the opportunity to receive a disease-specific treatment (A. Y. L. Chan et al., 2020) Although, several international definitions of RD have been proposed and used there is no single, unified and universally accepted definition of an RD (Richter et al., 2015). This lack of a universal definition is likely due to the numerous stakeholders involved, such as scientific societies, patient groups, regulatory agencies, industry, reimbursement and healthcare agencies, payers, decision-makers and policymakers. From a payer point of view, RDs are considered from a healthcare spending and resource utilisation angle, which is opposite from the view of patient groups, that mainly seek treatment accessibility. This perspective also differs in policymakers, whose priority is to improve the healthcare delivery and the healthcare system efficiency. The United Kingdom (UK), in the 2021 Rare Disease Framework (Lord et al., 2021) and the, European Union (EU) (European parliament and of the Council, 1999) use qualitative and quantitative criteria to define RDs as being 'life-threatening or chronically debilitating diseases which are of such low prevalence (less than 5 per 10 000) that special combined efforts are needed to address them so as to prevent significant morbidity or perinatal or early mortality or a considerable reduction in an individual's quality of life or socioeconomic potential along with the fact that it would be unlikely that marketing the drug in would generate sufficient benefit for the affected people and for the drug manufacturer to justify the investment'. In the United States of America (USA), the Rare Diseases Act (RDA) of 2002, precisely defines RD according to prevalence: ''rare disease' means any disease or condition that affects less than 200 000 persons in the USA'. Meanwhile, the Food and Drug Administration (FDA) used both qualitative and quantitative criterion to define RD as 'any disease or condition that affects less than 200 000 people in the USA or affects >2 00 000 in the USA and for which there is no reasonable expectation that the cost of developing and making available in the USA a drug for such disease or condition will be



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recovered from sales in the USA of such drug' (US Code 21). Nevertheless, as each RDs individually affects a small number of people, the industry, has promoted the use of 'orphan disease' when referring to rare conditions. To address the therapeutic gap in the available treatment options for these conditions, several countries have established mechanisms to encourage pharmaceutical companies to invest in, and manufacture, orphan drugs (ODs) (Sharma et al., 2010). Pharmaceutical companies that apply for such a designation are incentivised by these regulatory bodies, given if their therapy fulfils the designation criteria (European Medicines Agency, 2022b; U.S. Food & Drug Administration, 2013). These incentives include: (i) a seven-year period of exclusive rights to market an orphan drug following FDA approval;, (ii) a 25% tax credit for clinical trial costs (recently lowered from a 50% tax credit); and finnaly (iii) exemption from user fees, required for FDA review under the Prescription Drug User Fee Act ( this corresponds to $2.5 million per new drug application in 2019). These incentives contributed to the development and commercialization of over 600 drugs and biologics to treat RD (Chan et al., 2020). However, the upsurge in the number of orphan drugs approved in recent years, combined with the extremely high prices for these drugs (four times more expensive than a non-orphan drug), have triggered concern (Abozaid et al., 2022).

As of 2021, 72 FDA-approved indications met the inclusion criteria for treatment of dermatologic conditions, which constituted roughly 10% of all approved orphan indications, since passage of the Orphan Drug Act (ODA) in 1983. In other words, at least 7000 rare diseases have been identified, from these under 1000 are estimated to be dermatological diseases. Thirty-seven of the 72 orphan indications were approved for skin-related cancers (51.4%); 21 for hereditary disorders (29.2%); 7 for infectious diseases (9.7%), 3 for inflammatory disorders (4.2%); 2 for immunologic diseases (2.8%), and 2 for other diseases and conditions (2.8%) (Karas et al., 2019). Skin cancers are covered under a separate section, but as can be seen dominate amongst dermatological orphan drug approvals however some examples of none cancer rare/ orphan skin diseases are described below:



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*i. Epidermolysis bullosa*

There are four main types of epidermolysis bullosa (EB) (National Organization for Rare Disorders (NORD), 2019a):

- Epidermolysis bullosa simplex (EBS) – The most prevalent type. This condition may range from mild, with less serious complications, to severe;
- Junctional epidermolysis bullosa (JEB) – This condition is a rare form of EB, that ranges from moderate/intermediate to severe;
- Dystrophic epidermolysis bullosa (DEB) – This condition can range from mild to severe (defined as Dominant to Recessive).
- Epidermolysis bullosa aquisita (EBA) – This condition is an acquired form of EB.

*Prevalence*

EB occurs in an estimated 1 out of every 50,000 live births. The disorder may manifest in every racial and ethnic group throughout the world, and the disease appears to affect both sexes equally.

*Symptoms*

The symptoms of EB vary depending upon type as follows:

**EBS**

The localized form of EBS is the most prevalent type. Clinically, the blisters are mainly located in the hands and feet, however they may also present spread throughout the body, with relatively mild internal involvement. Patients may also present thickened calluses on the palms and soles, oral blistering during infancy and rough, thickened fingernails/toenails. Symptoms can manifest from early childhood to adulthood, but usually heal without scarring. In the intermediate form of EBS, blisters are formed in response to friction or trauma, therefore their distribution may occur throughout the body. Furthermore, there are some patients that exhibit lesions in the mucous



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membranes (inside the nose, mouth and throat). As with localised EBS, adults may experience thickening of the skin on their palms and the soles of their feet, as well as thickened fingernails and toenails. The symptoms usually begin at birth or during infancy and can result in scarring and millia. In the severe form of EBS, children develop widespread blistering all over the body including the inside of the mouth and throat with up to 200 blisters in a day, furthermore the thickening or loss of finger and toenails may also occur. As a result, the skin becomes vulnerable to infection, and the blisters can affect an infant's ability to walk, talk and eat. The blistering frequency gradually decreases as the child gets older, so adults may only experience occasional blistering. However, it is common for the skin of the palms and soles to become progressively thicker with age, and this may make walking, or using the hands painful.

**JEB**

Junctional EB can be further categorized into Herlitz junctional epidermolysis bullosa and in Generalized non-Herlitz junctional epidermolysis bullosa. In the first type, the symptoms – skin blistering and erosions - usually begin at birth and rapidly became generalized. In these patients the skin is extremely fragile, and the healing process is slow. The involvement of mucous membranes, eyes and some internal organs is frequent, and responsible for a wide range of complications. These may include life-threatening stenosis or stricture, which may lead to children death due to overwhelming infection or from failure to thrive. Other manifestations include permanent hair loss, as well as difficulty in eating and urinating. Adult patients have an increased risk of developing squamous cell carcinoma, so a regular review by a dermatologist specialized in EB is highly recommended (Burns et al., 2008).

The second type of JEB - Generalized non-Herlitz junctional epidermolysis bullosa – displays a similar clinical profile than that of the Herlitz form, with patients having fragile skin and blistering throughout the body. The prognostic however is better, as individuals usually survive to adulthood (Burns et al., 2008).

**DEB**

Dominant DEB causes blistering throughout the body, with the most common affected body sites being the hands, feet, arms and legs. These blisters usually result in scarring and milia (tiny white



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spots). In addition, the nails usually become thickened and abnormally shaped, or even lost altogether. The mouth is also often affected. Some patients may alternatively display mild symptoms with few blisters. In such cases the only signs of the disease are the misshapen or missing nails. On the other hand, the dominant DEB symptoms usually develop at birth or shortly afterwards, however there are reported cases in which the disease solely manifested later in childhood. Recessive DEB can be one of the most severe types of EB, leading to severe and widespread blistering on the skin and mucous membranes. This causes the appearance of persistent ulcers and repeated scarring, causing loss of nails and hair, problems with speaking and eating. These in turn may result in anaemia, malnutrition and delayed/reduced growth, and vision problems. These patients also display a high risk of developing skin cancer, therefore regular consultations with dermatologists are essential. All such symptoms are usually present at birth and there may even exist areas with no skin in a newborn.

**EBA**

Like EB, EBA causes the skin, oral mucosa and digestive tract to blister, together with some of the previously mentioned associated complications described above. The symptoms do not occur until after the age of 40.

*Causes*

The inherited forms of EB are caused by autosomal dominant or by autosomal recessive inheritance. EBS is usually dominantly inherited and is mainly caused by an impaired function of Keratins 5 and 14 and plectin genes. Alternatively, JEB is recessively inherited, and the genes involved are Laminin 332 (previously known as Laminin 5), plectin, and a6b4integrin. These genes are responsible for the expression of several components of the junction between the epidermis and dermis. As previously referred, there are two major subtypes of JEB, Herlitz JEB and JEB- other (includes non-Herlitz JEB, and JEB with pyloric atresia and several other subtypes). For the first type (Junctional Herlitz EB), the mutations are observed in any of the three Laminin 332 chains.



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DEB can be either dominantly or recessively inherited and involves defects in Type VII collagen. There is also a EBA which is not inherited but is an acquired autoimmune disorder, however the cause remains unclear.

*Treatment*

Any trauma, even if minimal, is likely to cause tear or blisters in the skin. In order to avoid this, the following actions are suggested:

- Reduce friction: Extreme care should be employed in handling the patient skin.
- Replace the use of adhesive bandages and dressings, by appropriate non-adhesive dressings, which should then be loosely wrapped with rolled gauze. This can be secured by using a tubular dressing retainer.
- Mantain the skin cool: Hot water baths should be avoided, as well as prolonged exposure to ambient heat and humidity. If possible, preferably the patient should be exposed to air-conditioned environments.
- Managing blisters: These should be drained, since blisters in EB are not self-limiting, and are usually large and fill with fluid.
- Clothing: In younger children, diapers may require additional padding at the legs and waist. The use of loose-fitting garments should be prioritized. If blisters develop from the seams of clothing, these should be be removed. The use of losely fitted and padded shoes is thought best.
- Nutritional deficiencies: As previously mentioned, many children affected by this disease become anaemic, due to the chronic blood loss reduced nutritional intake and absorption of iron and bone marrow suppression, all caused by the chronic inflammation. As a result, treatment for iron deficiency anaemia is frequently required, in addition to supplementation with selenium and carnitine or vitamin D, as these deficiencies may lead to cardiomyopathy and osteoporosis. Many patients develop failure to thrive and require feeding gastrostomies. To better manage these nutritional deficiencies, patients are encouraged to seek advice from an experienced nutritionist.
- Cancer monitoring: SCC is the leading cause of death in EB, usually occurring after the 2$^{nd}$ decade of life. Patients with RDEB and JEB are at increased risk of developing skin cancers



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during their lifetimes and patient monitoring at least one time per year is essential.

In 2022 Filsuvez (Oleogel-S10), a topical gel containing birchbark extracts (triterpenes) was approved by the EMA as the first therapy for the treatment of wounds in dystrophic and junctional EB. However, the same product was rejected by the FDA around the same time. In 2023, Vyjuvek was approved by the FDA as the first topical gene therapy treatment for patients of 6 months of age and older, with dystrophic epidermolysis bullosa (DEB) who present mutations in the COL7A1 gene.

### *ii. Pachyonychia congenita*

*Prevalence*

The prevalence of Pachyonychia congenita (PC) is estimated to be between 5,000 to 10,000 worldwide, with about 30-40% of cases being a result of new spontaneous mutations with no previous family history. Nevertheless, the risk of an affected individual passing the abnormal gene to offspring is 50 % for each pregnancy (National Organization for Rare Disorders (NORD), 2018).

*Symptoms*

The symptoms and severity of PC can vary widely. Symptoms typically appear within the first months or years of life (National Institute of Arthritis and Musculoskeletal and Skin Diseases, 2021) The most common features of PC include:

Painful calluses and blisters on the soles of the feet. The development of palmoplantar keratoderma generally begins when a child starts weight bearing and walking. The appearance of blisters beneath the calluses is extremely painful and usually itches. Calluses and blisters may also form on the palms of the hands. Thickened nails are extremely common in PC, but they may not manifest in every nail. Nail dystrophy is typically noted within the first few months/years of life. Nails tend to either (a) grow to full length with an upward slant caused by prominent distal hyperkeratosis or (b) have a nail plate that terminates prematurely leaving a mild sloping distal region of hyperkeratosis and exposed distal fingertip. Nail infection is likely to occur and is often painful.



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- **Cysts** of various types, such as vellus hair cysts and steatocystomas frequently develop at puberty and continue into adulthood. In some patients, particularly those presenting mutations in the KRT17 (PC-K17) gene, the cysts can be the most painful and often present problematic characteristics.
- **Bumps around hairs at friction sites,** such as the waist, hips, knees, and elbows. These symptoms are most common in children and became less frequent during teenage years.
- **White film on the tongue and inside the cheeks.** Oral leukokeratosis is more common in patients with PC-K6a. This symptom manifests at birth or within the first few months of life. It can be misdiagnosed as a *Candida Albicans* infection, if no other symptoms of PC are apparent.
- **Angular cheilitis**, laryngeal involvement resulting in a hoarse cry or hoarse voice and 'first bite syndrome'. First bite syndrome is more common in young children, it causes intense pain near the jaw or ears during 15-25 seconds after beginning to eat or swallow.

*Causes*

PC is caused by a mutation in the genes encoding keratin, K6a, K16, K17, K6b and K6c (listed in decreasing frequency). Until now, 115 mutations have been described. The disease is autosomal dominantly inherited, and as previously mentioned a patient is 50 % likely to pass the disease to his children. The disease does not target a specific ethnic group, and equally affects both sexes. PC is often due to sporadic mutations during conception, when patients do not report a family history (Abeyakirth, 2020).

*Treatment*

Currently, there is no cure or specific treatment for this disease. Patients manage their symptoms in a variety of ways, either at home or with the help of professional care. The main clinical symptom is plantar hyperkeratosis, which is usually treated by paring/trimming/grinding/filing. In what concerns the thickened nail, the treatment usually consists of filing/grinding/clipping. Topical keratolytics, such as salicylic acid and urea, together with moisturizers, may provide some



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benefit in softening the skin. Retinoids are used to manage the callus in some patients; however, these products may eventually lead to more pain. Plantar injections of botulinum toxin aiming to reduce plantar pain and blistering are also employed in some patients. In young infants displaying oral leukokeratosis, the use of a soft nipple with an enlarged opening on a feeding bottle maybe useful. Cysts usually do not require treatment, however if the patient exhibits pains or any signs of an infection, the cysts should be incised and drained, or even surgically removed. Wicking socks and ventilated footwear can help with hyperhidrosis.

### *iii. Bullous pemphigoid*

*Prevalence*

Bullous pemphigoid (BP) is the primary blistering disorder of the skin, especially in adults over 70 years of age. This disease displays an estimated prevalence of 1/4,000 in Europe; however the incidence is reported to be increasing (2-22/1,000,000 worldwide). The disease is more common in the elderly population, with over 300 people per million over the age of 80 being affected (Castel & Joly, 2020).

*Symptoms*

Clinically the disease manifests by tight and often large blisters with a clear content, which primarily appear on the edge of erythematous plaques. Intense itching is common and 10 to 20% of patients may have mucosal involvement.

*Causes*

Immunologically, the disease is caused by the production of autoantibodies that target two structural proteins found in the dermal-epidermal junction and ensuring dermal-epidermal cohesion: BP antigen 1 (BPAG1 or AgBP230), and BP antigen 2 (BPAG2, AgBP180 or collagen XVII). This interaction induces an inflammatory cascade, which compromises the integrity of the dermal-epidermal junction. The inflammation cascade, releases several cytokines such as CCL-2, CCL-17, IL-5, IL-6, IL-8, IL17, IL1-ß, and TNFα (Khalid et al., 2021). Furthermore, there are



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some drugs associated with the onset of BP (diuretics, antiarrhythmics, neuroleptics, gliptins, immunotherapies).

*Treatment*

Antibiotics and corticosteroids are used to reduce inflammation and swelling, however excision of the tissue may be necessary.

### *iv. Hidradenitis suppurativa (Acne Inversa)*

*Prevalence*

Hidradenitis suppurativa (HS) often develops at puberty, being more common in 20 to 40 years old individuals. The disease manifests in women, being usually resolved at menopause. HS is three times more common in females than in males and is reported to have a global prevalence of 1% in the general population (Ngan & Oakley, 2021; Revuz, 2009).

*Causes*

Although 'hidradenitis' indicates an inflammatory disease of the sweat glands, it is now known if HS is, or not, an auto inflammatory syndrome. The main risk factors include:

- Family history of HS; 30–40% report at least one other family member affected;
- Metabolic syndrome, which is characterized by obesity and insulin resistance;
- African ethnicity;
- Cigarette smoking;
- Follicular occlusion syndrome: acne conglobata, dissecting cellulitis, pilonidal sinus;
- Inflammatory bowel disease, mainly Crohn disease;
- Other skin disorders: psoriasis, acne, hirsutism;
- Comorbidities: hypertension, diabetes mellitus, dyslipidaemia, thyroid disorders, arthropathies, polycystic ovary syndrome, adverse cardiovascular outcomes;
- Drugs: lithium, sirolimus, biologics;



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- In addition to these risk factors, there are also several syndromes that may lead to the appearance of HS. These include PAPA, PASH and PAPASH syndromes.

*Symptoms*

Although HS develops when hair follicles become blocked, the exact mechanisms are not fully disclosed. However, it is possible to identify the following contributors to HS development:

- Follicular occlusion;
- An abnormal cutaneous or follicular microbiome;
- Release of pro-inflammatory cytokines;
- Inflammation causing rupture of the follicular wall, destroying sebaceous and apocrine glands and ducts.
- As a result, the main symptoms of HS are: Open double-headed comedones;
- Painful firm papules and nodules;
- Pustules, fluctuant pseudocysts, and abscesses;
- Draining sinuses linking inflammatory lesions;
- Hypertrophic and atrophic scars.

*Treatments*

The general treatment regards weight loss, the use of loose-fitting clothing, absorbent dressings, as well as analgesics. Furthermore, patients are advised to stop smoking. The management of anxiety and depression is often required, in order to reassure to the patient that the condition is not infectious, or a result of poor hygiene.

Drug interventions include:

**Topical antibiotics:** Mild symptoms might be managed with a topical antibiotic in liquid or gel form. When the disease is widespread, oral antibiotics such as doxycycline (Monodox), clindamycin (Cleocin), rifampin (Rimactane) or both, may be prescribed.

**Steroid injections:** Triamcinolone (Aristospan, Kenalog-10) injected into the sores, reduces swelling and inflammation.



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**Immunomodulatory treatments**: systemic corticosteroids should be used in acute flares, and steroid-sparing agents such as cyclosporine, methotrexate and azathioprine can also be employed.

**Hormonal therapy:** Hormone pills, such as estrogen-containing combined oral contraceptives may be effective for people with mild hidradenitis suppurativa.

**Biologics:** Adalimumab is approved in various territories for treating hidradenitis suppurativa. Others are under investigation in clinical trials. Paraxoxically Adalimumab and other biologics have been reported to trigger acne inversa.

**Retinoids:** Oral retinoids might be useful in patients exhibiting acne like disease.

**Pain medication.**

**Other systemic medical treatments** used off-label: metformin, acitretin, dapsone, colchicine, and zinc gluconate.

- **Surgeries** Uncovering the tunnels;
- Incision and drainage of acute abscesses;
- Local excision of persistent nodules, abscesses, and sinuses;
- Deroofing and curettage of persistent abscesses and sinuses;
- Radical excisional surgery of an entire affected area;
- Laser ablation (CO2) of nodules, abscesses, and sinuses;
- Laser/light hair removal.

*v. Harlequin ichthyosis*

*Prevalence*

Harlequin ichthyosis affects males and females in equal numbers. This condition affects approximately one in 500,000 persons or about seven births annually in the United States. Approximately 200 cases have been reported throughout the world. Recurrence of this condition in the next pregnancy is 25% (National Organization for Rare Disorders (NORD), 2019b; Pietrangelo, 2018; Shruthi et al., 2017).



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*Causes*

Harlequin ichthyosis is inherited in an autosomal recessive pattern, being caused by mutations in the ABCA12 gene, which encodes for a protein necessary for normal skin cell development.

This gene is essential for the lipids transportation to the epidermis in order to create an effective skin barrier. When the ABCA12 gene is disrupted, the skin barrier is compromised. Harlequin ichthyosis patients also present an impairment in keratin expression. It has been demonstrated that cultured keratinocytes with the disease display an excessive cornification, alongside with a failure of desquamation (Burns et al., 2008).

*Symptoms*

The symptoms in new-borns are extremely severe. These patients are typically premature, and are encased in a rigid, taut, yellow-brown, adherent thick skin, also referred to as a hyperkeratotic 'coat of armour'. This hardened skin covers the whole-body surface and as a result, deep fissures occur prenatally over the scalp. After birth, this "coat of armour" splits at sites of stress, producing further red fissures and a skin pattern resembling a harlequin's costume (Burns et al., 2008). As a result, these patients exhibit a wide range of symptoms ranging from the inability to keep the mouth closed to the limited mobility in arms and legs. As such, all movements are restricted, and respiratory insufficiency is likely to manifest, due to the limited chest expansion and sometimes coexistent skeletal deformity (Burns et al., 2008). The absence of effective feeding is responsible in many cases for the development of hypoglycaemia, dehydration and renal failure.

Older children and adults_may experience a delay in physical development. A child born with Harlequin ichthyosis will likely have red, scaly skin throughout their life. Other clinical manifestations include:

- Sparse or thin hair as a result of scales on the scalp;
- Unusual facial features due to stretched skin;
- Reduced hearing from a buildup of scales in the ears;



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- Problems with finger movement due to tight skin;
- Thick fingernails;
- Recurring skin infections;
- Overheating due to scales that interfere with sweating.

*Treatments*

A newborn requires neonatal intensive care, which may include spending time in a heated incubator with high humidity. Tube feeding can help to prevent malnutrition, as well as dehydration. The eyes should be kept moist and protected. Skin moisturizers and barrier repair formulas containing ceramides or cholesterol, moisturizers with petrolatum or lanolin, and mild keratolytics (products containing alpha-hydroxy acids or urea) can help to maintain the skin moisturized, thereby preventing further cracking and fissuring, which may lead to infections. Removal of damaged tissue (debridement) from the fingers may be needed if they are constricted by bands of skin to avoid a loss of circulation. Other possible treatments include topical or oral retinoids, as well as topical antibiotics. Furthermore, covering the skin in bandages may also be useful to prevent infections.

### *vi. Erythropoietic Protoporphyria (EPP) and X linked Protoporphyria (XLP)*

*Prevalence*

The prevalence of EPP in Europe is between 1 in about 75,000 to 1 in 200,000 individuals, however the incidence is the US is unknown. Conversely, XLP accounts for about 10% of cases in the United States and is more likely to present in males. Females with XLP maybe asymptomatic(National Organization for Rare Disorders (NORD), 2022a).

*Causes*

EPP is a hereditary porphyria characterized by painful and lifelong photosensitivity, which is sometimes accompanied by liver disease. EPP results from a mutation in the FECH gene, which is responsible for producing ferrochelatase, the final enzyme of haem biosynthesis. Without enough



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ferrochelatase, protoporphyrin which are highly sensitive to sunlight, build up in the superficial blood vessels under the skin. When they absorb sunlight, they react causing severe pain and inflammation, thereby resulting in EPP symptoms.

Some symptomatic EPP patients present a genetic alteration in ALAS2, a X chromosome gene. In such cases, the condition is called X-linked protoporphyria (XLP). XLP is passed down through families in an X-linked manner.

*Symptoms*

The disease is usually diagnosed at age 4, however there are several reports in which the disease onset occurs later. The major symptoms regard severe pain on exposure to sunlight, or even upon exposure to some types of artificial light, such as fluorescent lights (phototoxicity). When exposed to sunlight, patients experience tingling, itching, burning of the skin. After continued exposure to light, the symptoms evolve to severe burning pain, which can last anything between an hour and several days. The most affected area are the hands, arms and face. In these patients' liver and gallbladder function complications may also occur.

*Treatment*

The avoidance of sunlight will be beneficial to these patients. The use of sun protective clothing, such as long sleeves, hats, and sunglasses should also be employed. The use of tanning creams which increase skin pigmentation or sunscreens with physical reflecting agents is advised. EPP and XLP patients may also benefit from window tinting or using films to cover the windows in their car or house. However, before tinting or shading car windows, the patients should check with their local Registry of Motor Vehicles if such measures do not violate any local codes.

In EPP, a high potency form of Lumitene (oral beta-carotene) is the most widely used treatment. It is postulated that this drug may scavenge free radicals involved in the phototoxic reaction, however recent studies were not able to support the benefit of this treatment.

In 2019, the FDA approved Scenesse (afamelanotide) for the treatment of adult patients with EPP. Scenesse regards injectable implant that works by increasing skin pigmentation, thereby providing an



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enhanced skin protection and improvement of sun tolerance. Scenesse was already available in Europe, before its approval in the United States.

Iron supplementation should also be considered whenever the patient exhibits iron deficiency. Prevalite (cholestyramine) or activated charcoal, may also be prescribed to suspend protoporphyrin circulation through the liver and intestines, in patients with liver disease.

### *vii. Gorlin syndrome*

*Prevalence*

Gorlin Syndrome is known by several names, including BCCNS, Gorlin-Goltz Syndrome, Basal Cell Nevus Syndrome, or Nevoid Basal Cell Carcinoma Syndrome ((National Organization for Rare Disorders (NORD), 2020a)

This rare complex genetic disorder affects 10,000 people in the United States. Patients are particularly prone to develop BCCs in areas of the body exposed to ultraviolet light.

*Causes*

**Gorlin Syndrome** is characterized by mutations in the tumour suppressor gene encoding Patched1 (PTCH1), which acts as the primary inhibitor of the hedgehog signalling pathway. Less frequently, patients may also exhibit mutations in *PTCH2*, instead of *PTCH1*; Furthermore, mutations in the *SUFU* gene, which also belongs to the hedgehog pathway, may also be observed.

*Symptoms*

Gorlin syndrome is characterized by a wide variety of developmental abnormalities, as well as by an enhanced predisposition to develop certain forms of cancer, particularly basal cell carcinoma. The specific symptoms and severity of Nevoid Basal Cell Carcinoma Syndrome (NBCCS) are highly variable from patient to patient, even when considering members of the same family. However, common symptoms include multiple basal cell carcinomas (hundreds especially on the face and sun-exposed areas), recurrent keratocystic odontogenic tumours of the jaws, pits of the palms and soles,



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and skeletal malformations. As multiple organ systems can become involved, some patients also present distinctive facial features**.** To date, 100 different recognized features of this disease have been identified.

*Treatment*

Treatment is patient specific and often requires a multidisciplinary team. With no FDA-approved drugs available for Gorlin Syndrome, the standard of care is surgery. People with severe Gorlin Syndrome may have as many as 30 surgeries per year for removal of lesions in addition cryotherapy, electrodissecation, curettage radiotherapy and photodynamic therapy, which can all be repetitive and scarring. Obviously, the topical treatments of BCC such as 5-fluorouracil and imiquimod also apply. Recently, the FDA has approved two orally administered targeted drugs that specifically counteract the effect of *PTCH1* mutations: Sonidegib (Odomzo®) and vismodegib (Erivedge®), both drugs inhibit the hedgehog pathway and can be prescribed for adults with advanced BCC that has spread to other parts of the body, or that has recurred following surgery. When considering topical drugs, Pellepharm is developing a novel topical gel of patidegib, designed to mitigate the tumour burden in patients with Gorlin Syndrome.

*viii. Neurofibromatosis Type 1 (NF1)*

*Prevalence*

NF1 is a rare disorder that equally affects males and females, as well as all races and ethnic groups equally. The disease has a prevalence of 1 in 2,500 to 3,000 births (National Organization for Rare Disorders (NORD), 2022b)

*Causes*

In about 50% of patients with NF1, the disorder is transmitted through autosomal dominant inheritance, therefore the disease is always passed to offspring. The remaining 50% of cases correspond to sporadic or random pathogenic variants in the gene responsible for NF1, that solely



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manifest in the child, but not the parents. The NF1 gene regulates the production of neurofibromin, a tumour suppressor protein, which inhibits the activation of the Ras/Raf/MEK/ERK pathway. Mutations in this gene, will lead to the production of a nonfunctional version of neurofibromin, or alternatively to a decreased expression of neurofibromin. These occurrences dysregulate the cellular growth and division.

A more localized form of NF1 (segmental NF1) is caused by a genetic change in the NF1 gene that is not inherited. This mutation occurs sporadically during embryo development (somatic mutation), and solely some cells will present the disease-causing NF1 pathogenic variant (genetic mosaicism), therefore signs and symptoms of the disease maybe circumscribed to that body part, thereby reducing the severity and distribution of the symptoms. Nevertheless, it should be pointed out that he NF1 pathogenic variant responsible for a segmental NF1, may be inherited by offspring and cause full NF1.

*Symptoms*

Almost all adults with NF1 develop cutaneous neurofibromas (cNFs), which are nerve tumors that form under the skin and manifest as nodules that can be disfiguring, painful and ultimately cause a multitude of complications.

*Treatments*

Currently for cNFs surveillance is the preferred strategy due to their benign nature. However, if surgery is necessary, radiation, laser or electrocautery treatment can be employed. In April 2020, an oral dosage form Koselugo (selumetinib) was approved by the FDA (and later the EMA) for the treatment of NF1-associated plexiform neurofibromas that are disfiguring or inoperable in children 2 years and older. This drug acts as a kinase inhibitor, by blocking a key enzyme that promotes cellular growth. Literature points out to a reduction of plexiform tumors in up to 70% of patients. However, NFlection is developing a topical gel containing NFX-179, a proprietary ametabolically labile MEK inhibitor (blocks the RAS pathway) for the reduction of tumor burden of persistently cNFs. Systemic side effects of MEK inhibitors include severe acneiform rash, diarrhea, nausea, vomiting, edema,



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fatigue, high blood pressure, cardiomyopathy, and retinal detachment. Hence NFlection have developed a soft drug formulation which delivers drug to the cNF in the dermis but degrades to an inactive form when systemically exposed thus reducing the risk of the side effects.

### *ix. Ichthyosis Vulgaris*

*Prevalence*

Ichthyosis vulgaris is a fairly common disorder that affects approximately one in 250 persons in the United States. Males and females are equally affected, and the disease usually manifests in the first year of life, however it is usually not present at birth (National Organization for Rare Disorders (NORD), 2008).

*Causes*

Ichthyosis vulgaris is transmitted through an autosomal dominant inheritance, however the specific mutations leading to this condition are yet to be identified.

*Symptoms*

In ichthyosis vulgaris, the skin cells are produced at a normal rate, however in the stratum corneum the cells do not shed normally and are not shed as quickly as they should be. Symptoms vary in severity, from mild to severe. The scale is usually fine and white. Only a portion of the body may be involved, however the lower legs are most affected. Scaling on the torso is less severe and usually the face is unaffected. If the face is affected, the scaling is mainly limited to the cheeks and forehead, with the sides of the neck and the flexural areas being usually spared. Often, the skin on the palms of the hands and soles of the feet is thickened and may present exaggerated lines.

*Treatment*

Topical moisturizers containing urea or glycerol are useful, as well as lotions containing alpha-hydroxy acids. However, some patients may also experience atopic dermatitis, and in such



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circumstances the use of alpha-hydroxy acids should be avoided. Symptoms usually decrease in warm humid climates, or during summer months.

*x. Elastoderma*

*Prevalence*

Fewer than 1 in 1,000,000 people worldwide have the condition (Murphy, 2020).

*Causes*

The exact cause of elastoderma is unknown, however it is thought to be the result of elastin overproduction, a protein that gives structural support to organs and tissues**.**

*Symptoms*

- Excess wrinkled, redundant, inelastic and sagging skin.
- Skin papules - A circumscribed, solid elevation of skin with no visible fluid, varying in size from a pinhead to less than 10mm in diameter at the widest point.
- Growth of abnormal tissue on or under the skin.

It can occur on any part of the body, but the neck and the extremities, especially around the elbows and knees, are the most affected areas.

*Treatment*

There is no cure or standard treatment for Elastoderma. Some cases have been treated with surgical excision (removal of affected skin), but hyperlaxity of skin often returns following the surgery.

*xi. Lamellar ichthyosis (LI)*

*Prevalence*

Lamellar ichthyosis is estimated to affect 1 in 200,000 individuals. This condition is more common in Norway, where an estimated 1 in 90,000 individuals are affected (MedLine Plus, 2022).



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*Causes*

LI can be caused by recessively inherited mutations in several genes with the most common gene being TGM1 with others including NIPAL4, ALOX12B, and CYP4F22. These genes provide the instructions to make enzymes and proteins which are important for normal development, function, and shedding of skin cells.

*Symptoms*

Newborns with LI are sometimes referred to as “collodion babies”, as they are covered by a clear membrane (collodion), which resembles a plastic wrap. The main symptoms include scaly and dry skin, which usually manifests over large areas. Patients exhibit pruritus and erythroderma, in addition sweating is severely impaired. The scale appearance may vary, from fine and white, to dark and brown, separated by deep cracks. (National Organization for Rare Disorders (NORD), 2020).
Additional clinical features include palmoplantar keratoderma, scarring alopecia, persistent ectropion, and congenital hypoplasia of aural and nasal cartilages. In severe LI cases, growth may be compromised, as nutritional and vitamin D deficiencies are commonly reported (Burns et al., 2008).

*Treatment*

The treatment of LI aims to repair the skin barrier, therefore topical formulations containing ceramides or cholesterol are useful. Moisturizers with petrolatum or lanolin are also employed, as well as mild keratolytics or topical retinoids. Oral retinoids may also be employed.

*xii. Xeroderma pigmentosum (XP)*

*Prevalence*

Xeroderma pigmentosum (XP) equally affects males and females however, there appears to be an increased prevalence in certain geographic locations, namely Japan, North Africa and the Middle East. In these territories there is a higher expression of XP related gene mutations, and consequently a higher prevalence of this disease. In the United States and Europe, prevalence of XP is about 1 in



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1,000,000. In Japan, XP is much more common, affecting 1 in 22,000. Areas of North Africa (e.g., Tunisia, Algeria, Morocco, Libya, Egypt) and the Middle East (e.g., Turkey, Israel, Syria) also show an increased prevalence (National Organization for Rare Disorders (NORD), 2021; Whelan, 2017).

*Causes*

XP is a rare auto recessive multisystem genetic disorder, caused by mutations in the following genes: DDB2 (XP-E), ERCC1, ERCC2 (XP-D), ERCC3 (XP-G), ERCC4 (XP-F), ERCC5 (XP-B), POLH (XP-V or variant), XPA and XPC. (NORD2021).

The proteins expressed from these genes are involved in DNA repair, therefore whenever these mutations are present, the individuals are unable to properly repair the damages arising from UV exposure, which will then lead to the manifestation of the symptoms.

*Symptoms*

The disease is characterized by an intensified sensitivity to the DNA damaging effects of ultraviolet radiation (UV), being typically diagnosed in infancy or early childhood. Affected babies and toddlers usually start to develop lentigos on sun areas exposed, such as the face, neck, arms and legs. These sunburns may result in redness and blistering that can last for weeks. Other symptoms include:

- Spider veins (telangiectasia);
- Scarring;
- Weakened, thin skin.

Approximately half of XP patients develop blistering burns on sun exposed skin after minimal sun exposure (sometimes less than 10 minutes in the sun). The remaining 50% of XP patients do not burn, but tan after sun exposure. In XP patients there is a higher probability for the development of precancerous skin lesions (such as, actinic keratosis) and skin cancers (see above)

*Treatments*

The prevention is the most effective treatment for this disease. *Rigorous sun (UV) protection is* necessary, as soon as the diagnosis is suspected, this will prevent the continued DNA damage, as well as the disease progression. Individuals with XP should avoid exposing the skin and eyes to ultraviolet



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(UV) radiation by wearing protective clothing such as hats, hoods with UV blocking face shields, long sleeves, pants, and gloves. High sun-protective factor (SPF) sunscreens, UV-blocking glasses with side-shields, and the use of long hair can also be useful. Furthermore, patients are advised to take vitamin D oral supplements, since this vitamin is synthetized by the interaction of UV with the skin.

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## IV. DRUG ABSORPTION ACROSS THE SKIN-THEORY AND PRACTICE

### *A. Introduction*

Following application to the skin surface, numerous steps or events occur before a topically applied molecule is delivered into the systemic circulation (Figure 8). Since only molecules can penetrate into and diffuse through skin, solid drug forms are seldom applied although powders are used for dusting (e.g. to reduce friction for intertriginous skin disorders) and powder-base injectors have been developed. However, most commonly, the drug is in a dissolved state in a vehicle, and these can range from simple solutions to gels, foams, multiple emulsions or a polymeric matrix as seen with transdermal patches. Vehicles are selected for multiple reasons including to ensure the drug remains stable, to promote patient compliance (for example selecting a vehicle with a good "skin feel" and which spreads well and is non-greasy) or to enhance therapeutic efficacy (for example when selecting a fatty ointment to occlude and thus hydrate dry skin conditions). On application, the molecules must initially partition from the vehicle into the outermost layer of the stratum corneum and hence the choice of vehicle, and the solubility of the drug in the vehicle (or more accurately its thermodynamic activity (see section IV.G.ii), can affect drug release and consequently this initial step in drug delivery to the skin. Further, the first molecules to enter the stratum corneum are those immediately adjacent to the tissue surface. Over time, this layer in intimate contact with the skin becomes depleted of drug which is then replaced by molecules further from the skin surface, diffusing through the vehicle. For a simple solution, diffusion of molecules through the vehicle tends to be uninhibited but with highly viscous vehicles, or for example a polymeric transdermal patch matrix, then diffusion of the drug through the vehicle to the skin surface can become a rate limiting factor in drug delivery. Clearly, in addition to the vehicle factors affecting drug release and hence topical delivery, the physicochemical properties of the drug likewise influence this early penetration into the stratum corneum since large molecular weight or hydrophilic molecules are less likely to penetrate into the stratum corneum lipid domains.



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Once in the outer layer of the stratum corneum, the molecule diffuses through this barrier which again is complex given the heterogeneous structure of this layer, described in section B. Permeation through to the systemic circulation is then characterised by further multiple partitioning and diffusion steps – for example from the stratum corneum into and through the viable epidermis, then into and across the epidermal-dermal junction, then into and through the dermis and again into the capillaries.

Drug permeation through the skin is passive; to date, no active transport carrier systems have been identified. However, the skin is far from a simple inert membrane and has a strong immune function, contains multiple enzyme systems and is host to a complex surface microbiome, all of which offer the potential for biotransformations of the active drug during its transit through the tissue. Indeed, such biotransformations could be beneficial where, for example, a hydrophilic drug which would tend to poorly penetrate into the stratum corneum lipids is conjugated (eg through an ester link) with a lipophilic moiety such as a fatty acid to enhance its lipophilicity, and so aid penetration into the stratum corneum before being cleaved (eg through esterases) in the viable epidermis to liberate the "free drug". The skin metabolic activity has also been recently exploited through 'soft' drugs which have been developed and which display a metabolically labile functionality; having exerted its activity in the site of action, the soft drug undergoes rapid metabolism, leading to inactive metabolites. The soft drug approach is finding widespread application in the dermatological field since it provides a means of localising the therapeutic effect in skin, while minimising systemic exposure. Drugs targeting sphingosine-1-phosphate receptor 1 (S1PR1), transient receptor potential vanilloid 1 (TRPV1), Janus kinase (JAK), caspase 1, and histone deacetylase (HDAC), for the treatment of skin inflammatory, autoimmune, and oncological disease are in development, whilst a similar approach was reportedly used in the development of Eucrisa (Crisaborole) a soft phosphodiesterase 4 (PDE4) inhibitor, for atopic dermatitis.

Beyond metabolism, other factors can affect the rate and extent of drug delivery through the skin. Again dependent on its physicochemical properties, a permeant may bind to various elements in the stratum corneum or deeper skin layers, commonly with the intracellular keratin. Clearly binding can



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alter the rate of delivery into the deeper skin layers, but can be beneficial in providing a reservoir effect for sustained drug delivery over time. Similarly, vehicle components will also penetrate into the stratum corneum and can thus alter the solvating capacity of the stratum corneum – perhaps increasing the amount of drug that can enter the tissue. This then provides a reservoir of drug molecules that, over time will be delivered to the dermis. Permeation pathways through the stratum corneum.

As explained earlier (section II.B.iii), there are 3 major routes for drug permeation across the skin layers which are: across the cells (intracellular or transcellular), between the cells (intercellular or paracellular) and through the hair follicles and associated sweat glands (transappendageal or shunt route) (Barry, 2001), (Figure 8) with the transcellular and paracellular routes often referred to as the transepidermal pathway. As there are no active transport mechanisms across the SC, all three pathways are not mutually exclusive and indeed can all be part of the permeation process but the proportion of delivery via each route is determined by the physicochemical properties of the drug (Flynn, 2002).

*i. Transappendageal transport (shunt route transport)*

As described previously, the dermis supports the pilosebaceous unit, eccrine and apocrine ducts that extend to cross the stratum corneum barrier and which offer a shunt route (or “short cut”) by which molecules could enter the lower layers of the skin without having to traverse the stratum corneum barrier.

Intuitively, since the shunt routes avoid the intact stratum corneum barrier, then it may be expected that these provide a significant pathway for the delivery of drugs into the deeper skin layers. However, in vivo, these are not simple “empty” channels; the eccrine glands secrete sweat – essentially an aqueous solution - periodically whereas the sebaceous glands secret the lipophilic sebum into the follicle and of course the follicle contains the hair shaft. Thus, for drugs to permeate via these shunt routes they must do so against and outflow of either a hydrophilic or lipophilic



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"solvent". Equally significant, the openings of these shunt routes at the skin surface are relatively small, and constitute a small fraction of the surface to which topical medicaments are applied. In an elegant study using cyanoacrylate glue to template the appendages within skin, Otberg et al (2004) showed significant variability in both the surface opening and volume of appendages. For example, hair follicles provide approximately 0.1 of the skin surface area of the forearm but up to 13% of the surface on the forehead. Likewise, there is also considerable variability in the density of eccrine sweat glands with body site ranging from ~ 250 per $cm^2$ over the volar aspect of the hand (the palm) and ~300 per $cm^2$ on the volar aspect of the foot (sole), to ~100 per $cm^2$ on the cheek or lateral thigh (Taylor & Machado-Moreira, 2013).

Despite these constraints, the shunt routes play at least a minor role in delivery of drugs into human skin. For example, Siddiqui et al., (1989) estimated that the shunt routes contributed 5-10% of the observed steady state flux of steroids through excised human skin. El Maghraby et al., (2010) and Essa et al. (2010) showed a potentially major role for the shunt routes in transdermal delivery of hydrophilic molecules although this seemed to be fundamentally influenced by the *in vitro* experimental design. It is generally agreed that the shunt routes are important in the early stages of drug delivery since permeation though the bulk intact stratum corneum generally demonstrates an appreciable lag time. It is also apparent that delivery via the shunt routes is more evident *in vivo* than *in vitro*, since experimental design can influence the proportion of drug delivery pathway; for example, by ensuring the stratum corneum is fully hydrated during an in vitro study, the tissue may swell and effectively close the follicular and eccrine / apocrine gland openings. Further, considering molecules with non-ideal physicochemical properties, such as hydrophilic permeants or ions, then transappendageal transport may provide the major route for their (albeit limited) delivery. It is also believed that the shunt routes are also important for delivering vesicular structures to the skin and for targeting their contents to the pilosebaceous units (Lauer et al., 1996).



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*ii. Transcellular route*

The transcellular ("through the cells") pathway through the intact stratum corneum is often considered to be a polar or hydrophilic route through the membrane. Due to terminal differentiation and to perform their function as an effective barrier the coenocytes that comprise the major cells of the stratum corneum are largely filled with alphakeratin bundles, a structural fibrous protein, with little intracellular lipoidal material. The intracellular keratin is hydrated, and so provides a hydrophilic environment. However, it should also be noted that the corneocytes have a cornified cell envelope of covalently cross-linked proteins and covalently bound lipids, and are bound to the intercellular multiple bilayered lipid domains.

The complexity of the stratum corneum structure, and the consequent environments for a drug to penetrate into and diffuse through the tissue, are challenging for both hydrophilic and lipophilic drugs. Thus, the tissue has the keratin filled hydrophilic domains (corneocytes) embedded in the continuous multiple bilayered lipid domains. For transcellular permeation, the molecule will initially enter the hydrated corneocyte according to its partition coefficient which will be favourable for water soluble and hydrophilic permeants which would generally have a logP octanol/water <1. After it has entered the corneocyte, the molecule diffuses through the keratin-filled cell, driven by the concentration gradient across the cell. Then, in order to leave the cell, the molecule must then enter the surrounding bilayer lipids, which (in contrast) will be favourable for lipophilic molecules with a log P octanol/water typically >1, before diffusion through the lipid lamella to reach the next corneocyte. Clearly, the multiple alternating hydrophilic and lipophilic domains that are encountered via the transcellular route provide significant obstacles for a given permeant with defined physicochemical properties such as its partition coefficient.

Whilst the transcellular pathway presents sequential obstacles to permeation, it role and the proportion of molecules traversing the stratum corneum via this route should not be ignored and indeed can be significant for highly hydrophilic permeants. As described earlier, the stratum corneum lipids are complex and heterogeneous and include various polar lipids. Sznitowska et al., (1998)



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extracted several classes of polar lipids from the intercellular bilayers resulting in an increase in flux of the hydrophilic zwitterionic drug baclofen; removal of less polar lipids had minimal impact on drug delivery and thus demonstrated that the polar lipids provided the principle barrier to baclofen permeation. As noted above, the authors also emphasised that the pathways for delivery through skin (intra- and intercellular permeation, and via the shunt routes operate concomitantly. More recently, Miller et al. (2017) explored the uptake and desorption of a range of hydrophilic materials in human stratum corneum. The data was consistent with a transcellular diffusion model in which the diffusion of highly hydrophilic permeants occured through lipid defects and/or desmosomes that connect the corneocytes. Again, the authors emphasised that the skin appendages play a role in both transient and steady-state absorption of hydrophilic compounds.

Then proportion of drug molecules that traverse the stratum corneum barrier via the transcellular route clearly depends on the physicochemical properties of the permeant and in particular its partition coefficient; the majority of the literature illustrates that the transcellular route is particularly relevant for highly hydrophilic molecules. However, for these water-soluble drugs, the intercellular lipid domains remain a substantial barrier to their permeation as evidenced by multiple studies showing that when the lipids are removed by solvent extraction, transport of hydrophilic drugs invariably increases. Given that molecules traversing via the transcellular route enter and diffuse through the keratin filled cells, then there is evidently potential for drug: keratin binding to occur.

*iii. Intercellular pathway*

Given that the multiple lipid bilayers provide the major barrier to permeation of most drugs through the skin, it is remarkable that this domain comprises ~1% of the stratum corneum diffusional area and ~5% of the stratum corneum weight. However, as the matrix in which the corneocytes are embedded, it is the only continuous phase within the stratum corneum. It follows from the above that, given the relative proportion of permeation is dependent on the permeants physicochemical properties, its



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formulation and posology. It is anticipated that the lipid domains provide a more suitable environment for lipophilic permeants to enter the tissue than the essentially aqueous domains of the coenocytes.

The role of the stratum corneum lipids in regulating loss of water from the body and penetration of substances into the skin is well- established. When the intercellular lipids are perturbed or removed (for example by penetration enhancers or solvent extraction) trans epidermal water loss increases, electrical resistance decreases and delivery of most permeants (hydrophilic and lipophilic) increases. Removal or perturbation of the intercellular lipids might typically increase flux through intercellular and transcellular pathways since the lipid domains provide a barrier in both cases. As was described in Section II, to simply state the stratum corneum lipids exist in multiple bilayers underplays the complexity of this matrix. Unlike most lipid bilayers in the body, the stratum corneum bilayers do not contain phospholipids and have various fatty acids and ceramides alongside cholesterol and cholesterol sulphate. The bilayers interdigitate through long chain ceramides and have differing periodicities. In terms of their packing motif, the bilayers are again heterogeneous and are variously packed into highly ordered and densely packed crystalline phases with an orthorhombic arrangement which provide a formidable barrier to drug diffusion, to less-densely packed phases in a hexagonal arrangement which tend to be more gel-like and consequently are more permeable through to disordered, and relatively highly permeable near “liquid” domains. Additional complexity arises at interfaces with the corneocyte envelopes. Thus, the simple “multiple lipid bilayers” themselves offer a range of environments and associated diffusivities for drug permeation.

When considering the length of the pathways for permeation, the transcellular route of sequential penetration into and diffusion through corneocytes and then the lipid domain is generally taken to be the thickness of the stratum corneum with permeation directly across the tissue. In contrast, for a lipophilic molecule permeating via the continuous lipid domains, the diffusional pathway is not simply the stratum corneum thickness as transport is relatively tortuous and around the embedded corneocytes. Seen most clearly from the relatively long lag time to pseudo steady state flux, the



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intercellular permeation pathway is estimated to be between 150 and 500 μm, significantly greater (by an order of magnitude) that the tissue thickness (typically 20-40 μm) though again the route taken through the matrix will depend on the permeants physicochemical properties. Naturally, such studies can be confounded transcellular and shout route delivery occurs simultaneously but, given that the "true" pathlength taken by a permeant is unknown, then resultant parameters that characterise a permeant such as its permeability coefficient (with units of distance/time) or diffusion coefficient (distance$^2$/time) are apparent values.

### *B. Influence of permeant physicochemical properties on route of absorption*

From the above it is clear that the relative contribution of the three potential pathways through the stratum corneum will vary depending on the nature of the permeant as detailed below.

#### *i. Partition coefficient*

As is apparent from the above, the partition coefficient of a permeant is a critical determinant of its entry into and permeation through the stratum corneum and beyond into the viable epidermis, dermis and then to enter the systemic circulation. Given that the corneocytes are essentially a keratin filled aqueous matrix then it is tempting to simply expect that a hydrophilic molecule will simply partition into these cells from where it will traverse the tissue via the transcellular route, whereas lipophilic materials will enter the multiple lipid bilayers and so transit via the intercellular route. This approach again underestimates the complexity of the components in the stratum corneum where desmosomes and proteins are associated with the bilayered lipids, which additionally possess aqueous domains around the polar lipid head groups, or that the corneocyte membrane has lipids associated with it. Considering this heterogeneity, a "mixed permeation model" is generally accepted and states that most (usually lipophilic) drugs permeate largely via the continuous intercellular lipid domains, but both the lipid and polar regions of the bilayers could provide the micro-routes depending on the partition coefficient of the permeant (M. Roberts, 1995). For molecules with intermediate partition coefficients showing some solubility in both oil and water phases (log P (octanol/water) of approximately 1-4), the intercellular route probably predominates. For more highly lipophilic



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molecules (log P >4), then the intercellular route will be almost entirely the pathway used to traverse the stratum corneum. However, for these molecules a further issue is the ability to partition out of the stratum corneum into the essentially aqueous viable epidermal tissues. For more hydrophilic molecules (log P <1), the transcellular route becomes increasingly significant, yet there are still lipid bilayers to cross between the keratinocytes. For highly hydrophilic (and charged) molecules, the appendageal pathway may also become significant.

*ii. Molecular size*

A second major factor in determining the flux through human skin is the size and shape of the molecule. When considering the influence of molecular size on permeation, molecular volume is the most appropriate measure of permeant bulk. However, for simplicity, the molecular weight is generally taken as an approximation of molecular volume, with an inherent assumption that most molecules are essentially spherical.

It has long been appreciated that, in simple isotropic media, molecular weight influences the diffusion coefficient of a molecule with increasingly bulky molecules showing decreasing diffusivities (Crank, 1975). Despite the heterogeneity within human skin, this relationship also exists. It is evident that most commercially successful topical and transdermal products contain active ingredients with a molecular weight of < 500 Da, for example estradiol (272 Da), fentanyl (336 Da) and nicotine (162 Da). Though there are a few exceptions, such as the macrolactam immunosuppressives pimecrolimus (810 Da) and tacrolimus (804 Da) that are used topically to treat atopic dermatitis, the available data has led to the so-called “500 Dalton Rule”, which states that for a drug to be deliverable through native/healthy skin, it must have a molecular weight of less than 500 Daltons (Bos and Meinardi, 2000). However, there is a growing body of evidence showing that larger molecular weight biomacromolecules are able to penetrate into, and to some extent, diffuse through human skin, especially when diseased.



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xiii. *Solubility / melting point*

It is accepted that most organic materials with high melting points and with high enthalpies of melting have relatively low aqueous solubilities at normal temperatures and pressures (i.e., under the typical conditions in transdermal drug delivery). Thus, there is an obvious link between melting point and solubility and there are several theoretical models available that estimate solubilities from melting point data.

As described above it is clear that lipophilic molecules tend to permeate through the skin faster – have shorter lag times - than more hydrophilic molecules. Thus, solubility within the intercellular lipids (usually described by the partition coefficient) can be correlated with the permeability coefficient for a homologous series of compounds. However, whilst moderately high lipophilicity is usually a desirable (or required) feature of dermal candidates, it is also necessary for the molecule to exhibit some aqueous solubility since the deeper layers of the skin are more hydrophilic. Indeed, the steady state flux of a drug traversing the tissue is the result of the permeability coefficient and the applied concentration. Whilst lipophilic permeants may afford a relatively high permeability coefficient, their lipophilicity will usually prescribe that the aqueous solubility (and hence concentration in an aqueous formulation) will be relatively low with a resultant influence upon drug flux.

One further factor to consider is the potential for depletion of the permeant from a donor system. If the drug has relatively low water solubility and is delivered from a saturated or sub-saturated aqueous formulation, then the amount of drug present in the formulation will be relatively low. As a lipophilic permeant, the molecule will rapidly enter the stratum corneum, which could quickly deplete the drug within the formulation. As the drug concentration falls, so the thermodynamic activity of the drug in the formulation will fall and hence the driving force for diffusion diminishes and consequently the flux will rapidly decrease.

As with most of the formulation factors within the field of dermal drug delivery, a compromise in candidate solubility properties is required. Some drug lipophilicity is desirable allowing uptake into



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the stratum corneum and transport though the intercellular lipids yet with some aqueous solubility to provide enough permeant within an aqueous formulation to minimise the effect of donor depletion over the time course of application.

xiv. *Ionisation*

Considering the nature of the stratum corneum barrier to dermal delivery, residing largely within the lipid bilayer domains, it is widely believed that ionisable drugs are poor permeants. Indeed, many of the arguments against using weak acids and weak bases that will dissociate to varying degrees depending on the pH of the formulation (and on the pH of the stratum corneum) are founded in the pH-partition hypothesis. According to this hypothesis, developed for absorption of drugs through the gastrointestinal tract, only the unionised form of the drug can permeate the lipid barrier in significant amounts. However, with the complex structure of human skin, this model cannot be directly applied, and it is notable that many drugs under development for dermal use are weak acids or weak bases.

As described previously, permeation across human skin can be due to a number routes, none of which are mutually exclusive, and all of which probably operate for most molecules traversing the skin. Some appendages provide a mainly aqueous pathway across the stratum corneum, although with a limited cross-sectional area. The transcellular route is considered to possess intermediary properties whereas the intercellular pathway is essentially a lipophilic route. Thus, it is likely that charged permeants (ionised drugs) can cross the membrane by the shunt route, but that their permeability coefficient may be significantly lower than if the species were unionised and were to pass largely via the lipoidal intercellular route. Indeed, electrically assisted transdermal drug delivery essentially drives ions – iontophoresis – through the shunt routes.

Further complexity can be introduced if the relative aqueous solubilities of the ionised and unionised species are considered. As described previously, drug flux is the product of the permeability coefficient and effective drug concentration in the vehicle. Whereas the permeability coefficient of the unionised species through the lipid membrane may be high, its aqueous solubility could be low. In



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contrast, for an ionised species, then the permeability coefficient may be low, but the aqueous solubility will likely be high. It is conceivable that the resultant fluxes from these two situations may be equivalent and hence the simplistic "rule" that only unionised species can be effectively delivered into and through skin must be regarded with significant caution.

xv. *Other factors*

Drug binding is a factor that should be borne in mind when selecting drugs. The diverse nature of skin components (lipids, proteins, aqueous regions, enzymes etc.) and the variety of permeants (weak acids / bases, ionised species, neutral molecules etc.), and numerous potential interactions between drug substances and the skin produces a multiple of interactions. Binding can vary from hydrogen bonding to weak Van der Waals forces and the result of drug binding (if any) on the lag time (the time taken for the molecules to traverse the stratum corneum / skin membrane), drug reservoir formation in the tissue and flux across the tissue will vary depending on the permeant (M. B. Brown & Williams, 2019).

Contingent on the type of formulation selected, other factors may be important in a dermal delivery system. For example, if the drug is suspended, then the particle size may become a key regulator of flux; if the drug has a low solubility in the vehicle and dissolution of the particles is thus rate limiting in flux, then decreasing the particle size will increase the rate of drug dissolution in the vehicle and hence promote dermal delivery. However, it is important to note that when a drug is suspended in a drug solution there are two dosage forms and the physical stability and thus quality of any such formulation may well be compromised in the during shelf life. If a drug is racemic, it may be possible to improve delivery by selection of lowest melting (i.e. highest solubility) enantiomers. The effects of melting point depression on drug delivery to and through the skin are considered in more detail elsewhere (Brown & Williams, 2019).



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### *C. Physicochemical modifications for modifying skin penetration*

To enhance skin permeation, various approaches have been taken to modify (improve) the physicochemical properties of a permeant; the reader is directed to Brown & Williams (2019) for a more detailed review. However, one example is the use of prodrugs and the approach relies on dermal enzymes (e.g., esterases or phosphatases) converting the prodrug to the therapeutically relevant form.

Another such method of physicochemical modification is the use of a eutectic system. Eutectic systems are composed of two compounds which form a complex which mutually inhibit the crystallisation of the other by lowering the melting point which increased the solubility of the permeant in the skin lipids (Stott, 1998). Lidocaine and prilocaine has been used in a clinically relevant eutectic mixture (EMLA cream) and is a well-known method for pain-free anaesthesia (Nyqvist-Mayer et al., 1986).

Another powerful concept in the physicochemical modification of the permeating drug molecule is termed “ion-pairing”. The concept of ion-pairing is an important consideration when employing pharmaceutical salts in dosage forms.

### *D. Penetration enhancers*

Perhaps the most common method of promoting the absorption of permeants into and through the skin is through employing penetration enhancers in the vehicle. Penetration enhancers encompass a wide range of compounds which act on the stratum corneum in a number of different ways to promote drug delivery. Such mechanisms include increasing lipid fluidity (thereby increasing drug diffusivity) by disrupting lipid packing/extracting lipids) or interacting with the hydrophilic corneocytes. Importantly, drug penetration enhancers may also modify the solubility parameter of the stratum corneum such that it becomes closer to that of the permeant (A. C. Williams & Barry, 2004).

An increasingly broad range of chemicals have been tested - or designed, synthesised and tested. Below are examples of materials that have been researched in some detail, with comments on their proposed mechanisms of action (Figure 9). Greater detail on individual chemical enhancers, reviewing their efficacies can be found in the Chapters in Dragicevic and Maibach (2016).



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Nevertheless, to distinguish chemical penetration enhancers from other excipients, ideally an enhancer would:

- Have no pharmacological activity within the body.
- Be non-toxic, non-irritating and non-allergenic.
- Work rapidly, and the scale of enhancement and its duration should be predictable and reproducible.
- Have reversible action so that the skin barrier returns rapidly and fully.
- Be suitable for formulation into topical preparations and so be compatible with drugs and excipients.
- Be cosmetically acceptable with appropriate skin "feel".

Water is often overlooked as a dermal penetration enhancer. By hydrating the stratum corneum it enhances the permeability of most solutes (Potts & Francoeur, 1991; M. S. Roberts & Walker, 1993). This hydration is mostly achieved by occlusion as is the case with emollients (A. C. Williams & Barry, 2004). The water content of human stratum corneum, under normal conditions is around 15 to 20% of the tissue dry weight (although this varies depending on the external environment / humidity). Occlusion of the tissue reduces or prevents TEWL and hence increases the water content of the stratum corneum up to ~400% of the tissue dry weight thus promoting drug diffusivity through the membrane for both hydrophilic and lipophilic permeants. Water is the safest and most widely used chemical penetration enhancer and when used in typical formulations generally satisfies the above ideal characteristics for an enhancer, although prolonged occlusion can induce irritation.

A large number of different fatty acids are thought to induce phase separation and lipid defluidisation within the membrane. Saturated alkyl chain lengths of $C_{10}$-$C_{12}$ linked to the carboxylic acid group (as in lauric acid) appear to be optimal as enhancers, whereas for unsaturated chains, a $C_{18}$ chain length (as in oleic acid) appears to be preferable (Santoyo & Ygartua, 2000). Lauric acid is not approved for topical use in the US however, oleic acid is present in a range of topical products, such as emulsions, gels and solutions (FDA inactive ingredient



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database, 2022). Oleic acid induces enhancement at low concentrations (< 10%) and can be incorporated with propylene glycol, acting synergistically with it to bring about its enhancing effects (A. C. Williams & Barry, 2004). Another fatty acid, based on myristic acid, is isopropyl myristate (IPM) which is an aliphatic ester and has been widely used as a safe penetration enhancer in commercial dermatological formulations (e.g., Andro-Gel®). Studies have been performed that indicate that IPM works to extract stratum corneum lipids made possible by its incorporation into the lipid matrix (Engelbrecht et al., 2012).

Dimethylsulfoxide (DMSO) is an extensively investigated and potentially aggressive chemical penetration enhancer which has been shown to interact with the lipid polar head groups and disorder their fluidity and packing. It is also implicated in keratin denaturisation which although may aid drug penetration can also cause sensitisation and irritation (Kligman, 1965; Notman et al., 2006). In addition, DMSO is metabolised via dimethylsulphide which produces a foul odour to the breath. Thus, though the agent is a powerful and potentially valuable penetration enhancer, its use in commercial preparations is limited (Pennsaid is the exception) and so researchers have sought other, chemically related, sulphoxide penetration enhancers.

Ethanol appears to enhance drug flux through delipidisation (lipid extraction) or via dehydration and desiccation of the membrane after prolonged exposure (A. Williams & Barry, 1992; A. C. Williams & Barry, 2004). The volatile nature of ethanol means that enhancement in permeation can also be attributed to the induced supersaturated state of the formulation vehicles post-evaporation (M. B. Brown & Williams, 2019). As with most permeation enhancers, ethanol (and other alcohols) can work to increase drug permeation using a combination of the above methods, or independently.

Polar solvents like propylene glycol and dipropylene glycol are frequently proposed as penetration enhancers and are believed to change the solvent properties of the stratum corneum and synergistically affect the solubilization of the constituents within the SC hence promoting the lipid-modulating effect. Generally, they appear to work best when used in combination with another penetration enhancer such as ethanol or oleic acid. However, permeation of the glycols



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themselves through the tissue can also alter the composition and hence thermodynamic activity of the drug in the remaining vehicle, which could in turn can modify the driving force for diffusion. Likewise, Transcutol is another commonly investigated penetration enhancer and at certain concentrations causes the solubilization of drugs within the membrane.

Pyrrolidone derivatives have also been investigated as potential penetration enhancers based on the presence of pyrrolidone derivatives as humectants within the skin, since they are components of Natural Moisturising Factor (NMF). The most widely studied pyrrolidone or pyrrolidines are N-Methyl-2-pyrrolidone (NMP) and 2-pyrrolidone (2-P). Both are polar aprotic solvents with high miscibility in both water and ethanol.

### *E. Physiological factors affecting transdermal and topical drug delivery*

There are a number of physiological factors that affect the structure of healthy human skin, some of which may consequently influence topical and transdermal drug delivery. These are covered extensively in Brown & Williams (2019) but can be summarized as follows:

#### *i. Gender*

A number of differences between male and female skin have been reported. Many of these discrepancies are under hormonal control and arise from the sex steroid differences between males and females. Gender differences in gross skin thickness (dermis and epidermis) have also been reported with male skin thicker than female at all ages. Female skin has been reported to be approximately 10% thinner post-menopause and ovarectomy is associated with thinning of the skin whereas estrogen therapy thickens skin in females. Whilst these gender-related differences, particularly in pore size and surface pH could theoretically affect topical and transdermal drug delivery, in practice most studies report no differences in permeability between male and female skin. Similarly, TEWL is invariant between the sexes.



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*ii. Skin age*

Changes to human skin on aging have been extensively studied, both for drug delivery and for the use of pharmaceutical and cosmetic preparations. It is widely recognised that skin undergoes structural changes on aging with both the dermis and epidermis thinning; levels of collagen and elastin decline with age. Blood flow (dermal clearance of molecules traversing the tissue) tends to decrease with age and this could, in theory, reduce transdermal drug flux. In addition to these intrinsic biological changes, lifetime environmental exposure to solar radiation and exogenous chemicals (including soaps, cosmetics or pharmaceutical active ingredients) can also affect the structure and barrier performance of skin.

xvi. *Body site*

Variations in skin structure, and in particular the stratum corneum, are seen with body site. The stratum corneum is thicker on the load bearing areas of the body such as the palms of the hands and soles of the feet and is relatively thin on "sensitive" sites such as the lips. But the permeability of different skin sites is not simply related to stratum corneum thickness since different permeants have been shown to permeate to different rank orders through different skin. In addition to stratum corneum thickness, the corneocyte surface area varies and follicular density and the volume of pores differs from site to site as described earlier. Regional variations in cutaneous blood flow and TEWL have also been reported. Notwithstanding, a generalised rank order of site permeabilities is:

Genitals > head & neck > trunk > arm > leg.

xvii. *Damaged skin*

Whilst the stratum corneum is compromised in many different diseased states, exposure to radiation, irritant chemicals and allergens, as well as trauma, can reduce the barrier and increase topical drug delivery. Naturally, for conditions where the skin barrier is impaired, then as treatment progresses the



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barrier function of the tissue may be restored to that approaching uninvolved tissue and consequently, drug delivery decreases. Further problems are introduced when topical medicines that are used to treat skin disorders affect the stratum corneum barrier themselves; for example, corticosteroids and retinoids tend to reduce the thickness of the stratum corneum and its barrier function. To date these changes are not really considered in topical formulation development.

A reduction in the stratum corneum barrier function is commonly reported for patients with skin disorders. For example, those with atopic dermatitis tend to demonstrate increased TEWL – at both involved and non-involved sites – compared to that of normal healthy subjects. Similarly, TEWL increases at affected sites for patients suffering from psoriasis. However, whilst barrier impairment seen for psoriatic skin may increase drug absorption, depending on the severity of the disease the presence of thickened hyperkeratotic plaques may reduce permeation of some drugs; indeed increased drug uptake into the psoriatic plaques compared to uptake into uninvolved stratum corneum could be attractive to target delivery to only the disordred skin sites (Anigbogu et al., 2011).

xviii. *Other factors*

Historically, there has been little literature examining the influence of ethnicity on differences in the stratum corneum barrier function or drug delivery into or through the skin. However, there are significant differences in the stratum corneum water content between races (Berardesca et al., 1991) and it would be anticipated that these differences in hydration would cause differences in drug absorption, as the level of hydration of the stratum corneum can have a significant effect on drug permeation through the tissue. Since the levels of Natural Moisturising Factor decline with age, a reduction in this natural humectant can affect not only the pliability of the skin but also potentially reduce delivery of polar molecules. Null mutations of the filaggrin gene reduce the levels of NMF in the tissue and is related to dry skin conditions, which can also affect topical drug delivery.

As diffusion through the stratum corneum is a passive process, increasing temperature clearly increases the permeant diffusion coefficient as through any membrane. Naturally the internal body temperature is ~37°C but the external skin surface is exposed to the external environment and so the



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outer skin temperature is in the order of 32°C. The application of moderate heat to the skin, for example with heating pads of self-heating patches, has several effects, predominantly to increase drug release from a formulation and to increasing drug diffusivity within the stratum corneum (Wood et al., 2012) both of which tend to increase drug delivery. Elevating the skin temperature further (>55°C) can cause structural alterations in the stratum corneum lipids and denature proteins, and these modifications can radically increase diffusion through the tissue. In general, however, minor variations in skin or environmental temperatures tend to have minimal effects on topical drug delivery.

### *F. The mathematics of skin permeation*

Since drug absorption across the stratum corneum is a passive process, with no active transport mechanisms having been reported to date (Brown & Williams, 2019), relatively simple models drawn from standard diffusion theory have been applied by transdermal and topical drug delivery researchers. Despite the complexity of skin as a highly heterogeneous structure, and the variable pathways for permeation, approaches such as Fickian diffusion theory tend to work remarkably well for this tissue. Commonly, drug delivery to and through the skin from different formulations is assessed with *in vitro* drug diffusion experiments. In essence, a membrane (e.g. full thickness skin) separates a donor compartment (where the formulation is applied) from a receiver compartment (where drug permeating across the membrane can be analysed). The data generated can usually be plotted as the cumulative amount permeated per unit area (μg $cm^2$) against time and produces a characteristic profile depending on whether steady state drug permeation (infinite dosing, Figure 10-A) or transient permeation (finite dosing, Figure 10-B) has been achieved. Additionally, to understand the relative importance of the drug partitioning and diffusion processes to total drug permeation, the data obtained from these *in vitro* (or *ex vivo*) diffusion studies can be mathematically modelled according to Fick's laws of diffusion.



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*i. Steady state permeation*

In order to control variable and to explore the inherent permeation of a drug, an infinite (non-depleting) dose can be applied to the skin surface; clearly this doesn't reflect the situation where a patient may dose, for example, a steroid cream for local irritation but does represent the situation when a transdermal patch is applied. This approach ensures that a constant drug concentration is applied to the skin surface even though a relatively small amount of the drug may permeate the skin tissue over time. Advantageously, under these conditions, drug permeation reaches a constant rate, often referred to as (pseudo) 'steady state' and is easily identifiable as the linear portion of the profile (Figure 10-A). The lag time ($T_L$) can be calculated by linear extrapolation of the 'steady state' region back to the x axis intercept from which, according to Fickian diffusion theory, steady state flux occurs after 2.7 times the lag time.

Fick's first law of diffusion states that "the rate of transfer of a diffusing substance through a unit area of a section is proportional to the concentration gradient measured normal to the section" and can be described by **Equation 1**, where J is mass transfer per unit area, D is the diffusion co-efficient, C is the concentration of the diffusing species and X is the space co-ordinate measured normal to the section (Crank, 1975).

$$J = -D\frac{\partial C}{\partial X} \quad \textbf{Equation 1}$$

The concentration gradient across the membrane ($\partial$C) is calculated as the change in concentration between the outermost layer of the membrane ($C_o$) and the innermost layer of the membrane ($C_i$). The distance across which a permeant travels ($\partial$X) is assumed to be the thickness of the membrane (h), but note the earlier discussion that, for intercellular pathways for drug delivery, the actual distance travelled by a lipophilic molecule may be an order of magnitude greater than the simple thickness of the tissue. Thus, **Equation 1** can be simplified to:

$$J = \frac{D(C_0 - C_i)}{h} \quad \textbf{Equation 2}$$



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Fick's first law was developed using homogeneous isotropic membranes, ideal solution and gas phase experiments. Clearly the same conditions do not apply for skin permeation studies given that three permeation pathways (intercellular, transcellular and shunt routes) operate concurrently, to differing degrees, that the stratum corneum matrix is far from homogeneous (cells, lipid matrix, complexity of lipid phases) and that pathlength is unknown. However, the literature demonstrates that Ficks first law can be used reproducibly to give meaningful data that usually shows good correlation as evidenced by regulatory requirements to undertake such studies.

It is essential that sink conditions are maintained in the receiver fluid for the duration of the experimental period to ensure that permeation is not limited by increasing concentrations of the permeant in the receiver solution. Under these conditions, it is expected that the concentration of the permeant in the innermost layer of the membrane (in direct contact with the receiver fluid) is negligible ($C_i$ is essentially zero), thus **Equation 2** can be further simplified to;

$$J = \frac{DC_0}{h}$$ **Equation 3**

Practically, it is very difficult to measure $C_0$, the concentration of the drug in the most superficial layer of the stratum corneum which is in direct contact with the donor solution (Brown & Williams, 2019). However, the concentration of the permeant in this outermost layer ($C_0$) can be related to the concentration of the permeant in the vehicle ($C_v$) using **Equation 4**, where P is the partition co-efficient of the permeant between the vehicle and the membrane.

$$P = \frac{C_0}{C_v}$$ **Equation 4**

Since the concentration of the permeant in the donor solution is usually known (or can be easily assayed) rearranging **Equation 4** **Equation 4** for [Co] and substituting into **Equation 3** results in the widely used form of Fick's first law to estimate the flux of a permeant under 'steady state' conditions **(Equation 5).**

$$J = \frac{DPC_v}{h}$$ **Equation 5**



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The lag time can be obtained from extrapolation of the pseudo steady-state portion of the permeation profile to the intercept on the time axis. As a useful approximation, pseudo steady-state permeation for most drugs is achieved after around 2.7 times the lag time (Barry, 1983). Crank, (1975) showed that the lag time (L) can be related to the diffusion coefficient (D) by:

$$L=\frac{h^2}{6D}$$ **Equation 6**

**Equation 6** shows that it is feasible to determine the diffusion coefficient of a molecule in the membrane by measuring the lag time. Indeed, this approach has been used for studies with simple isotropic physical membranes such as polymers. However, skin is not a simple isotropic membrane. Difficulties are encountered when measuring the membrane thickness; for example, is the rate determining barrier the stratum corneum alone, or does it include the viable epidermal tissue? Application of various vehicles can expand the membrane, and indeed a simple measure of thickness does not account for the tortuous intercellular pathway for diffusion. As the membrane thickness is a squared factor in equation 6, any minor errors in this parameter rapidly magnify. Additionally, lag times obtained from permeation experiments with human skin tend to be highly variable and can be strongly influenced by permeant-skin binding.

An alternative approach to determine the apparent diffusion coefficient of a permeant from a pseudo steady-state permeation profile requires rearrangement of Equation 5 to;

$$\frac{Jh}{PC_v}$$ **Equation 7**

This expression allows calculation of the apparent diffusion coefficient without requiring an accurate lag time value but does still require an approximation for the membrane thickness (though not squared). The partition coefficient (stratum corneum / vehicle) for the permeant can be obtained from separate experimentation.



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As a result of the difficulty in accurately assessing membrane thickness, a composite parameter, the permeability coefficient (kp) is often used and is given as;

$$K_p = \frac{PD}{h} \quad \textbf{Equation 8}$$

which can be substituted into **Equation 5** to give;

$$J = k_p C_v \quad \textbf{Equation 9}$$

The pseudo steady-state flux, J, is simply obtained as the gradient of the linear portion of the permeation profile (**Equation 9**), and if the concentration of the permeant in the applied vehicle is known then the permeability coefficient can be determined. It is this parameter, which is often used to characterise the permeation of drugs through skin; other parameters such as the diffusion coefficient can be used but are more difficult to calculate accurately due to difficulties estimating, for example, membrane thickness.

Though the above equations, which are relatively simple and easy to use, there are some important assumptions made in their derivations which are questionable. For some experimental designs the equations become invalid as detailed below:

- The stratum corneum is the major rate-limiting barrier and that the primary rate-determining step is permeation through the stratum corneum and not partitioning into or out of the membrane. This assumption appears to be valid for most permeants, though for very lipophilic or very hydrophilic molecules then partitioning behaviour may become rate limiting.

- Permeation through the appendages (shunt routes) is negligible compared to permeation through the bulk of the stratum corneum. This will be the case for most permeants, but for ions and large molecules the shunt routes may be highly significant. Clearly, the above equations only apply at pseudo steady state and do not hold for permeation during the early time course of the process.

- Permeation through the stratum corneum is solely by passive diffusion. To date, no active transport mechanisms have been reported.



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- The nature of the stratum corneum is not altered by the application of the vehicle. This is clearly not the case if dry (or partially hydrated) stratum corneum is used as the membrane and an aqueous donor solution is applied. Similarly, some vehicles (such a propylene glycol) have been shown to partition into the stratum corneum thus altering the solvent nature of the tissue hence affecting partitioning of the permeant into the membrane.

- The drug is in solution within the stratum corneum. Given the varied nature of the tissue, then this appears a reasonable assumption for most drugs.

- The diffusion coefficient of the permeant is independent of concentration, time or distance. This is a questionable assumption since many permeants would be expected to bind to some extent to the tissue, effectively making diffusion coefficients dependent upon concentration. Additionally, permeants may exist in different states of self-association dependent upon the applied concentration – dimethylsulphoxide can exist in many self-assembled states including dimers and hydrates depending on the environment in which it is placed. Thus, the diffusion coefficients calculated from transdermal permeation experiments must be regarded as apparent diffusion coefficients since the errors inherent in using the above equations prevent absolute values from being generated.

- **Equation 6** (relating lag time to diffusion coefficient) is only applicable to situations where there is no binding between the permeant and the tissue.

- Fickian diffusion theory was developed for isotropic media. The stratum corneum is a heterogeneous membrane, yet we assume that it is uniform in character.

Despite these caveats, it is surprising that such simple equations fit experimental skin permeation data so well. More complex models and equations have been developed, some mathematically based, others using simulated data or compartmentalised models. However, the majority of literature reports



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use Fickian theory to describe experimental data and so the utility of alternative data manipulations for a wide range of permeants remains to be validated.

*ii. Thermodynamic activity*

From the above, it is clear that Fickian diffusion is dependent on a concentration gradient between the formulation and membrane, and where a permeant moves from high concentration to low concentration. Accordingly, the concentration of the permeant in the vehicle ($C_v$) is directly proportional to flux across the membrane (J), as described in Equation 10. Therefore, it follows that when the permeant is diluted in the formulation, there would be a proportional decrease in the flux of the permeant. However, whilst the use of concentrations works well under most circumstances, the true driver for diffusions through a membrane is the thermodynamic activity of the permeant in the formulation. Thermodynamic activity can be simply described as the 'escaping or leaving tendency' of a permeant from the formulation or vehicle; by definition, as a solid is regarded as having a thermodynamic activity of 1 (unity), so a saturated solution, which is in equilibrium with its solid (ie any more permeant added would form a crystal or precipitate) has maximum thermodynamic activity (also 1) (Brown & Williams, 2019). molecules with a high thermodynamic activity in the formulation (eg saturated) will be released from the vehicle and favourably partition into the stratum corneum, thus drug thermodynamic activity is related to both drug concentration in the vehicle and the partition coefficient (term '$KC_v$' in Equation V-5).

The relationship between thermodynamic activity and drug flux across a membrane is described by the Higuchi equation (Equation V-6; Higuchi (1960)) where α is the thermodynamic activity of the drug within the formulation and γ is the effective activity coefficient of the drug in the membrane. Thus, **Equation 5** can be rewritten as:

$$J = \frac{\alpha D}{\gamma h}$$ **Equation 10**

A saturated solution has maximal thermodynamic activity and, equally, has the greatest concentration gradient across a membrane into a perfect sink containing no drug – hence their interchangeable use in



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the above equations (supersaturated states can have higher transient concentrations and thermodynamic activities). However, as concentration moves down from its saturation maxima, thermodynamic activity may not fall linearly.

Practically, the equation shows that a given drug delivered from a “good” solvent and saturated at 100 mg/mL would provide the same flux as when delivered from a poorer solvent, saturated at 1 mg/mL, assuming that the drug does not deplete significantly within the donor vehicle Similar arguments can be used for any component of a topical formulation including penetration enhancers. Nevertheless, it is important to note that practically when a drug is formulated at too high a thermodynamic activity quality issues as a result of physical instability are likely to occur.

xix. *Transient permeation (finite dosing)*

In most clinical situations it is not possible to administer an infinite dose to a patient and in these circumstances of a finite dose application, pseudo-steady state permeation is unlikely to be achieved. In contrast to the infinite dose permeation profile shown in Figure 11, the cumulative permeation data when a finite dose has been applied increases to a plateau beyond which the amount permeated remains constant unless further doses are applied to the membrane surface (Figure 11). If the instantaneous flux values are plotted against time, then a peak in the profile is observed corresponding to the maximum flux that was achieved. Useful information can be gained from a cursory examination of instantaneous flux profiles from finite dose applications. For example, the breadth of the peak can indicate the extent of binding within the membrane with broadly spread data suggesting that some of the dose is retained within the tissue for extended periods of time, possibly as a reservoir.



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## V. TOPICAL FORMULATION DEVELOPMENT

### *A. Introduction*

When developing any medicine, it is important to keep in mind that the patient is not given a drug, but a drug product. There are many historical examples in the pharmaceutical history of drugs that could have become the next blockbuster if only a formulation could have been developed in order to deliver the molecule to the pathological site in an effective and safe manner. Moreover, topical drug delivery to the skin presents even greater challenges as patients have a choice as to what they apply to their skin and therefore they should be at the forefront of a formulator's mind. Topical products developed many years ago that were greasy, with a bad odour, and stained clothes are no longer acceptable; cosmetics and aesthetics of the final product are almost as important as the product's efficacy. As an example, topical foam products have become popular despite not always providing real benefits in terms of drug delivery but offer a more elegant alternative to familiar semisolids such as creams and ointments (Brown & Williams, 2019).

When preparing topical formulations, many options are available. There are a variety of elegant and innovative drug delivery systems available for formulating skin preparations, as well as several where extravagant claims may be unfounded. Rather than covering all of these formulations, this section of the review focuses on the principal formulations used to deliver medicaments effectively to the skin.

### *B. The requirements of a target product profile*

When embarking on a development program it is important to understand the end goal. Is that goal to develop a simple prototype formulation, a ‘Proof of Concept’ approach, or is the aim to develop a ready for market commercial product. The proof-of-concept approach is typically quicker in the short-term and can be useful if the aim is to generate data to acquire additional funding. However, there are questions on how relevant the data is and if such an approach limits the chance of success without the necessary optimisation of the product. A final optimised product might initially take longer but it is likely quicker



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and more cost-effective overall with a lower risk of failure. The desired approach will ultimately inform the target product profile.

At the start of a formulation development program a target product profile (TPP) needs to be defined highlighting the key requirements for the product. The diagram in Figure 12 shows design considerations for topical product development. The drug, formulation and final product are intrinsically linked. The selection of a vehicle for topical application is influenced by the physicochemical properties of the drug, the disease to which it is applied and the patient. Thus, in any formulation the stability and compatibility of the excipients with the drugs and with themselves must be considered, whilst ensuring that the preparation is cosmetically acceptable with a good skin feel, texture and fragrance and easy application.

An example of some of the considerations to include when preparing a TPP are shown in Figure 13. It is quite typical that not all of the information will be available at the beginning of the project and the TPP will evolve as the program develops. Participation of various stakeholders including a toxicologist, marketing, medicinal chemist amongst others is recommended. Many of the requirements are interlinked and help to inform the selection of excipients and then guide the route of development. Some key considerations from the development outset are:

- **Route of delivery** as requirements are different for a dermal when compared with other routes such as transdermal, ophthalmic, nasal or oral and the approved excipients available also differ.
- **Target populations**: for example, if the product is intended for children, certain excipients should be avoided.
- And **Therapeutic indication,** as it will define the most suitable formulation type, have implications in excipient selection (suitability of excipients on the disease state of the skin) and provide an understanding of whether any specific packaging/devices will be required.

Ultimately, the specifics of a TPP for a formulation applied to the skin will vary depending on its final



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purpose. However, there are key aspects of most TPPs that are common for the majority of formulations/routes. For example, the stability of the drug/formulation allowing adequate shelf-life. The extent and rate of release of drug from formulation throughout stability needs to be understood. Sufficient drug delivery to the site of action and the cosmetic elegance and patient compliance should be considered.

### *C. The pharmaceutical development processes*

A typical formulation development process from pre-formulation development to the identification of the final lead formulation candidate is outlined in the flow chart shown in Figure 14. The remainder of this section will provide an overview of some of the steps and considerations/challenges that a formulation scientist should overcome to develop an optimised product for the drug, formulation, disease, and consumer.

## *D. Quality by design*

The US Food and Drug Administration (FDA) and the guidelines of the International Conference on Harmonisation (ICH) Q8 (R2), define pharmaceutical QbD as "a systematic approach to product development that aims to ensure the quality of pharmaceutical products by employing statistical, analytical and risk-management methodology during the design, development and manufacturing" (European Medicines Agency, 2017). Ultimately, the aim is to identify Critical Material Attributes (CMA) and Critical Process Parameters (CPP) which require control and monitoring to obtain a product meeting the critical quality attributes (CQA).

A risk based QbD approach in drug product development is encouraged by the FDA. In its initial implementation the QbD approach focused on the manufacturing process, however, more recently the focus has shifted upstream to include preformulation and formulation development stages. QbD fundamentals should be included in the abbreviated new drug application (ANDA). Furthermore, as the



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industry and regulatory bodies harmonize their approach. a transition to more complete QbD filings is expected.

Implementation of this approach requires the definition of the following:

1. Define an objective by determining the Quality Product Profile (QPP).

2. Determine Critical Quality Attributes (CQAs) by a criticality assessment.

3. Perform a risk assessment to link raw Material Attributes (MA) and Process Parameters (PPs) to CQAs.

4. Develop a Design Space (DS) to identify a Control Space (CS) where the Design of Experiment (DoE) can be defined.

5. Design and implement a control strategy to ensure that CQAs are met.

6. Continual improvement during the product lifecycle.

To better understand the regulatory framework, the reader is encouraged to refer to the following guidelines: ICH, Q8 (R2 (Pharmaceutical Development), Q9 (Quality Risk Management) and Q10 (Pharmaceutical Quality System). Furthermore, ICH Q1WG on Q8, Q9, and Q10 Questions and Answers; the ICH Q8/Q9/Q10 Points to Consider; and the ICH Q11 (Development and Manufacture of Drug Substance) have been published by the European Medicines Agency (EMA) and FDA.

E. *Analytical method development*

To support the topical pharmaceutical development process, “phase-suitable” analytical methods are required. During preformulation and formulation development, the analytical method is required to be stability indicating, allowing the resolution/separation of impurities to allow not only stability



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determination by drug assay but also by assessment of impurity/related substance growth. Forced degradation experiments for the drug substance (in solution) at the outset (later for the lead drug product) are typically performed to not only confirm the method is stability indicating but also to assess drug degradation pathways. Due to the complexity of topical products (multiple excipients and low drug levels), existing analytical methods for the drug substance usually need further development to allow the detection and quantification of the drug and related substances in these matrices.

For the performance testing experiments that aid the selection of drug candidates and screening of formulations to identify a lead, the main challenge is developing methods with suitable sensitivity; considering the low levels of drug that require detecting. Thus, these experiments usually necessitate different analytical methodologies such as LC-MS/MS to those used for assaying the drug and related substances in the drug product.

Prior to clinical studies, phase suitable validated methods meeting the requirements of the International Conference on Harmonisation (ICH) guidelines ICH Q2A, (European Medicines Agency, 1995; The European Agency for the Evaluation of Medicinal Products, 1995) and Q2B (European Medicines Agency, 2022a) must be implemented for the drug and its related substances for the characterization and stability testing of the final lead formulations.

### *F. Preformulation*

According to Akers “Preformulation testing encompasses all studies enacted on a new drug compound in order to produce useful information for subsequent formulation of a stable and biopharmaceutically suitable drug dosage form”. (Akers, 1976). It is in this stage of research and development that the drug's physicochemical properties, desired dosage form, mechanism of action, and target disease are taken into consideration. The preformulation for topical semisolid drug products usually includes studies of drug solubility and drug-excipient compatibility. In such studies, critical parameters are identified that may impact product development. Some of these parameters include poor solubility and drug concentration, inherent drug instability, potential excipient/drug incompatibility, and/or excipient/excipient



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incompatibility. Additionally, preformulation studies aim to develop and explore methodologies for improving the defined issues so that the formulation dosage form achieves its target profile. Therefore, pre-formulation studies serve as the foundation for rational formulation design.

*i. Excipient selection*

Excipients play a crucial role in how the drug is presented and delivered. Whilst, traditionally, excipients have been considered as 'inert' materials, more recently the development of drug delivery systems has benefited from excipient–drug interactions to produce formulations with specific properties and performance. The selection of the excipients to be included in preformulation and formulation development experiments will depend on the physicochemical properties of the drug, the intended vehicle for drug delivery, their suitability for the disease state of the skin and the intended market territories. Generally, excipients with the following functions are to be included in such experiments:

- Solvents to allow the incorporation of drug at the target concentrations (e.g. water, polyethylene glycol, propylene glycol, ethanol, isopropyl alcohol or oils for lipophilic drugs);
- Humectants (e.g glycerol, propylene glycol, allantoin);
- Penetration enhancers (e.g diethylene glycol monoethyl ether, isopropyl myristate, propylene glycol);
- Antimicrobial preservatives to prevent contamination and microbial growth in aqueous formulations (e.g benzyl alcohol, benzoic acid, phenoxyethanol);
- Antioxidants and chelating agents, where the drug has shown susceptibility to oxidation in forced degradation experiments (Butylhydryanisole, Butylhydroxytoluene, propyl gallate, EDTA);
- Polymers (e.g carbomers, celluloses, poloxamers);
- Thickening agents (cetyl alcohol, cetostearyl alcohol, stearic acid, waxes);
- Surfactants (Polysorbates, sorbitan esters).



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A minimalistic approach to the inclusion of excipients is recommended where the inclusion of each excipient and their levels should be justified.

*ii. Solubility*

The importance of solubility in formulation development cannot be overstated since insufficient solubility in the formulation will hinder drug development if the target dose cannot be achieved. Similarly, highly soluble drugs may pose a different challenge where a formulation cannot be optimised for drug release. Therefore, the solubility of a drug may require the selection of a combination of solvents and non-solvents tailored to the drug and dosage form. A highly lipophilic drug, for example, is less likely to be formulated as an aqueous gel than as a cream, ointment, or non-aqueous gel.

When designing preformulation studies, excipients are selected from a library of acceptable excipients based on the intended dosage form. The use of cosolvents, pH adjustment, complexation, surfactants and combinations thereof are used to solubilise drugs in topical formulation development. Of these approaches, the use of cosolvents is possibly the simplest and most widespread method to increase the solubility of poorly soluble compounds in aqueous systems, when sufficient solubility cannot be achieved in a single excipient. When selecting a suitable cosolvent, the miscibility with solvents, should be considered to avoid phase separation. In order to increase the thermodynamic activity of highly soluble drugs at the desired concentration, solvents and non-solvents are combined in co-solvents systems.

For the development of topical semisolids, up to 20 excipients are typically selected for solubility screening during an initial pre-formulation stage. Excipients are initially selected based on the physicochemical properties of the drug and the desired dosage form or product profile. Upon completion of this initial investigation, it will be determined if alternative solvents need to be investigated or if the target dose can be achieved. If the latter is feasible, co-solvent systems are developed and tested, along with the drug, for compatibility between drug/excipient and excipient/excipient.



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*iii. Drug and excipient compatibility*

In order to develop a drug product, it is paramount to understand any interaction between excipients and the drug substance.

Excipient/drug or excipient/excipient compatibility can be classified as physical, chemical, or physiological, and such interactions may have implications for drug stability, product manufacture, product adhesion, drug release, product efficacy, therapeutic activity, and side-effect profiles. While the main approach is based on accelerated temperature studies, there are several approaches to conducting excipient compatibility screenings depending on the dosage form. The timepoints (typically over 2 over 4 weeks) and conditions (typically between 25-50 °C) to be assessed are dependent on the inherent drug stability based on previously available data and/ or forced degradation experiments performed during analytical method development, furthermore, the level of acceptable risk and timelines are also considered. Although the majority of initial drug/excipient compatibility studies for topical dosage forms are performed on solvents and excipients existing as liquids at room temperature, consideration (for example the use of co-solvents/binary systems) has to be taken for excipients which are in the solid form at room temperature.

*G. Topical (Dermal) formulation options*

Topical dosage forms have been generally classified as liquids, semi solids and solids and typical examples are summarised in Figure 15. The selection of a vehicle for topical application will be influences by the disease state of the skin intended to be treated and the physicochemical properties of the drug. Whilst there may be an indication of a preferred vehicle in the target product profile, it is possible that following preformulation experiments the choice of vehicle is restricted, and further excipients require to be tested. For example, the TPP might indicate a cream as the preferred vehicle for the delivery of a certain drug, however, if following excipient compatibility studies the drug proves to have poor stability in water, alternative non aqueous vehicles such as non-aqueous gels,



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ointments or ointment foams should be considered. The following section describes some of the most frequent formulation options in dermal drug delivery.

*i. Liquid formulations*

Liquid formulations include simple single-phase solutions, two phase systems and complex multiphase systems. The former can be aqueous, prepared from hydrophilic solvents and their mixtures or oils. Oil in water (o/w), water in oil (w/o) thin emulsions (lotions) and suspensions are examples of two-phase systems. Oil-in-water-in-oil (o/w/o) or water-in-oil-in-water (w/o/w) systems constitute the latter. Emulsions are most commonly used in creams and the classification of an emulsion between a liquid or a semi-solid is somewhat arbitrary.

Single- phase liquid preparations have poor residence time on the skin, limiting the drug delivery to short time frames and thus their application as topical vehicles. By means of packaging, one phase systems can be made into pump sprays, which have the advantage of ease of application and ability to cover large areas. Furthermore, the incorporation of film formers, allows increased residence time, thus expanding their applicability. Metered dose aerosols (MDA) have been rapidly gaining popularity as formulation devices for topical delivery with numerous advantages over other formulations based on ease of use and a metered dose without the need for manual manipulation post application and the potential to generate transient in situ supersaturation. MDAs utilise a delivery system that is dependent on the packaging (in particular the valve) and the liquid propellant within the canister. An MDA typically contains an API as a suspension or solution, volatile solvents, non-volatile solvents/non-solvents and a volatile liquid propellant. It can contain a film-forming polymer.

Two phase and multiphase liquid systems have been adopted more broadly as a vehicle for topical drug delivery. Lotions can provide a cooling sensation as water evaporates from the skin surface. This sensorial effect is enhanced by the presence of alcohol; however, alcohol should be avoided for indications with broken or dry skin. The drug can be dissolved or suspended in a lotion (for example, calamine lotion), for the latter, the evaporation of the vehicle deposits a film of the drug as a powder onto



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the tissue surface. Thus, the residence time is improved, however, the deposited powder must dissolve prior to penetrating the stratum corneum.

*ii. Semisolid formulations*

Semisolid formulations constitute the majority of topically applied formulations; they can be used to deliver drugs over a long period time due to their residence on the skin. Moreover, formulators have numerous options when preparing semisolids. Nevertheless, there is a lack of clarity in the classification of these topical dosage forms, depending on the literature cited. This is clearly illustrated by the differences in the pharmacopeial definitions of creams and ointments. the EP and BP define ointments as 'single-phase basis in which solids or liquids may be dispersed' and creams as 'multiphase preparations consisting of a lipophilic phase and an aqueous phase' (BP) (EP) whilst the USP and defines ointments and creams as 'a semisolid dosage form containing one or more drug substance dissolved or dispersed in a suitable base'(USP, 2022). A classification of topical products and their definitions, including semisolids has been proposed by Buhse et al., 2005).

*Ointments*

Ointments are semi-solid preparations applied to the skin, with drug solubilized in the vehicle or suspended as a microcrystalline solid. Different types of ointment bases are available, including hydrocarbon, absorption, emulsifying, and water-soluble bases. Hydrocarbon bases are occlusive and useful for dry scaling conditions but have limited application as drug delivery vehicles due to the poor drug solubility. Absorption bases can absorb water or aqueous secretions while maintaining a semi-solid consistency and are useful for stratum corneum hydration. Emulsifying bases create oil-in-water systems, making them easy to wash off, the emulsifying agent's ionic nature (anionic, cationic or non-ionic) determines the categorization of the base, and their suitability will be dependent on the ionic state of the drug. Water-soluble bases (polyethylene bases) mix well with skin secretions, have good chemical stability and are easily spread, but they are incompatible with certain drugs (i.e. penicilins) and high-



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water levels. The excipient selection including grade is key, various grades and qualities of the base materials (polyethylene glycols and hydrocarbons) are available with differing chain lengths following alternative refinement processes. These discrepancies will alter the physicochemical properties of the materials, resulting in modifications in drug release and absorption. The potential for sensitization of certain excipients such as wool fats and alcohols, should also be considered in the excipient selection. Ointments are generally prepared by fusing the components to a temperature above the melting point of all materials, and then cooling them whilst stirring. Heating temperature should be kept to the minimum to avoid degradation and reduce manufacturing costs, order of addition, stirring and cooling speeds usually constitute critical processing parameters. It has long been proposed that the occlusive benefits of ointments leading to increased epidermal hydration and thus drug permeation are often overlooked when developing medicines for particularly challenging dry skin conditions as a result of the unattractive organoleptic properties of some of the bases. However, recent developments in the appearance and feel of some new excipients mean that such formulation approaches are being reconsidered.

*Creams*

Creams can be defined as semisolid emulsions for application to the skin or mucous membranes. Patients tend to prefer creams over ointments because they are less greasy, easier to apply, and can usually be washed off with water. Due to their reduced occlusivity compared to hydrocarbon ointments, they tend to be less effective at hydrating the stratum corneum.

Emulsions can be classified by the diameter of the droplets of the disperse phase into coarse emulsions, fine emulsions (ca. 5μm) and microemulsions (>10 nm). Similarly, they are classified in terms of the nature of the dispersed and continuous phase. Namely, an oil-in-water (o/w) emulsion, if oil droplets are dispersed in water or water-in-oil (w/o) emulsion if water droplets are dispersed in a continuous oil phase. Multiple emulsions can also be formed where, for example, water globules are dispersed within oil globules in a continuous water phase. Emulsions are thermodynamically unstable systems, spontaneously the dispersed droplets tend to collide, flocculate, coalesce to form ever-larger globules that would



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ultimately separate into the oil and water phases, a phenomenon termed cracking. Thus, they necessitate emulsifiers to improve their physical stability. Emulsifiers can prevent flocculation separation via various mechanisms, including steric or electrostatic repulsion. The viscosity of the continuous phase can also be increased in order to stabilize an emulsion to some extent, thereby reducing the mobility of the droplets and preventing globule collisions. Surfactants, polymeric hydrocarbons, alcohols, proteins, and even finely divided powders like bentonite can act as emulsifiers.

Dermatological creams can be w/o or o/w emulsions. The former is more occlusive and leave a protective oil layer on the skin following water evaporation, they are mostly employed as emollients or cleansers. The latter are more widely used and are termed “washable” or “vanishing” creams; the evaporation of the aqueous phase offers a cooking sensation and whilst they do not deposit a continuous oily layer on the skin surface, they can deliver lipophilic and hydrophilic materials to the skin.

When developing creams, the solubility of the API and certain key components such as preservatives in both the aqueous and oil phases should be considered. Partitioning of the preservative into the oil phase can result in loss of preservative efficacy, the composition of the oil phase can have a big impact on this phenomenon as illustrated by (Herbig et al., 2023), where phenoxyethanol was observed to preferably partition into oil phases containing medium chain triglycerides. Similarly, the levels of surfactants can impact the partitioning behaviour of APIs and excipients, this is particularly relevant for lipophilic APIs sensitive to hydrolysis, for example Betamethasone, as increasing partitioning into the aqueous phase can impact the chemical stability of the formulation (Puschmann et al., 2019).

Due to the dynamic nature of emulsions, understanding the drug delivery of drug from creams is complex; the cream changes as the continuous phase evaporates and as the emulsion cracks. As water evaporates from an o/w cream containing a water-soluble permeant, the degree of saturation of the permeant can increase. Provided the oil phase prevents crystallization a supersaturated state can therefore form with a consequent increase in permeation. Conversely, from a w/o cream the permeation of a hydrophilic permeant could be decreased by the formation of the oily layer deposit on the skin surface



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which would act as a barrier to permeation. In addition, when an emulsion cracks, micelles formed from the emulsifier can trap the drug.

*Gels*

Gels are typically formed from a liquid phase that has been thickened with other component(s). The liquid essentially forms a continuous phase with the thickening agent providing a porous scaffold to maintain the semisolid consistency.

The liquid phase can be a polar solvent, such as water, an alcohol or a non-polar solvent, and often solvents will be blended to provide the continuous phase. Numerous thickening agents are available, with the selection partly governed by the physicochemical properties of the permeant and by compatibility with the continuous phase. Simple gels can be prepared from water thickened with natural polymers such as carageenans or pectin, or by using refined or synthetic polymers such as hydroxypropylmethylcellulose or Carbopol. A range of grades and molecular weight fractions of these polymers is available, but they are typically used at between 1 and 5 % in the formulation.

In terms of drug delivery from gels, the continuous liquid phase allows free diffusion of molecules through the polymer scaffold, and hence release should be equivalent to that from a simple solution. Drug release from the polymer can be modified for very large molecules (macromolecules), for highly viscous gels and in particular, for drugs that bind to the polymer in the gel.

*Foams*

Foams are dispersions of gas in a liquid (liquid foams) or solid (solid foams) with the liquid or solid continuous phase containing the drugs and excipients (USP, 2022). While solid foams have found uses in pharmaceutical technology in oral dosage forms, liquid foams are used in topical applications to the skin (Hoc & Haznar-Garbacz, 2021).

Foams can be obtained from a variety of vehicles combined with a propellant (typically hydrocarbons like butane, propane, isobutane, and their mixtures or tetrafluoroethene) in a pressurized container. Vehicles can range from simple solutions or gels (for example, aqueous foams, hydroethanolic foams, oil



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foams) to emulsions (hydrophilic and lipophilic foams) and ointments (ointment and hydrophilic ointment foams), their attributes and applications have been discussed extensively by Tamarkin, (2016).

While most medicated foams are formed from the dispersion of a liquid propellant phase in an immiscible liquid or solid, some can be obtained via the mechanical incorporation of air with specialized packaging. The latter are frequently used in medicated hand or facewashes (for example BACTI-Foam) and other over the counter uses (for example Theraworx).

Foams have become an increasingly popular dosage form as they tend to be favored by patient to conventional semisolids (Kimball, et al., 2008), the relative easier spredability makes them suitable for extensive areas of application particularly for irritated skin (Feldman, et al., 2000). Their favorable organoleptic properties make foam a particularly befitting vehicle for drug with poor water solubility or stability which would require non aqueous vehicles such as ointments or oily solutions; as the foam format of these vehicles tend to have wider patient acceptability (Tamarkin, 2016). A marketed example is Enstilar foam for the treatment of psoriasis containing betamethasone and calcipotriene in a hydrocarbon ointment propelled with dimethyl ether and butane as the propellants.

Additionally, foams are an attractive option for lifecycle management if suitable formulations are achieved with minimal modifications to the original product, it may accelerate the regulatory approval pathway.

The complexity in manufacturing foams is largely dependent on the base vehicle utilized, however, specialized equipment is required to seal the canisters and fill them.

*Colloidal carriers*

Colloidal drug delivery systems have been playing an increasingly important role in topical delivery, mainly in academic research. Colloidal drug delivery systems can be an attractive option to formulate drug candidates with poor solubility, stability and or permeability through the skin.

Figure 16 summarises the classification of colloidal drug delivery systems along with some examples of systems that have been employed for topical and transdermal drug delivery. Detailed reviews exploring



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various aspects of these systems can be found in the scientific literature, the present section intends to provide a brief overview of some commonly used colloidal drug delivery systems in dermal and transdermal applications rather than a comprehensive review of the subject.

***Emulsion-based drug delivery systems***

Emulsion-based colloidal drug delivery systems used in topical drug delivery include multiple emulsions, microemulsions and nanoemulsions. Multiple emulsions are polydisperse systems of great complexity where oil in water and water in oil emulsions, stabilised by lipophilic and hydrophilic surfactants, simultaneously coexist (Yaqoob Khan et al., 2006). The use of multiple emulsions is somewhat limited as they can be complex to formulate and physically unstable. However, they can present an attractive option where incompatible drugs are required to be administered from the same vehicle, and to prolong drug release from the vehicle without increasing trans-dermal permeation (Laugel et al., 1998; Raynal et al., 1993; Singh et al., 2021).

Microemulsions and nanoemulsions are translucent/clear fine dispersions with mean droplet sizes of 100-400 nm and 1-100 nm, respectively. Whilst the former are considered thermodynamically stable, the latter (due to the higher free energy) are considered thermodynamically unstable and require higher levels of cosurfactant and energy to form (Cimino et al., 2021). Nevertheless, nanoemulsions are considered kinetically stable due to intense Brownian movement (Souto et al., 2022). Microemulsions allow the solubilisation of hydrophilic and lipophilic drugs, this solubility increase is not only dependent on the solubility of the drug in the different components but also on the organisation of the phases in the microemulsion which creates solubility regions. Drugs (for example azoles and 8-Methoxsalen) have demonstrated increased cutaneous absorption when applied in microemulsions compared to conventional vehicles (Baroli et al., 2000; Garg et al., 2020). The physical stability of microemulsions, along with their solubilisation capacity and favourable drug delivery, make them an attractive vehicle for dermal drug delivery. However, due to the relatively high levels of oils surfactants and co-surfactants required for the formation of microemulsions, careful consideration of the components should be given to avoid the



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potential for skin irritation This has led to the development of single surfactant (e.g lecithin) microemulsions which although better tolerated are only formed in narrow specific concentration ranges compromising their stability or the replacement of medium chain alcohol as cosurfactants in favour of those with improved tolerability e.g triglycerol diisostearate (Kreilgaard, 2002). Similarly to microemulsions, nanoemulsions have been used to increase the solubility and enhance topical delivery of a variety of drugs including antioxidants, non-steroidal anti-inflammatory drugs and antimicrobial amongst others (Wu et al., 2013). Their ability to accumulate in the follicular recesses of the skin have made them particularly appealing vehicles for the treatment of acne (Najafi-Taher & Amani, 2017). AMELUZ® (aminolevulinic acid hydrochloride) topical gel which is indicated for the treatment of actinic keratosis (in combination with photodynamic therapy) is an example of a marketed product which utilises nanoemulsion technology. Formulating as a nanoemulsion resulted in an improvement in the chemical stability of aminolevulinic acid (ALA) preventing head-to-head condensation, allowing a shelf life of 36 months (Schmitz et al., 2016). Furthermore, in-vitro model using eyelid skin developed by the same author, the nanoemulsion containing 10% ALA triggered significantly higher Protoporphyrin IX concentrations than a 20% compounded ALA formulation used in clinical setting, suggesting a potential increase in efficacy.

***Vesicles:***

Liposomes are essentially lipid vesicles containing an aqueous core and thus can contain hydrophilic molecules within their core or incorporate lipophilic molecules in their membrane. Diverse lipid molecules have been used to form liposomes, most commonly employed are phospholipids, with or without cholesterol. The lipid composition affects the properties of the liposome that are formed, so, for example, the addition of relatively small amounts of cholesterol tends to stabilise the membrane and hence the liposome would be more rigid than a non-cholesterol containing vesicle. Varied methods are available to produce liposomes, for example by depositing a thin film of the lipid(s) on the surface of a rotary evaporator flask and then rehydrating with water or buffer. The formed liposomes may contain one



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or more bilayers, and can be extruded to reduce their size and lamellarity, and drug can be incorporated during manufacture or loaded subsequently by diffusion into the vesicle. Liposomes are generally classified based on their preparation method, size, or lamellarity (Brown & Williams, 2019).

Studies on liposomal drug application to the skin suggest that the drug remains localized in the outer skin layers, i.e. the stratum corneum and possibly the viable epidermis. Limited drug penetration into deeper skin tissue or systemic circulation is observed with liposomal formulations compared to solutions. Various mechanisms can contribute to the enhanced uptake of drugs into the stratum corneum from liposomes. One mechanism involves the adsorption and fusion of liposomes with the skin surface whilst another involves the penetration of liposomes into the stratum corneum, followed by fusion with the lipids present in the stratum corneum. An alternative proposed mechanism involves liposomal targeting of the pilosebaceous units of the follicular route. The structure, composition, and size of liposomes will influence which of these mechanisms or combination of mechanisms are responsible for facilitating drug delivery. (Brown & Williams, 2019.

The first marketed topical liposomal formulation (Pevaryl Lipogel containing 1% Econazole) was approved in 1988 (Schaller et al., 1999). Reduced damage if the epidermal barrier and toxicological effects in uninfected reconstructed human epidermis has been reported for the liposomal gel compared to a commercial econazole cream. Moreover, *candida albicans* specific damage was reduced in the model of human cutaneous candidiasis to a greater extent for the liposomal formulation, highlighting the promise of this drug delivery vehicle. (Schaller, et al. 1999).

Niosomes are analogous to liposomes but are composed of non-ionic surfactants rather than phospholipids. Again, niosome properties can be modified by incorporation of excipients, such as cholesterol, into the membrane and can have one or more lipid bilayers encapsulating an aqueous core. Sucrose ester surfactants and polyoxyethylene alkyl ether surfactants are amongst the many materials that have been used to produced niosomes. In comparison to ionic surfactants, nonionic surfactants tend to have relatively low toxicities and are less damaging to skin and other membranes. Non-ionic surfactants



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are readily available and can be sourced at the quality and levels required for clinical manufacture. (Brown & Williams, 2019).

As with phospholipid liposomes, the potential mechanisms by which niosomes can improve transdermal drug delivery across of the stratum corneum are varied: 1) Disruption of the stratum corneum lipids by the surfactants, 2) Vesicle penetration/fusion into the outer layers of the stratum corneum, and 3) Increase of the thermodynamic activity of the drug or formation additional barrier layer on the tissue surface that could inhibit drug flux following the vesicle collapse on the skin surface. The physicochemical properties of the permeant, vehicle, and surfactants employed will govern the mechanism or combination of mechanisms responsible for the modulation in drug penetration. (Brown & Williams, 2019).

***Particulate based drug delivery systems***

Nanoparticles can be defined as "solid colloidal particles ranging in size from 1–1000 nm, consisting of macromolecular materials that can be used as drug carriers in/to which the active is either dissolved, entrapped or encapsulated or sorbed" (Kreuter, 1994). Progress in exploiting nanosystems or nanoparticles for delivering drugs to and through the skin has been slow, though literature report are increasing. Examples of nano- and micro-scale particles that have been explored for topical application include dendrimers, quantum dots, metallic nanoparticles, nanoemulsions, carbon nanotubes, lipid-based colloidal nanosystems (e.g. solid lipid nanoparticles), and flexible nanovesicles (M. B. Brown & Williams, 2019).

The mechanisms underlying the ability of nanoparticles to facilitate drug penetration into and through the skin are still questioned. However, several properties of nanoparticles, including their size, shape, charge, surface characteristics, and aggregation state, may contribute to improved drug delivery across the stratum corneum or uptake by hair follicles. Additionally, the formulation vehicle and the skin's disease state at the site of application may also play a role.

Among nanoparticle systems, lipid nanoparticles (LNs) have garnered significant attention. LNs are colloidal dispersions consisting of a dispersed lipid phase stabilized by an emulsifier or emulsifier



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system, with a size ranging from 10 nm to 10 μm. When solid lipids are used, they are referred to as solid lipid nanoparticles (SLNs), while a mixture of liquid lipids leads to the formation of nanolipid carriers (NLCs). Both of these colloidal systems can enhance drug solubility, increase drug bioavailability, protect drugs from degradation caused by light or oxygen, and offer a means to achieve improved drug delivery. However, due to the incorporation of a fluid phase, NLCs possess a spatial structure that allows for greater drug loading and protection compared to SLNs. NLCs and SLNs are reported to enhance drug permeation through a similar mechanism, primarily through occlusion and interaction with skin lipids. Nevertheless, the presence of liquid lipids in NLCs enables increased solubilization, leading to enhanced drug release and penetration into the stratum corneum, resulting in superior delivery compared to SLNs.

Both NLCs and SLNs can be obtained by scalable manufacturing process like high-pressure homogenization, microemulsion technologies, and ultrasonication. Additionally, they are biocompatible and non-toxic and can be formulated using Generally Recognized as Safe (GRAS) or Inactive Ingredients Database (IID) excipients. However, despite their potential as platforms for reformulating established drugs to extend their product lifecycle, the costs associated with the ingredients and manufacturing of these systems in comparison to more conventional dosage forms may explain why the number of marketed products is still low.

***Micellar based drug delivery systems:***

Micelle drug delivery systems encompass various types such as polymeric micelles, reverse micelles, and mixed micelles. Polymeric micelles are formed by the self-assembly from copolymers consisting of both hydrophilic and hydrophobic monomer units (block copolymer) (Abolmaali et al., 2017; Deshmukh et al., 2017) Polymeric micelles have gained popularity in the drug delivery field their CMC is several 1000-fold lower than for classic micelles, in water they form spherical micelles with a hydrophobic core, allowing the solubilisation of hydrophobic (Abolmaali et al., 2017; Deshmukh et al., 2017). The mechanism of permeation of these carriers in the skin and their fate has been investigated and remains controversial (Makhmalzade & Chavoshy, 2018). These carriers have been investigated in the literature



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for the treatment of conditions like acne vulgaris, psoriasis, and fungal infections amongst others with higher bioavailability, improved stability, controlled drug release and higher efficacy (Parra et al., 2021).

*iii. Solid formulations*

Solid formulations are rarely used to deliver materials to and through the stratum corneum since the drug must undergo a dissolution step prior to permeation. Without the co-application of a solvent, TEWL or skin exudates are generally unable to dissolve the applied powder. Topical sprays are available for depositing powders onto the skin surface, but these generally include a volatile solvent that dissolves some of the powder prior to evaporation (with consequent solute supersaturation and hence elevated drug delivery). Dusting powders are used to reduce friction between skin surfaces and antiseptic dusting powders are available.

*H. Formulation characterisation.*

Whilst in-depth characterization of formulations is usually performed once a lead has been selected, to support the development of these formulations and monitor their stability at ambient and accelerated temperatures, formulations undergo physicochemical characterization. Typical parameters monitored for topical semisolids are discussed below.

*i. Macroscopic/ microscopic appearance and odour*

Product visual appearance is characterized during initial development and to monitor stability. Colour, flowability, application and appearance (e.g., phase separation of semisolid formulations on storage) should be monitored. microscopic appearance can give an indication of drug precipitation or changes in the droplet size of emulsions which are indications of poor physical stability. typically, optical microscopy is used, however, more advanced techniques such as raman microscopy which can help identify precipitates or chemical distribution within the sample can be employed. Development of odour



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overtime can be an indication of instability of certain excipients (e.g. triglycerides that are prone to hydrolysis or microbial contamination.

*ii. Apparent pH*

The pH of the product influences the solubility and stability of the drug in the formulation and can impact its potential to cause skin irritation. as such, during development the pH is adjusted within a narrow range and is monitored during stability. changes in pH can be indicative of chemical degradation (for example hydrolysis) and can lead to changes in the viscosity/ or rheology of formulations (for example in gels containing carbomers).

*iii. Accelerated physical stability.*

For multiphase systems such as emulsion and suspensions, physical stability is paramount. During development formulations can be screened and optimized based on their ability to withstand centrifugal forces in a simple centrifuge. More advanced dispersion analysers such as the LUMiSizer® and LumiFuge® are useful tools which monitor the changes in the transmission of light of samples, whilst they undergo centrifugation. This allows the calculation of an instability index and can aid in predictions of shelf-life (LUM, 2022).

*iv. Viscosity/ Rheology*

The rheology of semi-solid formulations is usually a key quality attribute as it can affect the physical stability, manufacturability, retention on the skin and the patient experience. Given that most topical formulations display non-Newtonian behaviour, absolute rheometers are used to understand the rheological profile of formulations. To support submissions, the EMA requires the following: Flow curve (viscosity vs. shear rate or shear stress vs. shear rate, yield stress, creep testing and characterization of the linear viscoelastic region under various shear stress and frequency (European Medicines Agency, 2018). Whilst some of these tests can be used to support early development, full rheological characterisation is



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usually performed once a lead candidate has been selected or during formulation optimization. Once the rheological behaviour has been characterized is it possible that simpler tests like the use of a rotational viscometer can be developed and used to monitor product quality in a Quality control setting.

*I. Formulation Short-term stability and selection*

Once prototype formulations are developed based on the preformulation data and target product profile, their chemical and physical stability is assessed over time (usually up to 4 weeks but might require longer timepoints for very stable drugs) under ambient and accelerated conditions. The storage conditions (typically 25 and 40 °C) will vary depending on the climatic zone of the target registration territory (as per Q1 A (R2) Stability Testing of New Drug Substances and Products), and the stability of the drug substance for example for biologics or very unstable molecules refrigerated conditions are often assessed. Assay and related substances of the drug in the formulations along with the macroscopic and microscopic appearance and apparent pH are assessed as a minimum.

The selection of a lead formulation will consider formulation stability, drug release, permeation testing, cosmetic acceptability and on some occasions the use of preclinical models. Suboptimal performance in any of these assessments may lead to one or various rounds of formulation optimization, several approaches can be used to optimize a product during the development stage; examples include using factorial design, single-factor approach, or a systematic approach.

Once all such assessments and formulation optimisation has been completed, the aim is to select a lead and preferably a backup formulation with greater chances of success in the clinic to take forward to process development.

*J. Device/Packaging*

Devices and packaging for topical products come in many different formats from a simple tube right through to metered dose devices to ensure that the patient is receiving the required dose upon application. Considerations during development vary depending on the complexity of the device or packaging and



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whether the final product is considered a drug and device combination. The type of formulation/device and/or packaging will determine how early in the development process packaging is assessed. On many occasions initial prototype formulations will be assessed in glassware however, if the device is critical in delivering the product it should be included in the development work from the outset.

The simplest packaging types for topical formulations are tubes or jars although these are not considered drug/device combinations the packaging material compatibility with both the drug and the excipients present in the product both on storage and in-use, protection from the environment and mechanical hazards as well as size/volume, applicator and outer packaging appearance should be considered.

While glass vials/jars are relatively inert and resistant to chemical substances with a reduced risk of leaching it is important to select the correct type of glass depending on the drug degradation pathway, excipients present and pH of the product. For example, while general purpose soda glass may be sufficient for non-aqueous systems if aqueous systems are stored it may result in an increase in pH for the product. However, because glass vials can break easily and are heavier for transportation, tubes are more prevalently used for topical pharmaceutical products.

When selecting a tube both the outer material (type of plastic or aluminum/tin) and the inner lacquer/material need to be considered. The outer material of a metal tube provides protection of the metal from the environment for example from corrosion while the inner material protects the product from the metal and vice-versa any excipients which may leach or corrode the metal. Typically, inner materials are resins with different cross-linking agents. Plastic tubes are also widely used and are often preferred by patients as they are more resistant to wrinkles and cracking as the product fill volume decreases. Different plastics provide different levels of resistance for example high density polyethylene (HDPE) has greater chemical resistance compared with low-density polyethylene (LDPE) but LDPE tubes are more flexible. Similarly, HDPE has less moisture and oxygen permeability compared with LDPE however the permeability of plastic tubes to environmental factors is much greater when compared



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with metal tubes. Aluminium barrier tubes offer the best of both worlds as they give the appearance of a plastic tube with the barrier properties of a metal tube.

Other more advanced packaging types are dependent on the product and application area are also available. Pump applicators more commonly used in cosmetics can be used to apply a known amount of formulation taking out the complication for a patient of knowing how much to dose or the use of dosing cards. These can either be a measured dose which delivers an approximate amount of formulation with the required number of pumps or a metered dose which delivers an exact amount of product. Many of these more complex packaging types constitute a drug device combination rather than container closure system and therefore developers need to be aware of the additional requirements and evaluations needed to both confirm accuracy of the device but also consider human factor studies. Airless pumps have the added advantage of metered dosing with a lower chance of both contamination and drug degradation by air as the product inside does not come into contact with the air. Airless tubes are also available with similar benefits to preservation and chemical stability.

Aerosol packaging can be used for dispensing propellant-based sprays or liquid sprays such as spray on films. They can be developed with both metered dose or continuous dose valves and the canisters used are typically aluminium or stainless steel with internal lacquers/anodized or plasma treatment to protect the product from the container.

Other packaging available are sachet/pouches, dosing syringes and tubes/bottles with different applications for example roll-on, brushes, or droppers.

*K. Process development and scale up*

Once the lead formulation has been optimised and selected for studies that require larger scale manufacture i.e. animal safety studies or human clinical studies the most risk adverse approach is to optimise the manufacturing process using process development/scale-up activities. The aim of process development is to establish optimal process parameters/ conditions to manufacture the pharmaceutical



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product to meet quality and regulatory standards robustly and reproducibly both initially and during scale-up. Process development should give an understanding of the following:

- Critical Processing Parameters (CPP) and their impact on the Critical Quality Attributes (CQA) of the finished product;
- Critical Material Attributes (CMA) influence on the CPP and CQA, for example how do materials of differing specifications/grades affect product quality;
- How robust the process is and within what limits (Design Space);

The level of process development required is dependent on the stage of development which can range from early phase development which is recommended to have sufficient understanding of the process so as not to risk manufacture and supply of clinical trial material right through to full process validation which is a requirement by the regulators.

The tools and approaches during process development are described below:

*i. Process risk assessment*

A rolling document prepared prior to experimental work and updated throughout development used to assess the impact of identified risks:

- One example of such an approach is an FMEA (Failure Modes and Effects Analysis) where initially identified generic risks become more defined with mitigation strategies as knowledge is acquired. Some considerations are:
  - Finished product CQAs;
  - Formulation composition and potential CMAs;
  - Preliminary CPP's based on prior formulation experience, composition and complexity and CQA's;
  - Hazards of the product;

The different stages of the risk assessment are:



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i. Risk identification - what might go wrong and may negatively impact a finished product CQA (failure mode);

ii. Risk analysis – what is the probability of occurrence, severity and likelihood of detection of the risk. For example, in an FMEA, this score is referred to as the risk priority number (RPN;

iii. Risk evaluation – high, medium and low risk and is such a level of risk deemed to be acceptable or is immediate action required;

iv. Risk control, which includes risk reduction (the process of reducing the risk to an acceptable level) and risk acceptance;

v. Risk communication - sharing of information.

Other tools used to aid risk assessment preparation are:

- A manufacturing process flow chart which indicates the material flow, processes, in-process checks and various processing stages.

Input, Process, Output (IPO) assessment examples are:

- Input – drug
- Process – stirring of drug in excipient mixture
- Output – Clear solution obtained
- Failure mode – incomplete drug dissolution causing inhomogeneity in the product
- Input material specifications – the specifications of excipients which can impact product quality and how closely controlled are they

The product risk assessment process is used to identify the CQAs and potential CPPs. Most of the CQAs are eventually listed on the product release and shelf-life specification and their limits established during process development and stability studies. Some potential CPP's and CQAs include (but are not limited to):

CPP's



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- Dissolution/hydration/solvation times
- Homogenisation time/speed
- Cooling rate
- Mixing speed and times
- Depending on scale paddle size, orientation and/or homogenization screens
- Filling temperature

CQA's

- Drug product appearance
- Microscopic appearance
- pH/ apparent pH
- Apparent viscosity
- Full rheological testing
- Drug assay and content uniformity/uniformity in container.
- Drug related substances
- Antioxidant/preservative or other key excipients assay (where applicable).
- *In vitro* performance of the drug product including drug release/dissolution and drug skin penetration/permeation testing.
- Microbial quality

If any of the above CQA's (or outputs) fail it could indicate either chemical, microbiological or physical stability concerns. The CQA's can be impacted by the CPP's and therefore it is critical to ensure that a manufacturing process can reproducibly and robustly result in a product that falls within specifications to maintain Safety, quality and efficacy.



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*ii. Manufacturing process and considerations*

In order to reproducibly manufacture a pharmaceutical product a set of established and eventually validated processes are required. Topical formulations are typically prepared using processes which include dissolution, hydration/solvation and gelation, emulsification. The process should be designed taking into consideration the drug and excipients (physico-chemical properties such as degradation pathways, solubility and melting point). For example, an emulsion process may require heating and if the drug or some excipients degrade at the temperatures required to melt and incorporate the oil and water phase they may need to be added at a later stage and the process designed to accommodate this.

*Manufacturing equipment*

Equipment selection is dependent on a number of factors including:

- Batch size
- Formulation type
- Formulation characteristics such as viscosity
- Manufacturing process steps

*Batch size and equipment capacity/energy*

When performing process development or any manufacturing the operational ranges of the manufacturing equipment should be considered in addition to the energy required for the process (dependent on batch size and physical properties of the product throughout the process).

Manufacturing equipment operation ranges dictate the appropriate batch size for the equipment examples are; for a 10 Kg batch a 12-15 L vessel may be appropriate however, much larger vessels (e.g. 300 Kg) may have a lowest operating range of around 85 Kg. If a product is manufactured below the lower operating range this could lead to inconsistency of the product (due to improper product flow) impacting product CQA's. Similarly, at early stage development where independent vessels and paddles are quite



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often used the mixing paddles should be of the correct dimensions for the vessel used to ensure product consistency, such issues are not typically encountered at commercial scale as the vessels will be fitted with purpose built paddles for material flow.

Product viscosity can impact the power draw required by the equipment and mathematical equations such as the Reynolds equation used to establish the ratio of the inertial force against the viscous force to determine whether the flow is laminar or turbulent. Such an approach can also be used to evaluate the magnitude of shear thinning or thickening which might occur at high shear rates (for example during homogenization) and such modelling can be used to predict optimal processing methods to achieve the required CQAs.

*Key processing steps involved in manufacturing topical formulations.*

When initiating scale-up operations from a laboratory scale right through to commercial scale it is important to consider each process of product manufacture, early assessment of the different steps will save time and effort at the later stage. As such important processing steps and specific points to consider are:

- API dissolution
- Drug solubility in excipient/excipient blends
- Drug particle size and polymorphic form
- Order of addition
- Mixing/Blending
- Vessel geometry
- Mixing mechanism (paddle shape and size) – dependent on product type and microstructure
- Motor power
- Initiation and completion point in the process
- Homogenization



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- Speed and time
- Initiation temperature
- Number of passes when using an inline homogenizer
- Heating/cooling rates and initiation and completion points in the process
- Filling temperature

The requirement for each of the step above is dependent on the formulation type and composition examples of which are shown below:

- Droplet size and distribution can be considered a CQA for a cream/emulsion formulation. This can be largely attributed to the surfactant used and its concentration but it is also dependent upon the viscosity ratio of the continuous phase to the dispersed phase and can be altered by the application of high shear using a homogenizer (ultrasonic disruption, high pressure or mechanical means) to uniformly reduce the droplet size of the dispersed phase resulting in a more stable emulsion.
- Heating and cooling rates.
- Some formulations (such as creams and ointments) contain solid components in formulations which require heating in order to melt prior to mixing with liquid based excipients. Slower heating rates and therefore longer heating times can result in volatile loss due to evaporation. Heating too fast can result in degradation of ingredients at the contact point of the heat exchange.
- During cooling an increase in product viscosity occurs as the excipient mixture begins to solidify.
- The rate at which this cooling occurs influences the product viscosity and rheological behaviour and ultimately the formulation microstructure.
- Polymer hydration or solvation.



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- Hydration or solvation of the gelling agent is important for the formation of a gel and to build the viscosity of a solution.
- Different type of gelling agents will hydrate and gel differently. For example, carbomer hydration and gelation is pH dependent (where the carboxyl group is activated generally above pH 5 by neutralization to form cross links and gel) but that of HEC (hydroxyl ethyl cellulose) is pH independent.

*Designing the optimum process*

There are various considerations when designing an optimized process which can impact chemical stability of the drug, physical stability of the formulation and speed and efficiency of the process. A number of these have been summarized below:

- Protection of API during manufacturing:
    - Consideration of the drug degradation pathway
    - Filtered light or closed manufacturing systems with light sensitive compounds.
    - Inert gas blankets for compounds prone to oxidation
- Order of addition:
    - To aid in the speed of dissolution of solid components of the formulation
    - To aid physical stability (surfactants in cream formulations)
    - Pre-dispersion of gelling agents prior to hydration or solvation
    - Assessment of processing steps and observations – in-process controls
    - The minimum steps necessary to achieve a robust product is preferred
- Definition of expectations during and at the completion of each processing step for example
    - Visual homogeneity upon mixing completion
    - Complete drug dissolution by visual assessment or microscopy
    - Defined droplet size for the formulation dispersed phase post homogenization



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The expectations can be used to determine proposed durations of the different steps and in-process controls (IPCs) can be agreed.

- IPCs - critical within the process to ensure the product has not deviated from expectations examples of which are:
    - Drug content uniformity at various points of the bulk prior to filling.
    - pH check to assess uniformity of any pH adjustment steps.
    - Formulation viscosity assessment to confirm within range.
    - IPCs are typically a sub-set of the final product specifications.

*iii. Process optimization, Design of Experiments (DoE) and the Design Space (DS) and Stability*

*CPPs and CQAs:*

Although CPPs are listed earlier it is important to revisit during the process optimization stage to take into account any learnings from earlier in the development pathway and translate these learnings to the next scale of manufacture. Commonly evaluated CPPs for topical formulations are provided in Table 6 with an explanation of why they are considered critical.



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*Design of Experiments and the Design Space*

Design of Experiment (DoE) is a statistical tool that can help reduce the number of experiments required to achieve at an optimal process parameter range. In the past, process optimization experiments would be performed by changing one factor at a time (OFAT). DOE efficiently reduces the number of experiments required to generate the optimal solution and can also be used to establish if there are any factors that have synergistic effects. Selecting the right numeric factors, with the necessary level of sensitivity to reflect changes in the process (CPP and the quality characteristic measured or response), for a DOE to optimise the process is critical. There is also a balance between the number of factors to be investigated and the risk, cost and time of the process optimization. Sufficient understanding the optimal process parameter ranges will also help in defining the Design Space (DS) or edge of failure of the process. Confirmatory batches prior to any GMP or commercial manufacture within the design space by way of technical batches can be manufactured to give confidence in the process.

*Stability studies*

It is important to monitor the stability of the product, both in terms of intermediate stages throughout the manufacture (hold time studies) and of the finished, filled product to ensure that there are no chemical or physical changes when increasing the scale of manufacture. Stability studies are performed in line with the ICH guidelines (ICH Q1A(R2)) and the stability specification should take into account all CQAs and any stability data generated to date.



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## VI. PERFORMANCE TESTING: REDUCING THE RISK OF CLINICAL FAILURE USING *IN VITRO*/*EX VIVO*/*IN VIVO* PERFORMANCE TESTING MODELS

One of the biggest challenges for topical formulations is overcoming a protective barrier that serves to protect the human body but also severely limits drug delivery into and through the skin; thereby restricting the therapeutic targets that can be accessed topically. Relatively few molecules have the appropriate physicochemical and therapeutic properties to allow topical and transdermal administration. Topical and transdermal products are unique to other drug products where the vehicle is a critical part of the final drug product and must interface with the skin surface to achieve clinical efficacy. The ability to measure drug release, delivery, and activity from the drug product in a reproducible and reliable manner is critical. For obvious reasons, it would be unethical and cost prohibitive to use human volunteers to monitor drug delivery and/or local activity *in vivo* during formulation development and optimization. As such, there is a need for *in vitro*/*ex vivo* product performance tests that can be used to assess release, delivery, and activity throughout the development process. Performance tests refers to the preclinical assays used to assess topical formulations and is an integral part of any formulation development program and can be used strategically throughout the development process to mitigate development risks and failures due to inadequate product performance.

Performance tests for topical and transdermals are *in vitro* or *ex vivo* assays to measure drug release (dissolution), drug delivery (pharmacokinetics), and drug activity (pharmacodynamics). *In vitro* release testing (IVRT) is essentially a dissolution assay measuring rate of release for topicals involves the use of a synthetic membrane used as a support for semisolid formulations or dissolution chambers for transdermal patches. *In vitro* penetration/permeation testing (IVPT) uses skin (ideally human) to measure the cutaneous pharmacokinetics or drug delivery into and through the skin. These IVPT studies are not able to confirm the bioavailability as this assay typically uses excised skin that has been stored frozen, therefore is not biologically viable. The function of an IVPT is to assess passive



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delivery through the non-living barrier (stratum corneum). To confirm bioavailability and target engagement, skin can be kept in culture to maintain biological function up to 4 weeks therefore allowing delivery and activity to be assessed establishing a local pharmacokinetic/pharmacodynamic (PK/PD) relationship.

Fundamental guiding principles should be used when designing experimental protocols for dermal drug delivery research; the literature contains multiple examples of inappropriate experimental designs that can provide misleading data which can be highly problematic if mistranslated to a clinical situation. Thus, regulatory, and advisory bodies produce guidelines and recommendations for methods to use when evaluating topical and transdermal drug delivery which are continuously being updated and the current guidance/recommendations for IVRT, IVPT, and the use of PD skin disease models should always be consulted.

### *A. IVRT*

*In vitro* release testing (IVRT) experiments are used to assess the drug release from a formulation across a non-rate limiting synthetic membrane. The resulting release profiles are not only correlated with the thermodynamic activity (physicochemical properties) of the drug but also the composition and manufacturing process of the formulation (Langley et al., 2019). IVRT experiments are often conducted using VDCs, also known as Franz diffusion cells which typically consist of a donor chamber separated from a receptor chamber by a synthetic membrane which is clamped in between the two chambers (Franz et al 1975). The receptor chamber contains receptor solution (RS), and the receptor chamber portion of the VDC is submerged in a water bath or heat block to ensure the membrane surface temperature is maintained at 32 °C to mimic *in vivo* skin temperature. An infinite dose of the topical formulation is applied to the membrane surface in the donor chamber, and the top of the donor chamber is occluded. Over time, the drug diffuses across the formulation and through the membrane into the RS contained within the receptor chamber. Samples of the receptor solution are



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manually collected over time, the concentration of drug is quantified, and the release profiles can be generated.

*i. Synthetic Membrane*

IVRT is generally a reliable and precise tool to assess the release of drug from a formulation. One of the reasons for this is due to the use of a synthetic membrane. Unlike other performance tests, such as *in vitro* permeation testing (IVPT), IVRT utilizes a non-rate limiting synthetic membrane instead of skin which decreases the variability in which skin can introduce. It should be noted that IVRT is not meant to mimic that of *in vivo* studies; however, the drug release profiles resulting from the IVRT experiments can provide valuable information about the performance and quality (e.g. thermodynamic effects of drug particle size and dissolution, drug solubility, partition coefficient, and pH and drug-excipient interactions) of a formulation prior to ever introducing the formulation to skin.

Commonly used membranes include mixed cellulose esters, nylon, polysulfone, and polyethersulfone and generally consist of a 0.45 μm pore size. Using alternate membranes, such as dialysis membranes, may be suitable; however, the aforementioned membranes are readily available, easy to use, and are compatible with most drugs. In addition, membranes with different pore sizes or molecular weight cut offs may be useful for drugs that have a particularly small or large molecular weight.

Regardless of the membranes selected for assessment, compatibility of the membrane with the drug should be evaluated to ensure that the release of the drug into the RS is not limited by drug binding to the membrane itself. Additionally, the back diffusion of the RS into the donor chamber should be evaluated. It should be noted that back diffusion cannot be eliminated, only minimized. Back diffusion can be minimized by reducing the amount of organic and/or surfactant content in the RS and/or saturating the membrane in the RS prior to the experiment. The back diffusion assessment is often subjective; however, in addition to observations, measuring the movement of the RS in the sampling arm overtime can aid in determining whether back diffusion will significantly impact drug release.



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*ii. Diffusion Cells*

The US Pharmacopeia (USP) outlines multiple apparatuses that can be used for drug release/dissolution testing for different types of dosage forms. For transdermal dosage forms, these methods often include USP Apparatus 5 (Paddle over Disk Method), Apparatus 6 (Rotating Cylinder Method), and Apparatus 7 (Reciprocating Holder Method) (USPC 2021). However, the most commonly used method for topical dosage forms is *in vitro* (drug) release testing (IVRT) in vertical diffusion cells (VDCs). The VDC is often used in IVRT experiments; although, alternative cell designs, such as immersion cells, can be used as described in USP-NF ⟨1724⟩ Semisolid Drug Products - Performance Tests. (USP-NF, 2022).

As previously described, the VDC has two chambers: the donor chamber which contains the dosing surface area (synthetic membrane) and the receiver chamber which contains the receptor solution and stirring bar in which the released drug is collected. VDCs are often hand-blown glass, and the orifice of the donor chamber (dosing area) is ~2 $cm^2$, and volume of the receiver chamber (receptor solution) is ~5 – 11 mL. Both the orifice of the donor chamber and the volume of the receiver chamber should be appropriately qualified prior to use.

*iii. Analytical Method*

The most commonly used analytical method for IVRT is ultra performance liquid chromatography (UPLC) or high-performance liquid chromatography (HPLC) coupled with an ultraviolet (UV) detector. As the resulting drug concentrations are typically in the microgram per millilitre (µg/mL) range, the sensitivity of (U)HPLC-UV is often sufficient. This methodology is also generally compatible with the types of RSs that are often used in IVRT experiments (≥ 30% organic combined with ≤ 70% aqueous). Alternate detectors, such as the diode array detector (DAD) or fluorescence detector (FLD), may be utilized or required for detection of certain drugs.



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*iv. Experimental Design*

As described, IVRT experiments often utilize VDCs where the formulation is dosed on a synthetic membrane, the drug from the formulation is released into the RS, and the collected RS is evaluated *via* analytical methodology to quantify the amount of drug released. Under a set of specified method conditions and parameters, IVRT is a valuable tool to assess the release of a drug from a formulation and can provide meaningful information about the drug and/or formulation. However, if the method parameters and/or conditions vary over the course of a study, the drug release rates and/or profile could change resulting in less reliable or profound results. As such, careful consideration should be taken when selecting a synthetic membrane, RS, and other method parameters for use in IVRT. Regulatory agencies, such as the Food and Drug Administration (FDA) and European Medicines Agency (EMA), have detailed criteria and recommendations that should be incorporated when developing an IVRT method for regulatory submissions but can also be utilized as a general guideline in research and development. The following method parameters and conditions should be assessed during IVRT method development:

*Dose*

Steady-state release of the drug into the RS is needed in order to calculate the drug release rates; therefore, an infinite dose of formulation should be utilized in IVRT studies so that there is an excess supply of drug throughout the duration of the experiment. Steady-state release is characterized by the linear portion ($r^2 \geq 0.97$ recommended by the FDA) of the drug release profile when plotted as the amount of drug released versus the square root of time. Ideally, the amount of dose depletion should not exceed 30%; however, there are instances in which steady-state release can be maintained beyond this depletion level. (CDERa_, 2022)



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The amount of formulation that qualifies as infinite is not explicitly defined as it can vary among formulations; however, dose amounts as low as 300 mg and high as 1 g have been used to achieve steady-state drug release.

In addition to the dose amount, the dose application procedure should be assessed in IVRT studies. Dose application procedures generally involve simple techniques and equipment but can greatly impact the release rate if not taken into consideration. One of the most common applicators is the positive displacement pipette. Other applicators include disposable harvester (commonly used with formulations that are susceptible to shear stress), spatula, and syringe (e.g. insulin syringe), though this list is not exhaustive. Alternatively, an applicator may not be necessary, and the formulation may be dosed directly onto the membrane. The type of applicator (or lack thereof) chosen often depends on the formulation. Regardless of the applicator chosen, the formulation should be dosed in a manner to ensure that the membrane is uniformly and entirely covered, and that introduction of air pockets is minimized.

*Receptor Solution*

The most commonly used receptor solutions are a combination of organic and aqueous components. The organic component is usually ≥ 30% of the solution and is often ethanol, acetonitrile, isopropanol etc. In addition to back diffusion (discussed in a previous section), the stability and solubility of the drug in receptor solution should be assessed.

As drug release could be limited due to the solubility of the drug in the receptor solution, ensuring the drug is sufficiently soluble is a crucial step in IVRT method development. Ideally, the solubility of a drug should be an order of magnitude higher than the highest concentration of drug quantified in the receptor solution. Ultimately, the choice of receptor solution is dependent on the physiochemical properties of the drug, though the effect on other components of the study should be considered. Receptor solutions containing higher amounts of organic content (≥ 50%) may be necessary if the



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drug has low solubility in aqueous solutions. If this is the case, precautions may need to be taken to ensure the receptor solution does not evaporate (i.e. occluding the sampling arm of a VDC) over the course of the study. In addition, a higher organic content could result in significant back diffusion during the IVRT experiment and/or chromatography issues during sample analysis; however, organic contents > 50% have been implemented without these issues.

In addition to drug and receptor solution compatibility, stirring rate should also be assessed. Stir rates of 400 – 600 RPM are often utilized in IVRT studies, though lower or higher stir rates may be deemed appropriate. The stir rate should ensure a uniformly mixed receptor solution without creating a vortex throughout the duration of the study.

*System Equilibration*

Once the VDCs are assembled with the selected membrane, receptor solution, and stir bar, they should be submerged in a water bath or heat block and allowed to equilibrate (usually ~30 min) prior to the start of the IVRT experiment. During this time, the heat block/water bath temperature can be adjusted to ensure the membrane maintains a consistent temperature over the course of the study. The membrane should be inspected to ensure that there is minimal back diffusion and no changes (e.g. no swelling or wrinkling) in membrane appearance. In addition, the receptor solution should not contain any air bubbles, especially directly beneath the membrane. The membrane and receptor solution should also be evaluated throughout the duration of the study.

*Experimental Length and Sampling*

The study duration should be sufficient to capture the steady-state drug release from a formulation. During IVRT method development, the initial IVRT study may include timepoints up to 24 hrs. to obtain more information on the release profile. Generally, there will be a lag in drug release, followed by steady-state release, and finally the drug release will often plateau. It could then be determined if there is a lag phase and/or plateau phase in drug release and at what timepoints these phases occur, if



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applicable. More importantly, it could aid in determining the timepoints in which steady-state drug release occurs. In order to calculate the drug release rate, steady-state release will often include timepoints beyond 1 hr and up to 4 – 6 hrs.

For VDCs, partial sampling and replacement (sampling a set amount of receptor solution and replacing with fresh receptor solution) of the receptor solution is quite common. Sampling often occurs with a syringe or pipette, and sampling should occur within ± 15 min. or ± 2% of each timepoint. The order and timing in which each VDC is sampled should also be synchronized with that of dosing.

*Number of Replicates*

The number of replicates can vary depending on the type of IVRT study being conducted. Replicates of 6 or 12 per formulation or test group are the most common. However, during method development when various parameters are being assessed, it may be more appropriate to conduct studies where replicates of 3 – 4 per parameter are tested.

*Membrane Temperature*

While the IVRT method developed should be robust to small changes in membrane temperature, significant changes in temperature can greatly impact the drug release rate. As mentioned previously, IVRT is not meant to mimic that of *in vivo* studies; however, membrane temperature should resemble that of the surface in which the formulation is intended to be applied. For formulations that are meant to be applied to the top of the skin, a membrane temperature of 32 ± 1°C should be maintained throughout the duration of the experiment. For formulations that are meant to be applied internally (e.g. rectally or vaginally), a membrane temperature of 37 ± 1°C should be maintained (USP-NF, 2022).



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The membrane temperature can be monitored by measuring the membrane temperature from a non-dosed VDC that is submersed in the same water bath/heat block as the dosed VDCs. This is often measured with an infrared thermometer or camera.

*Data Reporting*

Once the drug concentration is quantified from each sample at each timepoint utilizing a liquid chromatography detection method (e.g. HPLC-UV), the percentage of the applied dose (%) of drug released over time can also be plotted and calculated. It can also be utilized to generate release rate profiles by plotting the mean cumulative amount of drug released per unit area (xmol/$cm^2$) versus the square root of time ($\sqrt{t}$) which can then be used to calculate the drug release rate (slope; xmol/$cm^2/\sqrt{t}$). The linearity of the timepoints in which the drug release rate was calculated should also be determined ($r^2 \geq 0.97$ recommended by the FDA). The release rates and/or percent applied dose can then be compared among formulations tested in the IVRT study.

For regulated IVRT studies in which bioequivalence or sameness is determined, the specific guidance (EMA and/or FDA) should be consulted as each regulatory agency has their own set of requirements and acceptance criteria.

*v. Regulated IVRT*

IVRT can be utilized from a research and development standpoint to optimize formulations for drug release. Additionally, it can also be used in regulatory submissions. As with classical tablet dissolution testing, *in vitro* release has been employed to demonstrate "'sameness"' for specific scale-up and post-approval changes to semi-solid products (CDER, 1997), and it is generally agreed that *in vitro* release testing is useful when characterizing some performance characteristics of a final topical dosage form, (i.e., semi-solids such as creams, gels, and ointments). Changes in the characteristics of a drug product (eg viscosity or droplet size) or to the thermodynamic activity of the drug(s) in a changed formulation are expected to lead to differences in drug release. Further, IVRT methods with greater



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levels of validation are increasingly used for routine quality control of product performance throughout quality by design (QbD), process development, and scale- up, and also for shelf-life studies. Recently, the FDA have produced a series of draft guidance documents with greater detail on the validation of IVRT methods, alongside guidance on *in vitro* and *in vivo* bioequivalence studies, to demonstrate equivalence between a branded product and a generic (Q1–-Q3) copy without the need for clinical studies.

*B. IVPT*

Given the difficulties, costs, and time associated with *in vivo* experimentation, most topical and transdermal formulators use *ex vivo* methodology in order to optimize and compare formulations. It is widely accepted that the use of *ex vivo* human skin in conjunction with a diffusion cell (static or flow-through) can correlate to human clinical studies (Franz et al., 2009; Lehman et al., 2011; Mohammed et al., 2014) The *in vitro* penetration/permeation test (IVPT) is a well-validated tool for the study of percutaneous absorption and the determination of the pharmacokinetics of topically applied drugs. The model uses excised human skin mounted in specially designed diffusion chambers that allow the skin to be maintained at a temperature and humidity that match real use conditions. The product/formulation is applied to the surface of the skin and the penetration of the compound is measured by monitoring its rate of appearance in the receptor solution flowing underneath the skin samples. Additionally, this model allows drug and metabolites within the different layers of the skin to be measured (i.e. epidermis or dermis). Additionally, this model has the potential for carefully controlling many of the variables involved in topical application, like dosing volumes, humidity, temperature, drug stability, skin thickness, etc. Thus, it is critical that the conditions and analytical techniques are carefully assessed or qualified to ensure the data is reproducible, free from potential artefact, and has the capability of translating to an in-use situation.

Some key experimental variables, major risks associated with IVPT and their mitigation are:



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xx. *IVPT Skin Sources and Preparation:*

The sources of skin used in IVPT can vary depending on the specific requirements of the study, ethical and legal constraints, ease of access, or laboratory capabilities. Some of the common sources of skin used in *in vitro* skin permeation tests are described below.

i. Human Skin: Human skin sourced from cadavers or elective surgeries (abdominoplasty or mammaplasty) is considered the "gold standard" for *in vitro*/*ex vivo* permeation studies, as it is the closest approximation to the *in vivo* situation. Human skin that is sourced directly from patients can be kept biologically active in culture while maintaining the barrier properties of the stratum corneum for several weeks. This tissue can be dermatomed (split-thickness) and immediately frozen to create a tissue bank for IVPT studies as the protective barrier (stratum corneum) is non-living and can withstand freeze-thaws. Some labs have optimized their tissue processing methodologies to minimize damage to the barrier that can occur from inadequate preparation and storage of these tissues which can result in increased variability. Surgical tissue obtained from tissue banks is often stored for longer periods of time and there is less control on how the tissue was procured, transported, processed and dermatomed. However, this tissue is often pre-screened for infectious disease which can be a requirement for many labs. Surgical tissue has limitations associated with anatomical site (abdominal or breast), most often female and within a narrow age range, thus caution must be taken when trying to translate drug delivery for different anatomical sites, sex, or age.

There are several well-established methods for the preparation of skin membranes. Most commonly, the excess fatty and connective tissue is removed from the excised skin and then a dermatome is used to control the depth of sectioning (typically 250-500 μm). This is the preferred preparation by regulatory agencies as it offers the least amount of manipulation prior to testing. Another technique that has been widely cited in literature, is epidermal sheets that have been prepared by separating the epidermis from the dermis at the epidermal-dermal junction. The dermatomed skin is immersed in 60 °C water for 1-2 minutes and then the epidermal membrane is peeled off from the underlying dermis.



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This technique requires technical expertise, the epidermal membrane has less structural integrity (e.g. tearing) and may lose some of the barrier integrity during processing. These epidermal sheets can be further processed to isolate the stratum corneum through a chemical process involving a solution of trypsin. The isolated stratum corneum can then be floated onto synthetic membranes for support and storage prior to use. Isolated stratum corneum represents the most amount of processing that impart potential artefacts that likely impact the barrier integrity and may result in higher experimental variability making the interpretation of results difficult. The guidances from the FDA do not recommend using isolated stratum corneum and have a strong preference for dermatomed (split-thickness) skin especially if used in regulatory filings (CDERb., 2022; USP-NF, 2022) .

ii. Animal Skin: Other primate skin is a potential alternative tissue, but this is more difficult to source than human skin and is fraught with ethical concerns. Pig skin is widely accepted as most closely resembling human skin. The stratum corneum of the pig is most similar in structure and in thickness to the human skin and has been able to show similar permeation profiles. However, there are some differences between pig and human skin that should be taken into consideration if used in IVPT. A more aggressive cleaning procedure will need to be employed to remove residual formulation from the stratum corneum as a more robust adhesive structure exists in the stratum corneum of pig skin. Pig skin is more difficult to separate the epidermis from the dermis and the follicular diameter is much larger which may result in physical holes in the tissue when dermatomed, especially if using the skin from the back. Rodent skin has also been used, but this tissue is much more permeable with an increased follicular density related to the fur of the animal. It's widely accepted that rodent models are unacceptable models for predicting human skin permeation and any data produced must be treated with caution.

iii. Reconstructed Human Skin: Another alternative to *ex vivo* human skin is reconstituted or reconstructed human epidermis (RHE) membranes which can be sourced ready-to-use. These offer differentiated tissue models that contain normal, human-derived epidermal keratinocytes that are



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cultured at an air-liquid interface on tissue culture inserts. RHE exhibits the structure and cellular morphology of human epidermal tissue with greater uniformity and reproducibility. RHE membranes are commonly used for formulation development, toxicity (irritation and corrosivity) testing, disease (mechanistic) modelling, barrier repair, and metabolism studies. However, at present, the stratum corneum of the RHEs are significantly more permeable than excised human tissue and lack all of the appendages (hair follicles and sweat glands).

iv. Synthetic Membranes: A wide variety of synthetic artificial membranes have been used for permeation studies. Synthetic membranes, such as silicone membranes or polymeric films, can be used to evaluate permeation properties and drug release kinetics. While synthetic membranes do not fully replicate the complexity of human skin, they provide a controlled and reproducible system for permeation testing. Aiming to better mimic the stratum corneum composition, the polymeric membrane can be loaded with lipids to model the intercellular lipid domain of the stratum corneum. More structured lipid matrices have also been prepared to copy the barrier properties of the lipid domains within the stratum corneum. For example, the Strat-M™ synthetic membrane comprises two layers of polyethersulfone (PES, more resistant to diffusion) on top of one layer of polyolefin (more open and permeable). The different polymeric layers provide a porous structure with a gradient in terms of pore size and diffusivity across the membrane. The membrane is then impregnated with synthetic lipids to generate “skin-like” properties. Studies have shown that permeation through Strat-M membranes can be correlated to permeation through human skin though such correlations tend to use approved topical drugs rather than novel agents or formulations. These simple membranes have been used in formulation optimization studies and for testing physicochemical principles, but clearly are a relatively simplistic representation of a highly complex biological organ.

*ii. IVPT Diffusion Cells*

The two most common types of diffusion cells used in IVPT studies are vertical diffusion cells (VDC) or flow-through diffusion cells (FDC). VDCs are also commonly referred to as static diffusion cells or



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"Franz Cells", which is derived from Dr. Tom Franz who pioneered these diffusion cells. FDCs can be referred to as Bronaugh Type which is derived from Robert Bronaugh who was at the FDA when he introduced this design. Both VDCs and FDCs employ a membrane (synthetic or biological) positioned between a donor chamber and a receptor chamber with a heating element to ensure the skin temperature is controlled. The main difference between the two systems is within the design of the receptor chamber for the receptor solution which serves as a reservoir for drugs as they permeate through the skin. Both FDCs and VDCs can provide excellent results if the methods are appropriately developed, qualified, and the limitations of the systems are clearly understood.

VDCs are usually used upright and are made of glass (often hand blown), with the donor compartment available for application of the formulation and a fixed volume receptor compartment. The receptor solution is stirred at controlled rates, most commonly with a magnetic bar, and a sampling arm allows removal of receptor fluid at required time intervals. The sampling of the receptor solution is typically a manual process that involves a scientist removing the receptor solution at controlled intervals with a syringe or microneedle which can make sampling of certain time points (middle of the night) difficult. The relatively large and open donor compartment of VDCs allows simple application of semi-solid formulations or transdermal patches to the skin. The donor chamber area can be easily modified for small dosing areas (<0.5 $cm^2$) to accommodate small tissue samples like skin biopsies or large dosing areas (> 25 $cm^2$) to accommodate large transdermal patches. The fixed volume of the receptor chamber can be used to help concentrate low permeating compounds by allowing the compounds to accumulate in the receiving fluid through delayed sampling. One of the major limiting factors associated with the fixed volume of the receiving chamber of VDCs is ensuring compound solubility is not rate-limiting to drug flux. This is especially challenging for more hydrophobic compounds where solubilizing agents in the receptor solution are required to ensure sink conditions are met.

In contrast to the VDCs, FDCs attempt to mimic *in vivo* conditions by perfusing the underside of the skin with a constant flow of receptor solution through the incorporation of a pump. Sink conditions are



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achieved by keeping the receptor volume relatively low (<0.5 mL) and adjusting the flow rate of the pump such that there is a complete clearance of the receptor chamber within minutes. Some of the newer FDC systems have been able to minimize the void volume of the receptor chamber in conjunction with optimized fluidics such that flow rates in the microliters per minute (i.e. 4-10 µL/min) can be used to achieve local clearance in attempt to mimic blood flow at rest. This gives these systems the distinct advantage where biorelevant receptor solutions (e.g. isotonic saline, PBS, etc) can be used with hydrophobic compounds while still achieving sink conditions. FDCs are machined with tighter tolerances, thus minimizing diffusion cell size variations. Similar to VDCs, temperature is tightly controlled and allows various formulation applications (occlusive or open application, finite or infinite dosing). The FDCs are typically attached to a fraction collector to enable automated sample collection directly into vials or 96 well-plates allowing for more frequent sampling and overnight collection possible.

*iii. Bioanalytical Methods for IVPT*

Highly sensitive analytical methods are required due to the low levels of the drug permeating through the skin into the receptor solution. Liquid chromatography coupled with tandem mass spectrometer (LC-MS/MS) tend to be the analytical method of choice. This analytical methodology provides the necessary sensitivity and selectivity often required, especially for the quantitation of drug in the receptor solution where the levels are often in the sub-nanogram per millilitre (pg/mL) concentrations. These instruments come with a higher cost (>$400K) and complexity, require highly skilled analytical chemists, higher maintenance costs, and specialized laboratories to house and maintain the instruments. Liquid chromatography coupled with ultraviolet (UV) detectors diode array (DAD), or fluorescence detectors (FLD) can also be used. This analytical methodology is more commonly found in analytical laboratories as it offers a lower cost (<$100K), less complexity to run and maintain, and is a more common analytical chemist skill set. However, LC-UV, LC-DAD, or LC-FLD are less



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sensitive and often unable to detect drugs in the receptor solution but likely have the analytical sensitivity to detect drugs in the different layers of the skin.

Historically, the use of radiolabeled compounds (tritium ($H^3$) and carbon 14 ($C^{14}$)) have been used in IVPT studies, where the detection and quantitation are performed using scintillation counting. This methodology offers some advantages where the sample analysis is automated, rapid, sensitive, and selective. However, the radiolabelled drugs have the distinct disadvantage associated with the stability of the label (H3 or C14), translation of labelled drug compared to unlabelled drug, expense of chemical synthesis, and the environmental impacts associated with radioactive use and disposal. Very few, if any, laboratories use this technique in IVPT studies.

Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy and Raman and confocal microscopy have been used to identify and quantify drugs that traverse the stratum corneum into the upper layers of the skin. This technique is less common due to the interference of the skin itself and limited number of drugs having the required spectrum for analysis. As more companies explore large molecular weight compounds, specific bioassays may be necessary. These bioassays often utilize common molecular biology techniques such as dual hybridization assay (DHA), enzyme-linked immunoassay (ELISA), or other post-conjugated procedures prior to analysis.

*iv. IVPT Methodology or Experimental Design*

*Dose*

It is difficult to generalize protocols for application of the formulations or patches during IVPT studies since these are dependent on the experimental rationale, therefore it is important to understand the thermodynamic activity of the drugs in the formulation, the rate and extent of potential permeation, and the metamorphosis of the formulation upon application. The dosing procedures should mimic as closely as possible the *in vivo* situation to provide appropriate data.



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Finite dosing (2-15 mg/cm$^2$) is the most common approach for topical semisolid formulations meant to mimic the clinical situation where the patient would apply and even layer drug product on the skin. Given the technical difficulties associated with applying a uniform layer on the surface of the skin with lower volumes (2-5 mg/cm$^2$), most IVPT studies employ 10-15 mg/cm$^2$ which also helps improve analytical sensitivity by increasing the total amount of drug applied. The finite dose should deplete on the skin surface and undergo formulation metamorphosis (generation of a residual phase on the skin surface) to mimic as closely as possible the *in vivo* situation.

Any dosing technique should have some level of confidence that there is consistency in dispensing the formulation and even spreading or coverage of the skin. This can be achieved through the use of positive displacement pipettes, pre and post-weight measurements, and the use of glass rods or syringe plungers to evenly spread formulations within the dosing area.

*Receptor Solution*

The receptor solution should ideally mimic as closely as possible the *in vivo* situation. *In vivo*, the dermis contains the cutaneous microvasculature and lymph vessels that readily remove compounds which have crossed the stratum corneum and viable epidermal layers, thus maintaining sink conditions to ensure the concentration gradient (or more accurately the thermodynamic activity gradient) across the membrane is maximized. The solubility of the drug in the receptor solution should therefore be sufficiently high to ensure that the permeation of the drug through the skin is not impacted by modification of the concentration gradient across the membrane. Generally, the solubility of the drug in the receptor solution should exceed the highest expected concentration from the experiment, ideally by an order of magnitude or 10-fold. Another rule of thumb that is commonly used is to ensure sink conditions states the concentration of the drug should not exceed 30% of its solubility in the receptor solution. The ultimate selection of a receiver solution largely depends on the physiochemical properties of the drug and on diffusion cell design. Flow- through systems generally minimize the accumulation of permeant in the receptor and so allow the use of aqueous receptor media even for



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moderately lipophilic materials. While static diffusion cells may require additives to ensure drugs do not exceed the solubility constraints associated with a fixed volume.

Aqueous or isotonic saline are commonly used receptor solutions for hydrophilic and moderately lipophilic compounds (< LogP =2) and are often pH adjusted depending on the compound. Many researchers will include small concentrations (<0.05%) of a surfactant (e.g. Volpo N20 or Brij 020) as a blocking agent to prevent non-specific binding to labware and the diffusion cells. Difficulties arise when the drug is much more lipophilic or where aqueous solubility is low. Consequently, many researchers will add small concentrations (0.1% - 1%) solubilizing agents (e.g. polyoxyethylene (20) oleyl ether) or proteins (e.g. bovine serum albumin) to the receptor solution. Some solubilising agents are relatively "aggressive" and thus, while a receptor solution must ensure clearance of the permeant, any adverse effects of the receptor on the barrier properties of the skin must be considered; when using solubilizing agents, it is essential to ensure that these additives do not alter the permeability of the skin (for example surfactants could interact with, or solvents may extract, stratum corneum lipids). It should be noted that regulatory guidance now advises against the use of organic solvents and alcohols in the receptor solution to the extent that it may invalidate the IVPT method if used in a regulatory submission. If longer duration IVPT studies (>24 hours) are performed, the addition of antimicrobial/antimycotic agents (e.g. sodium azide, gentamycin sulfate, etc) might be required to prevent microbial growth and tissue deterioration. These antimicrobial agents need to be considered in the analytical method as they may require additional sample preparation prior to analysis.

*Experiment Duration and Sampling*

IVPT experiments can vary in duration from a matter of minutes to days depending on how the data will be used. Occupational exposure studies may expose the membrane to hazardous materials for seconds before washing off and monitoring while once a week patch formulation may require the experiment to be carried out for several days. Regardless, it's critical that the membrane integrity be maintained throughout the experimental period.



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Academic researchers and regulatory agencies agree that the duration of the experiment should be sufficient to characterize a 'suitable permeation profile'. When using IVPT studies to support formulation development and optimization to rank order prototype formulation, this means sufficient time to determine the lag phase and pseudo-steady state flux. Recently, regulatory agencies have an interest in aligning IVPT studies to traditional pharmacokinetics or "cutaneous pharmacokinetics". As such, IVPT studies used to establish bioequivalence must have sufficient duration to characterize a complete flux profile that includes the maximum (peak) flux and a decline across multiple time points. The guidances are going so far as to request removal of the formulation, mid-experiment, if peak flux cannot be obtained within 48-72 hours. Obviously, the removal of a formulation mid-experiment imposes potential artefacts of redistribution of drug into the lipid layers of the stratum corneum that would not occur a clinical situation.

*Donors and Replicates*

There is a high degree of variability between and within skin donors. Although inter donor variability is a likely representation of the true variance in the population, it may be possible to reduce intra donor variability through refinement of IVPT methods. Regardless, data can only be claimed to be statistically valid where sufficient replicates have been performed and will depend on how the data will be used. With relatively simple formulation screening, 5-6 replicates on a single skin donor may be sufficient to rank order the formulations but may not provide sufficient statistical power to show significance. However, when the IVPT data is used to make decisions on clinical formulations or establish bioequivalence in regulatory submissions a much larger number of skin donors will be required. The current minimum for these situations is no less than 4 skin donors and 4-5 replicates per skin donor. Pivotal bioequivalence studies typically require a much larger number of skin donors (discussed later) which can range from 6 to 24 skin donors depending on the variability established in a well-controlled pilot study.



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*Skin Temperature*

It is well established that skin permeability (and diffusion processes in general) varies with temperature, and indeed at high temperatures the stratum corneum bilayer lipids become disordered leading to a marked increase in diffusivity of a drug. Temperature control during the experiment is thus essential and most studies control skin surface temperature to mimic the *in vivo* temperature of 32°C. To achieve this control, and dependent on the experimental protocol and equipment which influence thermal conductivity, the receptor solution may need to be heated beyond 37°C. The skin surface temperature should be monitored using temperature probes placed on the stratum corneum or a thermal camera to ensure the skin surface temperature is maintained over the duration of the experiment.

*Skin Barrier Integrity*

As the preparation of skin membranes is a complex surgical process, there is considerable scope for damaging the tissue. There are numerous proposed methods to assess the skin barrier integrity for IVPT studies. One approach is to monitor the permeation of a well-known compound (typically tritiated water) through the skin. This method has limitations associated with the environmental impacts of use and disposal of radioactive material. Very few laboratories use this procedure. Another approach is electrical impedance/conductance which characterizes the bulk flow of electrical current across the skin. This test is commonly referred to as Trans-Epidermal Electrical Resistance (TEER) skin barrier integrity tests. The test requires a small amount of ionic solution to be applied to the surface of the skin and acceptance criteria is based on comparing skin sections with a compromised barrier (e.g. tape strips or small holes from needles) from skin sections with a competent barrier. The TEER skin barrier integrity test can vary substantially depending on the meter and the technical procedures used for the test. The skin is hydrated during the testing process and usually dosed soon after the integrity check, which could change (enhance) the drug permeation. Therefore, the relevance of TEER measurements for permeation of organic molecules through skin membranes is questionable.



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Transepidermal water loss is the most commonly used and preferred method by regulatory agencies as it measures water loss through the skin is kept dry and in contact with ambient conditions which more closely mimic the *in vivo* condition. TEWL is relatively quick and convenient but relies on devices that may not be designed specifically for diffusion cells. Some labs have designed TEWL instruments that are specifically designed for the diffusion cells donor compartment, which allows readings to be taken simultaneously with built in equilibration and rapid monitoring (1-2 minutes) of up to 32 diffusion cells. TEWL has the advantage of not hydrating the skin prior to checking integrity. It's important to note, the measure of water traversing the skin may not directly correlate with permeation pathways for lipophilic materials. Regardless of the barrier integrity method used, its impossible to guarantee each skin section has a fully intact competent barrier and is free from damage that may have occurred during the collection, transport, processing, and mounting of the skin onto the diffusion cells prior to dosing.

*Skin Penetration (Distribution) and Mass Balance*

IVPT experiments have the added advantage of measuring drug content in the different layers and surface of the skin. As such, IVPT studies can measure drug concentration removed from the surface during the washing procedures, upper layers of the stratum corneum via tape strips, viable epidermis (includes partial stratum corneum), and the remaining dermis (upper dermis) in attempt to establish a mass balance. Skin penetration samples are a snapshot in time where the skin is cleaned, heat separated into epidermis and dermis, and the drug is then extracted from the different matrices. This is considered a sacrificial sample that is performed at the end of the experiment after the receptor solution time points have been collected and is typically performed at 24 hours post-application. Earlier or later skin penetration time points (e.g. 2, 4, 8, 48 hrs) can be performed but require an increase in the number of replicates and can quickly double the size of the experiment. The information from drug delivery to the different layers of the skin can be useful to researchers who are interested in gaining insights into local site-specific delivery especially as it relates to the site of



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action. For example, a mechanism of action may be specific to epidermal proliferation and/or differentiation, therefore having information on drug delivery to the epidermis could be more useful than permeation to the receptor solution. On the converse, some anti-inflammatory compounds will need to be delivered to the lymphocytes in the dermis, therefore having information on the concentration of drug in this layer as well as drug delivery into the receptor solution could provide information to help indirectly answer pharmacodynamic questions.

Undertaking a mass balance at the end of the experiment – to quantify drug that has permeated, that remaining in the skin and that remaining in the donor solutions – has benefits in assuring that the total dose is accounted for and that the permeant has not degraded during the experiment. However, in practice, it is often difficult to get accurate mass balances and values that account for over 90% of the applied dose though sensitivities tend to improve when using radiolabelled permeants as opposed to HPLC or mass spectrometry approaches. Losses occur at multiple stages of these analyses. The drug needs to be quantified on the skin surface, swabs, and tape strips, as well as residuals on the diffusion cell surfaces, and it is critical to ensure the extraction solvent systems are capable of extracting the drug from the different formulation types, swabs and tape. Oil- based formulations such as ointments and complex creams are particularly challenging and so recoveries in the region of 50–-80% are commonly seen with these types of formulations. Full mass balance requires extensive bioanalytical method development to ensure the extraction methods are sufficient, reliable, and free from matrix interference in the analytical instruments.

*Data Analysis*

The expression of the data will depend on the purpose of the study. Studies conducted in the early stages of drug development intended to select new chemical entities (NCE) or part of the formulation development optimization process will typically include skin penetration/distribution (epidermis and dermis) and skin permeation (receptor solution). The concentrations of drug in the different layers of the skin (epidermis and dermis) are commonly presented as total concentration (ng), percentage of



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applied dose (% applied dose), and can be corrected for weight if the tissues are weighed prior to drug extraction (ng/mg). The concentration of drug permeating (cutaneous pharmacokinetics) will be presented as pseudo steady-state flux with lag phase, percentage of applied dose at given time points, maximum or peak flux ($J_{max}$), cumulative amount over the duration of the experiment, and total cumulative amount and an area under the curve (AUC) calculated. It should be noted that permeation data tends to be reported arithmetic terms (ie assuming that the data is normally or follows a Gaussian distribution) – with means and standard deviation reported for data on permeability coefficients or fluxes. However, as a biological membrane, and in common with many biological systems, data from such studies is seldom normally distributed but shows a geometric distribution, though these can be transformed (log transformation) to yield a normal distribution ( Williams et al. ,1992)). For geometric distributions, a geometric mean and confidence intervals are more appropriate than the arithmetic mean and standard deviation.

Studies conducted to support bioequivalence or used to support regulatory filings that reference the guidances released by the EMA and FDA require specific data outputs, analysis, statistics, and acceptance criteria. In these situations, specific guidance should be used to define how the data is presented and translated.

*v. Regulated Bioequivalence*

In efforts to make high-quality, affordable medicines available to the public, regulatory agencies have released guidances to create a modular framework for *in vitro* bioequivalence (BE) testing of topical products in lieu of clinical trials. The FDA recently released guidance "*In Vitro* Permeation Test Studies for Topical Drug Products Submitted in ANDAs - Guidance for Industry" that details the expectations for IVPT method development, IVPT method qualification and validation, sample analytical method validation, and IVPT pivotal studies. The IVPT statistical analysis from the FDA relies exclusively on permeation (receptor solution) data and does not allow for skin concentrations to



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be compared. This guidance document needs to be used in conjunction with the updated USP-NF {1724} chapter to ensure the strict expectations by the FDA are met.

The European Medicines Agency (EMA) released a similar general draft guideline on quality and equivalence of topical products in 2018. This guidance provides a more general approach that is similar in its reliance on skin permeation (receptor solution) data to establish bioequivalence, however the guidance also requests that penetration & mass balance be collected. While EMA has a similar approach to establishing IVPT bioequivalence using permeation data, the guidance on how to analyse the data is far less detailed and does not provide guidance on how to handle highly variable drugs.

There has certainly been an under appreciation of the work required to meet these new standards, especially as the IVPT and bioanalytical methods need to be validated with current quality approaches. This volume of work comes with a cost and timeline that may have been originally underestimated, which can exceed $300K and take as long as 12-18 months for the completion of a single regulated IVPT study. These guidelines now serve as the 'gold standard' on how to conduct well controlled IVPT study.

### *C. Disease/Activity Models*

Animal models have been widely used to help understand human skin disorders and to estimate the pharmacokinetics and pharmacodynamics of existing and potential new topical therapeutics prior to clinical trials. Most of the animal models have focused on inflammatory dermatosis, which encompasses a wide range of skin disorders from contact atopic dermatitis to misdirected autoimmunity in alopecia, vitiligo, psoriasis, and rosacea representing the largest markets (Richmond & Harris, 2014), Animal models utilizing rodents and dogs have been employed for research into atopic dermatitis. The rodent models require genetic manipulation involving transgenic mouse strains or hypersensitization using hapten-induction to achieve the pathological response (Kitagaki et al., 1995; Schn, 2008). Unfortunately, the only animals known to recapitulate psoriasis are rhesus and



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cynomolgus monkeys, which presents significant cost and ethical concerns related to their use and application prior to human clinical trials. Similar to atopic dermatitis, the majority of animal models for psoriasis rely on rodents and can be categorized into four areas; 1) spontaneous, 2) genetically modified, 3) induced and 4) xenotransplantation. Spontaneous and genetically modified mice present the disease state without exogenous stimulation, whereas induced mice require induction of disease state usually by surgery, chemical or biological application. Xenotransplantation is considered the humanization of mice by surgical grafting of human lesional tissue to immunocompromised mouse strains. Since psoriasis is not naturally occurring in mice, these models reflect a slightly different mechanism of the disease where the majority cell population are dendritic cells and mice lack an influx of T cells and Langerhans cells which is widely known as the major cell populations involved in human psoriasis (Bocheńska et al., 2017). Beyond the complexity and ethical concerns with using animal models in research, the differences in immune function, follicular thickness, skin thickness, barrier properties and inherent permeability of animal skin compared to humans makes translation somewhat limited (Shiohara et al., 2004). In general, animal models can present pathologies that mimic human disease; however these significant physiological and anatomical differences create challenges when using such models to evaluate novel therapeutics. These models are well placed for mechanistic understanding in academic and early discovery research but may have limited use in preclinical product development of topical and transdermal products.

Reconstituted human epithelium (RHE) skin are well established models for topical testing in both pharmacotoxicology studies and the evaluation of dermal irritation. The RHE skin have become the primary models for the cosmetic industry primarily driven by the “3Rs alternatives” (replacement, reduction, and refinement) associated with animal testing. These models have been further advanced to include collagen, dermal fibroblasts, melanocytes and newer models are regrown using keratinocytes obtained from psoriatic patients. It is unclear how much of the gene expression associated with psoriasis is lost during culture and expansion of the regrown psoriatic human



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epidermis. The RHE skin is a useful model when studying certain compounds that affect keratinocyte differentiation and proliferations (e.g. retinoids and VitD analogues) to visualize genetic and morphological changes in the viable epidermis. In attempt to overcome the lack of immune cell types, these RHE models have had some success when co-culturing polarized Th1 and Th17 T cells producing similarities in a psoriatic genetic profile, but the immune cells appeared to be hindered by a uniform application and migration into the extracellular matrix (Desmet et al., 2017). At present, the RHE still lacks vascularization, innervation, appendages, and are much more permeable than intact human skin. As tissue engineering advances using microfluidics and a better understanding of skin biology is gained, reconstructed tissue remains an exciting area of research and development for the future.

The use of *ex vivo* human surgical skin in developing disease models has numerous advantages over the use of animals, synthetic alternatives, and reconstructed tissue models. Skin can be harvested immediately after surgery and the *ex vivo* skin transplants can be kept in culture for up to 4 weeks. This *ex vivo* tissue contains fully differentiated keratinocytes, appendages, fibroblasts and collagen matrix associated with human dermis, and a fully functional intact stratum corneum mimicking the clinical permeability of human skin. Perhaps the biggest advantage is the presence of other resident cell types that include dendritic cells, Langherhans cells, monocytes, and neutrophils (CDERb., 2022). Clark, et al, showed that there is a large pool of skin resident memory T cells ($T_{RM}$) in normal skin that can initiate and perpetuate immune reactions in the absence of T cell recruitment from the blood (Clark et al., 2006). These skin resident memory T cells ($T_{RM}$) have been shown to be important mediators of frontline immunity against infection and autoimmune disease. This has led to the recent development and characterization of the human *ex vivo* skin culture (HESC) model that utilizes human surgical skin where inflammatory mixtures (cytokines and antibodies) can be added to induce inflammatory conditions (e.g. Th1, Th2, and Th17) that have overlapping biology with atopic dermatitis and psoriasis (Jessica E Neil et al., 2022). Neil, et al showed that this HESC model can be



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used to simultaneously assess the pharmacokinetics and pharmacodynamics of known drugs that correlate to the gene expression of known biomarkers from skin biopsies obtained from atopic dermatitis patients (Neil et al., 2022) The HESC model has been shown to reflect an enhanced inflammatory state and the ability of non-immune biomarkers (e.g. DefB4, S100A12, and MMP12) to be regulated consistent with the known pathophysiology of these inflammatory diseases. The HESC model is considered the preferred model over reconstructed human epithelial models (RHE) to investigate the effects of environmental stress, aging and skin disease (Lebonvallet et al., 2010) Due to the abbreviated time in culture, the HESC model is not able to reproduce the phenotypic changes commonly seen in atopic dermatitis (e.g disruption of stratum corneum) or psoriasis (epidermal hyperplasia) (Hoffman et al., 2012). In addition, this model has the limitation of being relatively static besides the replacement of media and devoid of vascular clearance and immune cellular infiltration. Some of the immune cellular infiltration can be added through co-culturing techniques but requires advanced knowledge of hydrogels and culture media. These HESC models can be used to ensure compounds delivered topically are bioavailable, able to engage their target and can generate pharmacokinetic/pharmacodynamic (PK/PD) relationships. Importantly, this method allows for the skin barrier to remain intact while measuring immunological changes within human skin after a topical application. Additionally, through the selection of pivotal biomarkers the elucidation of signaling pathways, off-target effects, vehicle effects and new mechanisms of action (MOA) can be explored as this *ex vivo* tissue contains most of the relevant cell types.

*Ex vivo* skin (fresh or frozen) can be used to assess antimicrobial effects from topicals. The skin (stratum corneum, epidermal sheets, or dermatomed skin) can be mounted in specialized vertical diffusion cell (e.g. TurChub®) employing a gasket designed to prevent lateral diffusion from the topical application. A range of organisms (e.g. C. albicans, T. rubrum P. acne, S. aureus or S. epidermis) can be grown in an agar matrix in the receptor chamber directly under the skin. As the drug/formulation permeates across the skin into the agar it results in a clearance of turbidity (area of



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no growth) or zone of inhibition. This assay has the added benefits of not requiring traditional analytical chemistry (HPLC, UPLC, etc.) to quantify the drug as the measurement is against the organism itself and vehicle effects are captured through the incorporation of placebo vehicle formulations. A slight modification to this model involves infecting the skin in mounted in a specialized diffusion cell (RoMar), where the same organisms are grown superficially or on the underside of the skin depending on the clinical presentation. For example, p. acnes are infected on skin dermatomed roughly to the same depth of sebaceous glands and cultured in an anaerobic chamber. The antimicrobial effects can be assessed by cell viability (i.e. ATP), PCR, or viable cell counts to get an indication of MICs. There is ongoing work to combine the infection with the HESC model, to explore the multimodal mechanistic effects of the infection and associated inflammatory responses.

There is continuous research in the extension of the HESC model to assess wound healing. This model was previously limited by the inability to keep these tissues in culture past 7-10 days. However, a few labs have been able to extend the viability of these cultures out to 4-6 weeks, thus allowing the wound repair and healing mechanisms to be assessed with and without treatment. There have also been advances in the devices used to induce wounds, now allowing carefully controlled and reproducible sections to be taken from the skin further reducing the variation associated with traditional manual techniques like punch biopsies. Other areas of research utilizing HESC could be other disease states like vitiligo, rosacea, skin fibrosis, and eventually a multimodal acne model where a zit could be regrown and treated.

The U.S. Food and Drug Administration recently announced “$5 million in new funding for a comprehensive strategy for new, alternative methods for product testing. The budget includes funding to support a new, FDA-wide New Alternative Methods Program to reduce animal testing through the development of qualified alternative methods and spur the adoption of methods for regulatory use that can replace, reduce and refine animal testing. New alternative methods have the potential to provide



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both more timely and more predictive information to accelerate product development and enhance emergency preparedness." This presents a tremendous opportunity for drug developers to present their data to the FDA from nonanimal models to assess safety and efficacy. Albeit this is likely to be a slow process it is definitely a step in the right direction and is incumbent on the pharmaceutical and biotechnology industry to begin submitting these alternative models in parallel with the traditional animal data.

## VII. THE HUMAN FACTOR

### *A. Prescription adherence*

Adherence is defined as "the extent to which a person's behaviour (taking medication, following a diet, and/or executing lifestyle changes) corresponds with agreed recommendations from a health-care provider" (World Health Organization, 2003), highlighting the importance of patient-clinician discourse in the decision making process (Ahn et al., 2017). Patient adherence within dermatology varies depending on the condition and type of treatment. Non-adherence can be separated into two primary categories: one where the patient does not collect the prescribed treatment (poor initiation of therapy), and/or the patient does not take the medication as directed by their physician (poor implementation and discontinuation of therapy) (Nasimi et al., 2022; Vrijens et al., 2012). Beyond these two primary categorisations there are multiple factors affecting a decline in adherence including: socio-economic-related, disease-related, clinician-related, treatment-related and patient-related factors.

### *B. Socio-economic related factors*

Marital/co-habitation status, employment, education, and lifestyle choices such as having a balanced lifestyle that does not include smoking or drinking have all been associated with adherence to the use of medication (Larsen et al., 2019; Nasimi et al., 2022; Svendsen et al., 2021). For example, higher adherence to psoriasis treatments for married individuals compared to those who are single and employed compared to unemployed people has been suggest to indicate that adherence is improved



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for those who have a better support system and structured routine (Zaghloul & Goodfield, 2004). The financial burden of treatment is also believed to be an important factor, with greater adherence reported among groups who did not pay for medication compared to those bearing the cost burden for medication (Zaghloul & Goodfield, 2004). Similarly, Ellis et al., (2011) and Venkatesh et al., (2022) have reported high costs of treatments as one of the top barriers to adherence among dermatology patients. Poor treatment adherence can also be seen with children and elderly patients, which could be associated with a lack of autonomy, understanding or memory (Eicher et al., 2019). Age related hearing loss, visual impairment and reduced manual dexterity may negatively impact elderly patients' ability to adhere to dermatological treatment plans (Endo et al., 2013; Gupta et al., 2009). A worldwide study observing adherence to acne treatments reported lower rates of adherence to in young patients (those under 15 years of age) (Dréno et al., 2010). Similarly, a study observing adherence to topical medication across a number of skin conditions reported lower adherence in younger populations (Furue et al., 2015). For paediatric patients where treatment plans are implemented by parents or carers, adherence related treatment outcomes are likely to be affected by the socio-economic demographics of the parent or carer. However, these correlations may not be generalizable across, or indeed within, dermatological treatment groups as other studies have observed alternative or contradictory findings. For example, in their study of adherence to the treatment of different skin conditions in Japan, Furue et al (2015) did not find marital status, whether an individual was unemployed or had a university education was associated with adherence. Alsubeeh et al (2019), who investigated adherence across four treatment types (oral, injection, topical and phototherapy) and different skin conditions did not find that age, education or marital status affected adherence and highlighted that cultural differences between study populations may have contributed to their findings (Alsubeeh et al., 2019).



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*C. Disease-related factors*

The skin condition, chronicity and severity of disease can impact on adherence to treatment. (Storm et al., 2008) investigated primary adherence (collection and initiation of therapy) across skin conditions and reported considerable variation across patient groups. Increased non-adherence was observed in psoriasis and eczema patient groups compared to those with acne or skin infection (44.2 % and 31.4 % compared to 9.1 % and 12.2% non-adherence, respectively). The impact of a skin condition on a patient's quality of life (QoL) has also been associated with poor adherence. When a patient only has mild impact to their QoL they are less likely to regularly apply their medication, whereas skin diseases that creates a moderate reduction to someone's QoL typically improves adherence (Eicher et al., 2019)A study investigating the correlation between the severity of rosacea and adherence reported higher baseline adherence in cases of severe rosacea (73 %) compared to less severe rosacea (48 %), a trend which held over the three-month study period (Perche et al., 2023). This suggests that a patient's motivation to adhere to treatment may be guided by the severity of the condition and extent of impact on daily life. This improvement in adherence does not continue indefinitely with further decreases in QoL however, with those with more severe disease often having the worst adherence (Eicher., 2019). The severity of dermatological conditions may be different to a patient's perceived severity of their condition, both of which can impact adherence. A positive correlation exists between the patient's perception of disease severity and the visibility of affected areas in psoriasis. This subset of patients (psoriasis affecting the face and highly visible areas) have self-reported greater adherence to treatment recommendations compared to patients with psoriasis on non-visible areas (40.3% and 37.5% versus 28.9%, respectively (Visentin et al., 2015). Other studies that examined the impact of the location of psoriatic lesions have also determined that there was a correlation between facial and visible locations and high treatment adherence (Alsubeeh et al., 2019). These same authors also highlighted that patients who felt a stigma associated with product use, including those treating visible areas, were less adherent to therapy. This links to another, often overlooked, reason for low adherence with topical



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medication, which is patients' mental health. Patients can often feel embarrassed, ashamed or self-conscious to have medication on their skin, especially when the affected area is located facially or on high visibility areas. The psychological burden of skin conditions on patients has been widely investigated (Davis et al., 2022; Dieris-Hirche et al., 2017; Dréno et al., 2010; Halvorsen et al., 2011). A European wide study reported significantly higher prevalence of clinical depression, anxiety disorder and suicidal ideation among patients with common skin diseases compared to control groups (Dalgard et al., 2015). Psychiatric disorders, like depression and anxiety, have an adherence rate that ranges from 25% to 43% which is lower than average. When seeking to improve adherence rates to topical treatments, the implications of patients' mental health and ability to adhere to treatment plans should also be considered.

*D. Clinician related factors*

A good clinician-patient relationship is essential for developing shared treatment goals, particularly for dermatological conditions where the treatment choice is often influenced by patient preference and lifestyle. Establishing regular channels of communication with patients, setting realistic expectations for treatment outcomes and providing clear educational information can, in turn, empower patients to better adhere to agreed treatment plans and self-manage their condition. With there being a shortage of dermatologists, it is important to fully utilise the appointed time with patients and make them feel confident about managing their condition. Holding open conversations between patients and dermatologists before giving the prescribed medication (pretreatment education) has been seen by Strydom, (2018) to increase treatment satisfaction scores. Furthermore, implementing tailored training sessions to coach patients on the use of topical therapies can improve adherence, as can referrals to patient advocacy groups and support networks (Atwood et al., 2018; Caldarola et al., 2017; Patel et al., 2017). Numerous patients have stated that access to support groups for skin conditions could be extremely helpful with their continued treatment and overall attitude towards their illness and t has been reported that 8 in 10 dermatology patients access health information on the internet or social



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media platforms before contacting their dermatologist (Gantenbein et al., 2020). This may be because patients are in search of advice or shared experiences relating to their particular condition, however the content on these platforms can be misleading, dangerous and/or lack a scientific basis (Mueller et al., 2019). Thus, clinicians are optimally placed to signpost patients to high quality online content and support groups. This is particularly important within dermatology as the number of patients with skin conditions that are correspondingly clinically depressed, and/or suffer with anxiety is significant (Dalgard et al., 2015) Store-and-forward teledermatology has taken off since 2020, due to the Covid-19 outbreak. Holding patient-clinician meetings online has overall been successful due to it being more time efficient for both parties. Dermatologists can attend to more patients, and it reduces the effort required for patients who have to co-ordinate follow-up meetings with multiple dermatologists. Studies suggest that patients with early-stage melanoma were attracted with direct store-forward teledermatology due to it resulting in fewer follow-up visits. Despite this success, there have been a few notable shortcomings. So, whilst there are many benefits to this system, it is not suited to all individuals. Hence it is advisable that there should always be an option for a face-to-face meeting. Ideally, a tailored and multifaceted approach should be adopted to improve the patient's experience and engagement in the shared decision-making process.

*E. Treatment related factors*

Within dermatological treatments, topical medication is the least desired amongst patients. Eicher et al. (2019) states that patients prefer to take oral medication or an injection over topical treatments. Topical medications were found to have a negative overview from patients due to the belief that it is not an effective treatment and is commonly used for more minor skin conditions. When looking at the self-reported adherence rates, biologic therapies were the most successful at 100%, whereas the lowest at 73% was topical therapies (S. A. Chan et al., 2013).



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The complexity of treatment regimes, duration of treatment, formulation type and misconceptions surrounding treatments may impact adherence. For chronic dermatological conditions such as psoriasis or acne, treatment may span weeks, months or years, with slow rates of improvements. This can be perceived by patients as 'treatment failure', resulting in a loss of motivation to adhere to treatment plans and subsequently disease regression (Moradi Tuchayi et al., 2016). In the treatment of actinic keratosis, patients on longer term therapy are less likely to adhere to treatment than patients on short term treatment plans, with the inconvenience of daily therapy often cited by patients as a reason for poor adherence (Carr et al., 2013; Neri et al., 2019) Treatment plans designed with fewer applications, shorter treatment durations and fewer side effects can improve adherence (Feldman, 2013; Grada et al., 2021; Visentin et al., 2015). This highlights the importance of developing practical and realistic treatment plans to enable patients to self-manage their conditions alongside day-to-day responsibilities.

The complexity of topical treatment is increased when more than one product is used. This is common in conditions such as atopic dermatitis where both topical corticosteroids and emollients are regularly applied. As the pharmaceutical industry develop topical products separately there tends to be little evidence of how different products should be applied in relation to one another to achieve optimal patient outcomes. Clinical guidelines have been produced providing instructions that are based on expert opinion, however the guidelines from different sources vary and may even contradict each other on how topical products should be applied. Examples of this variation include, whether the TCS or emollient should be applied first, whether the sequence of application should be changed depending on whether the emollient is a ointment or a cream and the time interval between application of the products, which ranges from 'as soon as absorbed' to 60 minutes (Ersser et al., 2012; Flohr, 2004; Moncrieff et al., 2013; National Eczema Association, 2019; Ring et al., 2012). These application recommendations are a result of wanting to ensure emollients are used optimally, reducing the need for the topical corticosteroid but also with an awareness that applying the two products on the skin



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surface at similar times could effectively alter the topical corticosteroid formulation performance, affecting delivery of the drug. The guidelines' recommendations however can be time consuming and challenging for patients (or their carers) to follow and therefore negatively affect adherence. Recently ex-vivo studies on the effect of emollients on drug absorption into the skin have suggested that, in general, applying emollients before a topical corticosteroid is likely to have a greater impact, reducing drug delivery to the skin (Beebeejaun et al., 2023; Draelos et al., 2020). This is contrary to the recommendation to some current guidelines. Further research to understand how to optimise patient outcomes considering both treatment efficacy and adherence is warranted.

Clinicians may select topical products in discussion with patients based on a number of factors such as formulation type, patient preference, ease of product use and convenience. Poor cosmetic characteristics impact negatively on patient adherence. A European wide survey of 1281 psoriasis patients revealed that 'sticky' formulations which take 'too long to rub in' were two of the prevailing reasons that patients failed to adhere to topical treatment (Fouere et al., 2005). Patient preference may vary by dermatological condition, with washes, creams, and lotions reported as a preference for acne whilst creams, ointments, and foams may be preferred in psoriasis (Eastman et al., 2014). Acne is associated with increased sebum production leading to 'oily' skin. Addition of further 'oil' through e.g. an ointment formulation is typically less preferred. Moreover, some oily excipients contained within ointment and cream formulations may be comedogenic and make acne worse (Fulton et al., 1984). In contrast ointments show better acceptability with drier skin conditions and are the preferred option because of their superior occlusive properties in the management dry skin conditions (National Eczema Association, n.d.). Topical corticosteroid ointments may also be more potent than their respective cream formulation (National psoriasis foundation). In some cases preparations containing ingredients such as coal tar, dithranol or salicylic acid may be desired to be used as a treatment option of the basis of potential efficacy, however these ingredients are associated but are associated with less favourable patient acceptability characteristics through being irritant or having a strong smell or



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colour (Buckley, 2001).These properties can require a trade-off between selecting products for efficacy and patient preference and adherence. Sensitive skin may also impact on product preference with alcohol-based formulations or those containing particular excipients including fragrances, emulsifying agents or preservatives, having lower patient acceptability as a result of causing skin irritation. The body site at which the skin condition is present is also relevant, with more fluid/easily spreadable formulations such as lotions or foams being preferred for hairy areas such as the scalp. Other factors influencing choice can include the time of day of application, with patients preferring not to apply products that are oily, visible to others or interfere with make-up or clothing during the day. Thus, a personalized approach to product selection should be adopted as patients have diverse preferences often influenced by individual perceptions or experiences, which are difficult to predict (Felix et al., 2020; Hong et al., 2017; Iversen & Jakobsen, 2016).

Corticophobia, the fear and reservations towards corticosteroids and their side-effects, is a frequent cause of non-adherence in dermatology (Eicher *et al.*, 2019). Corticophobia related non-adherence has been reported in 28.3 – 87 % of patients prescribed topical corticosteroids (Aubert-Wastiaux et al., 2011; Mueller et al., 2017) and is also reported among healthcare professionals (Lambrechts, Gilissen and Morren, 2019). Common concerns among patients include skin atrophy, systemic effects, and impairment of the immune system (Aubert-Wastiaux et al., 2011; Mueller et al., 2017). An abundance of misinformation readily available online and through social media platforms may contribute to fears and reservations in patients (Finnegan et al., 2023; Smith et al., 2017). A randomized clinical trial reported decreased corticophobia in patient who were offered an educational intervention through provision of videos, patient-clinician conversations, demonstrations of fingertip unit applications and patient information leaflets (Choi et al., 2020). When corticosteroids are used and discontinued appropriately according to clinical guidelines, the adverse effects are minimal (Mooney et al., 2015). Thus, fears associated with treatments can be minimized if dermatologist provide clear and consistent instructions, emphasising how topical steroids do not have significant side effects when used



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correctly. Furthermore, regular patient-clinician conversations with an aim to improve the patient's knowledge of corticosteroids offers an opportunity to clarify confusions, dispel misconceptions and manage treatment expectations.

*F. Patient related factors*

In a majority of cases, patients are required to play a proactive role in implementing agreed treatment plans and self-managing their condition long term. A number of digital interventions have also been developed to support the knowledge transfer and management of dermatological conditions (Hewitt et al., 2022) These interventions can serve to remind patients to adhere to treatment plans and are achieved through several modes of delivery, such as reminder texts, emails, apps, online self-reporting tools and phone calls. Engagement with an adherence-supporting app over a four-week period has been shown to improve adherence to topical treatment when compared to adherence in patients not using the app (65 % vs 35 %, respectively) (Svendsen et al., 2018)). Though digital interventions have been shown to improve treatment habits, they are often self-directed and therefore the patient's motivations and ability to engage with these approaches should also be considered when deciding on appropriate interventional strategies.

A patient's understanding of their skin disease, awareness of the role of medicines in improving their condition and ability to communicate their treatment expectations to clinicians is therefore essential for a successful outcome. Health literacy considers a patient's ability to seek, understand and utilise health information (Buchbinder et al., 2011) and higher health literacy has been associated with greater self-management characteristics in patients with psoriasis (Larsen et al., 2019). Furthermore, educational interventions to improve health literacy, through patient understanding of their disease and treatment have been shown to improve adherence across a range of dermatological conditions (Cork et al., 2003; Miyachi et al., 2011; Moore et al., 2009; Myhill et al., 2017; Sandoval et al., 2014). One holistic approach to empower greater self-management of skin conditions is therapeutic patient



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education (TPE) which focuses on the transfer of skills, such as correct product application and treatment modulation, alongside knowledge to patients and carers (Barbarot et al., 2013; Stalder et al., 2013). Implementation of TPE through a 12 h structured education programme over several sessions has been found to improve disease coping strategies, QoL and atopic dermatitis severity in adult patients (Heratizadeh et al., 2017) These changes are likely to be a result of patients feeling more confident in their knowledge and ability to actively manage their condition, including capabilities to identify and respond to changes in the severity of their condition. Thus, it is beneficial for clinicians to consider the health literacy of patients and offer educational programmes as part of the treatment plan, where appropriate.

*G. The placebo effect*

Before the beginning of this century, most successful treatments were likely due to the placebo effect. The placebo effect is activated when the patient's positive expectations and beliefs around the treatment they are receiving results in the treatment success with patients experiencing fewer symptoms. In contrast, the nocebo effect has the opposite reaction and can lead to worsening symptoms and outcomes. The placebo effect can be effective in almost all types of physiological response systems and diseases. This effect mainly occurs due to encouraging conversations between patient and doctor, especially on topics such as effectiveness, side effects, usability, and safety. These verbal suggestions are not the only way to influence the placebo effect. Conditioning has also been seen to prompt these effects. Brain imaging technology, can gather more information about the biological and psychological mechanisms surrounding the placebo effect, confirming its occurrence. In some cases, the placebo effect can reduce the pain component more than the prescribed medication.

The context in which medication is prescribed to a patient can have a substantial effect on the outcome of the treatment. If the prescriber is open and positive about the treatment's effects and outcomes, then this can only aid in its effectiveness. One of the major conditions in dermatology that has been used to study the placebo effect is itch. One study observed how verbal suggestions can activate the placebo



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effect and influence the experience itchiness. On being told that the histamine application they will be using often increases levels of itchiness and pain, patients experienced nocebo effects, resulting in them enduring more pain and an increase in itch (Evers et al., 2018). The placebo effect was activated by verbal suggestion in a separate study with an experimental group of 46 healthy volunteers. Volunteers in the experimental group experienced a significant decrease in their itchiness, compared to another 46 healthy volunteers in the controlled group that did not undergo any noteworthy changes. Darragh et al., (2015) and Meeuwis et al.(2018) also reported on the placebo effect of explicit verbal suggestion on itchiness. When volunteers were advised that a placebo cream would cause reduced itching following histamine induced skin reactions, self-reported itch was also reduced compared to those receiving only the placebo cream. A recent general consensus among clinical experts reported that approaches to maximise placebo effects and minimizing nocebo effects can, in theory, lead to better treatment outcomes and a reduction in side effects experienced (Evers *et al.*, 2018). This suggests that verbal suggestions and conditioning protocols may have a role to play in clinical practice, however the underlying mechanisms and effectiveness of the approach in different dermatological conditions must first be elucidated.

## VIII. THE FUTURE OF TOPICAL DRUG DELIVERY

Up until about the millennium, most marketed drugs for all routes of delivery had relatively low molecular weights. However, more recent developments in drug discovery, medicinal chemistry and advances in genetic therapy and diagnostics have resulted in an increasing number of new drugs that are macromolecular and biologic in nature, with molecular weights above 1000 Daltons. This is reflected in how the number of biologics in the top ten drugs (by sales revenue) has increased over the last 5 years. With this change, the pharma industry specialising in localised topical drug delivery is frequently asking if such biologics can be delivered into and across the skin?



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As stated previously, the human skin protects the body from physical, mechanical, and chemical insults while preventing endogenous water loss. When intact, the stratum corneum protects the human body, but also severely limits passive drug delivery into and across the skin. The historical and theoretical understanding of the type of compounds that will permeate healthy skin has resulted in the '500 Dalton rule', where it is assumed that most drugs able to permeate the SC in therapeutically relevant amounts have relatively small molecular weights (that is, less than 500 Daltons), are moderately lipophilic (log P 1.0–3.5) and are highly potent. However, as ever there are contradictions to this 'rule of thumb', and there are some marketed topical products that contain larger molecules (500-1000 Da) but these are largely for use on skin that may have a compromised barrier function because of localised disease such as psoriasis and atopic dermatitis. Indeed, 93% of approved topical products contain actives below 500-600 Daltons and the remaining 7% of the products contain actives from of 600-1,000 Daltons (www.citeline.com/products/pipeline). However, it is also recognised by the Pharma industry that diseased skin with its damaged stratum corneum, differing turnover, structural and physicochemical properties must have unique barrier properties that could provide potential opportunities for larger molecule delivery.

Up until recently the lack of any marketed topical products where the active has a molecular weight of over 1000 Da. certainly supported the view that topical biologic delivery may not be possible. However, it is important to note that the classification of a 'biologic' is a general term and can be used to reference proteins, peptides, antibodies, oligonucleotides, genes and the delivery vector etc where characterisation by Log P, solubility and molecular weight may be an oversimplification. For passive delivery to and across the complex barrier that is the skin, other physicochemical properties like molecular volume and conformational structure may also be key discriminators which, along with potency, may ultimately determine the chances of success for topical biologics. It still remains the case that little if anything is known about how skin disease affects drug absorption.



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Nevertheless, there are several instances in the published scientific literature claiming the successful topical delivery of macromolecules/biologics. The type of molecules investigated in these publications include aptamers, hyaluronic acid, anti-sense oligonucleotides, viruses, genes, SiRNA and nucleic acids. An in-depth discussion of this work is not appropriate here but a critical evaluation of much of this literature (M. Brown & Lenn, 2022; J. Lenn, 2016) suggests that some of the methodologies used are misleading and contain experimental artifacts that may be responsible for reaching the conclusions made. For example, many researchers have used inappropriate animal models, such as rodents and/or minipigs, either *in vitro* or *in vivo*, to assess the passive delivery of large molecules. Furthermore, several of these studies have been shown to be misleading because of inappropriate experimental design and validation and/or a lack of controls, thus making the conclusions debatable. The current lack of translation of such studies into successful clinical trials and/or products further supports some of the current scepticism and has led to a large commercial and academic effort to find ways of influencing the barrier function of skin through the use of complex formulations such as micro and nano technologies and perturbation using physical and/or active techniques such as abrasion, microporation, magnetophoresis, ultrasound, the use of microneedles and iontophoresis (Tapfumaneyi et al., 2022). Companies engaged in this type of research and development have come and gone in quite large numbers over the last twenty years, with hundreds of millions of dollars of speculative investment being spent with, as yet, little or no return. Nevertheless, the fact remains that some studies that appear to demonstrate the passive delivery of certain biologics to the skin cannot be so simply dismissed, with hypotheses around such delivery being linked to biologic specific physicochemical properties, structural conformation, potency and the lack of understanding of the barrier properties of diseased skin. (J. D. Lenn et al., 2018). As such in 2019, 35% of dermatological drug development programs involved biologics and of these, 1 in 5 were topical.

Probably the most encouraging development over the last 75 years is in the treatment of the orphan disease Dystrophic epidermolysis bullosa (DEB). In 2023, Vyjuvek was approved by the U.S. Food



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and Drug Administration (FDA) as the first topical gene therapy treatment for patients 6 months of age and older with dystrophic epidermolysis bullosa (DEB) who have mutations in the COL7A1 gene. Aside from being the first drug to treat this disease, Vyjuvek is also the first topical gene therapy to gain approval by with regulators. The topical gel is a genetically modified herpes simplex type 1 virus that delivers copies of the COL7A1 gene when directly applied to DEB wounds. In a 31-patient clinical trial, 65% of Vyjuvek-treated wounds closed completely, compared with 26% of placebo-treated wounds, the FDA said in its release. 'The approval covers patients 6 months of age and older and is likely to facilitate further investment in this type of topical drug delivery for the orphan disease market of $125 billion' so watch this space.

Another interesting and futuristic area of study in topical drug delivery is the use of nanobots which are a type of robot operating at the nanoscale (50-100 nm) which can carry and target a drug payload and are already being investigated in cancer treatment due to their autonomous movement and navigation in biological media.

Whatever develops over the next few years all the signs are that the development of topical therapies for the treatment of skin disease will continue to grow as the patient population gets older. However, it is likely that the growing appreciation of the effect of orphan skin diseases on a patients QoL will likely mean that this will provide a further push to understand the how barrier properties of diseased skin differ from those of healthy skin and thus open up for a whole new area of how drug targets previously believed to be unreachable based on conventional theory may now be a possibility for drugs and biologics of varying physicochemical properties.



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## IX. DATA AVAILABILITY SENTENCE

The authors declare that all the data supporting the findings of this study are contained within the paper.

## X. FOOT NOTES

This work received no external funding

No author has an actual or perceived conflict of interest with the contents of this article

**Figure Legends**

Figure 1: Approximate timeline of ingredients, formulations or methods for topical or transdermal drug delivery.

Figure 2: A diagrammatical cross-section through human skin. From, Williams (2003).

Figure 3: Illustration of the distinct layers of the epidermis: stratum corneum, stratum granulosum, stratum spinosum and stratum basale. Or Illustration of the distinct layers of the epidermis: stratum corneum, stratum granulosum, stratum spinosum, and stratum basale (Brown & Williams, 2019).

Figure 4: Illustration with electron micrograph of the “Bricks and Mortar” structure of human stratum corneum.



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Figure 5: Lateral and lamellar packing of stratum corneum lipids, adapted from van Smeden et al. (2014) with permission from Elsevier.

Figure 6: Market volume. of drugs for dermatological diseases in Europe and North America. (Produced by authors from www.citeline.com/products/pipeline).

Figure 7: Market volume of topical drugs in Europe and North America. (Produced by authors from www.citeline.com/products/pipeline).

Figure 8: A representation of the principal mechanisms and pathways operating during transdermal and topical drug delivery. From Williams AC., 2003, with permission.

Figure 9: Representation of different mechanisms of action of penetration enhancers on the intercellular lipid matrix of the stratum corneum, from Williams and Barry 2004, with permission from Elsevier.

Figure 10: The cumulative amount of drug permeation with time following (A) an infinite dose and (B) a finite dose application of a product to human skin. Steady state drug flux (Jss) can be obtained from the gradient of the linear portion of the infinite dose profile. Maximal drug flux (Jmax) can be obtained from the pseudo steady state (nonlinear) portion of the finite dose profile.

Figure 11: Typical permeation profile for a finite dose application to human, from Brown & Williams (2019) with permission from MedPharm.



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Figure 12: Common considerations for topical product development, from Brown & Williams (2019) with permission from MedPharm.

Figure 13: Considerations for a Target Product Profile, from (Brown & Williams, 2019). with permission from MedPharm.

Figure 14: Flow chart summarising key events of a typical topical semi-solid formulation development program from Brown & Williams, (2019) with permission from MedPharm.

Figure 15: Summary of typical topical dosage forms adapted from Brown & Williams (2019)., with permission from MedPharm.

Figure 16: Classification of colloidal drug delivery systems, along with examples of systems that have been utilised as topical and or transdermal vehicles, adapted from Singh et al. (2021), with permission from MedPharm.



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**Tables**

Table 1: Examples of products dated to classical antiquity (700 BCE – 150 CE) and their corresponding indications.

| **Substance / Formulation** | **Indication(s)** | **Reference** |
|---|---|---|
| Animal Bile: bear, chicken, carp, dog, elephant, goat, horse, vipers, python. | Infected wounds, ulcers. | (Wang & Carey, 2014) |
| Starlight ('astral radiation') in combination with topically applied formulations (salves, balms, oils). | Not well defined, but possibly indicative of an understanding of chronopharmacokinetics. | (Wee, 2014) |
| Various shellfish, cephalopods and marine sponges. | Sore wounds, burns, depilation, anti-pruritic, cracked skin, anti-inflammatory, scorpion bites, warts, psoriasis. | (Voultsiadou, 2010) |
| Mandrake (Mandragora). | Topical analgesia. | (Finch & Drummond, 2015) |
| Cold therapy (ice, snow-salt mixture). | Topical analgesia and anti-inflammatory. | (Dasa et al., 2014) |
| Figs | Cutaneous anthrax. | (Ben-Noun, 2003) |
| Black nightshade and bearded iris. | Skin cancers. | (Iranzadasl et al., 2020) |
| Opium patch (Olympic Victor's dark ointment; OVDO). | Local and systemic pain relief. | (Finch & Drummond, 2015; Harrison et al., 2012) |
| Hot, hard boiled pigeon egg. | Pruritus ani. | (Liddell, 2006) |
| Donkey milk. | Skin peel. | (Ursin et al., 2018) |
| Seaweed | Sunburn. | (Mouës et al., 2009) |
| Coal tar. | Anti-inflammatory. | (McLean & Irvine, 2013) |
| Crushed horn from a lusty stag. | Skin brightening. | (Ali et al., 2013) |
| Three yellow lotion (radix scutellariae, rhizoma coptidis, cortex phellodendri) combined with heat. | Antiseptic. | (Zhao & Miao, 2017) |
| Snail secretions | Anti-inflammatory. | (Liu et al., 2017) |
| Pig fat cooked with willow bark | Anti-inflammatory, astringent and anti-bacterial for burns. | (Kopp et al., 2003) |



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Table 2: Examples of contemporary, dermatological agents derived from ancient medicines.

| Active Ingredient | Original organism or source. | Medical indication | Reference |
|---|---|---|---|
| Capsaicin | Capsicum spp. | Pain relief for neuropathic conditions. | (Markovits & Gilhar, 1997) |
| Canthradin | Cantharidin spp. | Wart and callus removal. | (Akdemir et al., 2011) |
| Dithranol | Araroba tree bark. | Psoriasis treatment. | (Sehgal et al., 2014) |
| Ingenol mebutate | Euphoria peplus (petty spurge) | Actinic keratosis. | (Heron Bs & Feldman, 2021) |
| Podofilox | Podophyllum spp. | Genital warts treatment. | (De Clercq & Li, 2016) |
| Podophyllotoxin | Podophyllum spp. | Genital warts treatment. | (Phillips & Dover, 1992) |
| Psoralen | Psotalea corylifolia. | Psoriasis and vitiligo treatment. | (Halpern et al., 2000) |
| Tretinoin | Cod liver oil, animal fats. | Acne and skin aging treatment. | (Webster, 1998) |
| Tricholoacetic acid | Derived from vinegar. | Skin peeling and warts treatment. | (K. C. Lee et al., 2019) |

Table 3: Differences between acute and chronic radiation dermatitis. Produced by authors from from Elston, 2010; Hegedus et al., 2017.

| | |
|---|---|
| Acute radiation dermatitis | Primary transient erythema, lasting three days<br>Persistent erythema, which reaches a peak in 2 weeks and is |



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| | |
|---|---|
| ↓<br>Within 90 days of initiation | painful.<br>Hyperpigmentation (about day 20)<br>Skin dryness<br>Hair loss<br>Dry desquamation<br>Skin scaling and flaking<br>Moist desquamation<br>Blistering erosions and ulceration may occur<br>Permanent scarring may occur |
| Chronic radiation dermatitis<br>↓<br>More than 90 days after initiation of radiation | Occurs after fractional but relatively intensive therapy with total doses of 3000–6000 rad.<br>Scars and hypopigmentation<br>Loss of all skin appendages<br>Epidermal and dermal atrophy<br>Fibrosis<br>Edema formation<br>Thickening of the dermis<br>After 2-5 years:<br>Hyper- and hypopigmentation (poikiloderma)<br>Telangiectasia<br>Superficial venules become ectatic<br>Hyperkeratoses (x-ray keratoses)<br>Necrosis and painful ulceration: Rare side effects that occur at accidental exposure or error in dose |

Table 4: Classical pattern of Rosacea progression.

| | |
|---|---|
| Early | Episodic flushing<br>Mild telangiectasia |



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| | |
|---|---|
| | Transient oedema |
| Progressive | Papules<br>Pustules<br>Sustained oedema<br>Extensive telangiectasia |
| Late | Induration<br>Rhinophyma |

Table 5: Ecological division of dermatophytes with representatives.

| Ecological division | Mode of transmission | Dermatophytes |
|---|---|---|
| Anthropophillic | Person-to-person transmission by fomites and by direct contact | *Trichophyton* spp.: *T. Rubrum*<br>*T. mentagrophytes (interdigitale)*<br>*T. schoenleinii*<br>*T. tonsurans*<br>*T. violaceum*<br>*Microsporum audouinii*<br>*Epidermophyton floccosum* |
| Zoophilic or geophilic | Animal-to-human by direct contact or by fomites | *Trichophyton* spp.*: T. equinum*<br>*T. mentagrophytes (*var. *mentagrophytes)*<br>*T. verrucosum*<br>*Microsporum. canis* |
| Geophilic | Environmental | *Microsporum spp.: M. gypseum, M. nanum.* |

Table 6: Potential Critical Process Parameters for topical formulations.

| CPP | Criticality and considerations moving through the different scales of manufacture |
|---|---|



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| | |
|---|---|
| Drug dissolution time | The dissolution step is of paramount importance and dependent on the intrinsic solubility of the API, energy (agitation and temperature) applied and particle size and polymorphic form of the API. As scale of manufacture increases so to is the time for complete drug dissolution |
| Order of addition | Important to ensure an efficient process and de-risk any stability issues. Consideration of the mixing vessels used at different scales and the potential for the same process to be followed for example a heat labile API that is present in a formulation phase for a short heating duration at laboratory scale may needed to be added later in the process should the heating duration increase at a larger scale |
| Agitation parameters | Both mixing and homogenization introduce energy by way of mobility and shear and can have a marked impact on formulation microstructure. The amount of impact is typically dependent on the mechanical power and therefore mixer/homogenization speeds and times are critical to be investigated. |
| Heating | The heating process and time should be sufficient to ensure the quality of the final product but for both environmental reasons and to mitigate the risk of degradation of components by heat this should be kept to a minimum while not impacting formulation microstructure. |
| Cooling rate | Cooling rates can have a significant impact on the formulation microstructure for formulations which contain excipients which thicken at room temperature and can be used to control the final product characteristics. Where crystallisation and the formation of matrices occur quickly, the resultant microstructure tends to be disorganised, less stable and more fluid. The greater order resulting from faster cooling and the commensurate higher energies implicated in phase transition generally lead to a more ordered structure, higher viscosity and greater stability. |
| Hold time | Each manufacturing process involves various stages. It is important to understand the duration of the time that each stage can be held before proceeding to the next stage of manufacturing without affecting the quality of the end product. |



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| | |
|---|---|
| | The hold time for different manufacturing stages is achieved through hold time studies |
| Fill temperature and product curing | Various factors may influence the filling of semi-solid product into its primary container. Some of the factors that need to be considered during scale-up include the rheology of the product and the flow behaviour of the product when subjected to stress and the physical stability and recovery of the product after being exposed to the filling stresses and pressures on a filling machine. For certain products this may mean filling at an elevated temperature and subsequent cooling and product formation within the tube. This can aid not only in physical stability of the product as the final cooling stage is rapid once the smaller amount is present in the container but also shortening the manufacture and fill time. |

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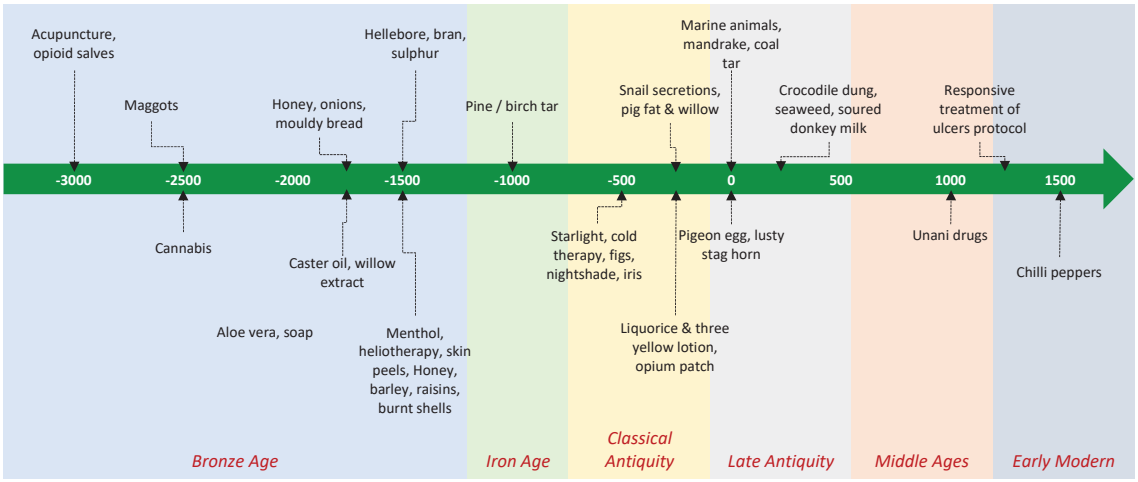


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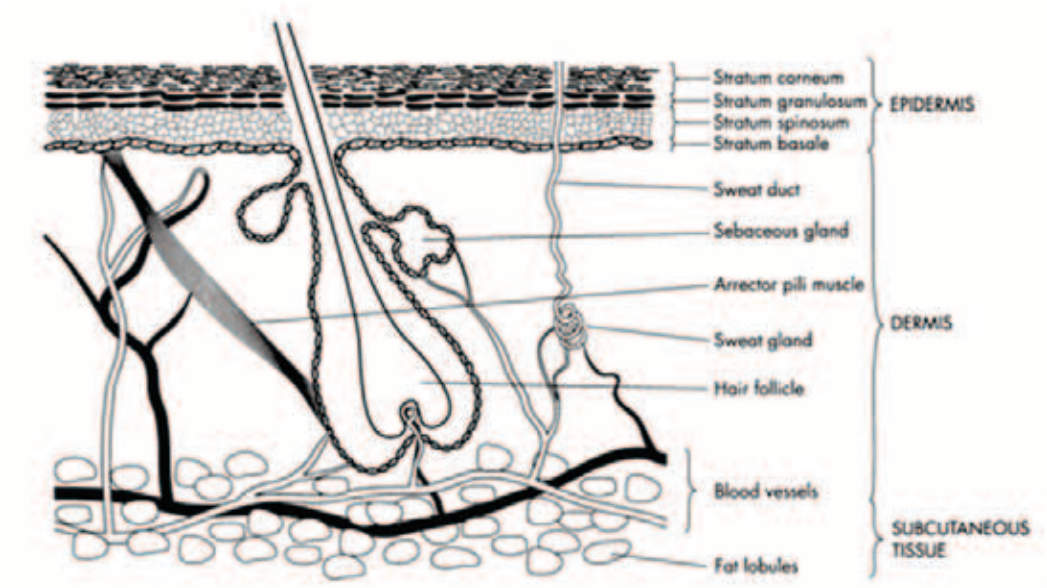


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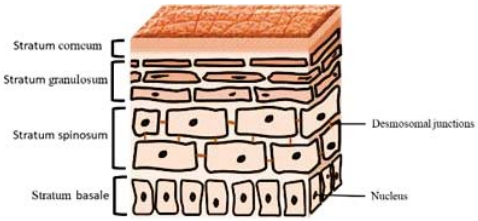


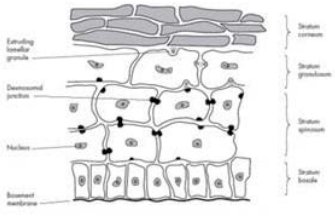

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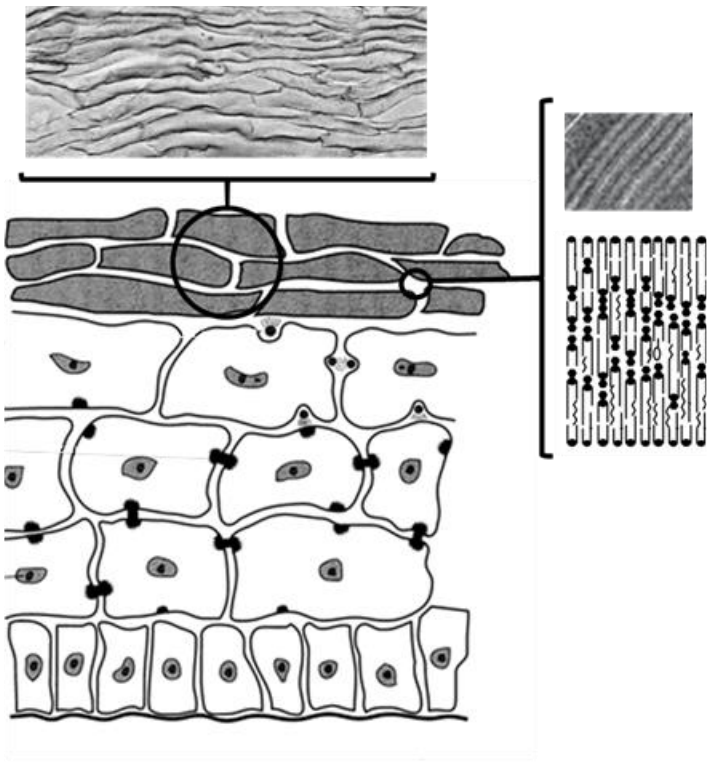

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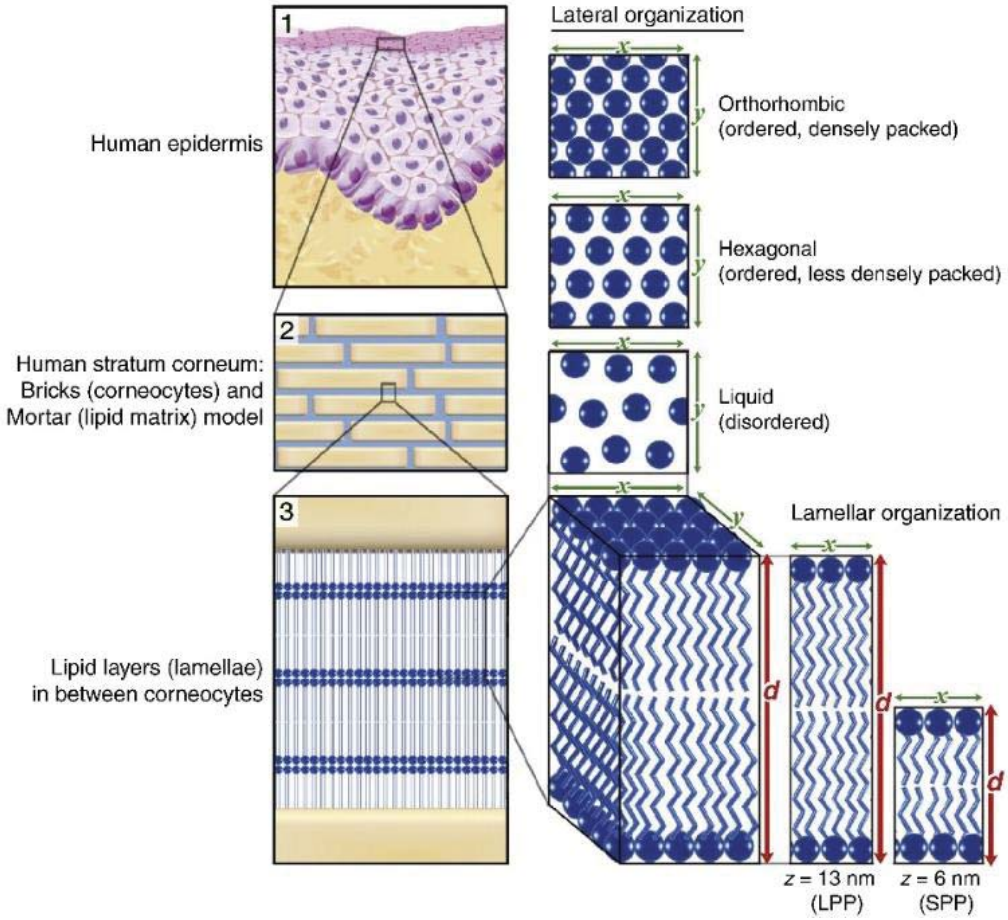


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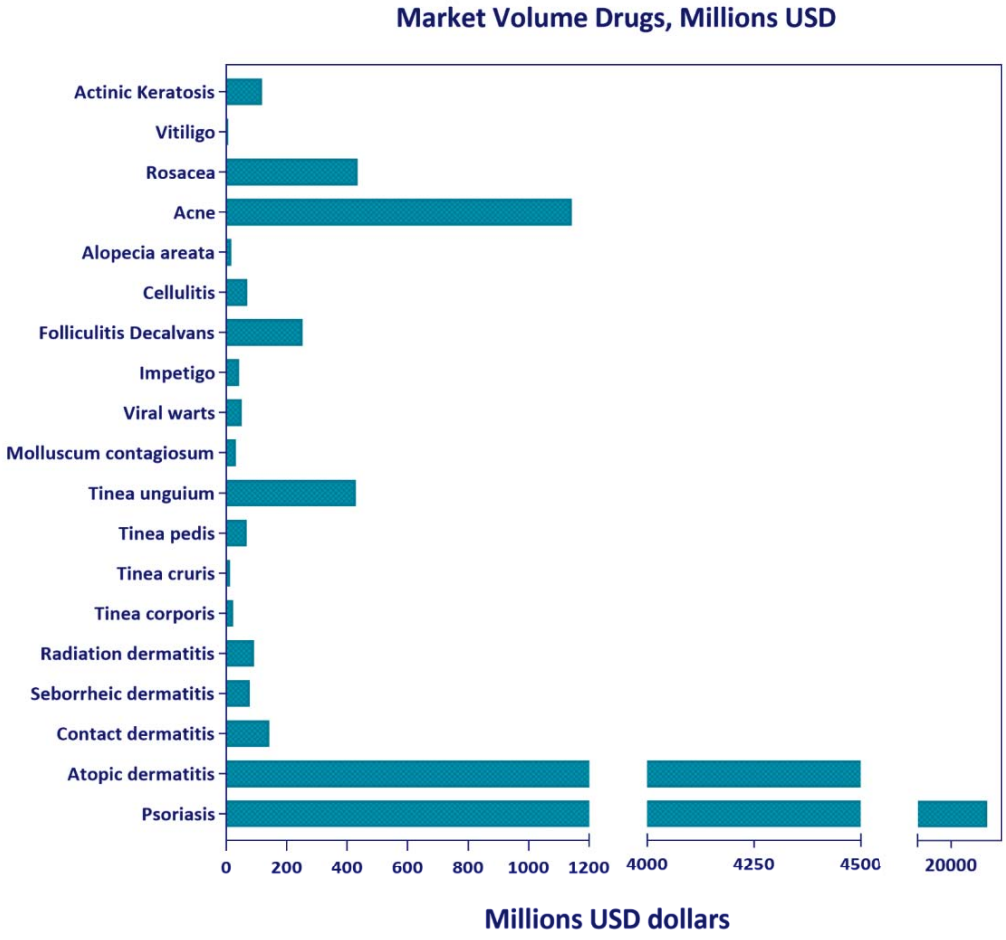


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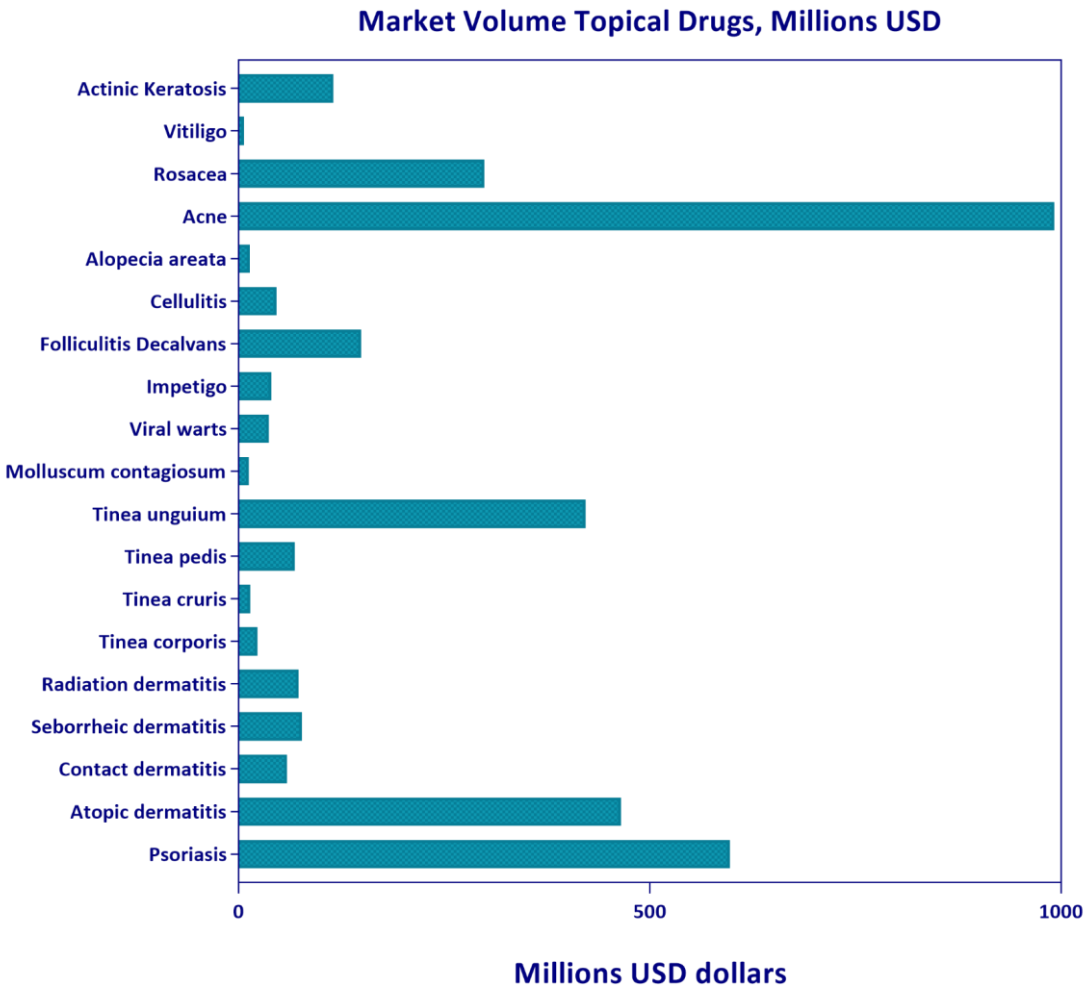
Fig 7

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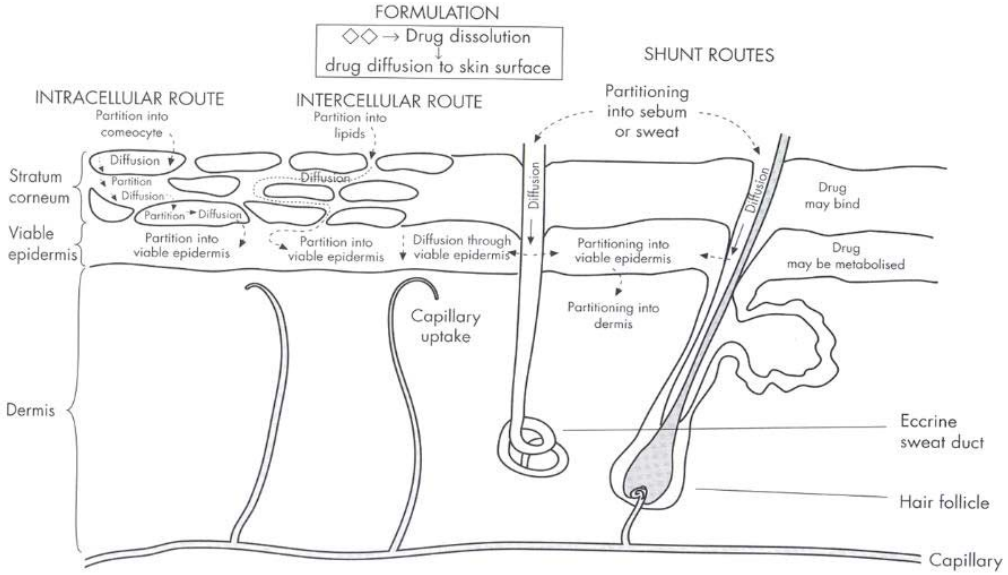


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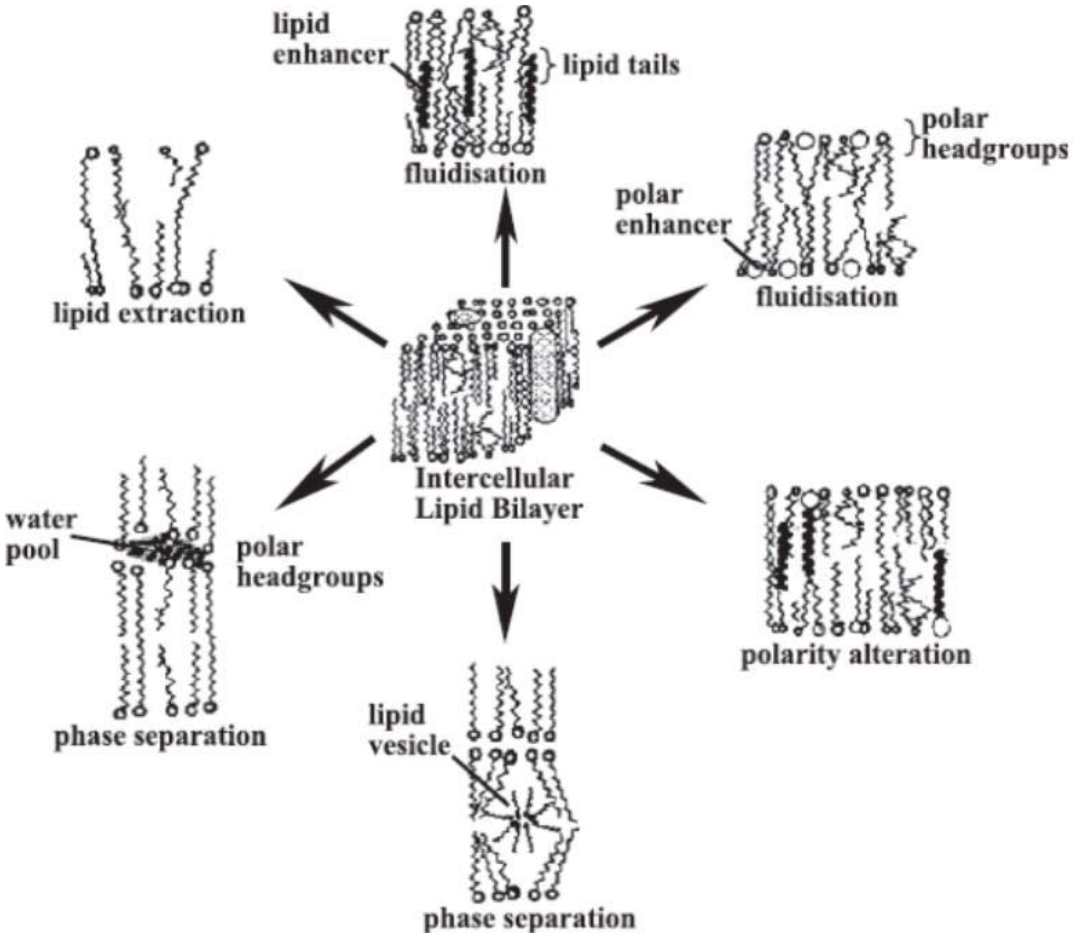


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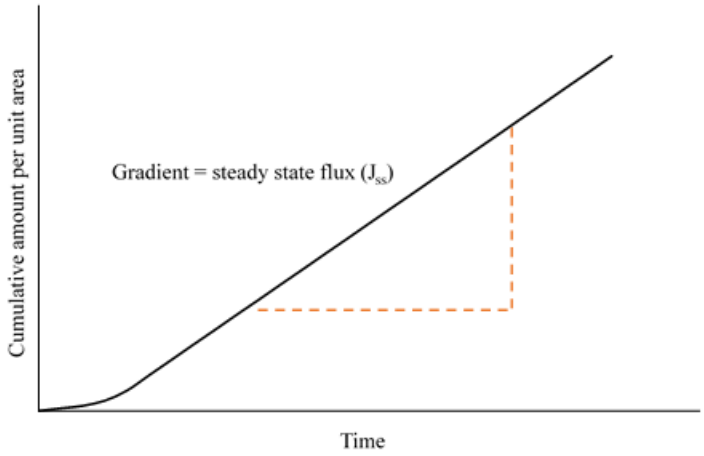
Fig 10

A¶




B¶

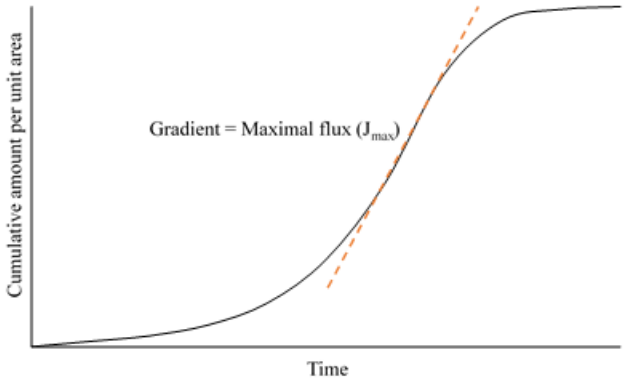



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Fig 11

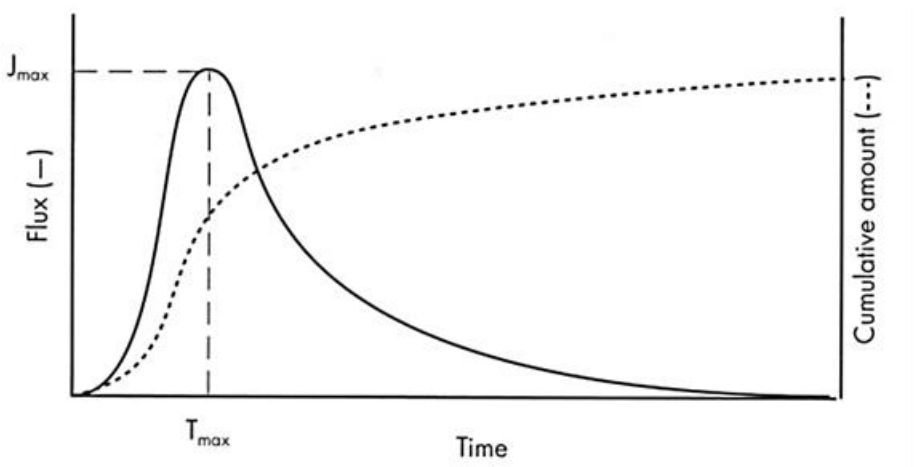


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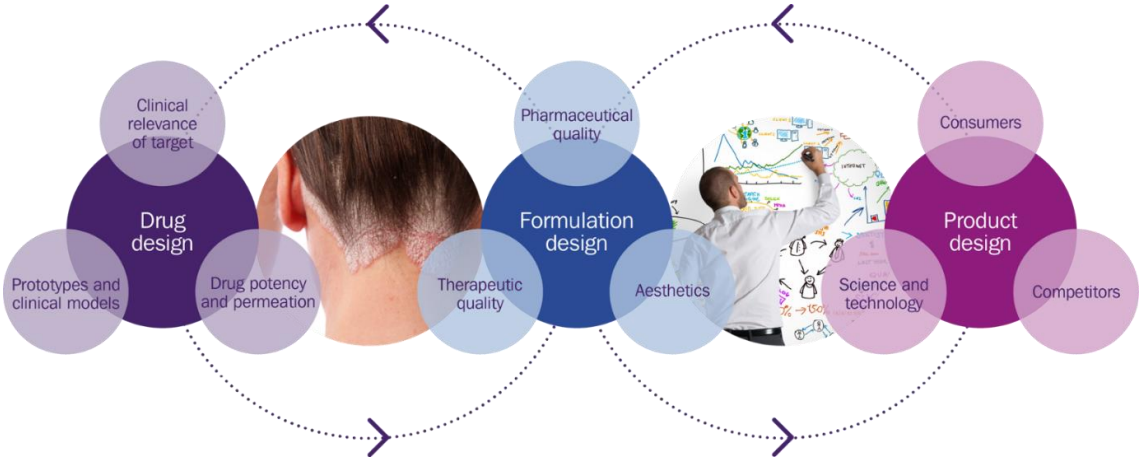
Fig 12

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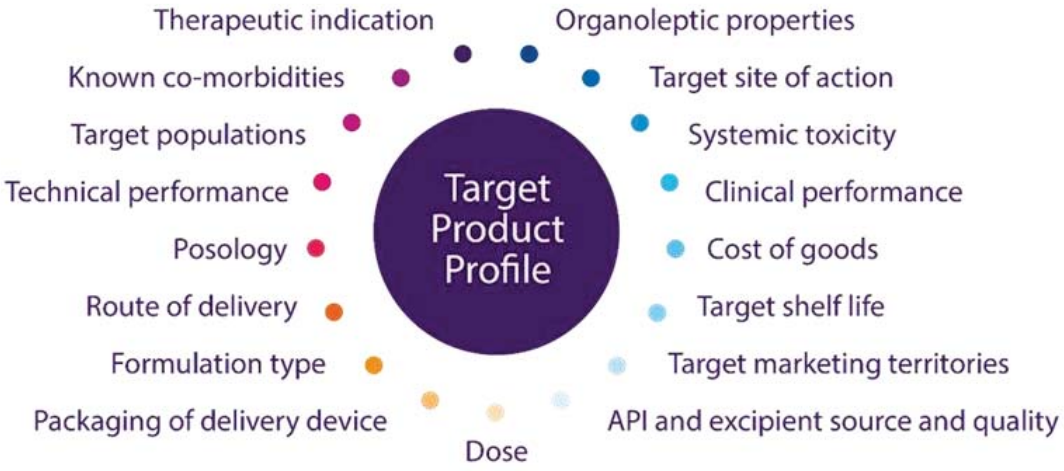


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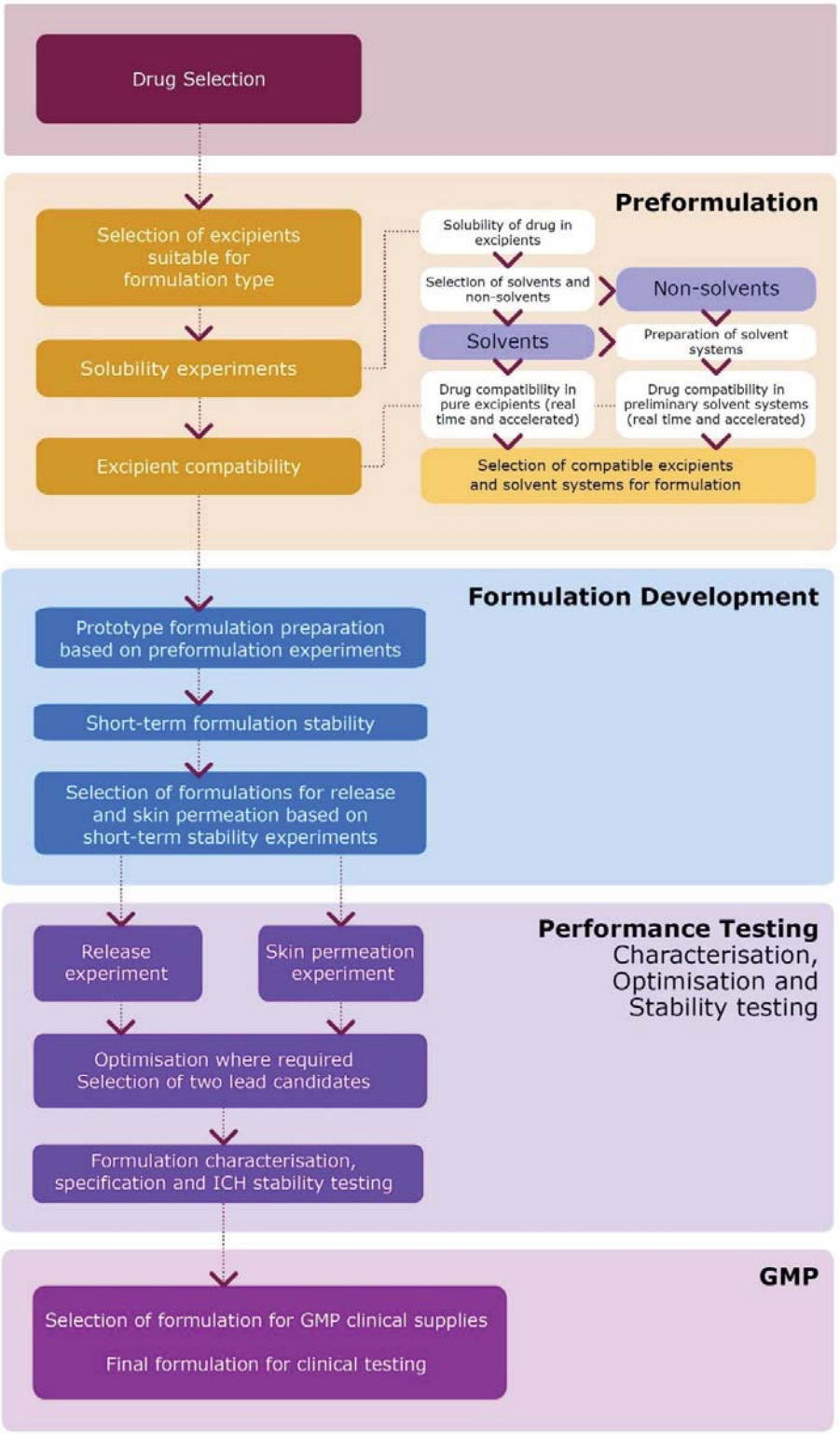


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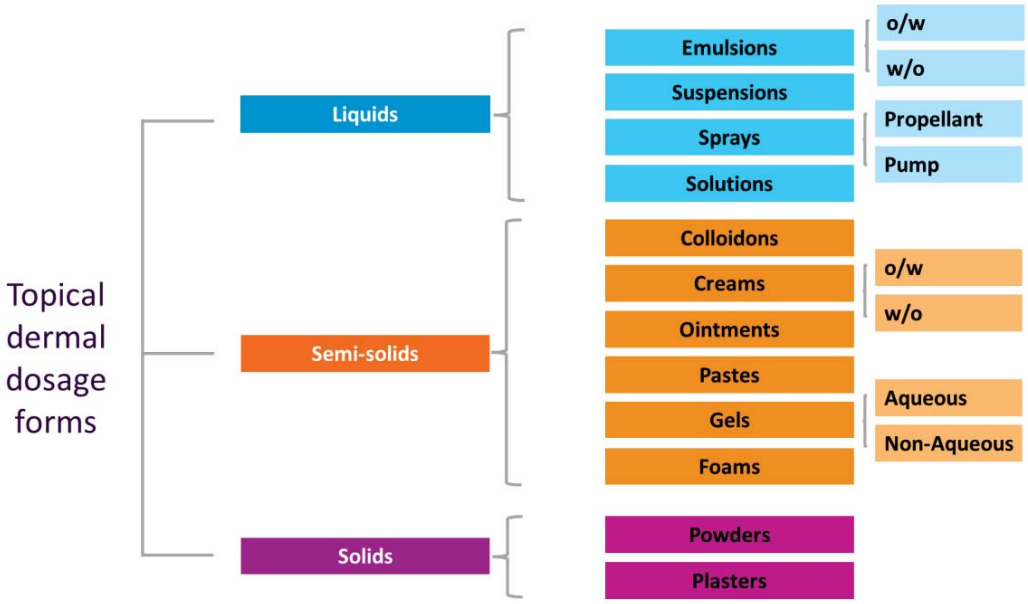


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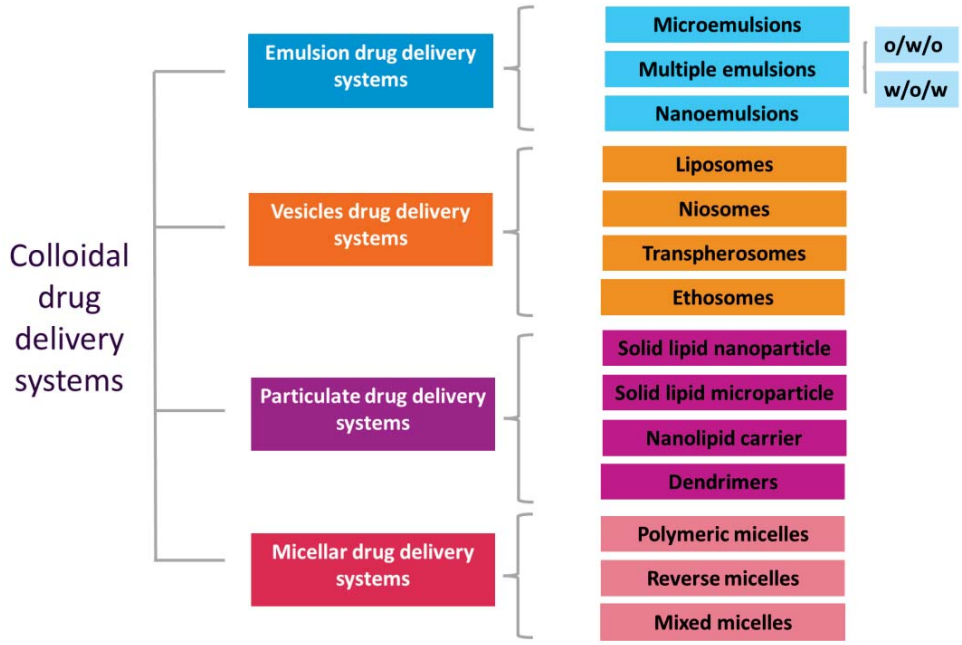


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