EGAS MONIZ SCHOOL of HEALTH & SCIENCE | INSTITUTO UNIVERSITÁRIO EGAS MONIZ

INSTITUTO UNIVERSITÁRIO EGAS MONIZ

MESTRADO INTEGRADO EM MEDICINA DENTÁRIA

POLYHYDROXYALKANOATES MEDICAL APPLICATIONS AND POTENTIAL FOR USE IN DENTISTRY

Trabalho submetido por

Rim Ben Abdeladhim

para a obtenção do grau de Mestre em Medicina Dentária

junho de 2024

EGAS MONIZ SCHOOL of HEALTH & SCIENCE | INSTITUTO UNIVERSITÁRIO EGAS MONIZ

INSTITUTO UNIVERSITÁRIO EGAS MONIZ

MESTRADO INTEGRADO EM MEDICINA DENTÁRIA

POLYHYDROXYALKANOATES: MEDICAL APPLICATIONS AND POTENTIAL FOR USE IN DENTISTRY

Trabalho submetido por

Rim Ben Abdeladhim

para a obtenção do grau de Mestre em Medicina Dentária

Trabalho orientado por

Prof. Doutor José Alexandre Reis

E co-orientado por

Prof.ª Doutora Ana Vieira

Prof.ª Doutora Catarina Dias de Almeida

junho de 2024

DEDICATION

This dissertation is dedicated to all that helped and supported me during this year.

In special to my parents Khaled and Faten, my husband Hichem, and my sons Aleskander and Hedi.

Without their tremendous understanding and encouragement, it would be impossible for me to complete my study.

ACKNOWLEDGEMENTS

First and foremost I am extremely grateful to my supervisor, Prof. Doutor José Alexandre Reis for his invaluable advice, continuous support, and patience during my thesis.

I would like also to thank Prof.ª Doutora Ana Vieira and Prof.ª Doutora Catarina Almeida, their immense knowledge and plentiful experience have encouraged me in all the time of my academic research.

I am grateful to the Instituto Universitario Egas Moniz and all its teachers and staff, for input and support throughout this programme.

RESUMO

Os polihidroxialcanoatos (PHA) são biopolímeros promissores devido à sua biodegradabilidade e biocompatibilidade, colocando-os como uma potencial alternativa aos polímeros sintéticos tradicionais. O mercado de PHA está em crescimento, principalmente na Europa, em resposta à crescente demanda por plásticos biodegradáveis e amigos do ambiente. Estes biopoliésteres são produzidos e degradados por uma variedade de microrganismos, tornando-os materiais ecológicos, ao mesmo tempo que oferecem benefícios como biocompatibilidade (quando adequadamente processados) e capacidade de biodegradação. A sua versatilidade estende-se a diversas áreas, desde a biomedicina até à agricultura e aos materiais compósitos, onde abrem caminho para inovações significativas.

No campo da medicina regenerativa, alguns PHA têm aplicações chave, nomeadamente em enxertos vasculares, regeneração de tecidos orais, tratamento de úlceras na mucosa bucal e desenvolvimento de polímeros auto-regenerativos. A sua utilização apresenta muitas vantagens, incluindo uma alternativa ecológica aos plásticos derivados do petróleo e uma solução amiga do ambiente.

Além disso, os PHA têm o potencial de serem utilizados na criação de materiais para implantes dentários. Alguns dos PHA apresentam compatibilidade com tecidos biológicos, capacidade de biodegradação e propriedades mecânicas, tornando-nos uma opção atraente para dispositivos médicos dentários. Os PHA também podem ser usados para encapsular medicamentos hidrofóbicos, proporcionando assim uma abordagem para tratamentos mais direcionados e eficazes.

Em suma, os polihidroxialcanoatos abrem novas perspectivas no campo da medicina, ao melhorar a veiculação de fármacos, e oferecer soluções ecológicas para dispositivos médicos. A sua versatilidade e propriedades únicas colocam-nos na vanguarda da inovação neste campo em constante evolução. O objetivo deste trabalho é apresentar as aplicações médicas e em medicina dentária dos PHA, suas vantagens, desvantagens e indicações, abordando temas como: produção, biocompatibilidade, utilização atual e perspetivas futuras através de uma revisão narrativa de literatura.

PALAVRAS-CHAVE

Polihidroxialcanoato, regeneração de tecidos, biomimético.

ABSTRACT

Polyhydroxyalkanoates (PHA) are promising biopolymers due to their biodegradability and biocompatibility, making them a potentially revolutionary alternative to traditional synthetic polymers. The PHA market is growing, especially in Europe, in response to the growing demand for biodegradable and environmentally friendly plastics. These biopolyesters are produced and degraded by a variety of microorganisms, making them environmentally friendly materials, while offering benefits such as biocompatibility (when adequately processed) and biodegradability. Their versatility extends to various areas, from biomedicine to agriculture and composite materials, where they pave the way for significant innovations.

In the field of regenerative medicine, some PHA have key applications, namely in vascular grafts, oral tissue regeneration, the treatment of mouth ulcers, and the development of self-healing polymers. Their use has many advantages, including an ecological alternative to petroleum-based plastics and an environmentally friendly solution.

In addition, PHA have the potential to be used in the creation of dental implant materials. Some of the PHAs have compatibility with biological tissues, biodegradability and mechanical properties, making them an attractive option for dental medical devices. PHA can also be used to encapsulate hydrophobic drugs, thus providing an approach for more targeted and effective treatments.

To summarize, polyhydroxyalkanoates open up new perspectives in the field of medicine by improving drug delivery, solving common problems and offering ecological solutions for medical devices. Their versatility and unique properties place them at the forefront of innovation in this constantly evolving field.

The aim of this thesis is to present the medical and dental applications of PHA; their advantages, disadvantages and indications, addressing topics such as: production, biocompatibility, current use and future prospects through a narrative literature review.

KEY WORDS

Polyhydroxyalkanoate, tissue-engineered substitutes, biomimetic.

GENERAL INDEX

INDEX OF FIGURES

INDEX TABLE

LIST OF ACRONYMS

ABS - Acrylonitrile Butadiene Styrene

ALG - Alginate

ALP - Alkaline phosphatase

APAC - Asian-Pacific American Council

ATR-FTIR – Attenuated total reflectance Fourier transform infrared

CAD - Computer Aided Design

Caff - Caffeic acid

CAGR - Compound annual growth rate

CAM - Chorioallantoic Membrane

CHA - Calcined hydroxyapatite

COVID - CoronaVirus Disease

CT - Computed Tomography

DNA – Deoxyribonucleic Acid

DSMZ - Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH

ECM - Extracellular Matrix

EU - European Union

FA - Folic Acid

FDA - Food and Drug Administration

FDM - Fused Deposition Modeling

GBL - Gamma-Butyrolactone

HA - Hydroxyapatite

HBMSCs - Human Bone Narrow Stromal Cells

HOBs - Human Maxillary Osteoblast

HVTE- Heart Valve Tissue Engineering

LPS – Lipopolysaccharides

lcl – Long chain length

mcl - Medium chain length

MRI - Magnetic Resonance Imaging

nBG - Nano-sized Bioactive Glass

NP - Nanoparticle

PCL - Poly(ε-caprolactone)

PEG - Poly(ethylene glycol)

PEP - Phosphoenolpyruvate

PGA - Poly(glycolic acid)

PHA - Polyhydroxyalkanoate

PHB - Polyhydroxybutyrate

PHBV - Polyhydroxybutyrate-co-valerate

P4HB - Poly(4-Hydroxybutyrate)

P3HB - Poly(3-Hydroxybutyrate)

P3HB4HB - Poly(3-hydroxybutyrate-co-4-hydroxybutyrate)

P3HO - Poly(3-hydroxyoctanoate)

PHV - Poly (3-hydroxyvalerate)

PLA - Polylactic acid

PLCL – Poly(L-lactide-co–caprolactone)

PLGA - Polylactic-co-glycolic acid

PLLA - Poly(L-lactic acid)

POSCO - Formerly Pohang Iron and Steel Company

PTFE - Polytetrafluoroethylene

PVA - Polyvinyl Alcohol

SBF - Simulated Body Fluid

SBR - Sequencing Batch Reactors

scl - Short chain length

SEM - Scanning Electron Microscopy

SLS - Selective Laser Sintering

β-TCP - β-Tricalcium Phosphate

TC - Tetracycline

Td - Decomposition temperature

Tg – Glass transition temperature

Tm - Melting Temperature

UK - United Kingdom

USA - United States of America

USD - United States Dollar

UV - Ultraviolet

5HV - 5 Hydroxyvaleric acid

INTRODUCTION

Polyhydroxyalkanoates (PHA) represent a class of biodegradable polymers that have garnered significant attention in the medical field due to their versatile properties and potential applications. PHAs are naturally occurring aliphatic polyesters synthesized by various microorganisms as intracellular carbon and energy storage compounds (Raza et al., 2018).

The green perspective of PHAs stems from their bio-based origin and biodegradable properties, positioning them as eco-friendly alternatives to petroleum-derived plastics. Studies have emphasized the compostability and minimal ecological footprint of PHAs compared to conventional plastics, aligning with sustainability and environmental responsibility principles (Behera et al., 2022).

Furthermore, their ability to interact well with biological systems, ability to break down naturally, and ability to have adjustable physical properties make them highly favorable options for a wide range of medical uses, such as creating new tissues, delivering medications, and wound healing (He et al., 2014).

Recently, research has increasingly concentrated on exploring the potential of PHAs in dentistry. The unique characteristics of PHAs, such as their ability to replicate the physical characteristics of organic tissues and their breakdown into harmless substances, make them particularly attractive for dental applications. From sutures to scaffolds for tissue regeneration in oral surgery, PHAs offer a sustainable and biocompatible alternative to traditional materials used in dentistry (Conte et al., 2017).

This thesis aims to present a comprehensive overview of the actual state of research on PHAs, emphasizing their medical applications and explicitly looking into their potential utilization in dentistry. By exploring the production methods, properties, degradation mechanisms, and existing applications of PHAs in medicine and dentistry, this thesis will elucidate the opportunities and challenges associated with harnessing these biopolymers for clinical use.

As biomaterials continue to evolve, PHAs stand out as a sustainable and innovative class of polymers with immense potential for transforming various aspects of medical practice, particularly in dentistry.

DEVELOPMENT

1 Overview of Polyhydroxyalkanoates (PHA)

1.1 Introduction to PHA

The increasing buildup of petrochemical plastic trash in the environment is becoming a significant worry. To find alternatives, some researchers have focused their attention on polyhydroxyalkanoates, which are entirely biodegradable polymers made from bacterial cells and share material properties comparable to some conventional plastics. Unlike traditional plastics, polyhydroxyalkanoates are biopolymers developed and broken down by a diverse range of microorganisms, which accumulate within microbial cells, mostly under conditions of nutrient imbalance (Shahid et al., 2021).

Currently, there have been more than 150 distinct monomers identified for the biosynthesis of PHAs, which makes them the most extensive category of natural polyesters. Table 1 provides representative instances of PHAs: (Mai et al., 2024).

Table 1: Illustrative instances of PHA homopolymers (Mai et al., 2024)

Chemical name	Abbreviation	Side groups
Poly(3-hydroxybutyrate)	P3HB	Methyl
Poly(4-hydroxybutyrate)	P4HB	Hydrogen
Poly(3-hydroxyvalerate)	P3HV	Ethyl
Poly(3-hydroxyhexanoate)	P3HHx	Propyl
Poly(3-hydroxyheptanoate)	P3HHp	Butyl
Poly(3-hydroxyoctanoate)	P3HO	Pentyl
Poly(3-hydroxynonanoate)	P3HN	Hexyl

The most frequently synthesized bacterial biopolymers belong to the PHAs family and their derivatives. These polyesters include short-chain monomeric components, such as 3HB, as well as medium-chain length monomeric components like 3HO and 3HD (Vicente et al., 2023).

Microorganisms generate polyhydroxyalkanoate (PHA) to build lipid inclusions that are used by cellular structures to store energy in granular forms. The investigation of polyhydroxybutyrate (PHB) can be traced back to 1925, when Lemoigne, a French scientist, discovered PHB in *Bacillus megaterium* (Lemoigne, 1926). Subsequently, over 90 families of bacteria, encompassing the two Gram-positive and Gram-negative types,

have been acknowledged as PHA developers, flourishing in anaerobic as well as aerobic environments (Tao et al., 2009).

Since 1925, multiple forms of PHA were discovered and are available in sufficient quantity for different applications. Some of the commonly known ones include (Ansari et al., 2021) :

- polyhydroxybutyrate,
- poly(3-hydroxybutyrate-co-3-hydroxyhexanoate),
- polyhydroxybutyrate-co-valerate,
- poly(4-hydroxybutyrate),
- copolymers of 3-hydroxybutyrate,
- 4-hydroxybutyrate.

Examination of isolated and treated PHAs uncovers intriguing characteristics such as the capacity to break down naturally and, when properly processed, the ability to interact well with living organisms, lack of cancer-causing qualities, and desirable thermal and mechanical characteristics. PHA has a significant level of structural variety since it has a large number of monomers available. Given these characteristics, this particular polymeric substance has significant practical worth in the field of healing and regenerating tissue (Peng et al., 2011).

1.2 Types of PHA

As shown in Figure 1, the basic structure of a PHA polymer molecule consists of monomeric units with an R side chain, linked by ester bonds. The side chain can be a saturated, unsaturated, modified, or branched alkyl group, depending on the molecule (Sharma et al., 2021).

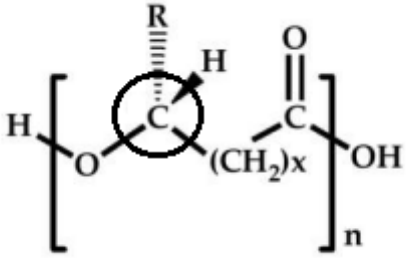

Figure 1: General chemical structure of biopolymer polyhydroxyalkanoate. (Adapted from Pulingam et al., 2022).

PHAs may be classified into three categories according to the amount of carbon atoms present in the monomers (Raza et al., 2018):

- short chain length PHA (scl-PHAs) consist of monomers containing less than five carbon atoms,
- medium chain length PHA (mcl-PHAs), ranging from five to fourteen carbon atoms,
- long chain length PHA (lcl-PHAs) with over fourteen carbon atoms; lcl-PHAs are rare and have been investigated to a lesser extent.

The type and microstructure of the produced PHA is partly related to the C-sources used for biosynthesis. Homopolymers, block or random copolymers, and even terpolymers can be synthesized (Cavalheiro et al., 2013).

In simple terms, a copolymer is a polymer made from more than one species of monomer. In fact, most of the copolymers are actually blends of various copolymers with different chemical composition. (Hagiopol, 2016).

Block copolymers consist of repeated sequences of monomers of the same type, while in random copolymers the monomers are randomly distributed throughout the polymeric chain (Feng et al., 2017).

1.2.1 scl-PHA

The monomeric building units of scl-PHA have three to five carbons each.

Most homopolymers of scl-PHA exhibit inadequate flexibility and fragility, and lack the exceptional mechanical characteristics required for medical and packaging material applications when used alone (S. Wu et al., 2007). Nonetheless this is not always the case. An exception to this trend is P(4HB), the first PHA to be approved by FDA (2007) to be commercialized for medical applications ("TephaFLEX®Absorbable Suture") (Carlson, 2007).

Some bacteria are able to produce different types of scl-PHAs. *Cupriavidus necator,* as do other bacteria, produces poly(3-hydroxyvalerate) (PHV), which is composed of monomers with 5 carbon atoms, and poly(3-hydroxybutyrate) (P3HB), which consists of monomers with 4 carbon atoms. A PHA copolymer with a short chain length, P(3HB-co-4HB), is categorized as an appropriate absorbable biomaterial for use in pharmaceutical and medical applications. Its specific applications include tissue engineering, orthopedics, wound healing, therapies, and drug administration. By adding 4HB monomer, copolymers with greater *in vivo* breakdown rates are produced, which improves biocompatibility without having negative side effects. The fact that P(3HB-co-4HB) may be modified to display a broad range of mechanical strengths from elastic rubber-like

materials to extremely crystalline plastics makes it applicable to several medical applications (Huong et al., 2021).

A more uniform surface for cell attachment is provided by P(3HB-co-4HB) with a high proportion of 4HB monomer. This suggests that the arrangement of the copolymer, particularly the proportion of 4HB monomers, can influence the surface characteristics, making it more conducive for cell adhesion. This property is crucial in tissue engineering applications where promoting cell attachment is essential for tissue growth and development (Vigneswari et al., 2019).

Applicability for the delivery of drugs is shown by the copolymer P(3HB-co-4HB), specifically for the encapsulation of hydrophobic drugs such as rifampicin. This indicates that P(3HB-co-4HB) could be used in the formulation development of nanoparticles for drug delivery purposes. The hydrophobic nature of the copolymer makes it suitable for encapsulating drugs with similar properties, which could have implications in controlled drug release systems (Chokshi et al., 2018).

Tensile strength and biocompatibility of P(3HB-co-4HB) make it suitable for synthesizing tri-leaflet prosthetic heart valves. This suggests that the copolymer has mechanical properties that are well-suited for applications in the cardiovascular field. Using P(3HB-co-4HB) in prosthetic heart valves implies that it can withstand the dynamic mechanical stresses in the cardiovascular system while being biocompatible, which is crucial for implantable medical devices (Salim et al., 2012).

1.2.2 mcl-PHA

The monomeric units of medium chain length PHA (mcl-PHA) have six to fourteen carbons. A specific instance is poly(3-hydroxyoctanoate), which is synthesized by *Pseudomonas mendocina* mcl-PHA exhibits high biocompatibility and biodegradability, sourced from renewable resources. In comparison to scl-PHAs, mcl-PHAs demonstrate improved mechanical properties, including reduced brittleness, lower crystallinity, a lower glass transition temperature, and higher melting temperatures, making them more flexible materials. In essence, these polymers behave like elastomers, suggesting their potential suitability for biomedical application (Dobrogojski et al., 2018).

1.2.3 lcl-PHA

PHAs with long chain lengths include monomers that build blocks with 15 or more carbons. For example, *Pseudomonas aeruginosa* produces poly (3-hydroxypentadecanoate) (Keskin et al., 2017).

The diverse composition of PHAs results in different polymer features, influencing biocompatibility, mechanical properties, and degradation times. Versatility makes PHAs suitable for various applications, including medical devices. Due to their distinct compositions, PHAs can undergo blending, surface modification, as well as combination with different polymers (Guo et al., 2022).

1.3 Properties of PHAs:

Depending on the composition of the polyhydroxyalkanoate, they can present different properties that influence their suitability for a range of applications.

1.3.1 Biodegradability

PHA exhibit unique biological properties, and one of their distinctive features is their degradability in various environments. PHA can undergo degradation through the action of mixed microbial populations present in different settings such as rivers, seawater, soil, and other environments. This degradation process can occur under both aerobic (oxygen-rich) and anoxic (oxygen-depleted) conditions (Volova et al., 2010). The microbial communities in these environments play a crucial role in breaking down PHA. Their secreted enzymes, called PHA depolymerase or hydrolases, act on PHA polymers. As a result of this enzymatic activity, PHA is broken down into low molecular weight monomers or oligomers that can then be assimilated by microbial cells as nutrients (Tang et al., 2020).

The study conducted by Qu and co-workers (2006) focused on examining the process of biological degradation of PHA in various conditions. The findings suggest that polyhydroxybutyrate and PHBV can be completely degraded in a number of circumstances (Qu et al., 2006).

Polymers may undergo biodegradation in either aerobic or anaerobic environments. In aerobic breakdown, oxygen serves as the ultimate electron acceptor. On the other hand, anaerobic decomposition employs other electron acceptors such as CO_2 and nitrates. The majority of biodegradable polymers exhibit signs of breakdown in both oxygen-rich and oxygen-poor conditions. Enzymatically degraded polymers are affected by temperature and need a temperature that is equivalent to or higher than their glass transition temperature in order to undergo efficient biodegradation. Organic carbon is converted via microorganisms in the presence of oxygen into CO_2, which is the main chemical formula for aerobic biodegradation. Anaerobic biodegradation results in the formation of methane, with the quantity of CO_2 generated being influenced by variables such as the remaining oxygen or the nature of the degraded substance (El Asri, 2022).

Industrial-scale anaerobic biodegradation has received less research attention compared to aerobic biodegradation, mostly because to the constraints associated with environmental management. Two primary anaerobic environments include biogas facilities, which capture methane for energy conversion, and landfills, where uncontrolled biodegradation can release methane. Landfills are a particular concern as they contribute significantly to methane emissions, and a large portion remains uncontrolled, contributing to environmental challenges given methane's potent greenhouse gas properties (Meereboer et al., 2020).

The degradation of PHA is subject to several factors, and the biodegradation rate is influenced by various parameters. The main factor is the PHA depolymerase enzymes' affinity for the ester linkages found in PHA polymers. Several factors affect this affinity, and they contribute to the overall degradation process. Some of these factors include (Numata et al., 2009):

- monomer type and composition;
- surface area;
- molecular weight;
- polymer conformation;
- melting temperature (Tm);
- crystallinity;
- environmental factors.

In summary scl-PHAs with a higher melting temperature and lower crystallinity degrade more rapidly than mcl-PHAs with the same characteristic. (Grigore et al., 2019).

1.3.2 Biocompatibility

The biocompatibility of PHA is a remarkable and distinctive biological characteristic that has garnered much interest. PHA has been shown to promote the proliferation of many types of tissue cells *in vitro*, such as chondrocytes, epithelial cells, and fibroblast. When inserted into live organisms, such as rats, human beings, and rabbits, PHA does not elicit a strong immunological reaction. Multiple studies have shown that PHA has exceptional biocompatibility and may even enhance cellular tissue attachment and proliferation (Wu et al, 2014).

Moreover, PHA's biocompatibility include its physiological and immunological reaction during breakdown. 3-hydroxybutyric acid (3HB), one of the monomers in the PHA polymerization chain, is a natural metabolite that is formed by the oxidation of fatty acids in the liver of humans. It is worth mentioning that 3HB is inherently found in human

blood in quantities that vary between 30 and 100 mg/L. The presence of this natural phenomenon in the body indicates that PHA is easily tolerated and embraced by the human system (Egan & D'Agostino, 2016).

3HB, a component of PHA, has additional beneficial effects. It has been found to diminish inflammation, facilitate tissue regeneration, and function as a source of energy for the body during times of necessity, such as during starvation. Ultimately, 3HB is excreted as carbon dioxide (Ang et al., 2020).

Despite other degradable polymers such as polylactic-co-glycolic acid and polylactic acid, which may cause the buildup of lactic acid products after being implanted, PHA keeps a stable pH level throughout the breakdown process. PHA's property of closely aligning with the normal metabolic processes in the human body makes it very favourable in terms of biocompatibility (Hu et al., 2009; Wu et al, 2014).

1.3.3 Non-carcinogenicity

Non-carcinogenicity is a crucial property for biopolymers intended for use in humans, and understanding the potential effects on cancer cell development is an important aspect of evaluating their safety. In the case of PHA, studies have looked at whether PHA promotes the growth of cancer cells while inhibiting their division (Peng et al., 2011; Yan et al., 2011).

Research by Peng et al., 2011 provided insights into this aspect by investigating the impact of various PHA types, including PHB, PHBHHx, and PHBV, on rat fibroblasts. The findings indicated that these PHA types did not induce carcinogenesis in the cultured rat fibroblasts. The observed accelerated cell growth on PHA was governed by the regular cell cycle. Furthermore, primary rat osteosarcoma cells cultivated in PHA did not change into tumor morphology; instead, they retained their normal shape (Tang et al., 2020).

Potential pathways via which PHA enhances tissue regeneration are believed to be associated with the response of tissue cells 3-HB and the interactions between materials and cells mediated by microRNAs. While these findings are promising, it's important to note that there is insufficient information to definitively establish that PHA does not have a cancer-causing impact on all types of cells. As a result, further research to investigate the non-carcinogenicity of PHA across various cell lines will be crucial for the continued development and application of PHA in biomedical contexts. (Peng et al., 2011).

1.3.4 Thermal properties:

The amorphous phase and the melting temperature (Tm) of the crystalline phase and the glass transition temperature (Tg) are often used to represent the thermodynamic

characteristics of PHA. The side chain length in PHA molecules has been shown to influence these thermodynamic properties (Tao et al., 2009).

Research has shown that the value of Tm generally decreases as the length of the side chain carbon increases, whereas Tg grows correspondingly. This leads to differences in thermodynamic properties between scl-PHA and mcl-PHA (Nagase et al., 2020).

For instance, P3HB has a rather high degree of crystallinity, ranging from 60% to 80%. It has a Tm of around 170°C and a decomposition temperature (Td) of roughly 200°C. Nevertheless, the little difference in temperature between Tm and Td leads to inadequate thermal stability throughout the processing phase. To address this, modifications, such as incorporating second monomers like 3HV, are often made to enhance thermal stability (Chuah et al., 2013).

On the other hand, mcl-PHA usually has a lower level of crystallinity, often about 40%. The Tg of mcl-PHA may exhibit a variety of values, spanning from -52°C to -25°C at ambient temperature. Additionally, the Tm can vary from 38°C to 80°C. Significantly, because to the low Tg of mcl-PHA, it exhibits elastic characteristics akin to rubber when exposed to suitable temperatures. When the temperature exceeds Tm, mcl-PHA undergoes a transformation into an amorphous state characterized by its viscous characteristics (Gopi et al., 2018).

In summary, the side chain length in PHA molecules significantly influences their thermodynamic properties, and modifications, particularly in mcl-PHA, can result in materials with unique elastic and viscous properties at different temperature ranges. These characteristics have implications for the processing, applications, and performance of PHA based materials in various conditions (Grigore et al., 2019).

1.3.5 Mechanical properties:

Carbon-chain length is one among the parameters that affects PHA's mechanical strength, structure, and composition. Different types of PHA exhibit varying mechanical properties, and adjustments can be made through compounding or synthetic copolymerization to suit specific applications (Singh et al., 2019).

PHB, a scl-PHA, is known for its rigidity and high strength, with a tensile strength exceeding 45 MPa (Singh et al., 2019). However, PHB has limitations such as insufficient processing speed, accelerated deterioration, and limited stretching ability. To address these drawbacks, PHB is often combined with other monomers like 4HB, 3HP, and 3HV, to decrease the level of crystallinity, enhance flexibility, and slow down the ageing process (Guo et al., 2022).

P4HB, another representative scl-PHA member, is highly thermoplastic and exhibits extensible ductility. P4HB has a maximum elongation at the point of rupture of 1,000%, which makes it one of the most elastic PHA homopolymer synthesized so far. It received FDA approval in 2007 for manufacturing surgical sutures (Williams et al., 2016).

The addition of different hydroxy acids to the copolymerization of 4HB may expand the variety of characteristics that can be achieved, allowing for the creation of resorbable elastomer compounds with a broad spectrum of durometer hardnesses, The development of these new elastomers is now underway for their application in absorbable medical devices (Williams et al., 2016).

mcl-PHA have lower tensile strength, greater elongation at break, and more elasticity than scl-PHA. mcl-PHA, as explained earlier, are copolymers consisting of monomers such as 3-hydroxyhexanoate (HHx) or 3-hydroxyhexanoate (3HO). The tensile strength of mcl-PHA varies from 5 MPa to 16.3 MPa, whereas the elongation at break varies between 88% to 356% (Gopi et al., 2018).

PHA has a broader range of applications compared to other polymers such as poly(ε-caprolactone) (PCL), polylactic acid (PLA), and polylactic-co-glycolic acid (PLGA). Moreover, the mechanical robustness of PHA may be modified by copolymerization or compounding following the demands of certain targeted settings, making it a flexible substance for many applications (Boesel et al., 2014).

1.4 Production and synthesis of PHA:

Lemoigne revealed in 1923 at the Institut Pasteur that aerobic spore-forming bacillus produced 3-hydroxybutyric acid in anaerobic suspensions. He conducted more research and accurately estimated the quantity of 3-hydroxybutyric acid produced. In 1927, he extracted a compound from Bacillus using chloroform and confirmed it was a polymer of 3-hydroxybutyric acid. However, commercial manufacture of poly(3-hydroxybutyrate) (P(3HB)) did not begin until the beginning of the 1960s. The pioneering work of Baptist and Werber at W.R. Grace & Co. (U.S.A) resulted in many patents for producing and isolating P(3HB). They started employing this polymer to make sutures and prosthetics. Their attempts were halted owing to poor fermentation yields and bacterial residues in the polymer. Additionally, the solvent extraction technique was expensive (Philip et al., 2007).

1.4.1 Fermentation :

PHA are produced and stored inside the cells of some bacterial strains, mostly when they experience disadvantageous development circumstances, such as a lack of phosphorus, nitrogen, oxygen, or magnesium, with an excess of carbon (Ojumu et al., 2004).

When selecting a microorganism for industrial production of PHA, several factors must be taken into account. These include the microorganism's ability to use a low-cost carbon source, its rate of growth, polymer production rate, and the maximum amount of polymer it can accumulate based on the substrate (Ojumu et al., 2004). To save costs, it is crucial to generate PHA with high efficiency. Various techniques, such as continuous cultivations and Fed-batch, have been used to enhance PHA's productivity (Ansari et al., 2021).

1.4.2 Fed-batch and Continuous Cultures:

In a fed-batch cultivation system, nutrients are introduced into a bioreactor (typically a stirred tank reactor) during the cultivation process. This method stands in contrast to a batch culture where all nutrients are added at the beginning of the cultivation. The fed-batch approach involves the gradual and controlled addition of nutrients, allowing for sustained growth and productivity. This cultivation process is considered a semi-open system. Although the bioreactor is sealed to maintain aseptic conditions, it is termed "semi-open" due to the ongoing, controlled nutrient additions. This flexibility in nutrient management contributes to optimizing culture conditions and improving the overall production process (Sanjogta Thapa Magar, 2021).

The primary objective of a fed-batch culture is to maximize biomass or product production. This is achieved by strategically supplying nutrients to support exponential growth over an extended period. The advantages of fed-batch cultivation include enhanced productivity, higher cell densities, and prolonged product synthesis (Sanjogta Thapa Magar, 2021).

The success of a fed-batch system relies on meticulous process control. Monitoring and regulating parameters such as nutrient concentrations, pH, temperature, and dissolved oxygen levels are crucial to creating optimal conditions for cell growth and product formation. To achieve high-cell density cultures and improve the productivity of the copolymer P(3HB-4HB), Raposo et al., 2017 conducted fed-batch cultivations of *Burkholderia sacchari*. They focused on optimizing the feeding strategy for glucose and γ-butyrolactone (GBL) to enhance volumetric productivity and increase the incorporation of 4HB into polymer chains. The study aimed to identify the most effective conditions

for optimal bacterial growth and copolymer production by adjusting nutrient addition timing, glucose amount, and GBL addition rate. The authors provide results from efficient fed-batch reactor conditions for P(3HB-4HB) copolymer synthesis in *Burkholderia sacchari* (Raposo et al., 2017).

1.5 Extraction Methods:

PHA extraction from microbial cells is a crucial step in the production of PHA products. The main options for extracting intracellular biopolymers like PHAs from bacterial cells are based in organic solvent extraction, aqueous extraction, and extraction using osmotic shock. Other emerging methods include enzymatic cell lysis and mechanical cell disruption, which are being explored to improve the efficiency and sustainability of PHA extraction. The choice of extraction method depends on factors like the bacterial strain, PHA content, desired purity, and cost considerations.

1.5.1 Organic solvent extraction

The predominant method for extracting PHA from microbial cells is solvent extraction Chloroform, ethylene carbonate, dimethyl sulfoxide (DMSO), and other suitable organic solvents are used to break up the bacterial cells and dissolve the biopolymer (Berger et al., 1989; Meneses et al., 2022).

In table 2 a brief description of the extraction of P(3HB) from biomass using organic solvent:

Table 2: PHA recovery with the use of organic solvent (Keshavarz & Roy, 2010; Lee, 2000)

Name	Example	Description
Chlorinated hydrocarbons	1.2- dichloroethane / chloroform	PHA exhibit solubility in the solvent. This process entails washing the cell with a hydrophilic solvent, followed by cycles of drying, grinding, and thawing
Cyclic carbonates	1.2-propylene carbonate / ethylene carbonate	One benefit of this method is its ability to dissolve large concentrations of PHA at elevated temperatures.

Azeotropic mixtures	Chloroform with ethanol, hexane, methanol or acetone	Methanol, acetone, water, or hexane are used as counter solvents to induce polymer precipitation after the formation of a clear solution of PHA in the primary solvent
Dispersion of chloroform and sodium hypochloride	Sodium hypochloride with chloroform	The inclusion of chloroform aided in avoiding polymer degradation.

The permeability of microbial cells can be enhanced to facilitate the release of PHA particles from inside the cell, which are then precipitated using solvents. The process typically involves the following steps:

- Cell permeabilization: Several techniques have been devised to enhance the permeability of microbial cell membranes, facilitating the liberation of internal PHA particles. These techniques include the use of solvents, detergents, enzymes, and other chemical substances that disturb the cell membrane (Dyrda et al., 2019; Flores Bueso et al., 2020; Schalck et al., 2021).
- PHA dissolution: After permeabilization of the cells, organic solvents such as chloroform are used to dissolve and remove the PHA particles from the cellular matrix. Chloroform is a very efficient solvent for PHA (Dyrda et al., 2019; Flores Bueso et al., 2020).
- PHA precipitation: Once PHA is obtained, non-solvent solvents such as methanol or ethanol are used to precipitate the PHA, resulting in a higher level of purification. These solvents are capable of mixing uniformly with chloroform, but they are unable to dissolve PHA, resulting in its separation from the solution (Dyrda et al., 2019; Flores Bueso et al., 2020).

The selection of solvents and extraction techniques is contingent upon variables such as the microbial strain, the specific kind of PHA synthesised, and the required level of purity for the finished product. Precise optimisation of the permeabilization and extraction procedures is essential to achieve the highest possible recovery and purity of PHA (Muheim et al., 2017).

A recent study has explored the production of 3D structures employing PHAs via the creation of microspheres using solvent extraction methods and solvent-water. In

addition, several methods like particle leaching, salt leaching, and heat induced solid-liquid phase separation have been adopted to create porous scaffolds with varying pore diameters based on the specific process and polymer utilised (Canadas et al., 2014).

Although this is an effective technique with high recovery rates, the use of organic solvents (some of them halogenated) raises financial, health and environmental issues. To avoid concerns associated with halogenated solvents, researchers have explored alternative extraction methods (Alfano et al., 2021; Aramvash et al., 2018).

1.5.2 Aqueous extraction:

The insoluble polymer granules are isolated from the non-polymer cellular components by selectively dissolving the cellular material in acidic or alkaline aqueous solutions or using surfactants. Although this method is more ecologically friendly, purity and recovery rates could suffer as a consequence (Ghosh et al., 2021). One of the simplest and most economical methods for PHA extraction is to use alkalis, such NaOH or KOH, or sodium hypochlorite, often known as bleach. Bleach is used to dissolve the cellular components that are not made of polymers, leaving behind the PHA particles, which may be subsequently retrieved (Flores Bueso et al., 2020; Samrot et al., 2021).

1.5.3 Enzymatic Digestion

The solvent method for PHA extraction includes the application of a number of solvents, which can be hazardous to the environment if employed on a widespread basis. Alternatively, the digestion process may be used for obtaining PHA from the cellular biomass. Enzymatic digestion is used in some extraction procedures, when proteases like trypsin, bromelain, cellulase, and lysozyme are used (Koller et al., 2013).

Enzymatic digestion is a biological process that utilises several proteolytic enzymes, such as lysozyme, alcalase, bromelain, and pancreatin. These enzymes hydrolyze intracellular components, particularly proteins, resulting in the preservation of PHA granules. Subsequently, these granules may be isolated using simple filtering and extracted using an appropriate solvent. Prior to enzymatic hydrolysis, a heat preliminary treatment of the biological material is sometimes conducted. Subsequently, the solution is subjected to surfactant treatment and decolorized using H2O2. In order to get PHA from B. megaterium with a high level of purity (90-93%), a proteolytic and caseinolytic enzyme derived from Streptomyces albus has been used (Kurian & Das, 2021).

1.5.4 Osmotic Shock

The use of osmotic shock to facilitate the release and extraction of PHA from microbial cells has been explored in several studies. By taking advantage of the halophilic bacterial strains' sensitivity to variations in salt content, osmotic shock may be used to break up the cells. Although this eliminates the need for harsh chemicals, it may not work for all species that produce PHA (Moradali & Rehm, 2020).

Osmotic shock can be used to enhance the permeability of microbial cell membranes, allowing the release of intracellular PHA particles. This is particularly relevant for halophilic or saline-tolerant strains, as they can accumulate PHA as an osmotic protectant (Koch, 1995; Obruca et al., 2020).

According to the research, hypo-osmotic shock (sudden decrease in external osmolarity) can trigger the opening of mechanosensitive channels like channel of large conductance in the cell membrane, leading to the release of intracellular contents, including PHA granules. (Poblete-Castro et al., 2020).

Overall, the use of osmotic shock to facilitate PHA extraction is a promising approach, particularly for halophilic or saline-tolerant microbial strains that accumulate PHA as an osmotic protectant. The optimization of the osmotic shock conditions and the engineering of the host strains can enhance the efficiency of this PHA recovery method. (Thu et al., 2023).

1.5.5 Processing Techniques:

- Porous Polymeric Matrices:

Different techniques, including evaporation, thermally induced phase separation, 3D printing, freeze-drying, solid-free fabrication, selective laser sintering, foam-coating, and solvent casting can be employed to create porous polymeric matrices from PHAs (Tao et al., 2009).

Copolymers P(3HB-co-3HHx), PHBV, and P(3HB-co-4HB) have shown a reduction in brittleness and an increase in flexibility, thus expanding the applications for three dimensional printing (Kovalcik, 2021).

In table 3 we detail the additive manufacturing technologies used for processing polymers:

Table 3: additive production techniques, polymer processing (Kovalcik, 2021)

Three dimensional printing	Polymer	Principle	Physical form
Selective laser sintering	Thermoplastics	Controlled the fusion of the powder using a CO2 beam (sintering, layer by layer mesh deposition)	powder
3D bioprinting	biomaterials with the suitable flowability and viscosity	The molten (or dissolved) polymer is extruded, layers are deposited using syringes or cartridges, and the mixture is then solidified.	Gel materials, Granules or paste
Fused deposition modeling	Thermoplastics	Melting filament extrusion (slow layer deposition and solidification)	Filament
Stereolithography	Photosensitive liquid polymer	Photopolymerization	Liquid

Fused deposition modelling (FDM) and selective laser sintering (SLS) are the most extensively investigated PHA-based 3D printing processes, with pilot experiments in stereolithography. As an illustration, the SLS technique has been used with a combination of calcium phosphate (Ca-P) and PHBV for scaffolds for bone tissue engineering. Although PHA has limited thermal stability in a 3D melt phase, it stays stable throughout the SLS process. Furthermore, it has been shown that the thermal characteristics and chemical composition of PHA remain unchanged during SLS, leading to an improved end product and the potential for recycling the initial material (Kovalcik, 2021; Mehrpouya et al., 2021).

FDM employs a process of layer-by-layer melt extrusion to build the ultimate 3D structure (Cano-Vicent et al., 2021). In order to achieve effective utilisation, it is

necessary to carry out further copolymerizations with monomers such as 3HV, which results in the formation of a polymer that is more resistant to heat degradation (PHBV) (Laycock et al., 2014). Adding up to 20% PHA to PLA has been shown to greatly enhance the ultimate flexibility of the PLA filament (Kovalcik, 2021).

In conclusion, the utilisation of stereolithography for 3D modelling with PHA is feasible, contingent upon the appropriate adjustment of the functional end groups and molecular weight of PHA to facilitate photopolymerization (Hegde et al., 2017). Recently, UV-curable PHA resins have been synthesised using a solvent-free technique consisting of two steps. The PHB oligomers exhibited solubility at a temperature of 90°C and were capable of undergoing photopolymerization when exposed to UV light in a time frame of 10 minutes (Park et al., 2024).

- Electrospinning*:*

Electrospinning is a technique used for developing scaffolds, which is the only one suitable for industrial applications due to its ability to be effectively scaled up (Kitsara et al., 2017). Composite nanomaterials and nanostructures are produced with this technique, and they may be coupled with other technologies to increase their usage in a variety of industries. Electrospinning is a potentially effective method for creating novel dressings to promote wound healing (Croitoru et al., 2020; Wen et al., 2023).

The process entails utilising a high-potential electric field, generated by a high voltage source (ranging from 1 to 30 kV), to spin a polymeric solution. This is achieved by employing either direct current or alternating current mode. The polymer is ejected from a blunt-ended stainless steel needle or capillary, with the assistance of a syringe pump. The polymer is then collected by a grounded collector, which can be either a flat plate or a rotating drum. Electrospinning may induce the formation of the β-form in the polymer chains. This is caused by the combination of fast evaporation of the solvent and the electrostatic force that elongates and aligns the fibres in the direction of the electrostatic field (Sanhueza et al., 2019).

2 Medical Applications of PHA

PHA homopolymers like PHB, P4HB and P3HO, and copolymers such as PHBV, P3HB4HB, PHBHHx, and composites including these polymers were used in the development of devices including repair devices, sutures, repair patches, slings, orthopaedic pins, adhesion barriers, cardiovascular patches, stents (Chen & Wu, 2005). In table 4 we detail the different medical applications of PHA

Table 4: PHA applications in medical fields (Park et al., 2024)

Areas	Applications	Key properties	PHA type
Tissue engineering	Porous nanocomposites are used in many applications such as 3D organ printing, bone-tissue engineering, scaffold engineering, and cardiovascular tissue engineering. These applications include aorta regeneration, as well as the production of screws, surgical mesh, staples, bone plates, skin replacements, nerve guides, and adhesion barriers.	Degradation rate, tissue integration, porosity, mechanical strength and biocompatibility	P4HB, PHB-co-4 HV, PHBV, P4HB, PHA, P3HO (poly-R-3-hydroxyoctanoate) blends with reinforcing PLA or ions.
Cell regeneration	Implanting a sciatic nerve conduit into removed sciatic nerves	Flexibility, biocompatibility, strength, nerve cell support	PHB/PHBV, PHBV, PHBVx, PHBV/collagen blends
Medical implants	Artery augments, tablets, vascular grafts, cardiological stents, and heart valves	Mechanical strength, , biocompatibility, corrosion resistance, durability.	P4HB, PHBV, PHB-co-4HV, PHBHHx, PHBV
Cell growth supporting matrix	Repair damaged nerves, scaffolds, long-gap nerve injury restoration implants, and foams used for tissue repair of bone marrow stromal cells, wound healing applications and bone regrowth	Cell adhesion properties, porosity, biocompatibility, tissue-specific support, degradation rate.	PHBV blends, degradation rate with organic compounds
Drug delivery	Enhancing drug delivery for controlled release involves the utilization of various formulations such as microcapsules, films, nanoparticles, and microspheres with porous matrices	Controlled release properties, biocompatibility	P4HV, PHBV/PEG blends

2.1 PHA as Materials for Medical Implants

The main need for a material to be considered biocompatible in a medical device meant for prolonged interaction with human tissues is that it does not damage those tissues; this is usually accomplished via chemical and biological inertness (Feres et al., 2015). However, in recent years, degradable implant materials, *in vivo*, including PHAs, became increasingly attractive. Using degradable implant materials like PHAs can avoid a second operation to remove the implant and the potential side effects of permanent implantations (Pulingam et al., 2022).

PHA has attracted considerable interest in the fields of biological tissue healing because of its varied structure and excellent physicochemical qualities. Its applications span across soft and hard tissue regeneration, including heart valve, nerve repair, and organ regeneration, as well as bone, vascular, and cartilage tissue engineering (Ali & Jamil, 2016).

As a result of their elastic properties and flexibility, mcl-PHAs are predominantly utilised in soft tissue sectors. These materials are often used for wound dressings, sutures, cardiac patches, cartilage tissue, heart valves, and nerve conduits, applications where flexibility and elasticity are crucial (Urbina et al., 2018; Vastano et al., 2015). The capacity to adjust the mechanical characteristics of PHAs by creating blends or copolymers has led to the development of composites with enhanced rigidity, making them appropriate for applications such as cartilage tissue. This has produced fascinating results (Butt et al., 2018).

On the other hand, PHB, a scl-PHA, is used in applications involving hard tissues like bone implants. This is because it has the required mechanical stiffness to create effective degradable scaffolds with suitable mechanical and physical qualities. Several composites and blends of PHA have been created in this sector (Sahana & Rekha, 2018).

2.1.1 Bone implants

Extremely vascularized bone tissue with remarkable regenerative capabilities can naturally heal minor fractures without surgical intervention (Singh et al., 2019).

However, significant bone abnormalities, especially those caused by the removal of bone tumours and severe fractures, need surgical treatment. When faced with these situations, many methods are used, such as allografts, xenografts, autografts, or bone implants made from biomaterials (Butt et al., 2018). PHAs have been extensively studied for their applicability in engineering bone tissue owing to their remarkable rates of biodegradation and improved mechanical characteristics.

Recently, researchers have created PHA films with antimicrobial properties specifically for use in bone regeneration. The combination of P(3HO-co-3HD-co-3HDD) and P(3HB)-based blends with Selenium-Strontium-hydroxyapatite was used to provide antibacterial characteristics without the use of antibiotics. Hydroxyapatite was added to promote the incorporation of tissue into bone and enhance the attachment and growth of osteoblasts. These films demonstrated significant antibacterial efficacy *E. coli* 8739 and against *S. aureus* 6538P resulting in the production of diverse films with distinct mechanical characteristics (Marcello et al., 2021).

Basnet and coworkers conducted research in 2021 where they created fibrous scaffolds made of poly(3-hydroxyoctanoate-co-3-hydroxydecanoate) (P(3HO-co-3HD)) and Poly(3-hydroxybutyrate) utilizing pressurized gyration. These scaffolds were intended for use in bone, nerve, and cardiovascular tissues. The osteoinductive capabilities of composite P(3HB)/fibers of HA were assessed utilizing a CAM *in vivo* model. In addition, they were inserted under the skin of mice with weakened immune systems to evaluate the development of bone tissue, the growth of blood vessels, and the invasion of host tissue. P(3HB) fibers incorporated with HA and implanted with Stro-1+ human bone marrow stromal cells (HBMSCs) exhibited the greatest degree of vascularization, a considerably higher quantity of vessels as compared to P(3HB) fibers seeded with HBMSCs alone, and increased collagen deposition. These positive outcomes highlight the potential of PHA composites for hard tissue engineering applications, given their osteoinductive properties and ability to promote angiogenesis (Basnett et al., 2021).

The composite scaffolds made of HA and PHB exhibited the capacity to stimulate a sustained bone tissue adaption response with no causing any foreign body inflammation. Studies *in vitro* have shown that the HA/PHB scaffold facilitated the attachment, differentiation, and growth of HBMSCs and human maxillary osteoblasts (HOBs). PHB/HA scaffolds, which were covered with type I collagen, stimulated the development of new bone tissue in nude mice models. The high levels of osteocalcin expression throughout the implantation period validated the osteogenic properties of the cell-seeded PHB/HA scaffolds, while the control group did not exhibit any discernible osteocalcin expression or osteoinduction (Basnett et al., 2021).

Incorporating a precise quantity of nano-sized Bioactive Glass (nBG) into the PHB scaffold resulted in a notable increase in the growth of human osteosarcoma cells (MG63) (Mačković et al., 2012). This also led to better ability to support bone formation and increased alkaline phosphatase (ALP) function (Shuai et al., 2017). Hajiali conducted

studies to assess the viability of utilizing PHB/nBG nanocomposites as scaffolds for bone tissue engineering. After submerging PHB/nBG scaffolds in simulated bodily fluid (SBF) for a period of one month, a thin layer of HA mineralization developed on the surface. This process improved the potential of the scaffolds to facilitate osseointegration and bone restoration (Amini et al., 2012).

Hydrogels were combined with PHB to create bioactive osteogenic scaffolds. Volkov et al. created Alginate (ALG) hydrogel-based PHB/HA scaffolds by a salt leaching process and using 3-D printing. The scaffolds provided strong support for the hydrogels, and their interconnected pore architectures promoted the proliferation of Mesenchymal Stem Cells (MSCs) and stimulated the development of bone tissue. The use of hydrogel-based PHB/HA composite scaffolds laden with MSCs successfully repaired radial bone defects in rat parietal bone at a high rate (94%) on day 28. This finding underscores the promising capabilities of bioactive polymer scaffolds in the field of bone tissue engineering (Volkov et al., 2020).

For bone tissue engineering, PHBV, was widely mixed with bioactive substances. PHBV demonstrated mechanical compressive strength that is similar to that of human bone, and it enhanced the experimental bioactivity of the PHA scaffold. Animal tests shown that the PHBV/HA composite significantly increased the thickness of newly produced bone from 130 μm to 770 μm over six months. This suggests that the composite has excellent properties for enhancing bone structural repair (Stevens, 2008).

MSCs and osteoprogenitor cells are stimulated to differentiate into osteogenic tissues by adenosine, another bioactive substance combined with PHBV. Zhong and collaborators conducted a thorough investigation of the osteogenic potential of composite nanofibers by integrating adenosine molecules in PHBV electrospun nanofibers, both in laboratory conditions and in living organisms. The electrospun fibres made from PHBV and loaded with adenosine shown significant efficacy in promoting bone regeneration when used to treat skull deformities in rabbits (Zhong et al., 2019).

In addition, Cool et al., 2007 conducted research to examine the osteogenic characteristics of PHBV combined with β-tricalcium phosphate (β-TCP) and calcined hydroxyapatite (CHA), respectively. The addition of nanoscale-reinforced phases to PHBV resulted in enhanced osteogenic characteristics and reduced inflammatory response (Cool et al., 2007).

In order to considerably promote the creation of apatite, Chernozem et al. (2019) presented a unique scaffold that combines PHB and PHBV with mineralized piezoelectric

bodies made of calcium carbonate. Minerals have the potential to greatly enhance the development of apatite surrounding PHB/PHBV scaffolds, hence promoting the creation of bone tissue. These investigations have introduced a novel therapeutic approach for using PHBV polymers in the regeneration of bone tissue (George et al., 2024).

2.2 PHA in Medical Device Manufacturing

2.2.1 Heart valve:

Surgical replacement of heart valves, whether biological, artificial, or mechanical, is a highly efficient treatment approach for treating heart valve diseases. However, these valves often do not expand and have limited durability, which may result in thrombotic issues caused by rough surfaces that encounter blood. Consequently, Heart Valve Tissue Engineering (HVTE) has emerged as a crucial strategy for addressing these challenges under dynamic conditions. Recent studies highlight PHA as a very suitable substrate for HVTE like shown in figure 2, a P4HB scaffold used to generate a HVTE (Chen et al., 2019).

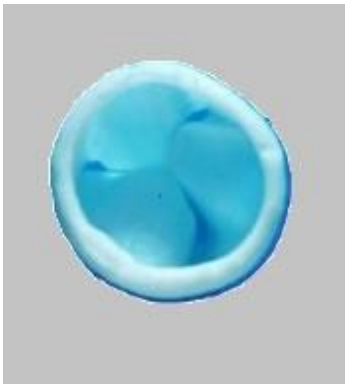

Figure 2: scaffold composed of poly(4-hydroxybutyrate) (P(4HB))/poly (glycolic acid) (PGA) was utilised to generate a tissue-engineered heart valve construct via in vitro tissue culture. (Adapted from Guo et al., 2022)

The tri-leaflet heart valve scaffold was created for the first time by Sodian et al. (2006) with the thermoplastic PHA polymer P3HB4HB. This scaffold, produced using stereolithography, facilitated the introduction of ovarian artery vascular cells, enabling the coordinated pulsation of the three-leaflet valves. The cells thrived and effectively generated an extracellular matrix (ECM) when exposed to pulsatile flow (Mai et al., 2006).

Later studies included replacing the original pulmonary valve in lamb animals with sheep carotid artery vascular cells injected onto the P4HB heart valve scaffold. The findings indicated that the P4HB heart valve scaffold was enveloped by healthy tissue without any blood clot formation, and the valve exhibited no notable backflow within 120 days after

the surgery. The pathological results revealed the existence of fibrous tissue with glycosaminoglycans as the ECM in the vicinity of the scaffold. Furthermore, PHA polymers have been used for the purpose of covering or patching heart valves. Decellularized porcine aortic valves that were coated with PHBHHx show enhanced resistivity, elevated tensile strength, and decreased calcification when tested in a living organism (Wu et al., 2007).

In another study made by Motta et al., 2019 they illustrated the potential to create a tissue-engineered heart valve using human cells. Furthermore, they introduced a unique valve design incorporating the Valsalva sinuses' anatomical characteristics, confirmed the practicality and safety of Transcatheter Pulmonary Valve Replacement using readily accessible tissue-engineered human cell-derived valves in experiments conducted on sheep (Motta et al., 2019).

A different example pertains to a heart valve with three leaflets that has a high level of porosity. This valve has a P4HB coating and is made of PGA. Perry et al. (2003) isolated and cultured Bone Marrow-Derived Mesenchymal Stem Cells (BMSCs) and then seeded them onto the PGA/P4HB composite scaffold. This led to the development of a tissue construct that exhibits biomechanical properties comparable to natural heart valves. The diameter of the valve scaffold increased in sheep after implantation for 20 weeks, suggesting the possibility of future expansion (Perry et al., 2003). The natural flexibility and malleability of P4HB enabled it to adjust to tissue development, while stem cells multiplied and specialised on the scaffold surface, creating an even coating of fused cells and extracellular matrix. Baghdadi et al. (2018) created a versatile cardiac patch utilising P3HO that has a mechanical strength similar to that of myocardial tissue (Bagdadi et al., 2018).

PHB composite scaffolds demonstrated a reduction in the inflammatory response when compared to other materials used for cardiac repair in infarcted rat hearts. Their induction of considerable angiogenesis renders them more advantageous for the healing and remodeling of heart tissue (Castellano et al., 2014).

2.2.2 Nerve engineering:

The manufacturing technique for nerve tissue aims to repair damage in the peripheral nervous system. It is acknowledged that natural polyesters are useful biomaterials for treating diseases of the peripheral nervous system. Biocompatible materials or biodegradable of biological origin are suitable for nerve path applications. mcl and scl-

PHA have been utilized to treat nerve tissue and regenerate nervous cells (Mohanna et al., 2003).

Contemporary methods for repairing peripheral nerves concentrate on creating substitutes for nerve autografts, such as nerve tubes, guides, or conduits. (Figure 3) These substitutes aid in the growth of axons and may span wider gaps in nerves (Lizarraga‐Valderrama et al., 2016).

Studies have indicated that PHBHHx is effective in Schwann cell regeneration, particularly when combined with PHB (Bian et al., 2009).

Additionally, blending PHBHHx and poly (DL-lactide) has shown promise for creating effective nerve path (Gao et al., 2006). Blended PHBHHx has demonstrated favorable outcomes, which can be attributed to the reduced crystallinity compared to pure PHBHHx. Another noteworthy approach involves blending PHBV microtubules with PLGA to enhance the flexibility of films used in nerve tissue engineering (Yucel et al., 2010).

Researchers have explored blended composites of PHBV with Collagen for nerve tissue regeneration. Mixed films or microspheres of PHBV/Collagen are preferred over pure polymers due to their higher proliferation ability for nerve cells (Prabhakaran et al., 2013). Masaeli et al. produced electrospun scaffolds by combining PHB and PHBV nanofibers for the redevelopment of myelinic cells (Masaeli et al., 2013).

In order to provide a historical perspective on the subject, all the references cited in this paragraph have been selected for their relevance despite their age. It should be noted that these references all date back more than 10 years, reflecting the evolution and continuity of debates in this field over time.

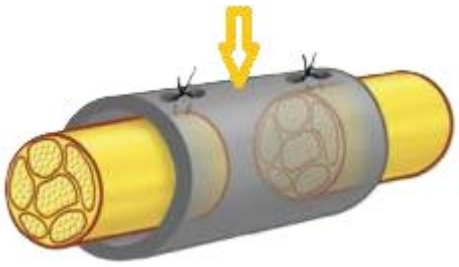

Figure 3: neural tube with a single lumen (Adapted from Lizarraga-Valderrama et al., 2016)

2.2.3 Sutures:

The skin can naturally regenerate, and minor injuries heal independently (Sahana & Rekha, 2018). However, in some instances, such as wounds with varying depths, severity, microbial invasion, and depending on the patient's health, external interventions like

sutures, wound dressings, and, for extensive defects, skin grafts or tissue-engineered skin may be necessary (Saniye Soylemez & Melis Kesika, n.d.).

Sutures, can be absorbable or non-absorbable, require materials that are antibacterial, biocompatible, possess high tensile strength, easily sterilizable, and can be securely fastened (Manavitehrani et al., 2016).

PHAs have proven to be advantageous for sutures, with P(4HB), P(3HB), P(3HB-co-3HHX), and P(3HB-co-3HV) (He et al., 2014). The FDA granted approval in 2007, commercially known as TephaFLEX® (Tepha, Inc. Lexington, MA, USA) (Figure 4) (scl-PHA), as suture material. P(4HB) stands out for absorbable sutures due to its less acidic degradation product compared to PGA and PLLA, as well as its faster degradation compared to PLLA, PCL, and other PHAs like P(3HB) (Chu, 2013). Subsequently, two more PHA-based suture materials MonoMax® (B. Braun, Tuttlingen, Germany)) and Phantom Fiber™ (Tornier Inc, Bloomington MN, USA.), both using P(4HB), received FDA approval (Manavitehrani et al., 2016).

Monomax® is an ideal thread for closing the abdominal wall due to its exceptional characteristics. Furthermore, Monomax® exhibits a much greater tear resistance in comparison to standard long-lasting tear-resistant sutures. It also provides physical support to the abdominal wall for a duration of six months, hence ensuring optimal performance (Odermatt et al., 2012).

Figure 4 : Tepha BBraun MonoMax suture (adapted from Https://Www.Bbraun.Fr/Fr/Products/B5/Monomax.Html, 2022)

Suture research employing PHAs is now focused on altering P(3HB-co-3HV) and P(3HB-co-3HHx) for suture applications (He et al., 2014). An experiment combining the low molecular weight polymers P(3HB-co-3HHx) and PLLA at a ratio of 20:80 was conducted to produce films. The results showed enhanced mechanical capabilities, greater

toughness, and faster degradation. This combination has been recognised as an exceptional option for producing "resorbable medical sutures" (Dydak et al., 2022).

2.2.4 Stents and vessels

A major issue linked with traditional metallic stents used in medical applications is the potential for restenosis, which involves the re-narrowing of the treated artery. To tackle this challenge, there's a need for the advancement of biodegradable stent technology. These stents are designed to perform their function of widening blocked arteries and subsequently degrade naturally, reducing the risk of restenosis. Recent advancements in this field include using biodegradable stents made from PLLA/P(4HB), which demonstrated encouraging outcomes when inserted into a porcine model. More precisely, when an oral atorvastatin medication was used in combination with these stents, there was a reduced level of narrowing compared to situations when permanent 316L (Stainless Steel) stents were used (Kischkel et al., 2016).

This progress indicates the potential for a better stent design, with PHAs emerging as an auspicious material for such applications. Due to their flexibility, which allows them to expand in response to blood flow pressures, elastomeric mcl-PHAs are especially attractive for blood arteries. Recently published research has shown that P(3HO) may be effectively melt-processed for tube extrusion by using bacterial cellulose nanofibers. The enhanced thermal and mechanical qualities obtained indicate a strong potential for using tissue-engineered blood arteries in live organisms (Panaitescu et al., 2017).

2.2.5 Wound dressings:

Wound dressings are crucial in promoting the process of healing for both chronic and acute wounds providing coverage and protection to the injured area (Dabiri et al., 2016). The selection of materials for wound dressings is essential, and they should be easy to use, non-adherent, sterile, and capable of preventing bacterial infections. The selected material must also support angiogenesis, facilitate gas exchange, maintain a moist environment, and promote the proliferation and migration of keratinocytes, fibroblasts, and epidermal cells. PHAs exhibit considerable potential for use in skin tissue engineering and wound dressings. Previous studies have demonstrated that fibroblasts and keratinocytes stick and multiply more effectively on PHA-based materials than other synthetic polymers (Shishatskaya & Volova, 2004).

Laboratory experiments conducted with murine fibroblasts have demonstrated that nanofibers made of P(3HB-co-4HB) incorporating collagen peptides promote the adhesion and growth of cells (Vigneswari et al., 2021). Animal studies conducted on rats

with full-thickness open excision-type skin wounds have shown that the utilization of P(3HB-co-4HB)/collagen nanofibers substantially accelerates wound healing, achieving a closure rate of 98% compared to the control treatment with gauze, which only achieved 63% healing (Vigneswari et al., 2016). In the Figure 5 we summarize all the possible applications in medicine of PHA

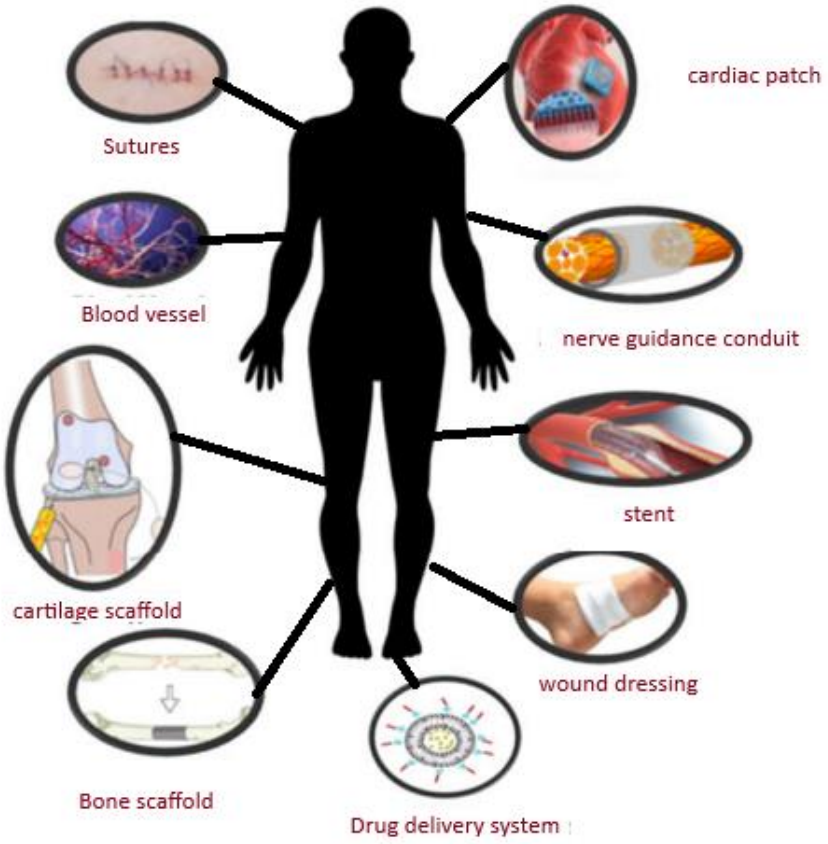

Figure 5: PHAs' use and progress in the biomedical sector, notably in the field of tissue engineering (Adapted from Pulingam et al., 2022).

2.3 PHA as Drug Delivery Vehicles:

PHAs find applications in drug delivery due to several advantageous properties (Pooja Basnett, 2014; Shrivastav et al., 2013).

One key advantage is their tunability, which is achieved through various production methods and allows for the controlled release of therapeutics over specific periods. Moreover, PHAs can be adjusted to focus on certain regions inside the body. Drug delivery systems include several methods such as injectable nanoparticles oral and

pulmonary administration, transdermal materials and devices, and drug delivery implants (Fenton et al., 2018).

PHAs have been employed in producing drug-delivery devices, incorporating diverse methods such as films, patches, and prototypes are used (Elmowafy et al., 2019). However, FDA has not yet approved any PHA-based nanomedicines for clinical applications, and their use is still limited to the experimental stage (Pecorini et al., 2023). Their tunable biodegradability is particularly valuable for these applications. This property allows PHA-based drug delivery systems to degrade at a controlled rate, aligning with the desired release profile of the therapeutic agent. Whether in injectable nanoparticles, transdermal patches, or other prototypes, PHAs offer versatility in tailoring drug delivery systems to meet specific needs regarding release kinetics and targeting within the body. biodegradability is particularly valuable for these applications (Adepu & Ramakrishna, 2021).

One major challenge in the treatment of cancer is balancing the anti-cancer effects while minimizing damage to healthy cells. A solution to this challenge involves developing non-toxic treatments targeting cancerous cells. Surface erosion observed in scl-PHAs makes them an excellent candidate for drug delivery. However, issues such as crystallinity and high hydrophobicity can result in the rapid release of medications without polymer degradation. In contrast, mcl-PHAs, with their low crystallinity and low temperature, have been reported to provide better drug delivery outcomes (Iqbal & Keshavarz, 2018; Rai et al., 2011).

A recent development in cancer treatment involves combining PHAs as carriers of hydrophobic photosensitizers (such as phthalocyanines, porphyrins, and related substitutions) for photodynamic treatment (Pramual et al., 2016).

These photosensitizers demonstrate strong propensity for accumulating in the membranes of intracellular organelles like mitochondria (Susan et al., 2011). Research has shown that porphyrin sensitizers induce an antineoplastic impact in myelocytic cell lines similar to the K562 cells after laboratory photodynamic treatment protocols (Neagu et al., 2007). They are using PHAs as carriers to present treatment options for various illnesses, overcoming the drawbacks of porphyrins, such as low photostability and high prices (Ion, 2017). This work shows the potential of PHAs in advancing cancer treatment strategies.

In 2024, E. Kilicay conducted a study on the utilisation of salicylic acid (SA) doped into PHB nanoparticles (NPs) to enhance the effectiveness of encapsulating caffeic acid and folic acid (FA) for the treatment of breast cancer, with the aim of increasing both the

encapsulation efficiency and anti-cancer activity. The nanoparticles (NPs) were generated via the process of solvent evaporation and then analysed using a range of different techniques. The findings indicated that the nanoparticles had excellent stability for a duration of 30 days, while also maintaining a continuous release for a period of 25 days. In summary, the research determined that FA-Caff NPs have great potential as a carrier system for the treatment of breast cancer (Kilicay et al., 2024).

According to another study conducted in 2020 by György Babos, dual drug-loaded nanotherapeutics have been found to effectively address medication resistance and mitigate negative effects caused by some drugs. The study utilized a specialized poly([R, S]-3-hydroxybutyrate) to co-encapsulate Doxorubicin and sorafenib through an emulsion-solvent evaporating technique. The nanoparticles were conjugated with poly(ethylene glycol) (PEG) to enhance the properties of medication release and make them more stealthy. The generated PHB-sorafenib-doxorubicin nanoparticles were 199.3 nm on average, and following PEGylation, they grew to 250.5 nm. While the attachment of PEG resulted in a minor reduction in the production of nanoparticles and the effectiveness of drug encapsulation, it had a positive effect on the release of Doxorubicin. Specifically, the drug was released more rapidly in a tumor-specific acidic environment compared to blood plasma. Although the PEG connection slowed down the release of both medications, sorafenib's release still occurred at a much higher pace, with an enhanced initial burst, compared to Doxorubicin. *In vitro*, the PEG-PHB copolymer exhibited more favourable drug release kinetics compared to the newly discovered PEGylated poly(lactic-co-glycolic acid) nanoparticles that contained the same medicines (Babos et al., 2020).

- **PHA as a sunscreen**

In 2019, Unilever and Bio-on introduced MyKai sunscreen, catering to consumers increasingly concerned with sustainability and responsible purchasing. The cosmetic ingredients, bioplastic micro powders, in the My Kai SPF Booster line are the product of Bio-on CNS's innovative research and development. These ingredients are manufactured at Bio-on's industrial facility in Bologna, Italy. Bio-on's biomaterials, including PHAs (polyhydroxyalkanoates) and PHBs (polyhydroxybutyrate), are derived from renewable plant sources. They offer the same thermo-mechanical properties as conventional plastics while being completely eco-sustainable and 100% naturally biodegradable. Unfortunately in 2023 the Bio-On group filed for bankruptcy (Bio-on, 2019; "Bio-On Is Back, Saved by the Maip Group," 2023).

3 Use of PHA in Dentistry

In dentistry, PHA might be used in various ways, such as developing biodegradable materials for dental applications, including temporary dental devices, sutures, or other materials used in oral and maxillofacial surgery. The biodegradable nature of PHA could be advantageous in specific dental applications, especially when temporary support structures are needed and can naturally break down over time (Kalia et al., 2023)

Table 5 provides a comprehensive overview of the in vivo dental applications of PHA.

Table 5 :PHA in vivo use (Chen & Zhang, 2018)

PHA	Applications	References
PHB scaffold with nanobioglass	By considerably increasing osteoblast-like MG63 cell proliferation, bone tissue engineering may lead to improved osteoconductivity.	(Karahaliloglu et al., 2015)
NaOH treatment of PHB, PHB mixture with PHBHHx, and PHB PEG scaffold	In cranio-maxillofacial and dental tissue engineering, bone tissue engineering has evolved into an antibacterial, osteoconductive directed bone regeneration membrane.	(Hajiali et al., 2012)
Tricalcium phosphate–PHB composite	Pre-existing cone-beam computed tomography images were utilised to 3D print customised TCP-PHB scaffolds for patients with clefts, according to their unique alveolar bone geometry.	(Wang et al., 2006)

3.1 PHA as a healing scaffold:

PHA has exceptional mechanical characteristics, biocompatibility, and degradation rates, making them very suitable for tissue engineering purposes. PHA, PHBV, PHB, P4HB, their composites, have use in diverse medical devices and bone tissue engineering. PHA scaffolds possess a mechanical compressive strength comparable to that of human bones, elicit a delayed inflammatory response, and promote more mineralization. PHA has been used in several formats, such as micro-grooved membranes, aligned nanofibers, and composite materials filled with carbon nanotubes. PHB has shown its ability to promote the development of human mesenchymal stem cells into bone tissue. Nevertheless, the

high crystallinity, extended degradation period, and hydrophobic properties of the material restrict its potential uses (Dwivedi et al., 2020). Various research investigations have made efforts to alter the material characteristics of PHB by combining it with other substances, such as a 50:50 mixture of PHB with PHBV, electrospun nanofibrous scaffolds, and electrospun nanofiber scaffolds made from a blend of PHB and cellulose acetate. PHA exhibits a significant in vitro cell adhesion, sustained proliferation for a duration of 10 days, enhanced differentiation, osteoblast regeneration, and provides cellular protection against stressors encountered during injection. When PHB scaffolds are covered with PHAgranule binding protein coupled with RGD peptide, they exhibit enhanced adhesion, proliferation, chondrogenic differentiation, increased synthesis of extracellular matrix, and dramatically improved cartilage-specific characteristics (Dwivedi et al., 2020).

PHA have proven effective in various tissue engineering areas, but their potential in regenerating oral tissue is largely unexplored. The oral cavity is home to many bacteria and microbes that can harm systemic health, leading to dental and periodontal conditions. Soft tissue deficiencies or post-implantation scenarios increase the risk of infections, therefore, there is a need for scaffolds with antibacterial properties to decrease the chances of wound infections and ameliorate the outcome rate of gingival surgery (Ansari et al., 2021).

Recent studies predominantly target on incorporating antibiotics into scaffolds, but limitations such as poor absorption, adverse reactions, and antibiotic resistance prompt the exploration of novel strategies. In addressing these challenges, the proposed solution involves the development of antibacterial scaffold with porosity based on flexible PHA embedded with: 3-hydroxybutyrate (3HB) and 4-hydroxybutyrate (4HB) (PAP34HB) shown in (Figure 6): A porous antibacterial scaffold made of P34HB, called PAP34HB, was developed to address complex oral soft tissue defects in healthy male Wistar rats. The scaffold had a unique asymmetrical interconnected porous structure that provides a barrier, which made it superior to commercial collagen membranes (CM) in terms of pro-healing, antimicrobial, and biocompatibility qualities. Even in the presence of infection, PAP34HB exhibits a significant healing impact on soft tissue anomalies in the mouth (Chen et al., 2023).

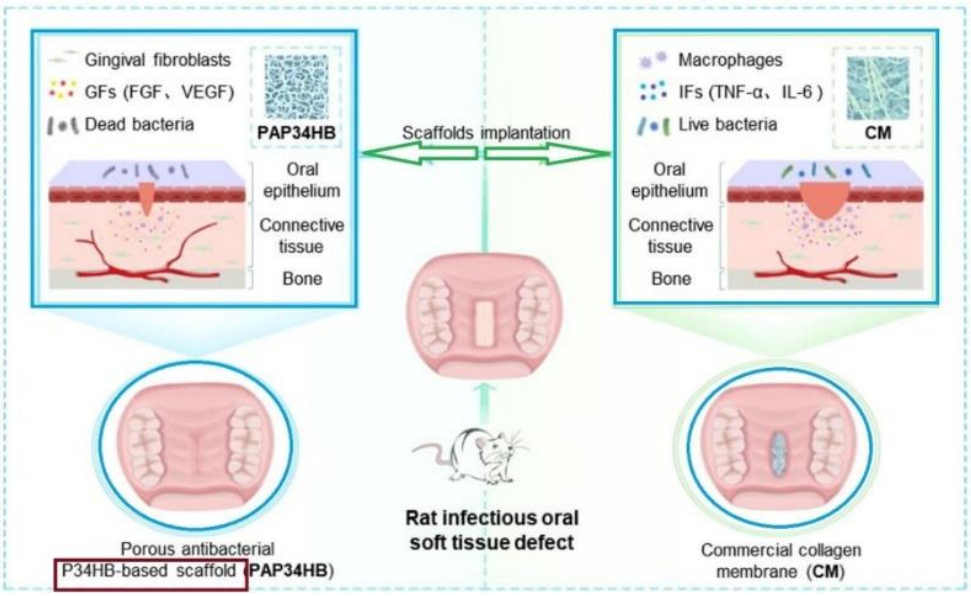

Figure 6: Oral soft tissue healing procedure (Adapted from F. Chen et al., 2023)

3.2 PHA for Oral Bone Regeneration

PHAs, when used as nanomaterials, demonstrate excellent potential as biomaterials for the development of bone scaffolds as previously explained, especially in combination with other biomaterials including cellulose acetate (CA), polylactic acid (PLA), polyL-lactide-co-caprolactone (PLCL), and hydroxyapatite (HA). Polyhydroxybutyrate (PHBHHx/P(3HO-co-3HHx)) and poly-lactide-co-caprolactone (PHBHHxPCL) are examples of PHA blends. Because of the planned micro- and macro-scaffold porosity, PHAs have proved effective in encouraging osteoblast cell adhesion (Puppi et al., 2017). Combined with HA, these PHA blends result in nanocomposites exhibiting impressive compressive strength like human bones. PHAs also demonstrate enhanced osteoblast regenerative properties, protect stem cells against tension, promote cell proliferation in damaged tissue areas, and exhibit improved adhesive properties compared to other biomaterials (M. Wang & Duan, 2011).

In a study made by Zinn et al., 2001, ten dogs underwent the removal of their third and fourth mandibular premolars on both sides of their jaws. The extracted teeth were then replaced with an occlusive barrier made of a PHBV membrane reinforced with polygalactin 910 (vicryl) fibers. After 8 and 12 weeks, researchers observed that P(3HB-co-3HV) films were more mechanically sound and elicited a better tissue response compared to other synthetic polymers like PLA and polycaprolactone (PCL) (Neves & Reis, 2016).

Membranes made of P(3HB-co-3HV) were compared to PTFE membranes to assess their tissue reaction post-implantation and to provide a barrier between bone and soft tissue. The study showed that P(3HB-co-3HV) membranes were superior to polytetrafluoroethylene membranes in promoting tissue regeneration and tolerance. Nonporous P(3HB-co-3HV) films effectively separated soft tissues and bone until the completion of the tooth transition, typically occurring around 24 weeks. The results showed that P(3HB-co-3HV) films exhibited superior mechanical characteristics and tissue responsiveness compared to PLA and PCL (Doineau et al., 2023).

Research on P(3HB-co-3HV) included its use in advanced regenerative dental treatments, including in the treatment of jawbone anomalies. The membranes serve as an obstacle to inhibit the infiltration of soft tissues, facilitate the full regeneration of newly formed bone in defects, and enhance the dimensions of the alveolar ridge. P(3HB-co-3HV) membranes were employed to study the therapy of mandibular anomalies and to enhance the thickness of the rat jaw. The findings indicated enhanced bone regeneration from 15 to 180 days, resulting in complete space fill with newly created bone within six months (Vilar et al., 2012).

In another research done by Seubsakul Phuegyod published in 2023, they produced three-dimensional porous scaffolds created utilising P(3HBco3HV) with a 50% hydroxyvalerate (HV) component (Phuegyod et al., 2023). The polymeric material, denoted as P(HB-50HV), was bioengineered from the bacterium *Cupriavidus necator H16*, and its potential for *in vitro* proliferation of dental cells in the context of tissue engineering was assessed. Compared assessments were performed using scaffolds made from PHB, PCL, and P(HB-12HV). All polymer films showed a hydrophobic character based on water contact angle studies. All the constructed scaffolds had a significant porosity of 90% and a sponge-like structure (Phuegyod et al., 2023).

Phuegyod's team discovered unique differences in compressive stiffness in the P(HB-50HV) scaffolds, as seen in Figure 7. These scaffolds demonstrated a lower rigidity compared to all other examined scaffolds in both dry and wet environments. Human gingival fibroblasts (HGFs) and periodontal ligament stem cells (PDLSCs) showed strong attachment, increased growth, and maintained a healthy cell shape when grown on the P(HB-50HV) scaffold. The results highlight the outstanding cells compatible with P(HB-50HV) scaffolds, indicating their possible use as biomaterials in stem cell-based treatments and periodontal tissue engineering (Phuegyod et al., 2023).

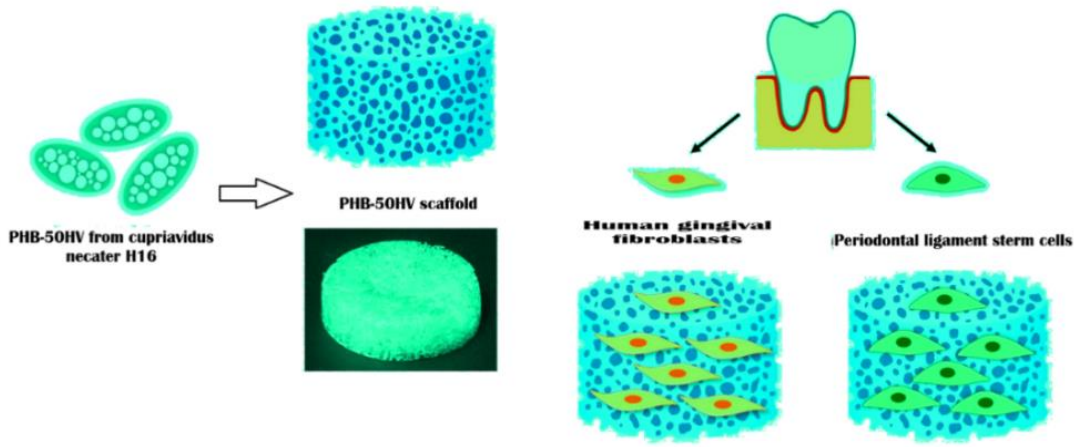

Figure 7: Biomaterial scaffold P(HB-50HV) for periodontal tissue engineering (Adapted from Phuegyod et al., 2023)

Galgut et al., 1991 examined the histological response of PHBV membranes, observing good toleration and minimal downgrowth of epithelial tissues (Galgut et al., 1991). Leenstra et al, in 1998 explored the use of nonporous PHBV films demonstrated superior mechanical properties and elicited a favorable tissue response in this procedure (G.Chen & Wu, 2005).

3.3 PHA in controlled drug delivery for oral disease

Oral mucosa ulceration is a common condition that may develop at any age. Therefore, pain can reduce the quality of life. The current options for treating a medical condition often involve using topical corticosteroids. Unfortunately, these options use poor drug delivery systems and need to provide adequate contact time for the medication to work effectively (Fitzpatrick et al., 2019).

In a recent research, Owji et al. (2021) developed a technique inspired by mussels that uses simple polydopamine chemistry to replicate their adhesive functionality (Figure 8). This technique allows for optimal adhesion and locally controlled medication delivery to the affected area. They coupled the polydopamine chemistry technique with PHA as the delivery system to achieve this. They focused on producing the PHA via *Pseudomonas mendocina CH50*, which is isolated from the bacterium and transformed into films using solvents. The polydopamine coating was applied by submerging the solvent-cast a film, made from a solution of polymerized dopamine (Owji et al., 2021).

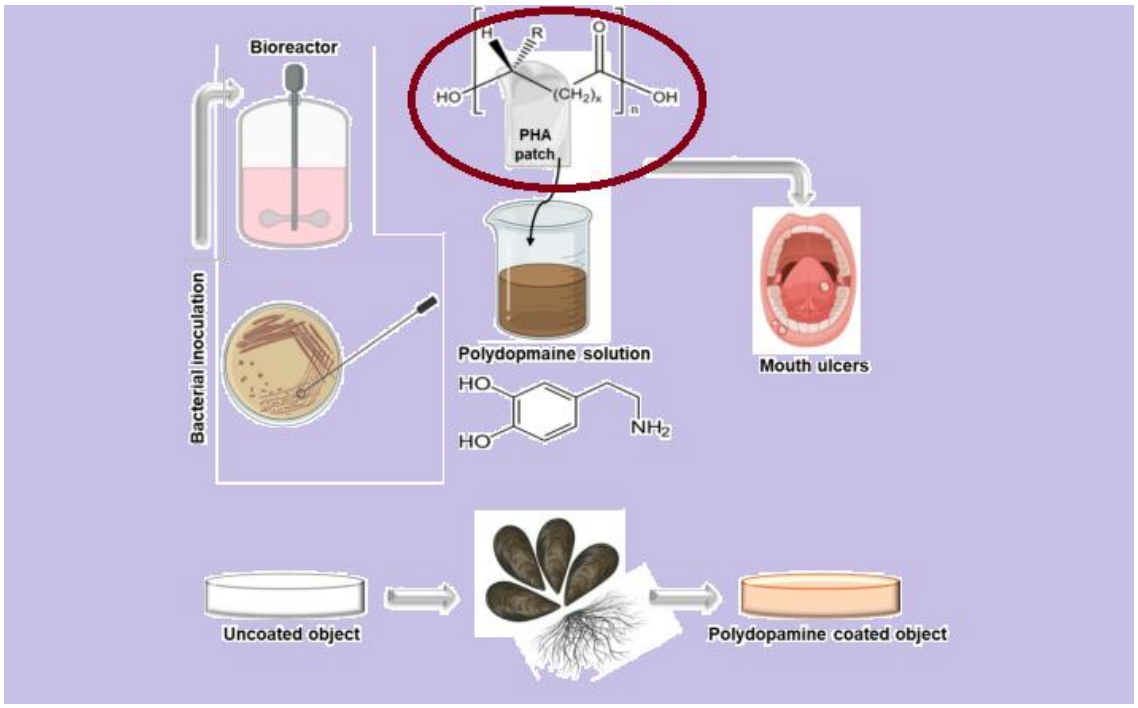

Figure 8: An example of PHA production is shown, followed by a polydopamine covering with chemistry inspired by superficial muscle (Adapted from Owji et al., 2021)

Periodontitis is defined as a condition affecting the gums and other structures that support teeth. The treatment of periodontal disease focuses on reducing infection by removing germs that cause periodontitis by mechanical cleaning and administering systemic antibiotics TC, a versatile antibiotic, is often used to treat periodontal conditions because of its ability to combat many types of bacteria responsible for periodontitis. Systemic use of antibiotics at large doses may cause various adverse effects such as gastrointestinal discomfort, hypersensitivity, and an elevated likelihood of bacterial resistance (Schou, 2008). The concern with systemic antibiotic administration is the potential adverse effects that may impact the patient's overall health. Hypersensitivity reactions can manifest as allergic responses, while gastrointestinal intolerance may cause discomfort and digestive issues. Additionally, the overuse of antibiotics can contribute to the development of bacterial resistance, reducing the effectiveness of these drugs over time, researchers and healthcare professionals are exploring alternative approaches to deliver antibiotics directly to the affected site to address these challenges and minimize systemic side effects. Localized delivery methods such as antibiotic-loaded gels, films, or other carriers allow targeted treatment, reducing systemic exposure and associated side effects (Weiser & Saltzman, 2014).

In this context, Panith et al., 2016 did research on the synthesis and characterisation of drug delivery vehicles using PHAs for the gradual release of TC in periodontal therapy. Four distinct PHA variations were used to create TC-loaded PHA microspheres. The authors investigated how various features of the microspheres were influenced by using varying molecular weights of polyvinyl alcohol (PVA) as a surface stabiliser, and found that the release speed of PHA microspheres was affected by the kind of PHA and the molecular weight of the PVA stabiliser. The research showed that TC-loaded PHB microspheres efficiently killed germs that cause periodontitis, indicating their potential use in curing periodontal disease (Panith et al., 2016).article size, drug loading, and drug release profile could be modified depending on the molecular weight of the PVA particle stabiliser, the core PHA polymer type, and the %HV content in PHA copolymers. An increase in the percentage of high-volatile content resulted in a reduction in particle size and lowered encapsulation efficiency. Microspheres with increased crystallinity had a slower drug release velocity, but those with a larger percentage of HV content showed a greater initial burst release and quicker drug release. The findings indicated that the TC released from PHA microspheres followed a Fickian diffusion process. The drug loading qualities and release characteristics were affected by the molecular weight of PVA, as shown in Figure 9. Low Mw PVA exhibited more adsorption to PHA particle surfaces and increased drug encapsulation efficiency. The research emphasised the potential of PHB: low molecular weight PVA microspheres for extended drug release and strong antibacterial effects against bacteria that cause periodontitis, indicating its suitability for future use in periodontal treatment (Panith et al., 2016).

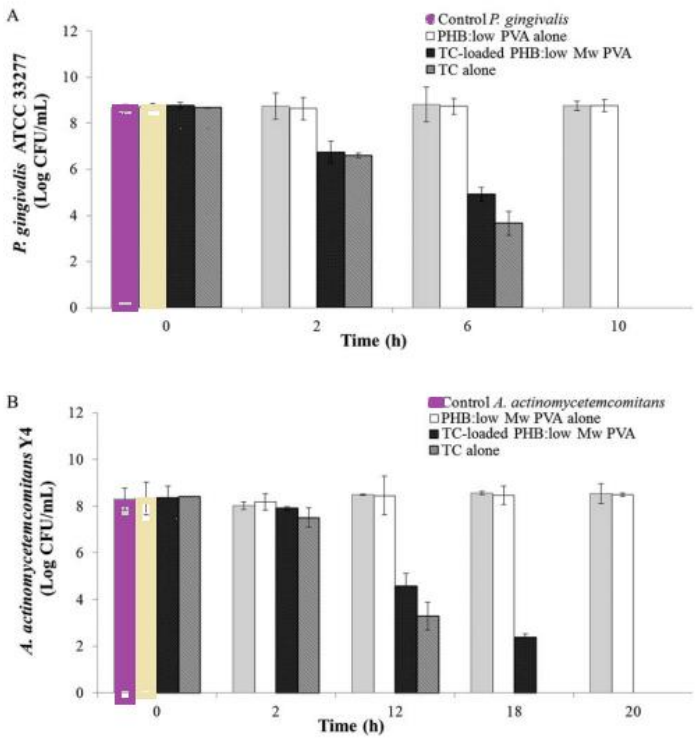

Figure 9: A) Porphyromonas gingivalis and B) Actinobacillus actinomycetemcomitan were the periodontal pathogenic bacteria that the TC-loaded PHB microspheres were shown to be effective against in vitro (Adaptated from Panith et al., 2016)

3.4 PHA in Dental Prosthetics and Devices

PHA have attracted growing interest in the medical field due to their exceptional biocompatibility, as demonstrated by several studies confirming their minimal toxic effects on cells and tissues, as well as their FDA-approved use including P4HB, this regulatory approval opens new prospects for PHAs in biomedicine, offering opportunities like tissue engineering and medical implant (Shrivastav et al., 2013).

PHA based sutures offer a promising alternative to traditional sutures in dentistry due to their biocompatibility, biodegradability, and potential for improved wound healing. Despite these advantages, there are also some challenges associated with the use of PHA based sutures in dentistry. One of the main challenges is the high cost of production, which can limit their commercialization and widespread use, additionally, the mechanical properties of PHA based sutures can vary depending on the composition of the polymer, and they can be affected by the degradation of the polymer and the environment in which they are used (Gregory et al., 2022).

Therefore, further research is needed to optimize the production and properties of PHA based sutures for use in dentistry (Gregory et al., 2022).

However, despite these advances, tooth regeneration remains a major challenge, because unlike some other parts of the body, teeth cannot regenerate due to age related changes that affect their ability to grow back naturally. To address this gap, dental implants such as endosteal and transosseous implants are commonly used in dental practice to replace missing teeth and provide support for fixed or removable partial teeth. The adequacy of the materials used for these dental implants is crucial, in terms of resistance to chemical attack, environmental stress, as well asining rigidity and thermal stability (Panchal et al., 2022).

At the same time research into materials that mimic the properties of natural dental enamel represents a promising pathway for the development of more effective dental implant. In this perspective, Gregory et al., 2023, focused on the development of various medical devices, including dental implants, bone materials, and repair patches for osteochondrial tissues. They opted for Fusion Deposit Modeling to design computer-assisted drawings for these devices, before implementing them with a 3D printer to validate their concept (Figure 10). Subsequently, these researchers successfully designed tailor-made dental implants for patients, using precise computed tomography images and applying mirror and mesh fusion techniques to reconstruct the missing parts. The use of materials such as polyhydroxyalkanoates and poly (lactic acid), common bioresorbable medical polymers, has enabled the creation of biocompatible dental implants. These implants are designed to withstand the strain of chewing, preserve the dental structure and harmonize with the aesthetics of other teeth (Gregory et al., 2023).

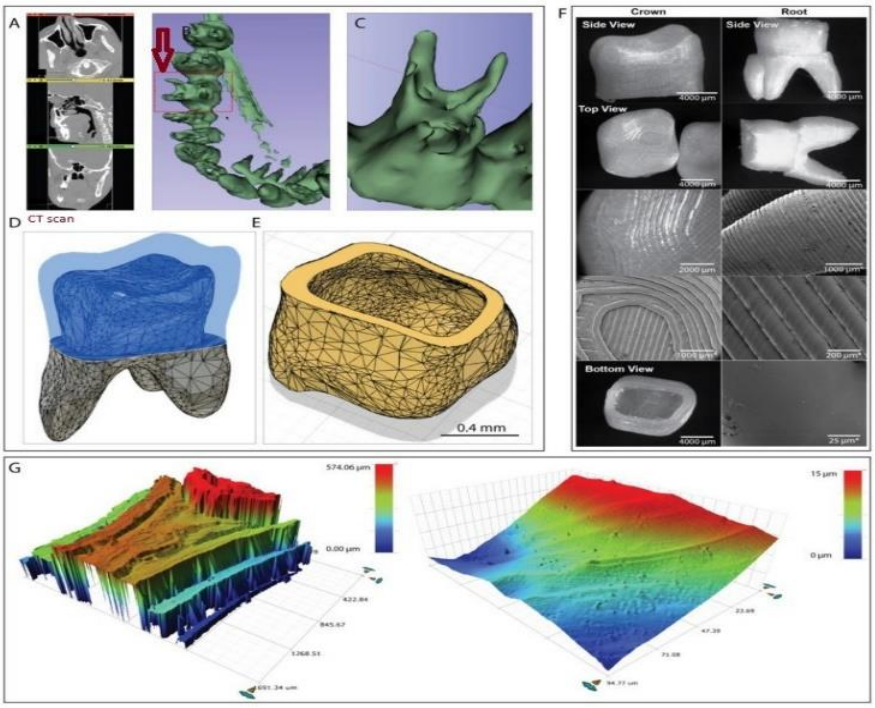

Figure 10: Creation of personalised 3D printed dental implants using actual CT scans (Adapted from Gregory et al., 2023)

Another possibility of the use of polyhydroxyalkanoates is the design of surgical guides for dental implantes that provides benefits in terms of biocompatibility and precision. Surgical guides printed in 3D, such as those made using Surgical Guide Resin, are sterile, biocompatible, ensuring patient safety throughout dental procedures. The creation of these guides includes numerically calculating patient dental models, precisely planning the placement of implants, and creating a surgical guide using a dental CAO software (P. Chen & Nikoyan, 2021).

Matthias Gielisch et al. conducted a study in 2022 to investigate the precision of surgical guides made of PLA/PHA and MED610 jet material in the implantation of fully guided dental implants before and after vapour sterilisatin Figure 11. The PLA was from a filament by Raise3D Inc (Lake Forest, Ca, United States). MED610 is a solid, transparent PolyJet™ (Stratasys, Eden Praire, MN, USA) material designed for medical applications. The study compares the precision of these surgical guides made by different materials and the difference between the planned and actual implant placements. The goal is to reduce the environmental effect of the medical industry by developing precise and biodegradable materials. After demonstrating levels of precision comparable to those of materials currently used in clinical practice, the research concluded that PLA/PHA surgical guides are a practical and environmentally friendly alternative (Gielisch et al., 2022).

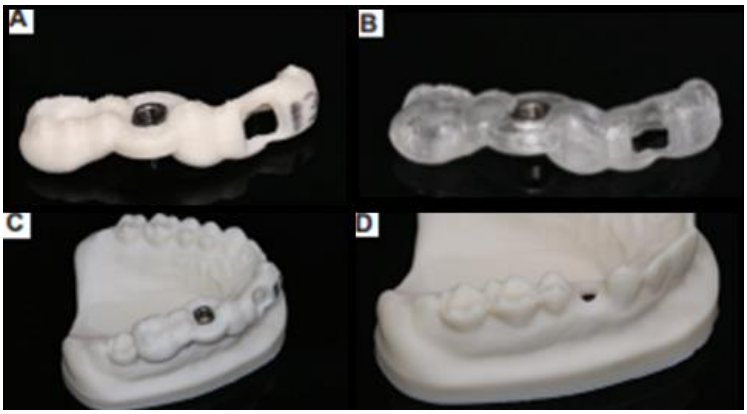

Figure 11: (A) Printed guide made from a polymer called polylactide/polyhydroxyalkanoate, with metal sleeves added (B) A translucent manual produced with MED610 (C) 3D-printed model accompanied with a surgical guide, prepared for the precise insertion of dental implants (D) Implant positioned in the space left by the upper jaw's first premolar (Adapted from Gielisch et al., 2022)

4 Critical Evaluation and Limitations

4.1 Analysis of the advantages and disadvantages of PHA:

PHAs are considered as a promising alternative to petrochemical polymers because they are derived from renewable resources. One of the critical advantages of PHAs is their environmental friendliness, as they are both biodegradable and biocompatible (Muhammadi et al., 2015).

Scientists studying microbiology, biochemistry, and bioengineering have focused their research on PHA's induced bioproduction. In order to achieve PHA concentrations in bioreactors that are suitable for industrial use, multiple substrates and bacteria are used. Separating polyesters and copolyesters with different chemical compositions and physical characteristics is another goal (Kumar et al., 2020).

PHA's tunable properties, which depend on microbial strains, carbon sources and specific enzymes' activity, open possibilities for a broad variety of industrial uses. The focus on optimizing bioproduction and isolation techniques is crucial for making PHAs commercially viable alternatives to traditional petrochemical polymers (Shao et al., 2018).

PHA is a versatile biodegradable polymer with several advantages over other implant materials approved by the FDA, such as PLGA and PLA. PHA has a weaker immune response and is suitable for use in medical implants. However, the *in vivo* biodegradation of PHA is slower than PLA and PLGA, making it unsuitable for rapid *in vivo* degradation applications (Li et al., 2012). To overcome this constraint and guarantee quick biodegradation, scientists have experimented with mixing PHAs with bigger and smaller molecular size. To hasten biodegradation *in vitro* and *in vivo*, they have also experimented with random or block copolymerization or mixing PHA with PLA or PLGA. Despite these encouraging results, the FDA has exclusively authorised poly-4-hydroxybutyrate, to be employed as a suture material. More people or businesses are unable to begin the clinical application procedure since it is time-consuming and costly. Moreover, the majority of PHA materials on the market aren't meant to be used in implant procedures. Rather, they want to penetrate the low-end recyclable packaging industry (Ma et al., 2016).

PHA has to be purified in order to get rid of bacterial contaminants, leftover proteins, and LPS before it can be used in implant applications. In order to guarantee the delivery of targeted PHA with the necessary constant composition, molecular weights, and purity, this purification method necessitates collaboration with a PHA manufacturer. Properties adjustments may be necessary for the requisite PHA throughout the development phase,

and this may only be done if a partnered producer is willing to provide the necessary PHA material (Ma et al., 2016).

PHA has many uses in medical implants despite these difficulties, including as injectable PHA, artificial blood arteries, heart valves, artificial nerve conduits, lithography, and drug delivery structures. Among these uses, 3D printing PHA is one that requires careful consideration, particularly when creating unique forms for certain use cases. However, PHA's sluggish crystallisation could be its biggest deterrent from becoming a viable material for three-dimensional printing. To further this field, scientists are investigating the creation of biocompatible chemicals that may quicken PHA crystallisation. PHA 3D printing has a large market, particularly in light of the growing need for plastic surgery alongside various medical applications (G.Chen & Hajnal, 2015).

The actual market cost of PHAs is roughly €5 per kg, which is over 6 times more expensive than the cost of traditional plastics (€0.8–1.5 per kg) (Acharjee et al., 2023). 50% of the total cost of manufacturing is attributed to the expense of the carbon source, which is the main cause of this high cost. Because of this, scientists are working to develop methods to use various waste products as a supply of carbon to lower the cost of producing PHA (Alavi, 2014).

In conclusion, while PHA offers various environmental and performance advantages, such as biodegradability and customizable properties, its high production cost and processing limitations are important drawbacks for its widespread adoption. Ongoing research might lead to more cost-effective synthesis pathways or ways to streamline the processing procedures. Additionally, emphasizing the long-term environmental benefits and the increasing consumer demand for sustainable alternatives could strengthen the case for wider adoption despite initial challenges (Alavi, 2014).

4.2 Current limitations of PHA use in medical and dental domains

Due to their remarkable hydrophobicity and brittle mechanical characteristics, the majority of PHA polyesters are not suitable for use as biomaterials. For biomedical applications, PHAs should, for instance, be more amphiphilic than hydrophobic, especially for drug delivery systems and tissue engineering (Yang et al., 2002).

For instance, the shorter carbon chain lengths of the scl-PHAs and PHB are shown to result in a greater crystallinity content, which limits their biological uses by making them rigid and inflexible (Brzeska et al., 2014).

The crystalline structure of scl-PHAs leads to inadequate mechanical characteristics and less biocompatibility. Copolymerizing PHB with PEG may address these issues by

eliminating PHB's stiffness and brittleness, resulting in a significant enhancement in the Young's modulus values to 21 MPa. The elongation at break values were greatly improved by up to 1912%. PHB's stiffness and hydrophobic nature allow for improvement, whereas PEG's flexibility and hydrophilic properties contribute to this possibility. By adjusting the length of the PHB and PEG blocks to match the needs of the application, the copolymer's crystalline structure and water absorption capability may be changed. The biodegradable amphiphilic PHB-PEG polymers shown favorable cell biocompatibility in cytotoxicity assessments (Loh et al., 2007). Moreover, enhancing the flexibility of PHB with low crystallinity may be achieved by adding long alkyl side chains like HB and HV, leading to the formation of copolymers such as P(3HB-co-4HB) and P(3HB-co-3HV) (Zembouai et al., 2013).

The aforementioned limitations also lead to the emergence of a new category of materials referred to as 'blends of PHAs.' This emerging material class is being extensively researched to enhance the thermal and mechanical characteristics of PHAs for biological applications (Volova et al., 2014). The blend of P(3HB) and PLA is comparatively the most studied blend, with mechanical properties intermediate between the individual components (Visakh P. M., 2014).

Zhao et al. (2013) studied the formation of P(3HB-co-3HV) and PLA blends, and the resulting blends showed improved mechanical properties (H. Zhao et al., 2013). Zembouai et al., 2013 made the discovery that augmenting the amount of PLA in P(3HB-co-3HV) and PLA blends could enhance their thermal stability. Mcl-PHAs exhibited decreased melting temperature (Tm), glass transition temperature (Tg), and crystallinity in comparison to scl-PHAs. By extending the length of the pendant chain, the physical properties of mcl-PHAs may be modified to change from semi-crystalline elastomers to amorphous sticky polymeric materials. This modification leads to a decrease in Tg. According to reports, mcl-PHAs with certain side chain lengths are valuable biopolymeric elastomers that have lower tensile strength and higher elongation at break. Increasing the length of the side chain in mcl-PHAs results in the production of a greater amount of sticky biopolymeric materials. (Zembouai et al., 2013).

These restrictions may be efficiently solved through research including blending or co-polymerization with other chemicals. Some of these compounds consist of natural and synthetic rubbers, as well as other polymeric components. For instance, mcl-PHA poly(3-hydroxy undecanoate) P(3HU) may be combined with PEG by copolymerization to adjust

the mechanical characteristics of the resultant copolymer, known as P(3HU)-co-PEG (Chung et al., 2003).

By increasing the quantity of PEG in the copolymer, the elongation at break increased by 16% and tensile strength were enhanced to 65 MPa, (Ghalia & Dahman, 2017) Similarly, the biocompatibility of P(3HU)-co-PEG was significantly improved in conjunction with PEG. Overall, scl-PHAs and mcl-PHAs are valuable polymeric materials that can be processed into elastomers for excellent medical applications (Ye et al., 2018).

The mechanical properties of PHB can also be improved when PHB is blended with P(3HB-co-3HHx) copolymer. The resulting blend, i.e. P(3HB-co-3HHx)/PHB blend polymers, showed an improved elongation at break value of 15 to 106% once the content of P(3HB-co-3HHx) copolymer in the blend was increased from 40 to 60% (J. Zhao, 2003).

The elevated crystallization content and quick crystallization rate of PHB create small holes and protrusions on its surface, forming a coral-like texture that may prevent the adhesion and proliferation of mammalian cells. The presence of P(3HB-co-3HHx) in PHB significantly decreased the crystallization content and rate of P(3HB-co-3HHx)/PHB films. It also resulted in a smooth surface that enhanced cell attachment and growth, leading to a notable enhancement in the biocompatibility of PHB. Increasing the proportion of P(3HB-co-3HHx) copolymer from 0% to 100% transformed the surface of P(3HB-co-3HHx)/PHB blend polymers from a coralloid appearance to a smooth, uniform surface without any openings, facilitating better cell adhesion and greater cell numbers. Additionally, treating PHB with lipase may transform the coral-like surface into a continuous one. The cells grown on the lipase-treated PHB polymer showed a 40-fold increase in number compared to those on the untreated PHB film. Transporting nutrients and waste products is crucial in three-dimensional permeable scaffolds. The scaffold made of a combination of P(3HB-co-3HHx) and PHB polymers in a 2:1 ratio exhibited enhanced biocompatibility compared to the pure P(3HB-co-3HHx) copolymer. The research demonstrated that P(3HB-co-3HHx)/PHB blend polymers are suitable for use as scaffolds in tissue engineering (K. Zhao et al., 2003).

While there have been numerous investigations into the effects of PHAs on cell multiplication and biocompatibility, further evidence is required to confirm the non-carcinogenicity of each cell line further research is required on every cell line to determine the carcinogenicity/non-carcinogenicity impact of PHAs (Ali & Jamil, 2016).

The biocompatibility of PHAs, including PHB, P(3HB-co-3HHx), and P(3HB-co-3HHx)/PHB blends, was evaluated *in vitro* utilizing the mouse fibroblast cell line L929 (MFCLL929). L929 cell growth was more efficient on PLA polymers and PHB. Yet, the restriction was resolved by creating composite films of P(3HB-co-3HHx) and PHB, which promoted the growth of L929 cells. The extent of enhancement varies based on the amount of P(3HB-co-3HHx) present in the blend films. The surface properties of the P(3HB-co-3HHx) copolymer were altered as the amount of 3HHx monomeric units increased. The most polished surface was obtained with the copolymer that included 20% HHx monomers. The PHB polymer exhibited the greatest hydrophilicity compared to all the evaluated PHB and co-polymeric P(3HB-co-3HHx). All copolymeric P(3HB-co-3HHx) also exhibited a strong attraction to protein molecules and had biocompatible characteristics (Y.-W. Wang et al., 2005).

Additionally, the biocompatibility of these polymeric materials was significantly enhanced by treatment with lipases and sodium hydroxide. It is noteworthy that the biocompatibility of PLA and treated PHB polymers was found to be almost identical, while P(3HB-co-3HHx) copolymers, as well as their dominant blends, exhibited greater biocompatibility than PLA (J. Zhao, 2003).

Wang et al., 2005 examined three-dimensional scaffolds made of PHB and co-polymeric P(3HB-co-3HHx) utilizing MFCLL929. The surfaces of PHB and co-polymeric P(3HB-co-3HHx) were more hydrophilic after lipase treatment. L929 cell proliferation on the lipase-treated scaffolds was double that of the control. A 40% decrease in cell proliferation was seen on PHA scaffolds coated with hyaluronan in comparison to the control. Although scaffolds are linked to enhanced hydrophilic characteristics, hyaluronan coating reduced cell adhesion and proliferation on PHAs. Lipase pretreatment enhanced cell growth on PHA scaffolds but did not enhance hydrophilic characteristics compared to hyaluronan coating (YWang et al., 2005).

Both hydrophilic and hydrophobic properties are essential for the biocompatibility of [P(3HB-co-3HHx)], particularly for promoting the growth of L929 cells on its surface.

PHA polymers, unlike FDA-approved implant substances such as PLA and PLGA, exhibit minimal immunoreactions and slow *in vivo* biodegradation (Qu et al., 2006).

Therefore, PHA polymeric materials isolated are unsuitable for applications that require rapid *in vivo* disintegration. However, combining higher and lower molecular weight PHAs may help maintain desirable mechanical assets while promoting rapid biodegradation (Shangguan et al., 2006). It is thought that random or block co-

polymerization, or even mixing PHA with PLA/PLGA, could speed up or improve the rate at which PHA breaks down in the lab and living things (Chen & Zhang, 2018).

The degradation rates of PHAs need to align with the rates of new tissue formation. The degradation processes of PHB and P(3HB-co-3HHx) scaffolds may be controlled by altering the ratio of substances with different molecular weights. However, it is regularly observed that the breakdown of these scaffolds occurs at a slower pace compared to the rate of new tissue formation (Young et al., 2002).

Additional research is required to develop PHA polymers that promote tissue regeneration and have a disintegration rate that matches the speed of tissue regeneration. In addition to these limitations, the production of PHA is hampered by their cost, in this context means have been put in place to reduce these costs, such as the utilization of cost-effective and sustainable materials for PHA manufacturing: The cost of major substrates contributes significantly to the overall production cost of PHAs. To reduce this cost, renewable waste materials and by-products from process industries can be used as substrates (Zytner et al., 2023).

These include molasses, palm oil, crude glycerol, waste vegetable oils, agricultural wastes, cheese whey, and wastewater. These materials are available in large quantities and have a high content of organic matter, making them promising substrates for economical PHA production (Zytner et al., 2023).

Metagenomics and molecular biology advancements may aid in creating improved strains for manufacturing PHA. An optimal industrial strain for PHA synthesis should possess the following traits: non-pathogenic, well-defined genomic background, delicate cell wall, simple genetic modification, easy aggregation, rapid growth in a specified medium, absence of toxin production, and potential cellulose utilization. The strains should possess a high capacity for PHA accumulation, be able to grow on mixed substrates, have a high substrate-to-polymer yield, and produce Short Chain Length and/or Medium Chain Length PHA. To efficiently extract PHAs, the genetically engineered strain should possess a weak cell wall, high cell size, and easily inducible flocculation (Khamkong et al., 2022).

PHA production process optimisation may be affected by inhibitory chemicals and variations in waste by-products composition, leading to fluctuations in final PHAs concentration, recovery yields, and content. To prevent these variations, it is advisable to use PHA optimisation measures at the laboratory level before moving on to pilot size testing (Cheng et al., 2017).

By implementing these strategies, it is possible to considerably decrease the production cost of PHAs, making them more commercially viable.

5 Future Perspectives

5.1 Emerging and future trends in PHA use

Recently, PHA is increasingly being used in many industries like biological healthcare, everyday chemicals, agriculture, food packaging and more (Acharjee et al., 2023b). PHA's future manufacturing and utilisation will mostly concentrate on the biomedical industry, requiring novel purification methods to maintain the stability and integrity of PHA polymers. PHA has specific features that set them apart from other biopolymers, including biodegradability, biocompatibility, thermal properties, and non-carcinogenic consequences (Tournier et al., 2020).

PHAs are frequently utilised in biomedical engineering for regeneration and tissue repair, such as in surgical sutures, bone and cartilage scaffolds, heart valves, and other applications. Yet, several technological challenges remain, and only a limited number of PHA products have been successfully brought to market and used in clinical settings. In the near future, PHA product development will primarily concentrate on high-value medical devices such interventional valves, artificial blood arteries and artificial bone joints. As the long-term trend shows a decline in the manufacturing cost of PHA (D. Tan et al., 2021), the product development focus will shift towards low-value medical consumables such surgical sutures and wound dressings. Then, PHA will possibly dominate the medical device industry because of its superior performance benefits. In the future, advancing PHA research in personalised tissue repair and regeneration might lead to innovative ways for creating outstanding performance biomaterials, exploiting the interdisciplinary nature of medical engineering (Cheng et al., 2017).

5.1.1 Market Growth:

The PHA market is expected to expand as a result of rising government laws globally that enforce limitations or prohibitions on the use of plastic bags, acting as a major catalyst for the PHA industry. The predicted market size for PHA is USD 195 million by 2028, with a compound annual growth rate (CAGR) of 15.9% (Danny Sharma, 2023).

In the packaging industry, PHAs are being explored as a biodegradable and cost-effective alternative to fossil fuel plastics. Research conducted in Cambridge, UK, has shown that PHA can serve as a material for various packaging styles. Companies like Cambridge Consultants have created fully compostable foodservice items made from PHA bioplastics, demonstrating the potential of this sector. Companies are working on scaling up the production of PHAs to make them more widely available in the market (Rick Lingle, 2018). As production volumes increase, PHAs are projected to become more cost-

effective, expanding their suitability for a broader variety of uses (Varžinskas & Markevičiūtė, 2020).

5.1.2 Application Diversification

PHA is being increasingly explored for various uses, such as packaging, medical devices, agricultural films, textiles, feeding additives, and even cosmetic beads. Its biocompatibility and biodegradability make it suitable for various biomedical and cosmetic applications. The versatility of PHAs makes them suitable for different industries, and researchers are investigating new ways to incorporate them into various products (Ray et al., 2022).

5.1.3 Technological Advancements:

Current research is focusing on developing new methods for producing PHA using alternative carbon sources such as methane, carbon dioxide, and methanol (Figure 12) (C1-substrates) or replacing pure C-sources by agro & industrial by-products (waste glycerol, sugar rich lignocellulosic hydrolysates, residual algal biomass). In this context, *Halomonas boliviensis* has been chosen due to its ability to produce high levels of P3HB under NaCl concentrations that discourage microbial contamination. Industrial residues from *Gelidium corneum*, containing significant carbohydrate content, are utilized as a cost-effective carbon source (Bondar et al., 2022).

Also, microalgae biomass, rich in carbohydrates and other macromolecules, presents a cost-effective alternative for PHA production. Bacterial PHA synthases are categorized into four classes, utilizing a range of substrates including starch, monosaccharides, and glycerol. Microalgae biomass contains proteins, amylopectin, starch, and cellulose, serving as valuable carbon sources for PHA synthesis, the most prevalent varieties of PHAs generated by bacteria utilising microalgal feedstock are are cited in the following table (Tan et al., 2022).

Table 6: The most prevalent varieties of PHAs generated by bacteria utilising microalgal feedstock (Tan et al., 2022)

Algae feedstock	Bacterial strain	Type of PHA produced	References
Defatted *Chlorella* biomass	Paracoccus sp. LL1	P(3HB-co-3HV)	(Khomlaem et al., 2021)

Corallina mediterranea	Halomonas sp.	P(3HB-co-3HV)	(El-malek et al., 2021)
Laminaria japonica biomass	Paracoccus sp. LL1	P(3HB-co-3HV)	(Muhammad et al., 2020)
Ulva sp.	Haloferax mediterranei	P(3HB-co-3HV)	(Ghosh et al., 2019)
Jatropha biodiesel waste	Halomonas hydrothermaliMTCC 5445	P(3HB-co-3HV)	(Bera et al., 2015)
Gelidium amansii	Bacillus megaterium	P3HB	(Alkotaini, 2016)

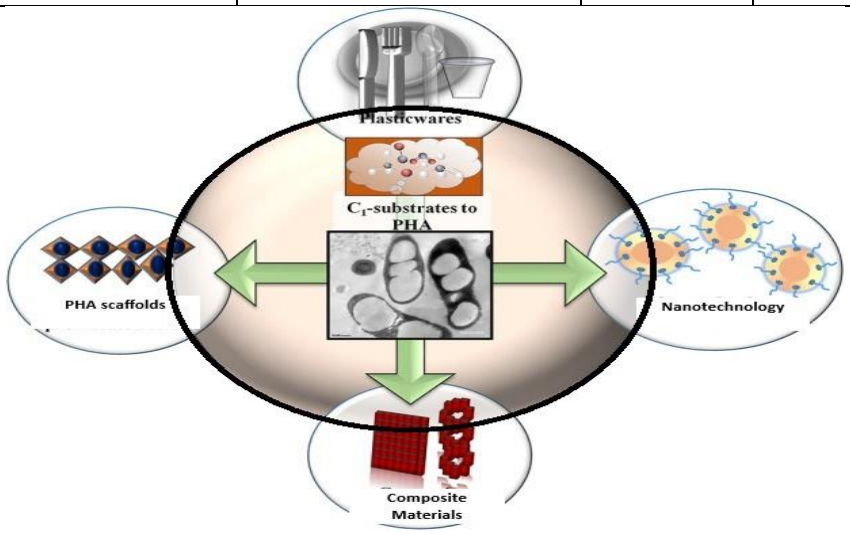

Figure 12: Manufacturing biopolymers from carbon substrates. Utilisations of PHA in various domains. (Adapted from Ray et al., 2022)

5.1.4 Industrial Production:

Polyhydroxyalkanoate polymers offer a broad spectrum of uses, but they are most used in bioplastic manufacturing. Despite this, bioplastics currently only make up 1% of the total plastics market. In order for PHA to replace synthetic plastics, the biopolymer market must become more competitive. There are many critical points in the issues with the PHA manufacturing process that need attention, such as cost, which is currently 2 to 5 times higher than synthetic plastic production. The demand for bioplastics has increased due to environmental pollution caused by plastics, which has been aggravated by the COVID-19 pandemic. Thus, bioplastic manufacturing is projected to grow in the next years. Researchers have been working to make the PHA production process more sustainable by improving upstream and downstream processes. Upstream processes focus

on cultivation and feeding strategies, while downstream processes involve polymer extraction from non-PHA biomass (Vicente et al., 2023).

Various methods have been used to make the process commercially viable, such as using industrial and agricultural by-products as carbon sources, extremophiles, transgenic plants, algae, and recombinant DNA technologies. Transgenic plants such as *Gossypium sp*, *Arabidopsis thaliana*, and *Camelina sativa* may produce PHAs at a reduced cost (Vicente et al., 2023).

- PHA production from CO_2 by cyanobacteria

CO_2 is a potent greenhouse gas emitter expected to increase by 60% by 2100 (Kumar et al., 2016). Microbial sequestration and fixation of CO_2 is a sustainable approach to mitigate CO_2 emissions and is more energy-efficient than catalytic conversion (Kumar et al., 2016). Cyanobacteria, hydrogen-oxidizing bacteria, and algae can consume CO2 and produce various valuable bioproducts, including PHA (Figure 13). Cyanobacteria can also tolerate high CO2 concentrations and are utilized in treating municipal and industrial wastewater (Arias et al., 2020). Under nutrient-limiting conditions, they produce different storage compounds, including PHA. Cyanobacteria perform oxygenic photosynthesis via the Calvin-Benson-Bassham cycle, which contains three phases: carboxylation, reduction, and regeneration. CO2 and HCO3 − are taken inside the cell via specific mechanisms, and CO2 transport occurs through Ci transporters at the plasma membrane. Several cyanobacterial species accumulate PHAs (Claassens, 2017).

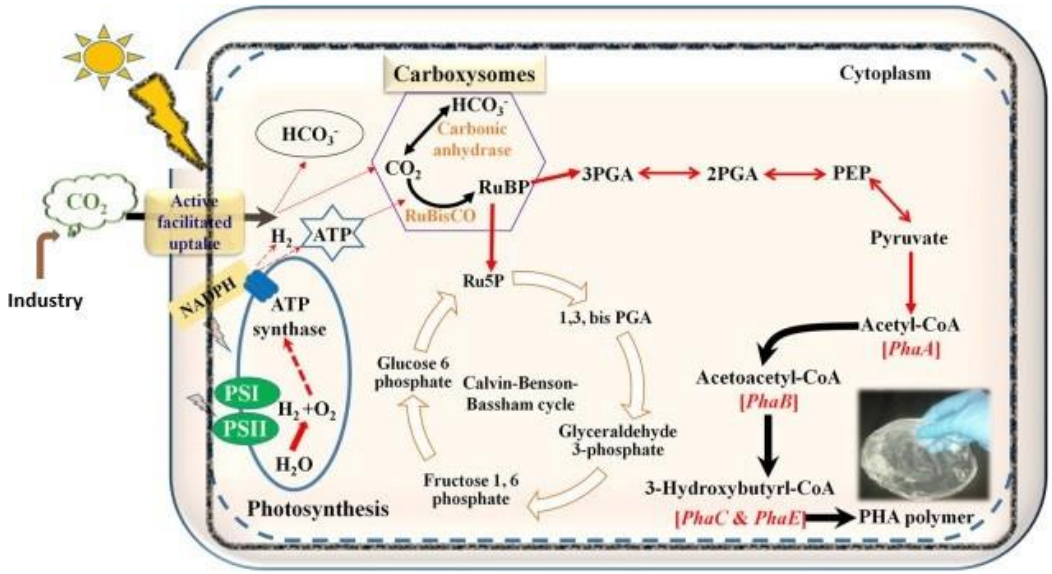

Figure 13: Schematic depiction of the PHA synthesis route in cyanobacteria using CO2 as a carbon source (Adapted from Ray et al., 2023)

- PHA production by hydrogen-oxidizing bacteria

Cupriavidus necator can utilize H_2 and O_2 as electron donors and acceptors with the assistance of ribulose biphosphate, which assists the bacterial cell in fixing CO_2 (Salehizadeh et al., 2020).

C. necator can convert carbon dioxide (CO_2) into PHA using different ratios of hydrogen (H_2) to CO_2. It may produce up to 75% PHB (Garcia-Gonzalez & De Wever, 2018). The PHA copolymer was cultivated using a fed-batch method, which yielded a dry mass of 65 g/L cell and a PHBV content of up to 27 mol% (Garcia-Gonzalez & De Wever, 2018). Under mixotrophic conditions, *C.necator* DSM 545 produced 1.14 g/L of P(3HB-co-3HV) via simultaneously using CO_2 and valeric acid. CO_2 was continuously passed through the medium in the form of bubbles to prevent substrate accumulation. Acetic acid may be an indirect CO_2 source that produces PHA homopolymers and copolymers. The ingestion of acetic acid was advantageous for the growth of *C. necator* and the synthesis of PHA (Ghysels et al., 2018).

- Bioconversion of methane to PHA:

Methane is the 2nd most common greenhouse gas, contributing 20% towards climate change and capturing ambient heat at a rate 25 times greater than CO2 (Mounasamy et al., 2020). Anthropogenic activities generate 63% of CH4, while coal mining, landfilling, and anaerobic wastewater treatment comprise the remaining percentage (Strong et al., 2016). Methane accounts for 90% of natural gas, and the global demand is assessed to inhance to 44.0% by 2040 (Liu et al., 2020).

Methane contributes to 20% of total greenhouse gas release (EU-28). This possibility has inspired academics to seek methods to mitigate greenhouse gases and environmental damage due to global warming worries (Cantera et al., 2018).

A particular strategy involves synthesising PHA from methane, serving as a sequestration method. The biotechnology technique is economically efficient and has little environmental consequences. This method utilises methanotrophs, which are microorganisms that transform CH_4 into CO_2 and H_2O by use O_2 as an electron acceptor. One carbon compound is conducive to the growth of methanotrophs, and in certain environments, certain species can even derive energy from multicarbon sources (Cantera et al., 2018; Semrau et al., 2010).

The aerobic methane-oxidizing bacteria known as γ-proteobacteria (Type I) and α-proteobacteria (Type II) are the well-researched methanotrophs. In order to assimilate carbon, they use the serine cycle and the ribulose monophosphate pathway (Cantera et al., 2018). Methane monooxygenase is the primary enzyme responsible for CH_4 oxidation and is present in either particulate or soluble forms. Non-methanotrophic bacterial consortia and anaerobic methanotrophic archaea work together to carry out anaerobic methane oxidation, converting CH4 into CH3OH (Pieja et al., 2017).

- PHA production from methanol

Methanol may be synthesized from biomass, industrial waste gas, and CO_2 hydrogenation methods. It may be employed as a feedstock in chemical and biological sectors to create compounds with increased value. Methanol in its liquid state is easy to manage and can be easily converted to PHA synthesis under environmental circumstances. Methylotrophic bacteria, particularly *Methylobacterium spp.,* are extensively researched for their production of polyhydroxybutyrate via the serine path. Mg^{2+}, NH^{4+},K^{+}, and PO_4^{3-} deficiencies are crucial for PHB buildup. Research focused on the manufacture of polyhydroxybutyrate and its copolymers from methanol by *Methylobacterium extorquens*. With fed-batch fermentation optimised, cell dry weight reached 115 g/L and polymer content reached 46% (w/w) (Borisut & Nuchitprasittichai, 2019).

The highest yield of PHB achieved using methanol as the carbon source was 0.18 g/g. Supplementing methanol with a co-substrate like n-amyl, or valeric acid, propionic acid may enhance the formation of PHB and its copolymer. Genetic modification might boost PHB production and output. Several data indicate that decreasing levels of malic acid, acetyl-CoA, and citric acid may enhance the formation of PHB. Additionally, adding NH^4+ and malic acid increased PHB production by *Methyloligella halotolerant* from methanol. Methanol was used by *Methylobacterium estrogens* DSMZ 1340 in order to produce 9.5 g/L of PHB, with a cell dry mass of 65.3% and a yield of 0.16 g/g. This was accomplished while the organism was subjected to nutritional constraints including nitrogen and magnesium (Strong et al., 2016).

44% (w/w) PHB was produced by fed-batch culture of *Methylobacterium extorquens* DSMZ. The polymer of *Methylobacterium extorquens* AM1 consists of PHA copolymers, namely 3HV, 3HB, and 3HHx, which were produced via recombinant technology. The CO2 content in the medium has an impact regarding both cell growth and PHA generation. A little quantity of CO2 enabled the attainment of a 3HV content of up to 6

mol%. The removal of the pccA gene led to an elevation in the proportion of 3HV in terpolymers (Ezhov et al., 2017).

Using methanol as its sole carbon source, Methylobacterium extorquens AM1 produced PHB at concentrations as high as 149 g/L. The media's composition was shown to be a crucial technique for enhancing cell biomass and PHB production. The use of methanol as a substrate in PHA synthesis is expected to result in reduced expenditures compared to using pure sugar, mostly due to its cheaper cost. However, it is worth noting that the yield of PHA is quite low when using other substrates. Organisms' tolerance should be increased to improve their suitability for industrial applications (Liao et al., 2016).

- PHA production from carbon monoxide

Several steel plant industries and thermal power plant generate substantial quantities of carbon monoxide, posing a threat if not managed correctly. For example, the South Korean steel firm POSCO produces almost 60 million cubic metres of carbon monoxide every day (Hwang et al., 2020). Although it is feasible to produce value-added goods from CO, there are certain constraints in the process. Carbon monoxide is an inert gas with poor process efficiency and a danger of explosion, limiting its use. Moreover, carbon monoxide is harmful to certain bacteria, but certain germs are capable of using it (Choi, Cho, et al., 2020).

One kind of bacteria known to be able to manufacture PHB in a two-phase whole-cell bio-catalytic operating system is *Acetobacterium woodii*, which uses CO as a substrate. *A. woodii* uses CO in the first stage and generates 53.1–60.7 mM of formate. During the second stage, a genetically modified strain of *Methylobacterium extorquens* utilises the formate produced in the first step to enhance PHB content by as much as 2.24%. This two-step procedure is an efficient method for carbon monoxide utilisation and has significant potential for use in biotechnological sectors. Further study is required to comprehend the fundamental process of converting carbon monoxide into polyhydroxyalkanoates. Once recognised, it might prove to be a great resource for the sector (Hwang et al., 2020).

- PHA production from formate

The electrochemical reduction of syngas hydration or carbon dioxide produces formate ($HCOO^-$) as a sustainable microbial feed substrate, generating methane, carbon monoxide, and ethylene. Certain microorganisms such as non-pigmented *Pseudomonas*, facultative autotrophs, methylotrophs, hypomicrobia, and heterotrophs can grow on formate using the serine or ribulose monophosphate path (Yoon et al., 2021). Formate is

used by *Methylorubrum extorquens* via the serine cycle and tetrahydrofolate pathway. The use of formate as substrate for *Escherichia coli* cultivations has been limited due to its slow growth (Kim et al., 2019). Still, *Methylobacterium extorquens* has shown promising results for producing various bioproducts from one carbon feed, such as proteins, PHAs, and dicarboxylic acids. *Methylobacterium extorquens* uses the Foc A transporter to utilize formate (Sun et al., 2019).

5.2 Opportunities for future research and innovation:

In 2023 the worldwide PHA market grown from its 2019 estimated value of USD 57 million to USD 98 million. The compound annual growth rate (CAGR) is 11.2% (Fior Markets, 2023). After North America and Asia-Pacific, Europe is presently PHA's biggest market. Short-chain length PHA is anticipated to dominate the market in terms of both value and volume. Increased awareness of the environmental effect of plastic waste has resulted in a higher demand for biodegradable polymers. This has led to an increased need for short-chain and medium-chain length PHA (Shahid et al., 2021). Europe is expected to continue to dominate the PHA market in terms of value during the forecast period. This is due to the presence of key players in the region, such as Biomer (Germany), Bio-on (Italy), Natureplast (France), and EarthBi (Germany), ColorFabb (Netherlands). Furthermore, the rising usage of biodegradable polymers in packaging and food service, biomedical, and agricultural applications powers the need for PHAs (Fior Markets, 2023). PHAs are promising biopolymers that provide an excellent opportunity to develop sustainable materials. They exhibit a wide range of copolymers and monomers with adjustable physical characteristics, which makes it a stimulating area of study for researchers. In order to maximise the output of biopolymers while lowering costs, researchers are looking at additional processing methods like 3D printing. Bioinformatics is a significant tool for improving PHA manufacturing by developing models based on trustworthy historical data to forecast properties and design materials (Pilania et al., 2019). Machine learning is also helpful in predicting chemical trends in biopolymers' mechanical and rheological properties. Based on the topology, charge/polarity, and shape of certain chemical units, data may be utilised in predicting structure and fingerprinting techniques (Bejagam et al., 2022).

By examining simulations of deformation, yield stress, and Young's modulus in various PHA types, for example, molecular dynamics simulations can forecast chemical trends in the mechanical and rheological characteristics of biopolymers. These predictions have shown that yield stress and Young's modulus diminish as the number of carbon atoms in

the side chain and polymer backbone rises. Functional groups in polymers enhance yield stress and Young's modulus. Bioinformatics offers guidance for developing systematic design principles to alter mechanical characteristics and perhaps customise biopolymers for industrial applications in the future (Bejagam et al., 2020).

The field is now able to anticipate the melting temperature of PHAs with efficiency and accuracy thanks to the introduction of machine learning. By merging curated data sets with molecular weights, polydispersity indices, and characteristics about the topology, shape, and charge/polarity of certain motifs, Bejagam et al., used machine learning to forecast the melting temperatures of PHAs. Simultaneously, other research groups also concentrated on glass transition temperature Tg values. This project will incorporate and improve systems biology and evolutionary engineering approaches to solve polymer design with multiobjective optimisation difficulties (Bejagam et al., 2020).

PHAs have a promising future since the worldwide market size for bioplastics is projected to grow from USD 9.2 billion to USD 20 billion by 2026. The worldwide bioplastics production capacity exceeded 2.1 million tonnes in 2020 and is expected to increase to 2.9 million tonnes by 2025. The genes responsible for PHA formation are well characterised, and foreign proteins may be attached to the N- and C-terminus to enable the attachment of one or more enzymes on the particle surface (Nanda et al., 2022).

PHA technique offers good manufacturing yields, monomer and polymer functionalization, stability, variety, and flexibility, despite certain unknown features of PHA particle formation. Different recombinant expression techniques may yield PHA, which has a longer shelf life and is more biodegradable. Nevertheless, some physicochemical characteristics, such particle size and dispersion, might be difficult to regulate. The current market pricing remains a challenge, thus, it is essential to establish conditions for creating new or improved producer strains. They may be accomplished via systems biology, protein engineering, synthetic biology, and evolutionary engineering (Dilkes-Hoffman et al., 2019).

PHA has a bright future in a variety of applications, including nanotechnology for making films with nanometric fibres and food packaging with antibacterial and breathable qualities, as well as medical devices such as medication delivery or mask material. Biomaterials have the potential to be the ultimate answer for the increasing need for plastic items and single-use healthcare materials. PHA is a fascinating biopolymer due to its ability to undergo spontaneous degradation. Thankfully, 3HB and 4HB, which are breakdown products of PHA, are naturally occurring metabolites in human beings. 3HB

is a constituent of the blood, whereas 4HB is extensively present in each of the major organs of the human body(Chen-Hui M et al., 2023; Lezcano et al., 2022).

Biopolymers, like PHAs, offer significant opportunities for commercialization due to their distinct features in comparison to other biopolymeric compounds. These biopolymers gradually degrade and are more easily sterilised compared to conventional plastic or metal devices (Kalia et al., 2023). This would be advantageous in situations when many surgical procedures are necessary or when a device has to be replaced because of deterioration. An eventual use of the technology is to create synthetic organs that can be surgically inserted into the human body. Furthermore, these devices may be used in prostheses to facilitate the restoration of functioning in impaired limbs. PHAs may function as templates for the production of recombinant proteins, which have the potential to be used as therapeutic agents for the treatment of several ailments. PHAs are used in the production of bone cement because to their robust mechanical characteristics. These examples represent only a small fraction of the many possible biological uses of PHA. Emerging technologies are being researched to expand the use of these substances in multiple uses and medical research endeavours (Kalia et al., 2023).

In summary, the outlook for PHA is optimistic both in the now and in the future. Although the production costs of PHA are still higher than petroleum-based plastics, gene engineering and bacterial production provide increased yields and the capability to modify and customise monomers and polymers to adjust thermal, mechanical, and rheological properties based on the monomer used. Further advancements in PHA manufacturing and processing will enhance its significance in the plastics sector and the creation of eco-friendly materials for diverse uses (Choi, Rhie, et al., 2020).

CONCLUSION

The exploration of PHAs within this thesis has highlighted their vast potential for medical applications, with a particular focus on the burgeoning field of dentistry. PHAs, as sustainable, biocompatible, and bioresorbable polymers, have demonstrated their capacity to serve as innovative materials for various dental applications, ranging from surgical sutures to dental implants.

The research presented has underscored the unique properties of PHAs that make them particularly suitable for dental use. Their ability to degrade in the body without leaving harmful residues aligns with the current demand for materials that can minimize long-term side effects and reduce the need for repeated surgeries. The mechanical strength of PHA copolymers, which surpasses that of homopolymers, is especially critical in dental applications where materials are subjected to considerable stress.

Furthermore, the potential of PHAs to sequester pathogenic microorganisms offers a novel approach to preventing and treating oral diseases, with applications in products such as toothpaste, mouthwash, and dental pastes. This antimicrobial property, combined with the ability to tailor PHA formulations for specific dental applications, presents a significant advancement in oral healthcare.

The green perspective of PHAs cannot be overstated. As bioplastics, PHAs represent a shift towards environmentally responsible materials that can be produced cost-effectively on a large scale. Their biodegradability and production from renewable resources contribute to a reduced environmental footprint, making them an attractive alternative to traditional, non-degradable dental materials.

In conclusion, the findings of this thesis suggest that PHAs hold a promising future in dentistry, offering a confluence of biocompatibility, mechanical robustness, and environmental sustainability. As research progresses, it is anticipated that PHAs will play an increasingly vital role in dental material innovation, leading to improved patient outcomes and a greener approach to oral health solutions. The potential of PHAs in dentistry is a testament to their versatility and the broader commitment to sustainable and advanced healthcare practices.

REFERENCES

Acharjee, S. A., Bharali, P., Gogoi, B., Sorhie, V., Walling, B., & Alemtoshi. (2023a). PHA-Based Bioplastic: a Potential Alternative to Address Microplastic Pollution. *Water, Air, & Soil Pollution*, *234*(1), 21. https://doi.org/10.1007/s11270-022-06029-2

Adepu, S., & Ramakrishna, S. (2021). Controlled Drug Delivery Systems: Current Status and Future Directions. *Molecules*, *26*(19), 5905. https://doi.org/10.3390/molecules26195905

Alavi, S. , T. S. , S. K. P. , K. N. , V. J. , & Y. S. (2014). *Polymers for packaging applications. CRC press.*

Alfano, S., Lorini, L., Majone, M., Sciubba, F., Valentino, F., & Martinelli, A. (2021). Ethylic Esters as Green Solvents for the Extraction of Intracellular Polyhydroxyalkanoates Produced by Mixed Microbial Culture. *Polymers*, *13*(16), 2789. https://doi.org/10.3390/polym13162789

Ali, I., & Jamil, N. (2016). Polyhydroxyalkanoates: Current applications in the medical field. *Frontiers in Biology*, *11*(1), 19–27. https://doi.org/10.1007/s11515-016-1389-z

Alkotaini, B. , K. H. , and K. B. S. (2016). *Production of Polyhydroxyalkanoates by Batch and Fed-Batch Cultivations of Bacillus megaterium from Acid-Treated Red Algae. Korean J. Chem*. *Eng 33*, 1669–1673.

Amini, A. R., Laurencin, C. T., & Nukavarapu, S. P. (2012). Bone Tissue Engineering: Recent Advances and Challenges. *Critical Reviews™in Biomedical Engineering*, *40*(5), 363–408. https://doi.org/10.1615/CritRevBiomedEng.v40.i5.10

Ang, S. L., Shaharuddin, B., Chuah, J.-A., & Sudesh, K. (2020). Electrospun poly(3-hydroxybutyrate-co-3-hydroxyhexanoate)/silk fibroin film is a promising scaffold for bone tissue engineering. *International Journal of Biological Macromolecules*, *145*, 173–188. https://doi.org/10.1016/j.ijbiomac.2019.12.149

Ansari, S., Sami, N., Yasin, D., Ahmad, N., & Fatma, T. (2021). Biomedical applications of environmental friendly poly-hydroxyalkanoates. *International Journal of Biological Macromolecules*, *183*, 549–563. https://doi.org/10.1016/j.ijbiomac.2021.04.171

Aramvash, A., Moazzeni Zavareh, F., & Gholami Banadkuki, N. (2018). Comparison of different solvents for extraction of polyhydroxybutyrate from Cupriavidus necator. *Engineering in Life Sciences*, *18*(1), 20–28. https://doi.org/10.1002/elsc.201700102

Arias, D. M., García, J., & Uggetti, E. (2020). Production of polymers by cyanobacteria grown in wastewater: Current status, challenges and future perspectives. *New Biotechnology*, *55*, 46–57. https://doi.org/10.1016/j.nbt.2019.09.001

Babos, G., Rydz, J., Kawalec, M., Klim, M., Fodor-Kardos, A., Trif, L., & Feczkó, T. (2020). Poly(3-Hydroxybutyrate)-Based Nanoparticles for Sorafenib and Doxorubicin Anticancer Drug Delivery. *International Journal of Molecular Sciences*, *21*(19), 7312. https://doi.org/10.3390/ijms21197312

Bagdadi, A. V., Safari, M., Dubey, P., Basnett, P., Sofokleous, P., Humphrey, E., Locke, I., Edirisinghe, M., Terracciano, C., Boccaccini, A. R., Knowles, J. C., Harding, S. E., & Roy, I. (2018). Poly(3-hydroxyoctanoate), a promising new material for cardiac tissue engineering. *Journal of Tissue Engineering and Regenerative Medicine*, *12*(1), e495–e512. https://doi.org/10.1002/term.2318

Basnett, P., Matharu, R. K., Taylor, C. S., Illangakoon, U., Dawson, J. I., Kanczler, J. M., Behbehani, M., Humphrey, E., Majid, Q., Lukasiewicz, B., Nigmatullin, R., Heseltine, P., Oreffo, R. O. C., Haycock, J. W., Terracciano, C., Harding, S. E., Edirisinghe, M., & Roy, I. (2021). Harnessing Polyhydroxyalkanoates and Pressurized Gyration for Hard and Soft Tissue Engineering. *ACS Applied Materials & Interfaces*, *13*(28), 32624–32639. https://doi.org/10.1021/acsami.0c19689

Behera, S., Priyadarshanee, M., Vandana, & Das, S. (2022). Polyhydroxyalkanoates, the bioplastics of microbial origin: Properties, biochemical synthesis, and their applications. *Chemosphere*, *294*, 133723. https://doi.org/10.1016/j.chemosphere.2022.133723

Bejagam, K. K., Gupta, N. S., Lee, K.-S., Iverson, C. N., Marrone, B. L., & Pilania, G. (2022). Predicting the Mechanical Response of Polyhydroxyalkanoate Biopolymers Using Molecular Dynamics Simulations. *Polymers*, *14*(2), 345. https://doi.org/10.3390/polym14020345

Bejagam, K. K., Iverson, C. N., Marrone, B. L., & Pilania, G. (2020). Molecular dynamics simulations for glass transition temperature predictions of polyhydroxyalkanoate biopolymers. *Physical Chemistry Chemical Physics*, *22*(32), 17880–17889. https://doi.org/10.1039/D0CP03163A

Bera, A., Dubey, S., Bhayani, K., Mondal, D., Mishra, S., & Ghosh, P. K. (2015). Microbial synthesis of polyhydroxyalkanoate using seaweed-derived crude levulinic acid as co-nutrient. *International Journal of Biological Macromolecules*, *72*, 487–494. https://doi.org/10.1016/j.ijbiomac.2014.08.037

Berger, E., Ramsay, B. A., Ramsay, J. A., Chavarie, C., & Braunegg, G. (1989). PHB recovery by hypochlorite digestion of non-PHB biomass. *Biotechnology Techniques*, *3*(4), 227–232. https://doi.org/10.1007/BF01876053

Bian, Y.-Z., Wang, Y., Aibaidoula, G., Chen, G.-Q., & Wu, Q. (2009). Evaluation of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) conduits for peripheral nerve regeneration. *Biomaterials*, *30*(2), 217–225. https://doi.org/10.1016/j.biomaterials.2008.09.036

Bio-on. (2019, January 28). *Unilever and Bio-on present My Kai the new line of ultra-green sun creams*.

Bio-On is back, saved by the Maip group. (2023, March). *Bioplastics MAGAZINE*.

Boesel, L. F., Meur, S. Le, Thöny-Meyer, L., & Ren, Q. (2014). The effect of molecular weight on the material properties of biosynthesized poly(4-hydroxybutyrate). *International Journal of Biological Macromolecules*, *71*, 124–130. https://doi.org/10.1016/j.ijbiomac.2014.04.015

Bondar, M., Pedro, F., Oliveira, M. C., da Fonseca, M. M. R., & Cesário, M. T. (2022). Red algae industrial residues as a sustainable carbon platform for the co-production of poly-3-hydroxybutyrate and gluconic acid by Halomonas boliviensis. *Frontiers in Bioengineering and Biotechnology*, *10*. https://doi.org/10.3389/fbioe.2022.934432

Borisut, P., & Nuchitprasittichai, A. (2019). Methanol Production via CO2 Hydrogenation: Sensitivity Analysis and Simulation—Based Optimization. *Frontiers in Energy Research*, *7*. https://doi.org/10.3389/fenrg.2019.00081

Brzeska, J., Heimowska, A., Janeczek, H., Kowalczuk, M., & Rutkowska, M. (2014). Polyurethanes Based on Atactic Poly[(R,S)-3-hydroxybutyrate]: Preliminary Degradation Studies in Simulated Body Fluids. *Journal of Polymers and the Environment*, *22*(2), 176–182. https://doi.org/10.1007/s10924-014-0650-2

Butt, F. I., Muhammad, N., Hamid, A., Moniruzzaman, M., & Sharif, F. (2018). Recent progress in the utilization of biosynthesized polyhydroxyalkanoates for biomedical applications – Review. *International Journal of Biological Macromolecules*, *120*, 1294–1305. https://doi.org/10.1016/j.ijbiomac.2018.09.002

Canadas, R. F., Cavalheiro, J. M. B. T., Guerreiro, J. D. T., de Almeida, M. C. M. D., Pollet, E., da Silva, C. L., da Fonseca, M. M. R., & Ferreira, F. C. (2014). Polyhydroxyalkanoates: Waste glycerol upgrade into electrospun fibrous scaffolds for stem cells culture. *International Journal of Biological Macromolecules*, *71*, 131–140. https://doi.org/10.1016/j.ijbiomac.2014.05.008

Cano-Vicent, A., Tambuwala, M. M., Hassan, Sk. S., Barh, D., Aljabali, A. A. A., Birkett, M., Arjunan, A., & Serrano-Aroca, Á. (2021). Fused deposition modelling: Current status, methodology, applications and future prospects. *Additive Manufacturing*, *47*, 102378. https://doi.org/10.1016/j.addma.2021.102378

Cantera, S., Sánchez-Andrea, I., Lebrero, R., García-Encina, P. A., Stams, A. J. M., & Muñoz, R. (2018). Carbon dioxide sequestration by chemolithotrophic oleaginous bacteria for production and optimization of polyhydroxyalkanoate. *Bioresource Technology*, *267*, 401–407. https://doi.org/10.1016/j.biortech.2018.07.057

Carlson, B. (2007). "Natural plastics" ripe with potential. *Biotechnology Healthcare*, *4*(3), 11–18.

Castellano, D., Blanes, M., Marco, B., Cerrada, I., Ruiz-Saurí, A., Pelacho, B., Araña, M., Montero, J. A., Cambra, V., Prosper, F., & Sepúlveda, P. (2014). A Comparison of Electrospun Polymers Reveals Poly(3-Hydroxybutyrate) Fiber as a Superior Scaffold for Cardiac Repair. *Stem Cells and Development*, *23*(13), 1479–1490. https://doi.org/10.1089/scd.2013.0578

Cavalheiro, J. M. B. T., Pollet, E., Diogo, H. P., Cesário, M. T., Avérous, L., de Almeida, M. C. M. D., & da Fonseca, M. M. R. (2013). On the heterogeneous composition of bacterial polyhydroxyalkanoate terpolymers. *Bioresource Technology*, *147*, 434–441. https://doi.org/10.1016/j.biortech.2013.08.009

Chen, F., Liu, X., Ge, X., Wang, Y., Zhao, Z., Zhang, X., Chen, G.-Q., & Sun, Y. (2023). Porous polydroxyalkanoates (PHA) scaffolds with antibacterial property for oral soft tissue regeneration. *Chemical Engineering Journal*, *451*, 138899. https://doi.org/10.1016/j.cej.2022.138899

Chen, G. Q., & Wu, Q. (2005). The application of polyhydroxyalkanoates as tissue engineering materials. In *Biomaterials* (Vol. 26, Issue 33, pp. 6565–6578). https://doi.org/10.1016/j.biomaterials.2005.04.036

Chen, G.-Q., & Hajnal, I. (2015). The 'PHAome.' *Trends in Biotechnology*, *33*(10), 559–564. https://doi.org/10.1016/j.tibtech.2015.07.006

Chen, G.-Q., & Zhang, J. (2018). Microbial polyhydroxyalkanoates as medical implant biomaterials. *Artificial Cells, Nanomedicine, and Biotechnology*, *46*(1), 1–18. https://doi.org/10.1080/21691401.2017.1371185

Chen, P., & Nikoyan, L. (2021). Guided Implant Surgery. *Dental Clinics of North America*, *65*(1), 67–80. https://doi.org/10.1016/j.cden.2020.09.005

Chen, Z., Fu, F., Yu, Y., Wang, H., Shang, Y., & Zhao, Y. (2019). Cardiomyocytes-Actuated *Morpho* Butterfly Wings. *Advanced Materials*, *31*(8). https://doi.org/10.1002/adma.201805431

Cheng, J., Nordeste, R., Trainer, M. A., & Charles, T. C. (2017). *Methods for the Isolation of Genes Encoding Novel PHA Metabolism Enzymes from Complex Microbial Communities* (pp. 237–248). https://doi.org/10.1007/978-1-4939-6691-2_15

Chen-Hui M, Xin-Ya Qi, Yan-Wen Ding, & Jing Zhou. (2023). Recent advances of medical polyhydroxyalkanoates in musculoskeletal system. *Biomater Transl*, *4*, 234–247.

Choi, S. Y., Cho, I. J., Lee, Y., Kim, Y., Kim, K., & Lee, S. Y. (2020). Microbial Polyhydroxyalkanoates and Nonnatural Polyesters. *Advanced Materials*, *32*(35). https://doi.org/10.1002/adma.201907138

Choi, S. Y., Rhie, M. N., Kim, H. T., Joo, J. C., Cho, I. J., Son, J., Jo, S. Y., Sohn, Y. J., Baritugo, K.-A., Pyo, J., Lee, Y., Lee, S. Y., & Park, S. J. (2020). Metabolic engineering for the synthesis of polyesters: A 100-year journey from

polyhydroxyalkanoates to non-natural microbial polyesters. *Metabolic Engineering*, *58*, 47–81. https://doi.org/10.1016/j.ymben.2019.05.009

Chokshi, N. V., Khatri, H. N., & Patel, M. M. (2018). Formulation, optimization, and characterization of rifampicin-loaded solid lipid nanoparticles for the treatment of tuberculosis. *Drug Development and Industrial Pharmacy*, *44*(12), 1975–1989. https://doi.org/10.1080/03639045.2018.1506472

Chu, C. C. (2013). Materials for absorbable and nonabsorbable surgical sutures. In *Biotextiles as Medical Implants* (pp. 275–334). Elsevier. https://doi.org/10.1533/9780857095602.2.275

Chuah, J.-A., Yamada, M., Taguchi, S., Sudesh, K., Doi, Y., & Numata, K. (2013). Biosynthesis and characterization of polyhydroxyalkanoate containing 5-hydroxyvalerate units: Effects of 5HV units on biodegradability, cytotoxicity, mechanical and thermal properties. *Polymer Degradation and Stability*, *98*(1), 331–338. https://doi.org/10.1016/j.polymdegradstab.2012.09.008

Chung, C. W., Kim, H. W., Kim, Y. B., & Rhee, Y. H. (2003). Poly(ethylene glycol)-grafted poly(3-hydroxyundecenoate) networks for enhanced blood compatibility. *International Journal of Biological Macromolecules*, *32*(1–2), 17–22. https://doi.org/10.1016/S0141-8130(03)00020-5

Claassens, N. J. (2017). A warm welcome for alternative <scp>CO</scp> 2 fixation pathways in microbial biotechnology. *Microbial Biotechnology*, *10*(1), 31–34. https://doi.org/10.1111/1751-7915.12456

Conte, R., De Luise, A., Petillo, O., Rengo, C., Riccitiello, F., Di Salle, A., Calarco, A., & Peluso, G. (2017). Biodegradable polymers for dental tissue engineering and regeneration. In *Biodegradable Polymers: Recent Developments and New Perspectives* (pp. 237–296). IAPC Publishing. https://doi.org/10.5599/obp.14.5

Cool, S. M., Kenny, B., Wu, A., Nurcombe, V., Trau, M., Cassady, A. I., & Grøndahl, L. (2007). Poly(3-hydroxybutyrate- *co* -3-hydroxyvalerate) composite biomaterials for bone tissue regeneration: *In vitro* performance assessed by osteoblast proliferation, osteoclast adhesion and resorption, and macrophage proinflammatory response. *Journal of Biomedical Materials Research Part A*, *82A*(3), 599–610. https://doi.org/10.1002/jbm.a.31174

Croitoru, A.-M., Ficai, D., Ficai, A., Mihailescu, N., Andronescu, E., & Turculet, S. (2020). Nanostructured Fibers Containing Natural or Synthetic Bioactive Compounds in Wound Dressing Applications. *Materials*, *13*(10), 2407. https://doi.org/10.3390/ma13102407

Dabiri, G., Damstetter, E., & Phillips, T. (2016). Choosing a Wound Dressing Based on Common Wound Characteristics. *Advances in Wound Care*, *5*(1), 32–41. https://doi.org/10.1089/wound.2014.0586

Danny Sharma. (2023, September). *Polyhydroxyalkanoate (PHA) Market: A Green Alternative to Traditional Plastics*.

Dilkes-Hoffman, L. S., Lant, P. A., Laycock, B., & Pratt, S. (2019). The rate of biodegradation of PHA bioplastics in the marine environment: A meta-study. *Marine Pollution Bulletin*, *142*, 15–24. https://doi.org/10.1016/j.marpolbul.2019.03.020

Dobrogojski, J., Spychalski, M., Luciński, R., & Borek, S. (2018). Transgenic plants as a source of polyhydroxyalkanoates. *Acta Physiologiae Plantarum*, *40*(9), 162. https://doi.org/10.1007/s11738-018-2742-4

Doineau, E., Perdrier, C., Allayaud, F., Blanchet, E., Preziosi-belloy, L., Grousseau, E., Gontard, N., & Angellier-Coussy, H. (2023). Designing poly(3-hydroxybutyrate-co-3-hydroxyvalerate) P(3HB-co-3HV) films with tailored mechanical properties. *Materials Today Communications*, *36*, 106848. https://doi.org/10.1016/j.mtcomm.2023.106848

Dwivedi, R., Pandey, R., Kumar, S., & Mehrotra, D. (2020). Poly hydroxyalkanoates (PHA): Role in bone scaffolds. *Journal of Oral Biology and Craniofacial Research*, *10*(1), 389–392. https://doi.org/10.1016/j.jobcr.2019.10.004

Dydak, K., Junka, A., Nowacki, G., Paleczny, J., Szymczyk-Ziółkowska, P., Górzyńska, A., Aniołek, O., & Bartoszewicz, M. (2022). In Vitro Cytotoxicity, Colonisation by Fibroblasts and Antimicrobial Properties of Surgical Meshes Coated with Bacterial Cellulose. *International Journal of Molecular Sciences*, *23*(9). https://doi.org/10.3390/ijms23094835

Dyrda, G., Boniewska-Bernacka, E., Man, D., Barchiewicz, K., & Słota, R. (2019). The effect of organic solvents on selected microorganisms and model liposome membrane. *Molecular Biology Reports*, *46*(3), 3225–3232. https://doi.org/10.1007/s11033-019-04782-y

Egan, B., & D'Agostino, D. P. (2016). Fueling Performance: Ketones Enter the Mix. *Cell Metabolism*, *24*(3), 373–375. https://doi.org/10.1016/j.cmet.2016.08.021

El Asri, O. (2022). Anaerobic Biodegradation: The Anaerobic Digestion Process. In *Handbook of Biodegradable Materials* (pp. 1–26). Springer International Publishing. https://doi.org/10.1007/978-3-030-83783-9_4-1

El-malek, F. A., Rofeal, M., Farag, A., Omar, S., & Khairy, H. (2021). Polyhydroxyalkanoate nanoparticles produced by marine bacteria cultivated on cost effective Mediterranean algal hydrolysate media. *Journal of Biotechnology*, *328*, 95–105. https://doi.org/10.1016/j.jbiotec.2021.01.008

Elmowafy, E., Abdal-Hay, A., Skouras, A., Tiboni, M., Casettari, L., & Guarino, V. (2019). Polyhydroxyalkanoate (PHA): applications in drug delivery and tissue engineering. *Expert Review of Medical Devices*, *16*(6), 467–482. https://doi.org/10.1080/17434440.2019.1615439

Ezhov, V. A., Doronina, N. V., Shmareva, M. N., & Trotsenko, Yu. A. (2017). Synthesis of high-molecular-mass polyhydroxybutyrate from methanol in Methyloligella halotolerans C2. *Applied Biochemistry and Microbiology*, *53*(1), 47–51. https://doi.org/10.1134/S0003683817010112

Feng, H., Lu, X., Wang, W., Kang, N.-G., & Mays, J. (2017). Block Copolymers: Synthesis, Self-Assembly, and Applications. *Polymers*, *9*(12), 494. https://doi.org/10.3390/polym9100494

Fenton, O. S., Olafson, K. N., Pillai, P. S., Mitchell, M. J., & Langer, R. (2018). Advances in Biomaterials for Drug Delivery. *Advanced Materials*, *30*(29). https://doi.org/10.1002/adma.201705328

Feres, M., Figueiredo, L. C., Soares, G. M. S., & Faveri, M. (2015). Systemic antibiotics in the treatment of periodontitis. *Periodontology 2000*, *67*(1), 131–186. https://doi.org/10.1111/prd.12075

Fior Markets. (2023). Biodegradable Plastics Market by Type (PLA, Starch Blends, PHA, biodegradable Polyester), End use industry, and region, Global Forecast 2028. *Biodegradable Plastics Market*.

Fitzpatrick, S. G., Cohen, D. M., & Clark, A. N. (2019). Ulcerated Lesions of the Oral Mucosa: Clinical and Histologic Review. *Head and Neck Pathology*, *13*(1), 91–102. https://doi.org/10.1007/s12105-018-0981-8

Flores Bueso, Y., Walker, S., Quinn, J., & Tangney, M. (2020). A novel cell permeability assay for macromolecules. *BMC Molecular and Cell Biology*, *21*(1), 75. https://doi.org/10.1186/s12860-020-00321-x

Galgut, P., Pitrola, R., Waite, I., Doyle, C., & Smith, R. (1991). Histological evaluation of biodegradable and non-degradable membranes placed transcutaneously in rats. *Journal of Clinical Periodontology*, *18*(8), 581–586. https://doi.org/10.1111/j.1600-051X.1991.tb00093.x

Gao, Y., Kong, L., Zhang, L., Gong, Y., Chen, G., Zhao, N., & Zhang, X. (2006). Improvement of mechanical properties of poly(dl-lactide) films by blending of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate). *European Polymer Journal*, *42*(4), 764–775. https://doi.org/10.1016/j.eurpolymj.2005.09.028

Garcia-Gonzalez, L., & De Wever, H. (2018). Acetic Acid as an Indirect Sink of CO2 for the Synthesis of Polyhydroxyalkanoates (PHA): Comparison with PHA Production Processes Directly Using CO2 as Feedstock. *Applied Sciences*, *8*(9), 1416. https://doi.org/10.3390/app8091416

George, A., Varghese, H., Chandran, A., Surendran, K. P., & Gowd, E. B. (2024). Directional freezing-induced self-poled piezoelectric nylon 11 aerogels as high-performance mechanical energy harvesters. *Journal of Materials Chemistry A*, *12*(2), 911–922. https://doi.org/10.1039/D3TA05911A

Ghalia, M. A., & Dahman, Y. (2017). Investigating the effect of multi-functional chain extenders on PLA/PEG copolymer properties. *International Journal of Biological Macromolecules*, *95*, 494–504. https://doi.org/10.1016/j.ijbiomac.2016.11.003

Ghosh, S., Gnaim, R., Greiserman, S., Fadeev, L., Gozin, M., & Golberg, A. (2019). Macroalgal biomass subcritical hydrolysates for the production of polyhydroxyalkanoate (PHA) by Haloferax mediterranei. *Bioresource Technology*, *271*, 166–173. https://doi.org/10.1016/j.biortech.2018.09.108

Ghysels, S., Mozumder, Md. S. I., De Wever, H., Volcke, E. I. P., & Garcia-Gonzalez, L. (2018). Targeted poly(3-hydroxybutyrate-co-3-hydroxyvalerate) bioplastic production from carbon dioxide. *Bioresource Technology*, *249*, 858–868. https://doi.org/10.1016/j.biortech.2017.10.081

Gielisch, M., Heimes, D., Thiem, D. G. E., Boesing, C., Krumpholtz, M., Al-Nawas, B., & Kämmerer, P. W. (2022). Steam-sterilized and degradable fused filament fabrication-printed polylactide/ polyhydroxyalkanoate surgical guides for dental implants: Are they accurate enough for static navigation? *International Journal of Bioprinting*, *9*(2), 655. https://doi.org/10.18063/ijb.v9i2.655

Gopi, S., Kontopoulou, M., Ramsay, B. A., & Ramsay, J. A. (2018). Manipulating the structure of medium-chain-length polyhydroxyalkanoate (MCL-PHA) to enhance thermal properties and crystallization kinetics. *International Journal of Biological Macromolecules*, *119*, 1248–1255. https://doi.org/10.1016/j.ijbiomac.2018.08.016

Gregory, D. A., Fricker, A. T. R., Mitrev, P., Ray, M., Asare, E., Sim, D., Larpnimitchai, S., Zhang, Z., Ma, J., Tetali, S. S. V., & Roy, I. (2023). Additive Manufacturing of Polyhydroxyalkanoate-Based Blends Using Fused Deposition Modelling for the Development of Biomedical Devices. *Journal of Functional Biomaterials*, *14*(1), 40. https://doi.org/10.3390/jfb14010040

Gregory, D. A., Taylor, C. S., Fricker, A. T. R., Asare, E., Tetali, S. S. V., Haycock, J. W., & Roy, I. (2022). Polyhydroxyalkanoates and their advances for biomedical applications. *Trends in Molecular Medicine*, *28*(4), 331–342. https://doi.org/10.1016/j.molmed.2022.01.007

Grigore, M. E., Grigorescu, R. M., Iancu, L., Ion, R.-M., Zaharia, C., & Andrei, E. R. (2019). Methods of synthesis, properties and biomedical applications of polyhydroxyalkanoates: a review. *Journal of Biomaterials Science, Polymer Edition*, *30*(9), 695–712. https://doi.org/10.1080/09205063.2019.1605866

Guo, W., Yang, K., Qin, X., Luo, R., Wang, H., & Huang, R. (2022a). Polyhydroxyalkanoates in tissue repair and regeneration. In *Engineered Regeneration* (Vol. 3, Issue 1, pp. 24–40). KeAi Communications Co. https://doi.org/10.1016/j.engreg.2022.01.003

Hagiopol, C. (2016). Copolymers. In *Reference Module in Materials Science and Materials Engineering*. Elsevier. https://doi.org/10.1016/B978-0-12-803581-8.01126-7

Hajiali, H., Hosseinalipour, M., Karbasi, S., & Shokrgozar, M. A. (2012). The influence of bioglass nanoparticles on the biodegradation and biocompatibility of poly (3-hydroxybutyrate) scaffolds. *The International Journal of Artificial Organs*, *35*(11), 1015–1024. https://doi.org/10.5301/ijao.5000119

He, Y., Hu, Z., Ren, M., Ding, C., Chen, P., Gu, Q., & Wu, Q. (2014). Evaluation of PHBHHx and PHBV/PLA fibers used as medical sutures. *Journal of Materials Science: Materials in Medicine*, *25*(2), 561–571. https://doi.org/10.1007/s10856-013-5073-4

Hegde, M., Meenakshisundaram, V., Chartrain, N., Sekhar, S., Tafti, D., Williams, C. B., & Long, T. E. (2017). 3D Printing All-Aromatic Polyimides using Mask-Projection Stereolithography: Processing the Nonprocessable. *Advanced Materials*, *29*(31). https://doi.org/10.1002/adma.201701240

https://www.bbraun.fr/fr/products/b5/monomax.html. (2022, September 26). B. BRAUN Surgical SA.

Hu, Y.-J., Wei, X., Zhao, W., Liu, Y.-S., & Chen, G.-Q. (2009). Biocompatibility of poly(3-hydroxybutyrate-co-3-hydroxyvalerate-co-3-hydroxyhexanoate) with bone marrow mesenchymal stem cells. *Acta Biomaterialia*, *5*(4), 1115–1125. https://doi.org/10.1016/j.actbio.2008.09.021

Huong, K.-H., Sevakumaran, V., & Amirul, A. A. (2021). P(3HB- *co* -4HB) as high value polyhydroxyalkanoate: its development over recent decades and current advances. *Critical Reviews in Biotechnology*, *41*(4), 474–490. https://doi.org/10.1080/07388551.2020.1869685

Hwang, H. W., Yoon, J., Min, K., Kim, M.-S., Kim, S.-J., Cho, D. H., Susila, H., Na, J.-G., Oh, M.-K., & Kim, Y. H. (2020). Two-stage bioconversion of carbon monoxide to biopolymers via formate as an intermediate. *Chemical Engineering Journal*, *389*, 124394. https://doi.org/10.1016/j.cej.2020.124394

Ion, R.-M. (2017). Porphyrins and Phthalocyanines: Photosensitizers and Photocatalysts. In *Phthalocyanines and Some Current Applications*. InTech. https://doi.org/10.5772/intechopen.68654

Iqbal, H. M. N., & Keshavarz, T. (2018). Bioinspired polymeric carriers for drug delivery applications. In *Stimuli Responsive Polymeric Nanocarriers for Drug Delivery Applications, Volume 1* (pp. 377–404). Elsevier. https://doi.org/10.1016/B978-0-08-101997-9.00018-7

Kalia, V. C., Patel, S. K. S., & Lee, J.-K. (2023a). Exploiting Polyhydroxyalkanoates for Biomedical Applications. *Polymers*, *15*(8), 1937. https://doi.org/10.3390/polym15081937

Kalia, V. C., Patel, S. K. S., & Lee, J.-K. (2023b). Exploiting Polyhydroxyalkanoates for Biomedical Applications. *Polymers*, *15*(8), 1937. https://doi.org/10.3390/polym15081937

Karahaliloglu, Z., Ercan, B., Taylor, E. N., Chung, S., Denkbaş, E. B., & Webster, T. J. (2015). Antibacterial Nanostructured Polyhydroxybutyrate Membranes for Guided Bone Regeneration. *Journal of Biomedical Nanotechnology*, *11*(12), 2253–2263. https://doi.org/10.1166/jbn.2015.2106

Keskin, G., Klzll, G., Bechelany, M., Pochat-Bohatier, C., & Öner, M. (2017). Potential of polyhydroxyalkanoate (PHA) polymers family as substitutes of petroleum based polymers for packaging applications and solutions brought by their composites to form barrier materials. *Pure and Applied Chemistry*, *89*(12), 1841–1848. https://doi.org/10.1515/pac-2017-0401

Khamkong, T., Penkhrue, W., & Lumyong, S. (2022). Optimization of Production of Polyhydroxyalkanoates (PHAs) from Newly Isolated Ensifer sp. Strain HD34 by Response Surface Methodology. *Processes*, *10*(8), 1632. https://doi.org/10.3390/pr10081632

Khomlaem, C., Aloui, H., & Kim, B. S. (2021). Biosynthesis of Polyhydroxyalkanoates from Defatted Chlorella Biomass as an Inexpensive Substrate. *Applied Sciences*, *11*(3), 1094. https://doi.org/10.3390/app11031094

Kilicay, E., Erdal, E., Elci, P., Hazer, B., & Denkbas, E. B. (2024). Tumour-specific hybrid nanoparticles in therapy of breast cancer. *Journal of Microencapsulation*, *41*(1), 45–65. https://doi.org/10.1080/02652048.2023.2292226

Kim, S.-J., Yoon, J., Im, D.-K., Kim, Y. H., & Oh, M.-K. (2019). Adaptively evolved Escherichia coli for improved ability of formate utilization as a carbon source in sugar-free conditions. *Biotechnology for Biofuels*, *12*(1), 207. https://doi.org/10.1186/s13068-019-1547-z

Kischkel, S., Grabow, N., Püschel, A., Erdle, B., Kabelitz, M., Martin, D. P., Williams, S. F., Bombor, I., Sternberg, K., Schmitz, K.-P., Schareck, W., & Bünger, C. M. (2016). Biodegradable polymeric stents for vascular application in a porcine carotid artery model. *Gefässchirurgie*, *21*(S1), 30–36. https://doi.org/10.1007/s00772-015-0011-z

Kitsara, M., Agbulut, O., Kontziampasis, D., Chen, Y., & Menasché, P. (2017). Fibers for hearts: A critical review on electrospinning for cardiac tissue engineering. *Acta Biomaterialia*, *48*, 20–40. https://doi.org/10.1016/j.actbio.2016.11.014

Kovalcik, A. (2021). Recent Advances in 3D Printing of Polyhydroxyalkanoates: A Review. *The EuroBiotech Journal*, *5*(1), 48–55. https://doi.org/10.2478/ebtj-2021-0008

Kumar, M., Gupta, A., & Thakur, I. S. (2016). Carbon dioxide sequestration by chemolithotrophic oleaginous bacteria for production and optimization of

polyhydroxyalkanoate. *Bioresource Technology*, *213*, 249–256. https://doi.org/10.1016/j.biortech.2016.02.038

Kumar, M., Rathour, R., Singh, R., Sun, Y., Pandey, A., Gnansounou, E., Andrew Lin, K.-Y., Tsang, D. C. W., & Thakur, I. S. (2020). Bacterial polyhydroxyalkanoates: Opportunities, challenges, and prospects. *Journal of Cleaner Production*, *263*, 121500. https://doi.org/10.1016/j.jclepro.2020.121500

Laycock, B., Halley, P., Pratt, S., Werker, A., & Lant, P. (2014). The chemomechanical properties of microbial polyhydroxyalkanoates. *Progress in Polymer Science*, *39*(2), 397–442. https://doi.org/10.1016/j.progpolymsci.2013.06.008

Lemoigne, M. (1926). Products of dehydration and of polymerization of β-hydroxybutyric acid. *Bull Soc Chem Biol*, *7*, 770–782.

Lezcano, M. F., Álvarez, G., Chuhuaicura, P., Godoy, K., Alarcón, J., Acevedo, F., Gareis, I., & Dias, F. J. (2022). Polyhydroxybutyrate (PHB) Scaffolds for Peripheral Nerve Regeneration: A Systematic Review of Animal Models. *Biology*, *11*(5), 706. https://doi.org/10.3390/biology11050706

Li, Y., Thouas, G. A., & Chen, Q.-Z. (2012). Biodegradable soft elastomers: synthesis/properties of materials and fabrication of scaffolds. *RSC Advances*, *2*(22), 8229. https://doi.org/10.1039/c2ra20736b

Liao, J. C., Mi, L., Pontrelli, S., & Luo, S. (2016). Fuelling the future: microbial engineering for the production of sustainable biofuels. *Nature Reviews Microbiology*, *14*(5), 288–304. https://doi.org/10.1038/nrmicro.2016.32

Liu, L.-Y., Xie, G.-J., Xing, D.-F., Liu, B.-F., Ding, J., & Ren, N.-Q. (2020). Biological conversion of methane to polyhydroxyalkanoates: Current advances, challenges, and perspectives. *Environmental Science and Ecotechnology*, *2*, 100029. https://doi.org/10.1016/j.ese.2020.100029

Lizarraga-Valderrama, L. R., Panchal, B., Thomas, C., Boccaccini, A. R., & Roy, I. (2016). Biomedical Applications of Polyhydroxyalkanoates. In *Biomaterials from Nature for Advanced Devices and Therapies* (pp. 337–383). Wiley. https://doi.org/10.1002/9781119126218.ch20

Loh, X. J., Wang, X., Li, H., Li, X., & Li, J. (2007). Compositional study and cytotoxicity of biodegradable poly(ester urethane)s consisting of poly[(R)-3-hydroxybutyrate] and poly(ethylene glycol). *Materials Science and Engineering: C*, *27*(2), 267–273. https://doi.org/10.1016/j.msec.2006.05.009

Ma, Y.-M., Wei, D.-X., Yao, H., Wu, L.-P., & Chen, G.-Q. (2016). Synthesis, Characterization and Application of Thermoresponsive Polyhydroxyalkanoate-*graft* -Poly(*N* -isopropylacrylamide). *Biomacromolecules*, *17*(8), 2680–2690. https://doi.org/10.1021/acs.biomac.6b00724

Mačković, M., Hoppe, A., Detsch, R., Mohn, D., Stark, W. J., Spiecker, E., & Boccaccini, A. R. (2012). Bioactive glass (type 45S5) nanoparticles: in vitro reactivity on nanoscale and biocompatibility. *Journal of Nanoparticle Research*, *14*(7), 966. https://doi.org/10.1007/s11051-012-0966-6

Mai, J., Kockler, K., Parisi, E., Chan, C. M., Pratt, S., & Laycock, B. (2024). Synthesis and physical properties of polyhydroxyalkanoate (PHA)-based block copolymers: A review. *International Journal of Biological Macromolecules*, *263*, 130204. https://doi.org/10.1016/j.ijbiomac.2024.130204

MAI, R., HAGEDORN, M. G., GELINSKY, M., WERNER, C., TURHANI, D., SPÄTH, H., GEDRANGE, T., & LAUER, G. (2006). Ectopic bone formation in nude rats using human osteoblasts seeded poly(3)hydroxybutyrate embroidery and hydroxyapatite-collagen tapes constructs. *Journal of Cranio-Maxillofacial Surgery*, *34*, 101–109. https://doi.org/10.1016/S1010-5182(06)60022-X

Manavitehrani, I., Fathi, A., Badr, H., Daly, S., Negahi Shirazi, A., & Dehghani, F. (2016). Biomedical Applications of Biodegradable Polyesters. *Polymers*, *8*(1), 20. https://doi.org/10.3390/polym8010020

Marcello, E., Maqbool, M., Nigmatullin, R., Cresswell, M., Jackson, P. R., Basnett, P., Knowles, J. C., Boccaccini, A. R., & Roy, I. (2021). Antibacterial Composite Materials Based on the Combination of Polyhydroxyalkanoates With Selenium and Strontium Co-substituted Hydroxyapatite for Bone Regeneration. *Frontiers in Bioengineering and Biotechnology*, *9*. https://doi.org/10.3389/fbioe.2021.647007

Masaeli, E., Morshed, M., Nasr-Esfahani, M. H., Sadri, S., Hilderink, J., van Apeldoorn, A., van Blitterswijk, C. A., & Moroni, L. (2013). Fabrication, Characterization and Cellular Compatibility of Poly(Hydroxy Alkanoate) Composite Nanofibrous Scaffolds for Nerve Tissue Engineering. *PLoS ONE*, *8*(2), e57157. https://doi.org/10.1371/journal.pone.0057157

Meereboer, K. W., Misra, M., & Mohanty, A. K. (2020). Review of recent advances in the biodegradability of polyhydroxyalkanoate (PHA) bioplastics and their composites. *Green Chemistry*, *22*(17), 5519–5558. https://doi.org/10.1039/D0GC01647K

Mehrpouya, M., Vahabi, H., Barletta, M., Laheurte, P., & Langlois, V. (2021). Additive manufacturing of polyhydroxyalkanoates (PHAs) biopolymers: Materials, printing techniques, and applications. *Materials Science and Engineering: C*, *127*, 112216. https://doi.org/10.1016/j.msec.2021.112216

Meneses, L., Esmail, A., Matos, M., Sevrin, C., Grandfils, C., Barreiros, S., Reis, M. A. M., Freitas, F., & Paiva, A. (2022). Subcritical Water as a Pre-Treatment of Mixed Microbial Biomass for the Extraction of Polyhydroxyalkanoates. *Bioengineering*, *9*(7), 302. https://doi.org/10.3390/bioengineering9070302

Mohanna, P. -N., Young, R. C., Wiberg, M., & Terenghi, G. (2003). A composite poly-hydroxybutyrate–glial growth factor conduit for long nerve gap repairs. *Journal of Anatomy*, *203*(6), 553–565. https://doi.org/10.1046/j.1469-7580.2003.00243.x

Motta, S. E., Lintas, V., Fioretta, E. S., Dijkman, P. E., Putti, M., Caliskan, E., Rodriguez Cetina Biefer, H., Lipiski, M., Sauer, M., Cesarovic, N., Hoerstrup, S. P., & Emmert, M. Y. (2019). Human cell-derived tissue-engineered heart valve with integrated Valsalva sinuses: towards native-like transcatheter pulmonary valve replacements. *Npj Regenerative Medicine*, *4*(1), 14. https://doi.org/10.1038/s41536-019-0077-4

Mounasamy, V., Mani, G. K., Ponnusamy, D., Tsuchiya, K., Reshma, P. R., Prasad, A. K., & Madanagurusamy, S. (2020). Investigation on CH4 sensing characteristics of hierarchical V2O5 nanoflowers operated at relatively low temperature using chemiresistive approach. *Analytica Chimica Acta*, *1106*, 148–160. https://doi.org/10.1016/j.aca.2020.01.060

Muhammad, M., Aloui, H., Khomlaem, C., Hou, C. T., & Kim, B. S. (2020). Production of polyhydroxyalkanoates and carotenoids through cultivation of different bacterial strains using brown algae hydrolysate as a carbon source. *Biocatalysis and Agricultural Biotechnology*, *30*, 101852. https://doi.org/10.1016/j.bcab.2020.101852

Muhammadi, Shabina, Afzal, M., & Hameed, S. (2015). Bacterial polyhydroxyalkanoates-eco-friendly next generation plastic: Production, biocompatibility, biodegradation, physical properties and applications. *Green Chemistry Letters and Reviews*, *8*(3–4), 56–77. https://doi.org/10.1080/17518253.2015.1109715

Muheim, C., Götzke, H., Eriksson, A. U., Lindberg, S., Lauritsen, I., Nørholm, M. H. H., & Daley, D. O. (2017). Increasing the permeability of Escherichia coli using MAC13243. *Scientific Reports*, *7*(1), 17629. https://doi.org/10.1038/s41598-017-17772-6

Nagase, K., Shukuwa, R., Takahashi, H., Takeda, N., & Okano, T. (2020). Enhanced mechanical properties and cell separation with thermal control of PIPAAm-brushed polymer-blend microfibers. *Journal of Materials Chemistry B*, *8*(28), 6017–6026. https://doi.org/10.1039/D0TB00972E

Nanda, S., Patra, B. R., Patel, R., Bakos, J., & Dalai, A. K. (2022). Innovations in applications and prospects of bioplastics and biopolymers: a review. *Environmental Chemistry Letters*, *20*(1), 379–395. https://doi.org/10.1007/s10311-021-01334-4

Neagu, M., Manda, G., Constantin, C., Radu, E., & Ion, R.-M. (2007). Synthetic porphyrins in experimental photodynamic therapy induce a different antitumoral effect. *Journal of Porphyrins and Phthalocyanines*, *11*(01), 58–65. https://doi.org/10.1142/S1088424607000084

Neves, N. M., & Reis, R. L. (2016). *Biomaterials from Nature for Advanced Devices and Therapies*.

Numata, K., Abe, H., & Iwata, T. (2009). Biodegradability of Poly(hydroxyalkanoate) Materials. *Materials*, *2*(3), 1104–1126. https://doi.org/10.3390/ma2031104

Odermatt, E. K., Funk, L., Bargon, R., Martin, D. P., Rizk, S., & Williams, S. F. (2012). MonoMax Suture: A New Long-Term Absorbable Monofilament Suture Made from Poly-4-Hydroxybutyrate. *International Journal of Polymer Science*, *2012*, 1–12. https://doi.org/10.1155/2012/216137

Ojumu, T. V., Yu, J., & Solomon, B. O. (2004). Production of Polyhydroxyalkanoates, a bacterial biodegradable polymer. *African Journal of Biotechnology*, *3*(1), 18–24. https://doi.org/10.5897/AJB2004.000-2004

Owji, N., Mandakhbayar, N., Gregory, D. A., Marcello, E., Kim, H. W., Roy, I., & Knowles, J. C. (2021). Mussel Inspired Chemistry and Bacteria Derived Polymers for Oral Mucosal Adhesion and Drug Delivery. *Frontiers in Bioengineering and Biotechnology*, *9*. https://doi.org/10.3389/fbioe.2021.663764

Panaitescu, D. M., Lupescu, I., Frone, A. N., Chiulan, I., Nicolae, C. A., Tofan, V., Stefaniu, A., Somoghi, R., & Trusca, R. (2017). Medium Chain-Length Polyhydroxyalkanoate Copolymer Modified by Bacterial Cellulose for Medical Devices. *Biomacromolecules*, *18*(10), 3222–3232. https://doi.org/10.1021/acs.biomac.7b00855

Panchal, M., Khare, S., Khamkar, P., & Suresh Bhole, K. (2022). Dental implants: A review of types, design analysis, materials, additive manufacturing methods, and future scope. *Materials Today: Proceedings*, *68*, 1860–1867. https://doi.org/10.1016/j.matpr.2022.08.049

Panith, N., Assavanig, A., Lertsiri, S., Bergkvist, M., Surarit, R., & Niamsiri, N. (2016). Development of tunable biodegradable polyhydroxyalkanoates microspheres for controlled delivery of tetracycline for treating periodontal disease. *Journal of Applied Polymer Science*, *133*(42). https://doi.org/10.1002/app.44128

Park, H., He, H., Yan, X., Liu, X., Scrutton, N. S., & Chen, G.-Q. (2024). PHA is not just a bioplastic! *Biotechnology Advances*, *71*, 108320. https://doi.org/10.1016/j.biotechadv.2024.108320

Pecorini, G., Ferraro, E., & Puppi, D. (2023). Polymeric Systems for the Controlled Release of Flavonoids. *Pharmaceutics*, *15*(2), 628. https://doi.org/10.3390/pharmaceutics15020628

Peng, S.-W., Guo, X.-Y., Shang, G.-G., Li, J., Xu, X.-Y., You, M.-L., Li, P., & Chen, G.-Q. (2011a). An assessment of the risks of carcinogenicity associated with polyhydroxyalkanoates through an analysis of DNA aneuploid and telomerase activity. *Biomaterials*, *32*(10), 2546–2555. https://doi.org/10.1016/j.biomaterials.2010.12.051

Perry, T. E., Kaushal, S., Sutherland, F. W. H., Guleserian, K. J., Bischoff, J., Sacks, M., & Mayer, J. E. (2003). Bone marrow as a cell source for tissue engineering heart valves. *The Annals of Thoracic Surgery*, *75*(3), 761–767. https://doi.org/10.1016/S0003-4975(02)03776-1

Philip, S., Keshavarz, T., & Roy, I. (2007). Polyhydroxyalkanoates: biodegradable polymers with a range of applications. *Journal of Chemical Technology & Biotechnology*, *82*(3), 233–247. https://doi.org/10.1002/jctb.1667

Phuegyod, S., Pramual, S., Wattanavichean, N., Assawajaruwan, S., Amornsakchai, T., Sukho, P., Svasti, J., Surarit, R., & Niamsiri, N. (2023). Microbial Poly(hydroxybutyrate-co-hydroxyvalerate) Scaffold for Periodontal Tissue Engineering. *Polymers*, *15*(4), 855. https://doi.org/10.3390/polym15040855

Pieja, A. J., Morse, M. C., & Cal, A. J. (2017). Methane to bioproducts: the future of the bioeconomy? *Current Opinion in Chemical Biology*, *41*, 123–131. https://doi.org/10.1016/j.cbpa.2017.10.024

Pilania, G., Iverson, C. N., Lookman, T., & Marrone, B. L. (2019). Machine-Learning-Based Predictive Modeling of Glass Transition Temperatures: A Case of Polyhydroxyalkanoate Homopolymers and Copolymers. *Journal of Chemical Information and Modeling*, *59*(12), 5013–5025. https://doi.org/10.1021/acs.jcim.9b00807

Pooja Basnett. (2014). *Biosynthesis of polyhydroxyalkanoates, their novel blends and composites for biomedical applications*. University of Westminster.

Prabhakaran, M. P., Vatankhah, E., & Ramakrishna, S. (2013). Electrospun aligned PHBV/collagen nanofibers as substrates for nerve tissue engineering. *Biotechnology and Bioengineering*, *110*(10), 2775–2784. https://doi.org/10.1002/bit.24937

Pramual, S., Assavanig, A., Bergkvist, M., Batt, C. A., Sunintaboon, P., Lirdprapamongkol, K., Svasti, J., & Niamsiri, N. (2016). Development and characterization of bio-derived polyhydroxyalkanoate nanoparticles as a delivery system for hydrophobic photodynamic therapy agents. *Journal of Materials Science: Materials in Medicine*, *27*(2), 40. https://doi.org/10.1007/s10856-015-5655-4

Pulingam, T., Appaturi, J. N., Parumasivam, T., Ahmad, A., & Sudesh, K. (2022). Biomedical Applications of Polyhydroxyalkanoate in Tissue Engineering. *Polymers*, *14*(11), 2141. https://doi.org/10.3390/polym14112141

Puppi, D., Morelli, A., & Chiellini, F. (2017). Additive Manufacturing of Poly(3-hydroxybutyrate-co-3-hydroxyhexanoate)/poly(ε-caprolactone) Blend Scaffolds for Tissue Engineering. *Bioengineering*, *4*(2), 49. https://doi.org/10.3390/bioengineering4020049

QU, X., WU, Q., ZHANG, K., & CHEN, G. (2006). In vivo studies of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) based polymers: Biodegradation and tissue reactions. *Biomaterials*. https://doi.org/10.1016/j.biomaterials.2006.02.015

Rai, R., Keshavarz, T., Roether, J. A., Boccaccini, A. R., & Roy, I. (2011). Medium chain length polyhydroxyalkanoates, promising new biomedical materials for the future. *Materials Science and Engineering: R: Reports*, *72*(3), 29–47. https://doi.org/10.1016/j.mser.2010.11.002

Raposo, R. S., de Almeida, M. C. M. D., da Fonseca, M. M. R., & Cesário, M. T. (2017). Feeding strategies for tuning poly (3-hydroxybutyrate-co-4-hydroxybutyrate) monomeric composition and productivity using Burkholderia sacchari. *International Journal of Biological Macromolecules*, *105*, 825–833. https://doi.org/10.1016/j.ijbiomac.2017.07.111

Ray, S., Jin, J.-O., Choi, I., & Kim, M. (2022). Recent trends of biotechnological production of polyhydroxyalkanoates from C1 carbon sources. *Frontiers in Bioengineering and Biotechnology*, *10*, 907500. https://doi.org/10.3389/fbioe.2022.907500

Raza, Z. A., Abid, S., & Banat, I. M. (2018). Polyhydroxyalkanoates: Characteristics, production, recent developments and applications. *International Biodeterioration & Biodegradation*, *126*, 45–56. https://doi.org/10.1016/j.ibiod.2017.10.001

Rick Lingle. (2018, April 10). *PHA bioplastics a 'tunable' solution for convenience food packaging*. Packaging Digest and PlasticsToday.

Sahana, T. G., & Rekha, P. D. (2018). Biopolymers: Applications in wound healing and skin tissue engineering. *Molecular Biology Reports*, *45*(6), 2857–2867. https://doi.org/10.1007/s11033-018-4296-3

Salehizadeh, H., Yan, N., & Farnood, R. (2020). Recent advances in microbial CO2 fixation and conversion to value-added products. *Chemical Engineering Journal*, *390*, 124584. https://doi.org/10.1016/j.cej.2020.124584

Salim, Y. S., Sharon, A., Vigneswari, S., Mohamad Ibrahim, M. N., & Amirul, A. A. (2012). Environmental Degradation of Microbial Polyhydroxyalkanoates and Oil Palm-Based Composites. *Applied Biochemistry and Biotechnology*, *167*(2), 314–326. https://doi.org/10.1007/s12010-012-9688-6

Sanhueza, C., Acevedo, F., Rocha, S., Villegas, P., Seeger, M., & Navia, R. (2019). Polyhydroxyalkanoates as biomaterial for electrospun scaffolds. *International Journal of Biological Macromolecules*, *124*, 102–110. https://doi.org/10.1016/j.ijbiomac.2018.11.068

Saniye Soylemez, & Melis Kesika. (n.d.). Biosensing Devices: Conjugated Polymer Based Scaffolds. *Encyclopedia of Polymer Applications, First Edition*.

Sanjogta Thapa Magar. (2021). Fed-batch Culture- Definition, Principle, Process, Types, Applications, Limitations. *Microbe Notes*.

Schalck, T., Bergh, B. Van den, & Michiels, J. (2021). Increasing Solvent Tolerance to Improve Microbial Production of Alcohols, Terpenoids and Aromatics. *Microorganisms*, *9*(2), 249. https://doi.org/10.3390/microorganisms9020249

SCHOU, S. (2008). Implant treatment in periodontitis-susceptible patients: a systematic review. *Journal of Oral Rehabilitation*, *35*(s1), 9–22. https://doi.org/10.1111/j.1365-2842.2007.01830.x

Semrau, J. D., DiSpirito, A. A., & Yoon, S. (2010). Methanotrophs and copper. *FEMS Microbiology Reviews*, *34*(4), 496–531. https://doi.org/10.1111/j.1574-6976.2010.00212.x

Shahid, S., Razzaq, S., Farooq, R., & Nazli, Z. i. H. (2021). Polyhydroxyalkanoates: Next generation natural biomolecules and a solution for the world's future economy. In *International Journal of Biological Macromolecules* (Vol. 166, pp. 297–321). Elsevier B.V. https://doi.org/10.1016/j.ijbiomac.2020.10.187

Shangguan, Y.-Y., Wang, Y.-W., Wu, Q., & Chen, G.-Q. (2006). The mechanical properties and in vitro biodegradation and biocompatibility of UV-treated poly(3-hydroxybutyrate-co-3-hydroxyhexanoate). *Biomaterials*, *27*(11), 2349–2357. https://doi.org/10.1016/j.biomaterials.2005.11.024

Shao, P., Niu, B., Chen, H., & Sun, P. (2018). Fabrication and characterization of tea polyphenols loaded pullulan-CMC electrospun nanofiber for fruit preservation. *International Journal of Biological Macromolecules*, *107*, 1908–1914. https://doi.org/10.1016/j.ijbiomac.2017.10.054

Sharma, V., Sehgal, R., & Gupta, R. (2021). Polyhydroxyalkanoate (PHA): Properties and Modifications. *Polymer*, *212*. https://doi.org/10.1016/j.polymer.2020.123161

Shishatskaya, E. I., & Volova, T. G. (2004). A comparative investigation of biodegradable polyhydroxyalkanoate films as matrices for in vitro cell cultures. *Journal of Materials Science: Materials in Medicine*, *15*(8), 915–923. https://doi.org/10.1023/B:JMSM.0000036280.98763.c1

Shrivastav, A., Kim, H.-Y., & Kim, Y.-R. (2013). Advances in the Applications of Polyhydroxyalkanoate Nanoparticles for Novel Drug Delivery System. *BioMed Research International*, *2013*, 1–12. https://doi.org/10.1155/2013/581684

Shuai, C., Guo, W., Gao, C., Yang, Y., Xu, Y., Liu, L., Qin, T., Sun, H., Yang, S., Feng, P., & Wu, P. (2017). Calcium Silicate Improved Bioactivity and Mechanical Properties of Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) Scaffolds. *Polymers*, *9*(5), 175. https://doi.org/10.3390/polym9050175

Singh, A. K., Srivastava, J. K., Chandel, A. K., Sharma, L., Mallick, N., & Singh, S. P. (2019a). Biomedical applications of microbially engineered polyhydroxyalkanoates: an insight into recent advances, bottlenecks, and solutions. *Applied Microbiology and Biotechnology*, *103*(5), 2007–2032. https://doi.org/10.1007/s00253-018-09604-y

Stevens, M. M. (2008). Biomaterials for bone tissue engineering. *Materials Today*, *11*(5), 18–25. https://doi.org/10.1016/S1369-7021(08)70086-5

Strong, P., Laycock, B., Mahamud, S., Jensen, P., Lant, P., Tyson, G., & Pratt, S. (2016). The Opportunity for High-Performance Biomaterials from Methane. *Microorganisms*, *4*(1), 11. https://doi.org/10.3390/microorganisms4010011

Sun, X., Atiyeh, H. K., Huhnke, R. L., & Tanner, R. S. (2019). Syngas fermentation process development for production of biofuels and chemicals: A review. *Bioresource Technology Reports*, *7*, 100279. https://doi.org/10.1016/j.biteb.2019.100279

Susan, M., Baldea, I., Senila, S., Macovei, V., Dreve, S., Ion, R. M., & Cosgarea, R. (2011). Photodamaging effects of porphyrins and chitosan on primary human keratinocytes and carcinoma cell cultures. *International Journal of Dermatology*, *50*(3), 280–286. https://doi.org/10.1111/j.1365-4632.2010.04700.x

Tan, D., Wang, Y., Tong, Y., & Chen, G.-Q. (2021). Grand Challenges for Industrializing Polyhydroxyalkanoates (PHAs). *Trends in Biotechnology*, *39*(9), 953–963. https://doi.org/10.1016/j.tibtech.2020.11.010

Tan, F. H. P., Nadir, N., & Sudesh, K. (2022). Microalgal Biomass as Feedstock for Bacterial Production of PHA: Advances and Future Prospects. *Frontiers in Bioengineering and Biotechnology*, *10*. https://doi.org/10.3389/fbioe.2022.879476

Tang, X., Westlie, A. H., Caporaso, L., Cavallo, L., Falivene, L., & Chen, E. Y. -X. (2020). Biodegradable Polyhydroxyalkanoates by Stereoselective Copolymerization of Racemic Diolides: Stereocontrol and Polyolefin-Like Properties. *Angewandte Chemie International Edition*, *59*(20), 7881–7890. https://doi.org/10.1002/anie.201916415

Tao, J., Song, C., Cao, M., Hu, D., Liu, L., Liu, N., & Wang, S. (2009). Thermal properties and degradability of poly(propylene carbonate)/poly(β-hydroxybutyrate-co-β-hydroxyvalerate) (PPC/PHBV) blends. *Polymer Degradation and Stability*, *94*(4), 575–583. https://doi.org/10.1016/j.polymdegradstab.2009.01.017

Tournier, V., Topham, C. M., Gilles, A., David, B., Folgoas, C., Moya-Leclair, E., Kamionka, E., Desrousseaux, M.-L., Texier, H., Gavalda, S., Cot, M., Guémard, E., Dalibey, M., Nomme, J., Cioci, G., Barbe, S., Chateau, M., André, I., Duquesne, S., & Marty, A. (2020). An engineered PET depolymerase to break down and recycle plastic bottles. *Nature*, *580*(7802), 216–219. https://doi.org/10.1038/s41586-020-2149-4

Urbina, L., Wongsirichot, P., Corcuera, M. Á., Gabilondo, N., Eceiza, A., Winterburn, J., & Retegi, A. (2018). Application of cider by-products for medium chain length polyhydroxyalkanoate production by Pseudomonas putida KT2440. *European Polymer Journal*, *108*, 1–9. https://doi.org/10.1016/j.eurpolymj.2018.08.020

Varžinskas, V., & Markevičiūtė, Z. (2020). Sustainable Food Packaging: Materials and Waste Management Solutions. *Environmental Research, Engineering and Management*, *76*(3), 154–164. https://doi.org/10.5755/j01.erem.76.3.27511

Vastano, M., Casillo, A., Corsaro, M. M., Sannia, G., & Pezzella, C. (2015). Production of medium chain length polyhydroxyalkanoates from waste oils by recombinant *Escherichia coli*. *Engineering in Life Sciences*, *15*(7), 700–709. https://doi.org/10.1002/elsc.201500022

Vicente, D., Proença, D. N., & Morais, P. V. (2023a). The Role of Bacterial Polyhydroalkanoate (PHA) in a Sustainable Future: A Review on the Biological Diversity. *International Journal of Environmental Research and Public Health*, *20*(4), 2959. https://doi.org/10.3390/ijerph20042959

Vigneswari, S., Chai, J. M., Shantini, K., Bhubalan, K., & Amirul, A. A. (2019). Designing Novel Interfaces via Surface Functionalization of Short-Chain-Length Polyhydroxyalkanoates. *Advances in Polymer Technology*, *2019*, 1–15. https://doi.org/10.1155/2019/3831251

Vigneswari, S., Gurusamy, T. P., Khairul, W. M., H.P.S., A. K., Ramakrishna, S., & Amirul, A.-A. A. (2021). Surface Characterization and Physiochemical Evaluation of P(3HB-co-4HB)-Collagen Peptide Scaffolds with Silver Sulfadiazine as Antimicrobial Agent for Potential Infection-Resistance Biomaterial. *Polymers*, *13*(15), 2454. https://doi.org/10.3390/polym13152454

Vigneswari, S., Murugaiyah, V., Kaur, G., Abdul Khalil, H. P. S., & Amirul, A. A. (2016). Simultaneous dual syringe electrospinning system using benign solvent to fabricate nanofibrous P(3HB-co-4HB)/collagen peptides construct as potential leave-on wound dressing. *Materials Science and Engineering: C*, *66*, 147–155. https://doi.org/10.1016/j.msec.2016.03.102

Vilar, G., Tulla-Puche, J., & Albericio, F. (2012). Polymers and Drug Delivery Systems. *Current Drug Delivery*, *9*(4), 367–394. https://doi.org/10.2174/156720112801323053

Visakh P. M. (2014). Polyhydroxyalkanoates (PHAs), their Blends, Composites and Nanocomposites: State of the Art, New Challenges and Opportunities. In *Polyhydroxyalkanoate (PHA) Based Blends, Composites and Nanocomposites* (pp. 1–17). The Royal Society of Chemistry. https://doi.org/10.1039/9781782622314-00001

Volkov, A. V., Muraev, A. A., Zharkova, I. I., Voinova, V. V., Akoulina, E. A., Zhuikov, V. A., Khaydapova, D. D., Chesnokova, D. V., Menshikh, K. A., Dudun, A. A., Makhina, T. K., Bonartseva, G. A., Asfarov, T. F., Stamboliev, I. A., Gazhva, Y. V., Ryabova, V. M., Zlatev, L. H., Ivanov, S. Y., Shaitan, K. V., & Bonartsev, A. P. (2020). Poly(3-hydroxybutyrate)/hydroxyapatite/alginate scaffolds seeded with mesenchymal stem cells enhance the regeneration of critical-sized bone defect.

Materials Science and Engineering: C, *114*, 110991. https://doi.org/10.1016/j.msec.2020.110991

Volova, T. G., Boyandin, A. N., Vasiliev, A. D., Karpov, V. A., Prudnikova, S. V., Mishukova, O. V., Boyarskikh, U. A., Filipenko, M. L., Rudnev, V. P., Bá Xuân, B., Việt Dũng, V., & Gitelson, I. I. (2010). Biodegradation of polyhydroxyalkanoates (PHAs) in tropical coastal waters and identification of PHA-degrading bacteria. *Polymer Degradation and Stability*, *95*(12), 2350–2359. https://doi.org/10.1016/j.polymdegradstab.2010.08.023

Wang, M., & Duan, B. (2011). Nanocomposite Scaffolds for Bone Tissue Engineering: Design, Fabrication, Surface Modification and Sustained Release of Growth Factor. *MRS Proceedings*, *1301*, mrsf10-1301-oo06-01. https://doi.org/10.1557/opl.2011.470

Wang, Y.-W., Yang, F., Wu, Q., Cheng, Y., Yu, P. H. F., Chen, J., & Chen, G.-Q. (2005). Effect of composition of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) on growth of fibroblast and osteoblast. *Biomaterials*, *26*(7), 755–761. https://doi.org/10.1016/j.biomaterials.2004.03.023

Wang, Z., Hu, H., Wang, Y., Wang, Y., Wu, Q., Liu, L., & Chen, G. (2006). Fabrication of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBHHx) microstructures using soft lithography for scaffold applications. *Biomaterials*, *27*(12), 2550–2557. https://doi.org/10.1016/j.biomaterials.2005.11.026

Weiser, J. R., & Saltzman, W. M. (2014). Controlled release for local delivery of drugs: barriers and models. *Journal of Controlled Release*, *190*, 664–673. https://doi.org/10.1016/j.jconrel.2014.04.048

Wen, Z., Chen, Y., Liao, P., Wang, F., Zeng, W., Liu, S., Wu, H., Wang, N., Moroni, L., Zhang, M., Duan, Y., & Chen, H. (2023). In Situ Precision Cell Electrospinning as an Efficient Stem Cell Delivery Approach for Cutaneous Wound Healing. *Advanced Healthcare Materials*, *12*(26). https://doi.org/10.1002/adhm.202300970

Williams, S. F., Martin, D. P., & Moses, A. C. (2016). The History of GalaFLEX P4HB Scaffold. *Aesthetic Surgery Journal*, *36*(suppl 2), S33–S42. https://doi.org/10.1093/asj/sjw141

Wu, C.-S. (2014). Mechanical properties, biocompatibility, and biodegradation of cross-linked cellulose acetate-reinforced polyester composites. *Carbohydrate Polymers*, *105*, 41–48. https://doi.org/10.1016/j.carbpol.2014.01.062

Wu, S., Liu, Y., Cui, B., Qu, X., & Chen, G. (2007). Study on Decellularized Porcine Aortic Valve/Poly (3-hydroxybutyrate-co-3-hydroxyhexanoate) Hybrid Heart Valve in Sheep Model. *Artificial Organs*, *31*(9), 689–697. https://doi.org/10.1111/j.1525-1594.2007.00442.x

Yan, C., Wang, Y., Shen, X.-Y., Yang, G., Jian, J., Wang, H.-S., Chen, G.-Q., & Wu, Q. (2011). MicroRNA regulation associated chondrogenesis of mouse MSCs grown on

polyhydroxyalkanoates. *Biomaterials*, *32*(27), 6435–6444. https://doi.org/10.1016/j.biomaterials.2011.05.031

Yang, X., Zhao, K., & Chen, G.-Q. (2002). Effect of surface treatment on the biocompatibility of microbial polyhydroxyalkanoates. *Biomaterials*, *23*(5), 1391–1397. https://doi.org/10.1016/S0142-9612(01)00260-5

Ye, H., Zhang, K., Kai, D., Li, Z., & Loh, X. J. (2018). Polyester elastomers for soft tissue engineering. *Chemical Society Reviews*, *47*(12), 4545–4580. https://doi.org/10.1039/C8CS00161H

Yoon, J., Chang, W., Oh, S.-H., Choi, S.-H., Yang, Y.-H., & Oh, M.-K. (2021). Metabolic engineering of Methylorubrum extorquens AM1 for poly (3-hydroxybutyrate-co-3-hydroxyvalerate) production using formate. *International Journal of Biological Macromolecules*, *177*, 284–293. https://doi.org/10.1016/j.ijbiomac.2021.02.092

Young, R. C., Terenghi, G., & Wiberg, M. (2002). Poly-3-hydroxybutyrate (PHB): a resorbable conduit for long-gap repair in peripheral nerves. *British Journal of Plastic Surgery*, *55*(3), 235–240. https://doi.org/10.1054/bjps.2002.3798

Yucel, D., Kose, G. T., & Hasirci, V. (2010). Polyester based nerve guidance conduit design. *Biomaterials*, *31*(7), 1596–1603. https://doi.org/10.1016/j.biomaterials.2009.11.013

Zembouai, I., Kaci, M., Bruzaud, S., Benhamida, A., Corre, Y.-M., & Grohens, Y. (2013). A study of morphological, thermal, rheological and barrier properties of Poly(3-hydroxybutyrate-Co-3-Hydroxyvalerate)/polylactide blends prepared by melt mixing. *Polymer Testing*, *32*(5), 842–851. https://doi.org/10.1016/j.polymertesting.2013.04.004

Zhao, H., Cui, Z., Sun, X., Turng, L.-S., & Peng, X. (2013). Morphology and Properties of Injection Molded Solid and Microcellular Polylactic Acid/Polyhydroxybutyrate-Valerate (PLA/PHBV) Blends. *Industrial & Engineering Chemistry Research*, *52*(7), 2569–2581. https://doi.org/10.1021/ie301573y

Zhao, J. (2003). Dual Subcellular Distribution of Cytochrome b5 in Plant, Cauliflower, Cells. *Journal of Biochemistry*, *133*(1), 115–121. https://doi.org/10.1093/jb/mvg009

Zhao, K., Deng, Y., Chun Chen, J., & Chen, G.-Q. (2003). Polyhydroxyalkanoate (PHA) scaffolds with good mechanical properties and biocompatibility. *Biomaterials*, *24*(6), 1041–1045. https://doi.org/10.1016/S0142-9612(02)00426-X

Zhong, L., Hu, D., Qu, Y., Peng, J., Huang, K., Lei, M., Wu, T., Xiao, Y., Gu, Y., & Qian, Z. (2019). Preparation of Adenosine-Loaded Electrospun Nanofibers and Their Application in Bone Regeneration. *Journal of Biomedical Nanotechnology*, *15*(5), 857–877. https://doi.org/10.1166/jbn.2019.2761

Zinn, M., Witholt, B., & Egli, T. (2001). Occurrence, synthesis and medical application of bacterial polyhydroxyalkanoate. *Advanced Drug Delivery Reviews*, *53*(1), 5–21. https://doi.org/10.1016/S0169-409X(01)00218-6

Zytner, P., Kumar, D., Elsayed, A., Mohanty, A., Ramarao, B. V., & Misra, M. (2023). A review on polyhydroxyalkanoate (PHA) production through the use of lignocellulosic biomass. *RSC Sustainability*, *1*(9), 2120–2134. https://doi.org/10.1039/D3SU00126A