INSTITUTO SUPERIOR DE CIÊNCIAS DA SAÚDE EGAS MONIZ

MESTRADO INTEGRADO EM CIÊNCIAS FARMACÊUTICAS

ANTIMICROBIAL SUSCEPTIBILITY OF BACTERIA ASSOCIATED WITH ORAL INFECTIONS

Trabalho submetido por
José Alexandre Fernandes
para a obtenção do grau de Mestre em Ciências Farmacêuticas

Outubro de 2015

INSTITUTO SUPERIOR DE CIÊNCIAS DA SAÚDE EGAS MONIZ

MESTRADO INTEGRADO EM CIÊNCIAS FARMACÊUTICAS

ANTIMICROBIAL SUSCEPTIBILITY OF BACTERIA ASSOCIATED WITH ORAL INFECTIONS

Trabalho submetido por
José Alexandre Fernandes
para a obtenção do grau de Mestre em Ciências Farmacêuticas

Trabalho orientado por
Pedro Oliveira

Outubro de 2015

To my grandfather and my cousin

Acknowledgements

I would like to acknowledge my grandparents and my parents, for giving me the opportunity to study further and for supporting me throughout my years in this university; my friends, for being there when I needed moral support; my fellow classmates, for giving me advice; and my supervisor, for his guidance and support during the writing of this dissertation.

Lastly, it is not without the efforts of many others that I completed this dissertation. Although they have not been personally mentioned, I would like to recognize their efforts, guidance and support throughout this dissertation.

Resumo

O envolvimento de doenças sistémicas e de resistência aos antibióticos com bactérias da cavidade oral está a aumentar. Desde a descoberta da penicilina em 1928, vários antibióticos foram desenvolvidos de modo a aumentar o espectro de acção e a combater infecções bacterianas. Os antibióticos conseguiram com que diversas doenças tivessem uma cura, melhoraram as condições nas cirurgias e aumentaram a esperança média de vida, por exemplo. No entanto, devido ao uso abusivo, a resistência aos antibióticos está a tornar-se mais difícil de combater. Apesar de estarem a ser desenvolvidos novos antibióticos, estes são mais caros que os que estão actualmente disponíveis no mercado, o que se torna um inconveniente. Uma nova abordagem é necessária para resolver estes problemas. A solução poderá passar por explorar as propriedades antimicrobianas de metais e a sua possível aplicação como nanopartículas.

Nesta dissertação é feita uma revisão dos antibióticos e anti-sépticos usados actualmente em Portugal no tratamento de infecções da cavidade oral assim como possíveis novos agentes antimicrobianos que estejam a ser desenvolvidos e que possam ser usados no futuro.

Palavras-chave: antimicrobiano; antibiótico; anti-séptico; infecção oral; nanopartícula.

Abstract

The evidence of the connection between systemic diseases and antibiotic resistance with oral bacteria is increasing. Since the discovery of penicillin in 1928, many new antibiotics have been developed to broaden the spectrum of activity and to combat bacterial infections. Antibiotics have helped cure various diseases, improved surgery conditions and elevate the life expectancy, for example. However, due to its abusive use, bacterial resistance is becoming harder to battle. Despite the development of new antibiotics, they are more expensive than the average available nowadays, which is an inconvenient. A new approach is needed to resolve these problems. The solution may reside in the antimicrobial properties of metals and their possible application as nanoparticles.

In this dissertation work, a revision is made about the antibiotics and antiseptics currently used in Portugal to treat infections in the oral cavity. It is also investigated the possible use of newly developed antimicrobial agents containing metallic elements.

Keywords: antimicrobial; antibiotic; antiseptic; nanoparticle; oral infection.

Table of contents

List of figures

List of tables

List of abbreviations

AgNPs	Silver nanoparticles
ATP	Adenosine triphosphate
CHX-HMP NPs	Chlorhexidine-hexametaphosphate nanoparticles
CuONPs	Copper oxide nanoparticles
Cu_2ONPs	Copper dioxide nanoparticles
DMAE-CB	Methacryloxylethylcetyl dimethyl ammonium chloride
DNA	Deoxyribonucleic acid
ENR	Enoyl-acyl carrier protein reductase
MBC	Minimum bactericidal concentration
MDBP	12-methacryloyloxydodecylpyridinium bromide
MIC	Minimum inhibitory concentration
MRSA	Methicillin-resistant *Staphylococcus aureus*
NAD+	Nicotinamide adenine dinucleotide
QPEI	Quaternary ammonium polyethylenimine
STD	Sexually transmitted disease
TiO_2NPs	Titanium dioxide nanoparticles
ZnONPs	Zinc oxide nanoparticles

Introduction

An antimicrobial agent is a compound that has a synthetic, semisynthetic or natural source and can inhibit the growth or kill microorganisms such as bacteria, fungi, viruses and protozoa. Antibiotics and antiseptics are antimicrobials (Cole, 2014).

The use of antibiotics to treat infections has increased since the discovery of penicillin in 1928 by Alexander Fleming. He discovered that a culture he had inoculated with *Staphylococcus aureus* had been contaminated with mold, which would then be named *Penicillium notatum*. The culture appeared to have transparent halos without bacterial growth. After a few tests to this mold, Fleming discovered that it produced a compound, penicillin, which had inhibition markers and antimicrobial properties capable of inhibiting and eliminate pathogenic bacteria. However, it was only after a few years that these results became a matter of interest to the scientific community (Ligon, 2004).

Since the discovery of penicillin and up until 1962, 20 new classes of antibiotics were developed and produced. The appearance of these new antibiotics made significant improvements to the health and life expectancy of the population. Without antibiotics, therapeutic interventions and treatments that are a routine nowadays (organ transplants, surgeries, for example) would not be possible (Coates, Halls, & Hu, 2011; Cole, 2014).

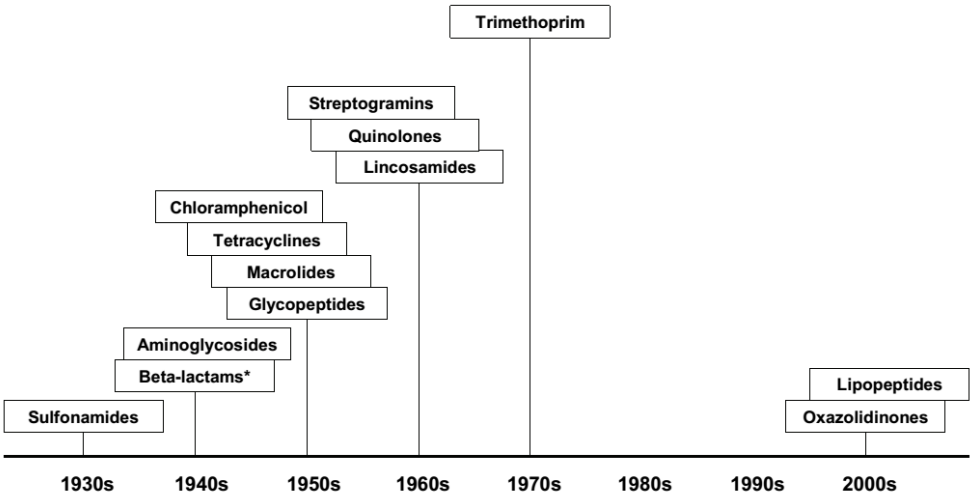

Figure 1: Timeline of the discovery of new antibiotics (Norrby et al., 2009).

However, in the recent years, the research and development for new antibiotics has decayed probably due to the commercialization of analogues being more profitable to the pharmaceutical industry, to the absence of new metabolic targets, to the declining number of pharmaceutical companies involved in the development of new drugs or even

to the insufficient funding for the development of new drugs (Aminov, 2010; Coates et al., 2011; Cole, 2014).

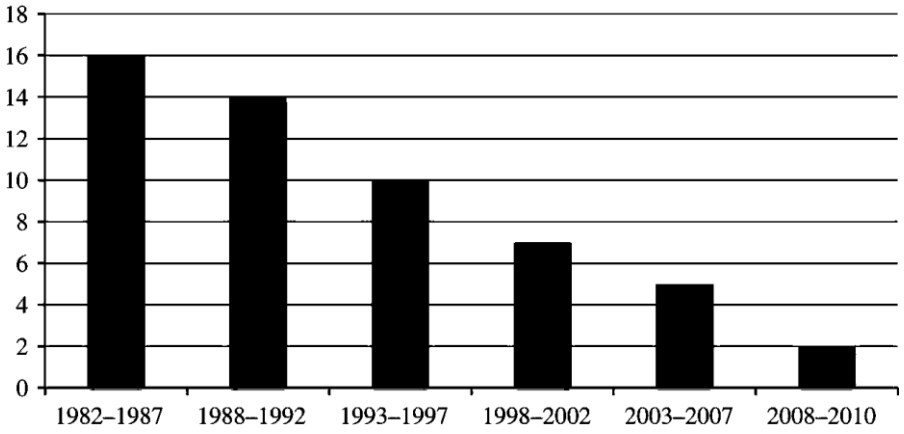

Figure 2: The number of antibiotics approved has been decreasing throughout the years (Cole, 2014).

Pathogenic bacteria in the oral cavity have been linked to antibiotic resistance (Laxminarayan, 2014). With the emergence of bacterial resistance to the currently used antibiotics, a new approach has been adopted to combat these resistances and to treat multi-drug resistant infections. This approach may consist in (Aminov, 2010; Cushnie, Cushnie, & Lamb, 2014; Nuñez-Anita et al., 2014):

- Modifying the antibiotics currently available in order to change their targets, to improve their activity, to decrease their toxicity and decrease the bacterial resistances;
- Using peptides, alkaloids and other compounds with antimicrobial properties;
- Incorporating metals, such as silver, titanium and zinc, in nanoparticles.

This dissertation will address the antibiotics and antiseptics that are currently used, in Portugal, to treat and to help prevent possible oral infections, such as dental caries, gingivitis and periodontitis. Since the use of antibiotics is being affected by resistant strains of bacteria, an investigation about possible new antimicrobials was conducted in order to evaluate their possible use in the dentistry field. For that, Google Scholar and PubMed databases were used to research the articles needed. The following keywords were used: “oral infection”; “gingivitis”; “periodontitis”; “caries”; “symptoms”; “side effects”; “antibiotic”; “amoxicillin”; “clavulanic acid”; “metronidazole”; “clindamycin”; “minocycline”; “antiseptic”; “chlorhexidine”; “triclosan”; “spectrum of activity”; “nanoparticle”; “silver”; “copper”; “gold”; “zinc”; “titanium”; “quaternary ammonium compounds”; “calcium phosphate”.

I. Oral Infections

Oral infections can be originated by bacteria, fungi and even viruses. They can be caused by external pathogens or by opportunist pathogens living in the oral cavity.

Bacterial infections in the oral cavity usually cause pain, fever and damage to the gingiva. The most common cause for these infections is bad maintenance of a proper oral hygiene since the patient may feel pain, see gingival bleeding or be clueless about oral health status (Lôbo & Martins, 2009). They are mostly caused by Gram-negative bacteria such as *Escherichia coli* and *Pseudomonas* spp.; and by oral colonizers such as *Streptococcus mutans*, *Streptococcus sanguis*, *Streptococcus mitis* and *Streptococcus oralis* (Table 1).

Anaerobic bacteria	**Facultative anaerobic bacteria**
Peptostreptococcus micros	*Streptococcus* spp.
Actinomyces spp.	β-hemolytic streptococci
Eubacterium spp.	*Streptococcus milleri*
Propionibacterium spp.	*Lactobacillus* spp.
Veilonella spp.	*Aggregatibacter actinomycetemcomitans*
Prevotella nigrescens	*Capnocytophaga* spp.
Prevotella intermedia	
Porphyromonas gingivalis	
Bacteroides forsythus	
Rothia dentocariosa	
Fusobacterium nucleatum	
Treponema denticola	
Treponema sokranskii	

Table 1: Pathogenic bacteria found in oral infections, adapted from Brook et al. (2005); Tancawan et al. (2015).

In 2010, the most common forms of bacterial infections in Portugal involved dental caries and periodontal disease (Ordem dos Médicos Dentistas, 2010). In order to treat and prevent bacterial infections in the oral cavity, it is necessary to promote a good oral health by changing and correcting unsuitable behaviors. These include smoking and the high consumption of carbohydrates and processed sugars. There is also the need to

eliminate dental biofilm by correctly educating the patient on how to properly brush and floss the teeth. If these strategies are insufficient, the intervention of a specialist is needed to prevent further complications (Varoni, Tarce, Lodi, & Carrassi, 2012).

Oral infections can also be caused by fungi and are mostly by *Candida albicans* and other species of *Candida spp* (Lôbo & Martins, 2009). They are common with patients that have head and neck cancers; are submitted to radiotherapy; wear dentures; and with leukemia and transplant patients (Waltimo, Sirén, Torkko, Olsen, & Haapasalo, 1997; Brown et al., 2012).

Viruses can also cause infections in the oral cavity. They are mostly caused by *herpes simplex* virus and *herpes zoster* virus and are common in immunocompromised and oncology patients (Lôbo & Martins, 2009).

I.1. Dental caries

Dental caries are a form of tooth decay that occurs when specific strains of bacteria produce cariogenic acids that destroy and damage the various layers of the tooth (Takahashi & Nyvad, 2011). The oral cavity is home to these bacteria and they can accumulate in the form of biofilms (also known as dental plaque) in certain areas: near the gum line; between the teeth; around dental fillings; on cracks, pits and rough surfaces; in areas with a low salivary flow (Moreau, Sun, Chow, & Xu, 2011).

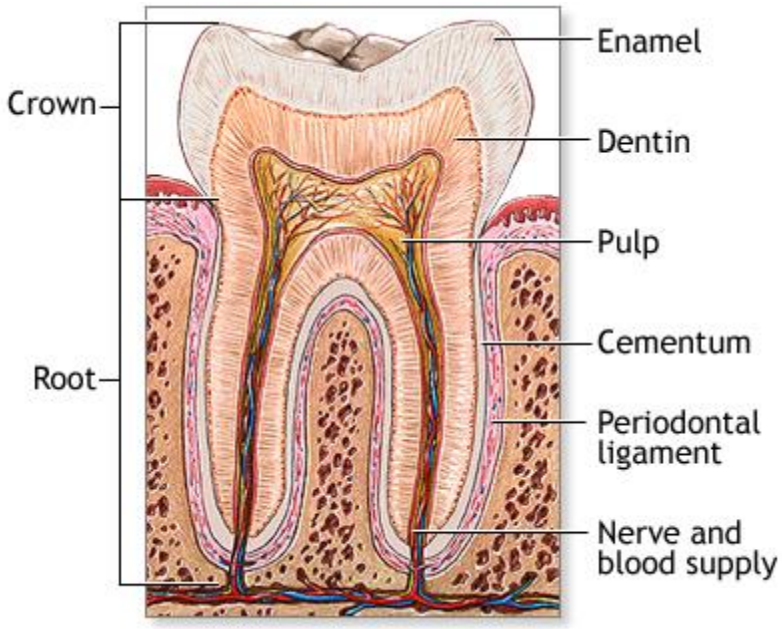

Figure 3: Structure of a healthy tooth. Cariogenic bacteria can damage enamel and dentin layers of the teeth (Heller, Zieve, & Ogilvie, 2014)

Most dental caries are caused by *Streptococcus mutans* but other strains of bacteria can also been found, such as *Streptococcus sobrinus*, *Veilonella spp.*, *Lactobacillus acidophilus*, *Eubacterium spp.*, *Streptococcus salivarius* and *Actinomyces spp*. (Aas et al., 2008). When in the presence of certain foods, such as carbohydrates, these bacteria can convert them into acids through fermentation. If these acids remain on the tooth, the acids will start to interact with the calcium and phosphate from the teeth (Melo, Guedes, Xu, & Rodrigues, 2013), therefore demineralizing it and beginning to form cavities on the surface of the teeth (Takahashi & Nyvad, 2011).

Saliva can neutralize the effect of the acids produced by cariogenic bacteria. However, it is also necessary to have a good oral hygiene to prevent the formation of cavities. One of the ways to do it is by brushing the teeth after meals and by flossing daily. Antibacterial mouth rinses can also be used since they are able to decrease the

levels of cariogenic bacteria. The use of toothpaste and mouth rinses containing fluoride, chlorhexidine and/or triclosan effectively reduces the formation of dental caries. Another way to prevent dental caries involves the use of fluoride to strengthen and remineralize the teeth and to inhibit the growth of cariogenic bacteria. Molar teeth can also be sealed in order to decrease or prevent their decay (Darby & Walsh, 2014).

Once the enamel is damaged, only a specialist can repair the tooth. Generally, the treatment involves the use of fillings, crowns or root canals. The specialist will start to remove the damaged parts with a drill or a laser (Park et al., 2014) and then replace it with restorative materials such as amalgam, acrylic resin, composites, gold or porcelain (Heymann, Swift Jr., & Ritter, 2014). If the tooth is extensively decayed, the use of fillings is not recommended as it can break the remainder of the tooth. In these cases, a crown made of porcelain or gold is usually used to protect the tooth (Powers & Wataha, 2014).

If the decay is too extensive and damages the nerve in the tooth, a root canal needs to be performed. The nerve, the pulp and the damaged parts of the tooth are removed and filled with restorative materials. A crown is then applied on top of the tooth in order to protect it against future infections (Powers & Wataha, 2014).

Sometimes, the decay can also reach the bone supporting the tooth, originating a dental abscess. If the tooth structure is damaged and has rifts on the enamel, bacteria can spread and infect the pulp, the root and then the surrounding bone tissue. Symptoms can include pain, fever, hypersensitivity, suppuration and erythema (Robertson & Smith, 2009; Powers & Wataha, 2014).

I.2. Periodontal disease

Periodontal disease is an inflammation of the gingiva that can also affect the bones surrounding and supporting the teeth. This inflammation is caused by toxins produced by bacteria in dental plaque, as described in chapter I.1.

Gingivitis is a mild form and the earliest stage of periodontal disease caused by the accumulation of dental plaque and tartar at the gum line. This accumulation can induce inflammation, irritation and redness and, if not treated properly, can evolve into a more severe form. The most common bacteria involved are *Actinomyces* spp., *Prevotella intermedia*, *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Fusobacterium nucleatum* and *Tanerella forsythia* (Tsaousoglou, Nietzsche, Cachovan, Sculean, & Eick, 2014; Yang, Zhang, Li, Yang, & Shi, 2014).

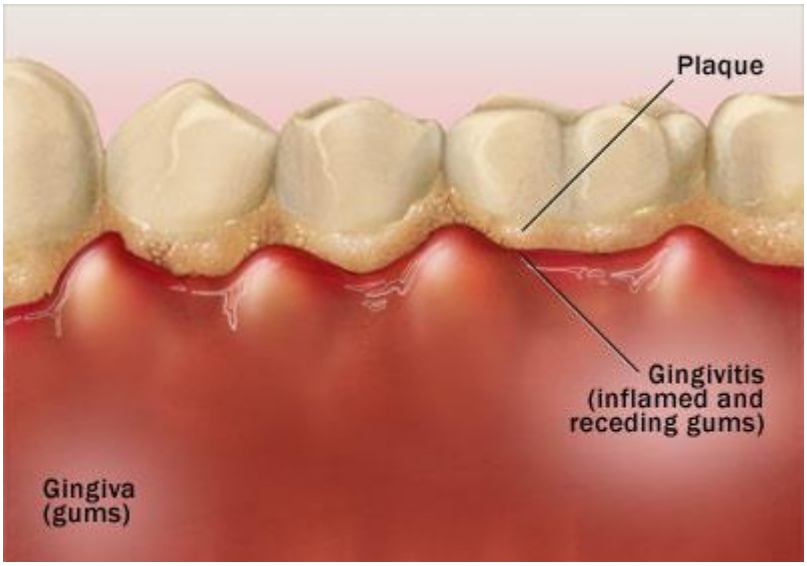

Figure 4: "Gingivitis can cause dusky red, swollen, tender gums that bleed easily" (Mayo Foundation for Education and Research, n.d.)

Some of the signs and symptoms of gingivitis include changes in the appearance of the gingiva as it may be swollen, tender, bleeding and with more accentuated red coloration (Figure 4). Other signs also include the formation of pockets with plaque in the gingiva, bad breath and receding gingiva (Cope & Cope, 2011).

The most common cause is a poor oral hygiene but the effects of gingivitis can be reversed if they are treated in the early stages. Maintenance of a good oral health is essential in order to remove the plaque and it can be achieved by brushing the teeth after meals, by flossing daily and by using mouth rinses (Powers & Wataha, 2014). If the plaque hardens (it becomes tartar), it can only be removed through a root planning (pro-

cess of smoothing a tooth's root by removing the affected part of the dentin and cementum) or a scaling (a professional cleaning that uses hand and ultrasonic instruments) (Darby & Walsh, 2014; Heymann et al., 2014).

If gingivitis is not treated properly, the inflammation can reach the area around the tooth and advance into periodontitis, a more severe form of periodontal disease. Periodontitis damages the soft tissue and can even destroy the bone supporting the affected teeth. The gingival symptoms present in periodontitis are identical to gingivitis. Other signs may also include deep pockets in the gingiva, loose teeth, receding gingiva, bad breath, pus between the tooth and the gingiva, and malocclusion (Cope & Cope, 2011; Anwar, Amir, & Khan, 2014).

The first part of the treatment for periodontitis also involves a scaling or a root planning in order to remove the plaque and tartar in the pockets. If the problem persists, antibiotic therapy is required. The recommended antibiotics are amoxicillin, erythromycin with clindamycin and metronidazole (Tancawan et al., 2015). In Portugal, the first line of treatment with antibiotics is amoxicillin combined with clavulanic acid (Direcção Geral da Saúde, 2014). Metronidazole, alone or in combination with amoxicillin can be used as the second choice antibiotic (Direcção Geral da Saúde, 2014). If the patient has an allergy to beta-lactamic antibiotics, clindamycin is recommended (Direcção Geral da Saúde, 2014). If the pathogen involved in the infection is *Aggregatibacter actinomycetemcomitans*, the prescription of a tetracycline, such as minocycline (Direcção Geral da Saúde, 2014) is recommended. However, if the infection persists, dental surgery is required. In this case, periodontitis is in an advanced form and can only be reverted through flap surgery, soft tissue graft, bone grafting, guided tissue regeneration and enamel matrix derivative application (Darby & Walsh, 2014).

II. Antibiotics

An antibiotic consists in an antibacterial agent produced by a microorganism that has the ability to kill or inhibit the growth of bacteria by disrupting their cellular functions (Norrby et al., 2009). They can be classified according to their spectrum of activity, their effect and their mode of action.

The mechanism of action of an antibiotic may consist in (Norrby et al., 2009):

- The disruption of cell membrane functions;
- The inhibition of DNA replication;
- Or the inhibition of the synthesis of new proteins and/or cell wall materials.

The spectrum of activity of an antibacterial agent can be classified as narrow or broad. A broad spectrum antibiotic covers most of the gram-positive and gram-negative bacteria whether a narrow spectrum antibiotic will only cover specific strains of bacteria.

Antibiotics also have a bactericidal and/or a bacteriostatic effect on bacteria, depending on the dosage and duration of the treatment. A bactericidal antibiotic can eliminate the intended bacteria whether a bacteriostatic antibiotic will only inhibit and delay the growth of bacteria.

In this chapter, the antibiotics that will be discussed are the ones that are used in Portugal (Direcção Geral da Saúde, 2014) to treat oral infections: amoxicillin, metronidazole, clindamycin and minocycline.

II.1. Amoxicillin in association with clavulanic acid

Amoxicillin is a semisynthetic beta-lactam antibiotic from the aminopenicillin family. Its mode of action consists in the inhibition of penicillin-binding proteins during the metabolic synthesis of the bacterial peptidoglycan. The inhibition of the peptidoglycan synthesis leads to the weakening of the bacterial cell structure, followed by cell lysis and the death of the bacteria (Dörr, Davis, & Waldor, 2015). However, amoxicillin is susceptible to beta-lactamases produced by resistant bacteria.

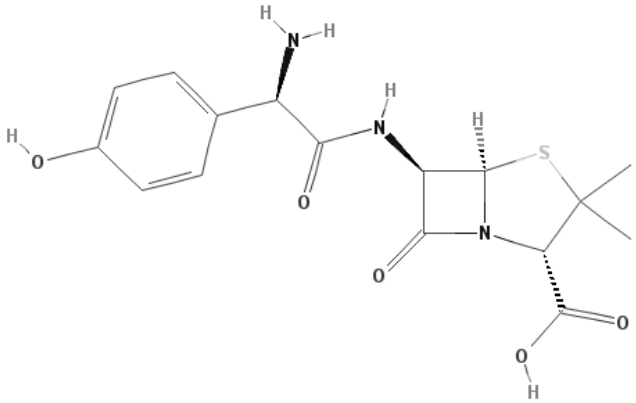

Figure 5: Chemical compostion of amoxicillin. (National Center for Biotechnology Information, n.d.-c)

Some of the most common side effects of amoxicillin include nausea, diarrhea and rash. Although renal side effects are rare, the dosage of amoxicillin needs to be adjusted if the patient has renal insufficiency (Manuel José Guedes da Silva Lda & Guia de Saúde: Edição e Comunicação Audio-Visual, 2014).

Amoxicillin has a broad spectrum of action that includes Gram-positive coccus, *Haemophilus influenza*, *Escherichia coli*, *Proteus mirabillis*, *Salmonella*, *Fusobacterium* spp. and *Shigella*. It is inactivated by staphylococci that produce beta-lactamases, *Enterobactereaceae*, *Bacteroides fragillis* and *Pseudomonas* (MJGSLda & GS: ECAV, 2014).

Clavulanic acid is a semisynthetic compound obtained from the fermentation of *Streptomyces clavuligerus* and normally used in combination with beta-lactam antibiotics in order to prevent their inactivation by beta-lactamases. Its mechanism of action consists in irreversibly binding with beta-lactamase enzymes so that susceptible antibiotics, such as amoxicillin, can induce their activity on the bacteria (Jensen, 2012). When used in combination with amoxicillin, the treatment may need to be monitored in patients with hepatic and renal insufficiency (MJGSLda & GS: ECAV, 2014).

The combination of amoxicillin with clavulanic acid expands the spectrum of activity since clavulanic acid inhibits bacteria that produce beta-lactamases. They are the first choice antibiotics to use when treating oral infections (Direcção Geral da Saúde, 2014). There are many dosages of this association that are used depending on the degree of the infection and/or the age of the patient. For adults, the dosage, expressed in mg of amoxicillin, can vary between 250 mg and 875 mg every 12 to 6 hours (Infarmed, 2015). If it is administered via IV therapy, the recommended dosage is 1 g to 2 g of amoxicillin every 8 or 6 hours. The treatment should not exceed 14 days without further evaluations of the patient's condition (MJGSLda & GS: ECAV, 2014).

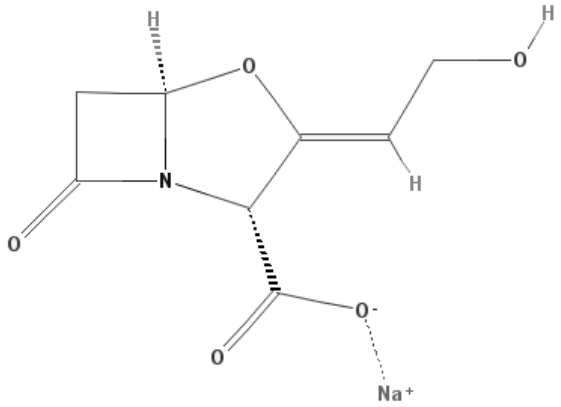

Figure 6: Chemical compostion of clavulanic acid (National Center for Biotechnology Information, n.d.-a).

However, if the patient being treated is allergic to amoxicillin, it can be replaced by clindamycin. Clindamycin can also be associated with clavulanic acid to show similar results and effectiveness (Tancawan et al., 2015).

II.2. Metronidazole in association with amoxicillin

When antibiotic therapy is required to treat gum disease, metronidazole, alone or in combination with amoxicillin, can be an alternative to the amoxicillin-clavulanic acid association (Direcção Geral da Saúde, 2014). It is a compound derived from nitroimidazole that can be used as an antibacterial or as an anti-parasitic agent (Soares et al., 2012). It is commonly used in the treatment of periodontitis, acne, rosacea, bacterial vaginosis, *Trichomonas vaginalis* infections, *Clostridium difficile* infections and amoebic dysentery (Finberg & Guharoy, 2012; Khodaeiani et al., 2012).

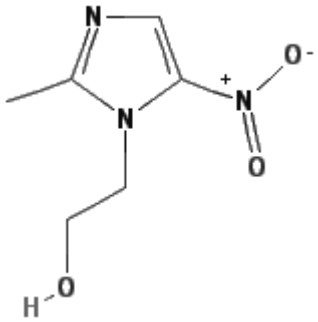

Figure 7: Chemical composition of metronidazole. (National Center for Biotechnology Information, n.d.-d)

Metronidazole has a bactericidal effect on bacteria. Its mechanism of action consists in inhibiting nucleic acid synthesis. The molecule enters the bacterial cell by passive diffusion and it is metabolized inside the cell. The metabolites bind to the DNA, preventing cellular replication and inhibiting enzymes responsible for energy production (Finberg & Guharoy, 2012; Soares et al., 2012). Metronidazole is active against some parasites, for instance *Trichomonas vaginalis*, *Helicobacter pylori* and *Giardia lamblia*. It is also active against anaerobic bacteria, such as *Clostridium* spp., *Fusobacterium* spp., *Prevotella* spp. and other anaerobic cocci and bacilli. Certain bacterial strains present in the oral cavity, that include *Actinomyces* spp. and *Propionibacterium* spp., are resistant to metronidazole (Finberg & Guharoy, 2012).

Some of the most common side effects of metronidazole include nausea, headaches, diarrhea, abdominal pain, metallic taste, rash, anorexia and candidiasis (Infarmed, 2015).

When used alone, the recommended dosage for metronidazole *per os* is 250 mg to 500 mg every 8 hours or 250 mg every 6 hours when treating infections caused by

Helicobacter pylori. For IV therapy, it is recommended a dosage of 500 mg every 8 hours (Infarmed, 2015).

Metronidazole can be combined with amoxicillin to improve its spectrum of activity. The use of this antibiotic association to treat oral infections has shown positive results and improvements in treatments when combined with scaling and root planning (Cionca, Giannopoulou, Ugolotti, & Mombelli, 2009; Powell, 2013). Both antibiotics have also shown antibacterial activity against *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia* and *Tannerella forsythia* (Soares et al., 2012; Yek et al., 2010). It also improves the periodontal conditions and reduces the inflammation (Berglundh et al., 1998). The usual recommended dose of this antibiotic association is 250 mg of metronidazole plus 375 mg of amoxicillin every 8 hours (Mani, Dalvi, & Mani, 2015).

II.3. Clindamycin

Clindamycin is a semi synthetic macrolide antibiotic with a bacteriostatic and bactericidal (if used in higher concentrations) effects on bacteria. If a patient is allergic to beta-lactamic antibiotics, such as amoxicillin, clindamycin is prescribed to treat periodontal disease (Direcção Geral da Saúde, 2014). It can be used to treat other conditions, such as amygdalae pharyngitis, acute sinusitis, *acne vulgaris*, acute otitis media, scarlet fever, intra-abdominal infections, endometritis, lung abscess and osteomyelitis. Its mode of action consists in binding to the 50s ribosomal subunits to stop the formation of peptide bonds, inhibiting protein synthesis (Brook et al., 2005).

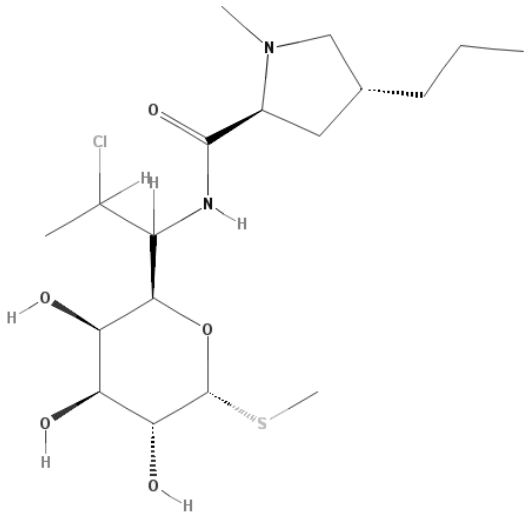

Figure 8: Chemical composition of clindamycin. (National Center for Biotechnology Information, n.d.-b)

Some of the most common side effects of clindamycin include abdominal pain, diarrhea, nausea, rash, anorexia, vomiting and flatulence (Finberg & Guharoy, 2012). There are also other side effects that despite not being common need to be accounted for. These include eosinophilia, neutropenia, pseudomembranous colitis and thrombocytopenia (Finberg & Guharoy, 2012; Tancawan et al., 2015).

Clindamycin is a broad-spectrum antibiotic with activity against anaerobic, Gram-negative and Gram-positive bacteria (Table 2).

Gram-positive bacteria	**Gram-negative bacteria**
β-hemolytic streptococci	*Bacteroides fragilis*
Actinomyces spp.	*Fusobacterium* spp.
Eubacterium spp.	*Porphyromonas* spp.
Lactobacillus spp.	*Prevotella* spp.
Peptostreptococcus spp.	*Veilonella* spp.
Propionibacterium spp.	
Staphylococcus spp.	

Table 2: Spectrum of activity of clindamycin, adapted from Brook et al. (2005).

The recommended dosage, for an adult and depending on the infection, is between 150 mg and 300/450 mg every 6 hours. For children older than 6 years old, the dosage (expressed in mg of clindamycin) also varies according to the degree of the infection: 2 to 6,3 mg/kg every 6 hours or 2,7 to 8,3 mg/kg every 8 hours (Brook et al., 2005; Tancawan et al., 2015). In a study conducted by Tancawan *et al.* (2015), the recommended dosages for clindamycin and clavulanic acid in association with amoxicillin (875 mg/125 mg every 12 hours) were compared to test their effectiveness in the treatment of oral infections. Both achieved clinical success after 7 days of treatment, proving that clindamycin can be a good alternative when a patient is allergic to penicillin.

II.4. Minocycline

Minocycline is a semi-synthetic antibiotic that belongs to the second generation of the tetracycline family. It is a bacteriostatic antibiotic that has a broad spectrum of activity against Gram-positive and Gram-negative bacteria. It is used in the treatment of respiratory tract infections, such as acute and chronic bronchitis, bronchiectasis, pulmonary abscesses and pneumonia; STD's, such as gonorrhea, chlamydia, non-gonococcal urethritis and pelvic inflammatory disease; acne; periodontitis, gingivitis and dental abscesses (Garrido-Mesa, Zarzuelo, & Gálvez, 2013).

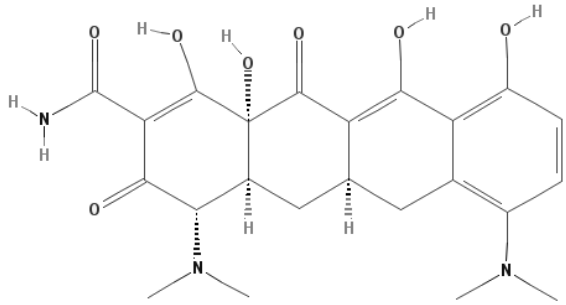

Figure 9: Chemical composition of minocycline. (National Center for Biotechnology Information, n.d.-e)

When in the presence of minocycline and other tetracyclines, the bacterial membrane uses an energy-dependent system that actively transports these antibiotics into the cytoplasm. Its mode of action consists in blocking protein synthesis by reversibly binding to the 30s ribosomal subunit of bacteria in order to prevent the connection to tRNA and inhibit protein synthesis (Garrido-Mesa et al., 2013).

The most common side effects may include nausea, dizziness and vertigo during the administration. Patients taking minocycline need to be monitored since it is a hepatotoxic antibiotic and it could lead to appearance of pigmentation and systemic lupus erythematosus (Infarmed, 2015).

Minocycline is mainly used to treat *acne vulgaris* and some STD's. Due to its anti-apoptotic, anti-collagenase and anti-inflammatory properties, it is also used in the treatment of periodontitis (Garrido-Mesa et al., 2013). When taken in doses between 100 and 200 mg per day for 7 to 14 days, minocycline has shown to effectively control the progression of periodontitis and promote the healing of the affected area. Therefore, the suggested dosage for the treatment is 200 mg initially followed by 100 mg every 12 hours. In children aged 12 or older, the recommended initial dose is between 2 to 4 mg/kg followed by 1-2 mg/kg every 12 hours (Garrido-Mesa et al., 2013; Infarmed, 2015).

III. Antiseptics

Oral antiseptics can help control the formation of bacterial biofilms in the oral cavity by reducing bacterial growth, delaying accumulation of dental plaque and eliminating bacteria. Antiseptics can be used on the skin and on the mucous membrane (Corbin, Pitts, Parker, & Stewart, 2011).

These antimicrobials have side effects that usually appear after long periods of usage. For example, the continuous usage of chlorhexidine can induce teeth staining, dysgeusia and xerostomia (Park et al., 2014).

There are many antiseptics that can be applied in the oral cavity in order to treat and prevent future infections (Lachenmeier, 2014; Parashar, 2015), for instance: alexidine; benzydamine hydrochloride; cetylpyridium chloride; chlorhexidine; essential oils, such as eucalyptol, menthol and thymol; ethanol; oxygenating agents, such as hydrogen peroxide and sodium peroxycarbonate; triclosan; povidone-iodine; sodium carbonate; sodium benzoate. In this chapter, the antiseptics that will be discussed are the ones most commonly used in dentistry: chlorhexidine and triclosan.

III.1. Chlorhexidine

Chlorhexidine is an antiseptic agent with antimicrobial and antifungal properties that belongs to the bisbiguanide family (Parashar, 2015). This compound is extensively studied and it is applied in the medical and dentistry fields as, for example, in pre-surgical cleansing agents, antibacterial mouth rinses, gels and eye drops. Chlorhexidine can help reduce the frequency of periodontal disease, reduce gingival inflammation and bleeding, and reduce dental plaque formation (Varoni et al., 2012).

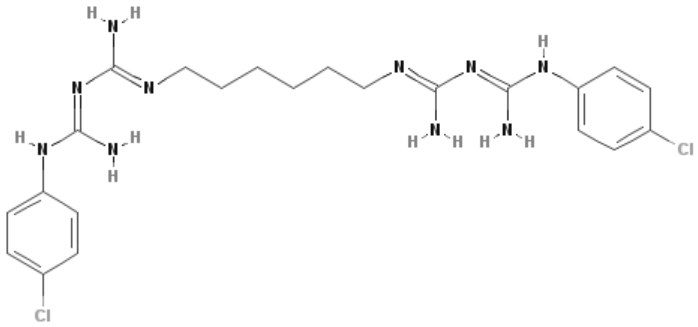

Figure 10: Chemical composition of chlorhexidine. (National Center for Biotechnology Information, n.d.-g)

Its mode of action targets different areas of the bacterial cell, depending on the concentration used. A bacteriostatic effect is shown when used in lower concentrations and targets the osmotic balance of the cell. At higher concentrations, chlorhexidine acts as a bactericidal antiseptic and targets the cell membrane, resulting in its rupture and leakage of its intracellular components (Varoni et al., 2012).

Chlorhexidine usually has a better antimicrobial activity when pH is between 7 and 8. It has shown a wide antibacterial activity against Gram-negative and Gram-positive bacteria, yeasts, virus and fungi. With regard to the bacteria responsible for infections in the oral cavity, it was shown that *Aggregatibacter actinomycetemcomitans*, *Streptococcus mutans*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis* and *Enterobacteria* were susceptible to chlorhexidine (Varoni et al., 2012; Parashar, 2015).

There are various concentrations and formulations available. Chlorhexidine can be presented as a gel, mouth wash, spray and aerosol with concentrations that can range between 0,02% and 0,3% (Varoni et al., 2012).

The side effects that follow a treatment with chlorhexidine are usually reversible once the treatment ends. These include mucosa and teeth staining, dysgeusia, xerostomia and hypersensitivity reactions (Varoni et al., 2012; Lachenmeier, 2014).

III.2. Triclosan

Triclosan (or 5-Chloro-2-(2,4-dichlorophenoxy) phenol) is a non-ionic antiseptic used in diverse consumer products in order to reduce and prevent contamination by bacteria and fungus (Varoni et al., 2012; Dhillon et al., 2015). It is commonly used in diverse products such as mouth washes, toothpastes, shampoos, deodorants, soaps, surgical cleaning treatments, textiles, among others. As an ingredient in dentistry products such as toothpastes and mouth washes, it has shown to reduce tooth cavities, dental plaque and gingival related diseases (Yazdankhah et al., 2006; Haraszthy, Sreenivasan, & Zambon, 2014).

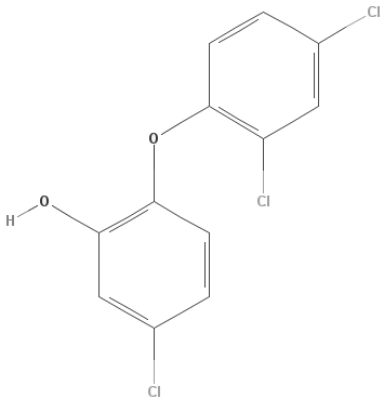

Figure 11: Chemical composition of triclosan. (National Center for Biotechnology Information, n.d.-f)

This compound acts as a biocide if used in higher concentrations and targets specific areas in the cytoplasm and membrane. If used in lower concentrations, such as in dentistry related consumer products, triclosan is essentially bacteriostatic. Its mechanism of action inhibits the bacterial synthesis of fatty acids. Triclosan binds to the ENR enzyme, creating a stable complex with NAD+. This complex will no longer participate in the fatty acid synthesis thus preventing the formation of cell membranes (Dann & Hontela, 2011; Dhillon et al., 2015). Triclosan can also inhibit the cyclooxygenase and lipoxygenase pathways by reducing the levels of leukotrienes and prostaglandins involved in the inflammatory process (Parashar, 2015).

The use of triclosan has been linked to side effects such as allergic sensitization to allergens, altered levels of thyroid hormones, rash and bacterial resistance to some antibiotics (Davison & Maillard, 2010; Li et al., 2013; Lachenmeier, 2014).

Despite its antibacterial activity, there has been some controversy about its use. In 2009, Denmark suggested that triclosan should be banned due to being linked to anti-

biotic and bacterial resistance, endocrine disrupting effects and environmental impacts (Davison & Maillard, 2010; Li et al., 2013; Miljøstyrelsen, 2014). Since then, the European Union has been reducing the use of triclosan in some products and restricting the maximum concentration that can be used. Triclosan can be used at a maximum concentration of 0,2 % in mouth washes and at 0,3 % in toothpastes, soaps, deodorants and other cosmetic products (European Comission, 2014).

IV. Nanoparticles as antimicrobial agents

Since the discovery of penicillin, antibiotics have been used and developed to treat bacterial infections. However, due to the abusive use and prescription, many bacteria are becoming resistant to these compounds, making it more difficult to treat some infections (Khan, Ahamed, Musarrat, & Al-Khedhairy, 2014). In spite of this resistance, scientists are trying to develop new antimicrobial and inorganic materials to mimic the same killing effect that antibiotics have.

Nanotechnology can be applied to the dental field in order to develop new materials with better antimicrobial properties (Melo et al., 2013). Nanoparticles made of metallic elements, such as gold, mercury, silver, titanium and zinc, are being developed due to their antimicrobial properties and spectrum of activity. These metals have been used for many years in the dental field (Fernandes et al., 2015) and to treat infectious diseases (Rizzoto, 2012). One of the first uses of mercury can be traced back to China in the 2nd century BC (Sloane, 2012). Before the discovery of antibiotics, mercury was also used to treat syphilis and skin diseases (The Trustees of Indiana University, 2002). In the 20th century, it was commonly applied as an antiseptic and a disinfectant but, due to its toxicity, there has been a decline in its use. Zinc also has antibacterial and antifungal properties and can be found in ointments to treat skin diseases (Gupta, Mahajan, Mehta, & Chauhan, 2014) and in oral products to decrease the formation of dental plaque (Allaker, 2010).

Nanoparticles have become a new area of interest. They have a smaller size than the average antibiotics. This provides the nanoparticles with unique physio-chemical properties (Oyar, 2014) and improves their access to certain parts of the body and gives advantages in the biomedical field (B. Sutariya & Pathak, 2015). The size of a nanoparticle can range between 1 nm to 100 nm (Oyar, 2014). However, nanoparticles ranging between 1 nm and 10 nm may offer a better bactericidal effect when compared to larger particles (Verran, Sandoval, Allen, Edge, & Stratton, 2007). Bacteria are less likely to resist because nanoparticles may have multiple microbial targets and there would have to be a large number of mutations in order to resist (Kim et al., 2007). Shape can also influence the activity of nanoparticles. For instance, silver nanoparticles (figure 12A) in the shape of truncated triangles show a better biocidal effect, in contrast to the nanopar-

ticles shaped like spheres (figure 12B) and rods (figure 12C), due to the proportion of active facets in the particles (Pal, Tak, & Song, 2007).

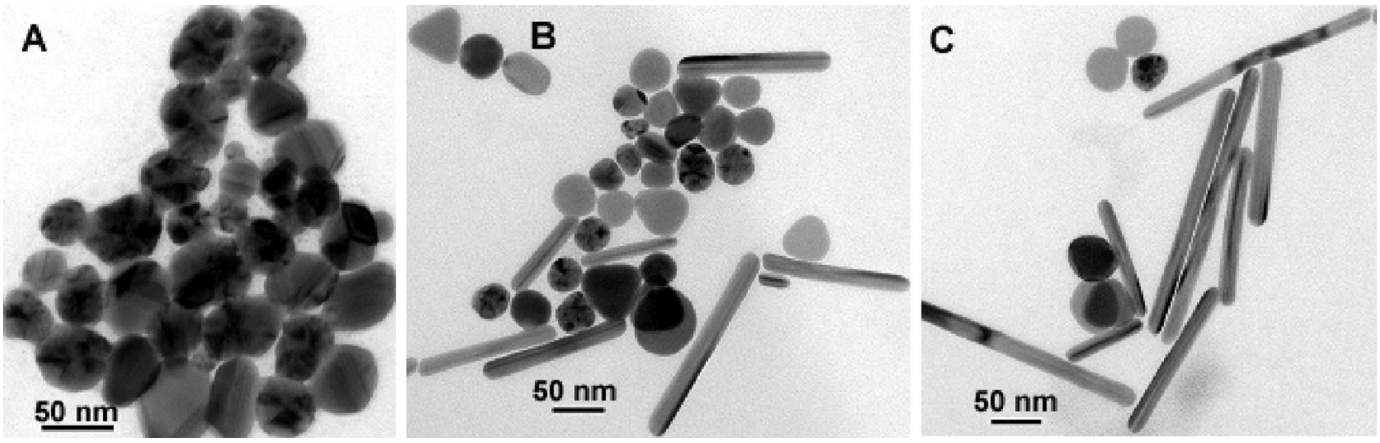

Figure 12: Silver nanoparticles shaped as truncated triangles (A), spheres (B) and rods (C), adapted from Pal et al. (2007).

Metal nanoparticles toxicity studies have brought new antimicrobial applications. In a study, copper, copper oxide, silver, titanium dioxide and zinc oxide showed a significant activity against *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Prevotella intermedia* and *Aggregibacter actinomycetemcomitans* with minimum bactericidal concentrations ranging 250 µg/mL and 2500 µg/mL. Activity against *Pseudomonas aeruginosa* and MRSA was also shown with minimum bactericidal concentrations between 100 µg/mL and 5 mg/mL (Vargas-Reus, Memarzadeh, Huang, Ren, & Allaker, 2012).

In this chapter, nanoparticles made of metallic elements, chlorhexidine and calcium-phosphate will be addressed. Despite calcium-phosphate not having antibacterial properties, it has improved the activity of those properties when combined with other nanoparticles.

IV.1. Biocompatibility

There is a considerable interest in the development and application of nanotechnology to the human body. Some nanoparticles could accumulate in certain organs, tissues and cells due to their small size, which is not verified by larger sized particles such as antibiotics (Oyar, 2014; B. Sutariya & Pathak, 2015). Although there are some suppositions about the mode of action of some nanoparticles, it is not enough. It is necessary to have a thorough knowledge about their absorption, distribution, metabolism, excretion, physiochemical properties and genotoxic and immunogenic potential in the human body in order to build a safety profile with the desired biological effects and the minimal potential toxicity (B. Sutariya & Pathak, 2015).

To safely use nanotechnology and to design nanoparticles for biological applications, it is necessary to know the interactions that occur between the human body and the nanoparticles, which are covered by the following: the surface/characteristics of the nanoparticle; the solid-liquid interface and the effects of its surroundings; and the contact zone with biological substrates (A. E. Nel et al., 2009). These components are influenced by several characteristics as it is shown in Table 3.

Surface of the nanoparticle:	Chemical composition; Hydrophilicity; Hydrophobicity; Number of sides; Porosity;	Roughness; Shape; Surface crystallinity; Surface function; Size heterogeneity.
Solid-liquid interface and effect of the surrounding media:	Ionic strength; pH; Presence of organic molecules/detergents; Temperature.	
Contact zone with biological substrates:	Dispersion state; Hydration; Nanoparticle aggregation;	Stability; Zeta charge.

Table 3: Nanoparticle characteristics that influence the interactions with biological systems, adapted from Long, Saleh, Tilton, Lowry, & Veronesi (2006).

However, data about the toxic effects of nanoparticles to humans are still limited to draw any conclusions in order to ensure a safe use (A. Nel, Xia, Mädler, & Li, 2006; Oyar, 2014). Some effects obtained in experiments can be found in Table 4. The research currently available is expensive and inconsistent in the matter of the preparation and dosimetry of the nanoparticles being used. Therefore, results may vary depending on the researcher and on the materials used to perform these experiments. There is a need to standardize these studies so that they can correctly evaluate the potential benefits and risks of existing and newly-developed nanoparticles (B. Sutariya & Pathak, 2015).

Experimental nanoparticle effects	**Possible outcomes**
Reactive oxygen species generation	Protein, DNA and membrane injury; Oxidative stress.
Oxidative stress	Phase II enzyme induction; Inflammation; Mitochondrial perturbation.
Mitochondrial perturbation	Inner membrane damage; Permeability transition; Pore opening; Energy failure; Apoptosis; Apo-necrosis; Cytotoxicity.
Inflammation	Tissue infiltration with inflammatory cells; Fibrosis; Granulomas; Atherogenesis; Acute phase protein expression.
Uptake by reticulo-endothelial system	Asymptomatic sequestration and storage in liver, spleen and lymph nodes; Possible organ enlargement and dysfunction.
Protein denaturation/degradation	Loss of enzyme activity; Auto-antigenicity.
Nuclear uptake	DNA damage; Nucleoprotein clumping; Autoantigens.
Neuronal tissue uptake	Brain and peripheral nervous system injury.
Perturbations in phagocytic function, particle overload and mediator release	Chronic inflammation; Fibrosis; Granulomas; Interference in clearance of infectious agents.
Endothelial dysfunction and effects on blood clotting	Atherogenesis; Thrombosis; Stroke; Myocardial infarction.
Generation of neoantigens and breakdown in immune tolerance	Autoimmunity; Adjuvant effects.
Altered cell cycle regulation	Proliferation; Cell cycle arrest; Senescence.
DNA damage	Mutagenesis; Metaplasia; Carcinogenesis.

Table 4: Experimental effects of the use of nanoparticles and their possible outcome, adapted from A. Nel et al. (2006).

IV.2. Silver nanoparticles

Silver has been known throughout history for its antimicrobial effects and can be used in different areas: in the treatment of burns, wounds and ulcers (Rai, Yadav, & Gade, 2009); making water potable (Chopra, 2007); and in dentistry, catheters, wound dressings and sutures, surgical instruments and prostheses (Kim et al., 2007; Corrêa et al., 2014). Silver also exhibits a good biocompatibility with the human cells, low bacterial resistance and a long-term antimicrobial action (Corrêa et al., 2014). It is also capable of inactivating specific microorganisms such as the human immunodeficiency virus type 1 (Elechiguerra et al., 2005) and the hepatitis B virus (Lara, Garza-Treviño, Ixtepan-Turrent, & Singh, 2011).

The mechanism of action of silver nanoparticles is not well known but it is thought that it might involve multiple targets. This inconsistency may be caused by the different physicochemical properties of the silver nanoparticles that are used in these studies (Markowska, Grudniak, & Wolska, 2013). Some studies suggest that the positive-charged nanoparticles establish an electrostatic connection with the negative-charged cell membrane of bacteria. The nanoparticles will then bind with proteins that contain either sulphur (thiol groups) or phosphorus and release silver ions into the microbial cell. These ions can stop the replication of DNA and inhibit respiratory chain enzymes and cellular proteins, releasing reactive oxygen species and compromising the levels of ATP (Figures 13 and 14)(Kim et al., 2007; Lara et al., 2011). These mechanisms can help improve the reduction of the wound burden since silver nanoparticles can release more ions than molecular silver (Habiboallah et al., 2014).

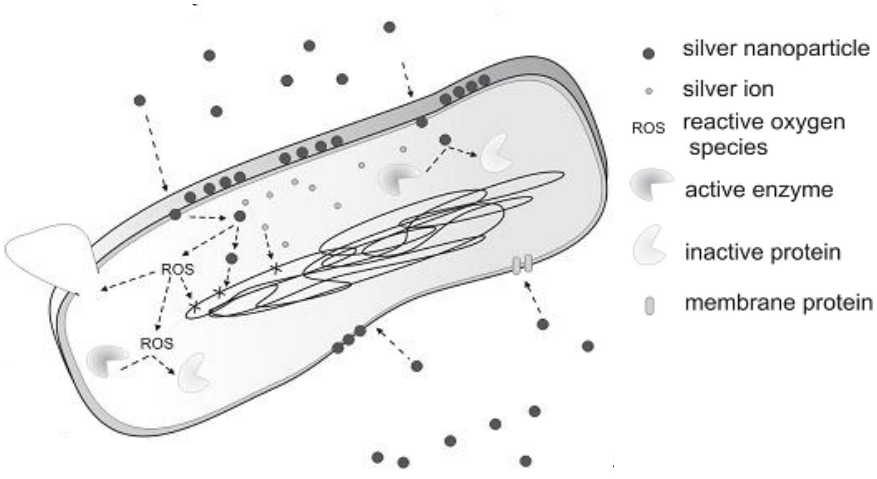

Figure 13: "Some studies have reported that nano-silver causes oxidative damage, leading to the production of reactive oxygen species" (Markowska et al., 2013).

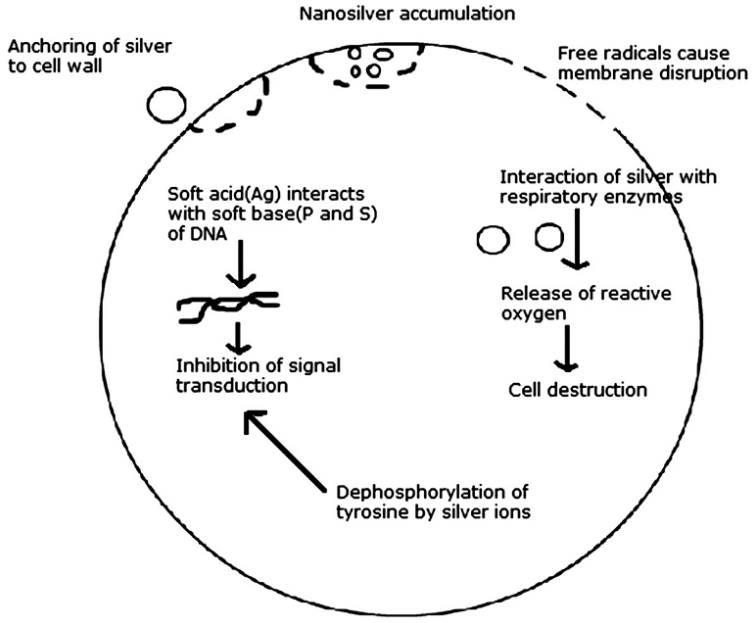

Figure 14: Some of the mechanisms of silver nanoparticles in the bacterial cell, adapted from Prabhu & Poulose (2012).

In a study conducted after surgery in the oral cavity of healthy rabbits, silver nanoparticles effectively reduced the inflammatory parameters during the four days after the surgery when using a high concentration of nanoparticles in the periodontal dressing. However, when determining the "dose-dependent histopathological and biological effects", researchers found that using a higher concentration of nanoparticles could cause mild cytotoxicity (Habiboallah et al., 2014).

When compared to other antimicrobials currently used (such as chlorhexidine), silver nanoparticles have shown a better antimicrobial action against strains of *Streptococcus mutans* with a MIC of 50 µg/mL (Besinis, De Peralta, & Handy, 2014). Silver nanoparticles also show activity against *Porphyromonas gingivalis*, with a MIC of 250 µg/mL; *Aggregatibacter actinomycetemcomitans* and *Fusobacterium nucleatum* with a minimum inhibitory concentration of 100 µg/mL (Table 5).

	MIC	MBC
Aggregatibacter actinomycetemcomitans	100	100
Fusobaterium nucleatum	100	100
Prevotella intermedia	100	100
Porphyromonas gingivalis	250	250

Table 5: Minimum inhibitory concentration and minimum bactericidal concentration values expressed in µg/mL of the tested bacterial species with AgNPs, adapted from Vargas-Reus et al. (2012).

In another study conducted by Lu et al. (2013), a lower MIC was obtained for *Aggregatibacter actinomycetemcomitans* and *Fusobacterium nucleatum*, probably due to the size of the nanoparticles that were used. The concentrations obtained for both bacteria and sizes used were 25 and 50 µg/mL. It has also shown activity against *Streptococcus sanguis* and *Streptococcus mitis*, with a MIC of 50 µg/mL (Lu, Rong, Li, Yang, & Chen, 2013).When associated with copper oxide and zinc oxide nanoparticles, silver nanoparticles can help improve their antimicrobial action (see chapters IV.4. and IV.5.).

IV.3. Gold nanoparticles

Gold is one of the oldest elements known to mankind and its first uses can be traced back to ancient China and Egypt in 2500 BC (Thakor, Jokerst, Zavaleta, Massoud, & Gambhir, 2011; Dykman & Khlebtsov, 2012). It was used to treat fevers and syphilis between the 17th and the 19th century. Currently, gold is used in cavity filling, prostheses, stents and ophthalmology. It can also treat some forms of arthritis such as rheumatoid arthritis and psoriatic arthritis. As a nanoparticle, it can be used in Raman Imaging, photothermal ablation, antitumor drug delivery, siRNA delivery, thermal imaging and as contrasting agents (Thakor et al., 2011; Rieznichenko et al., 2012).

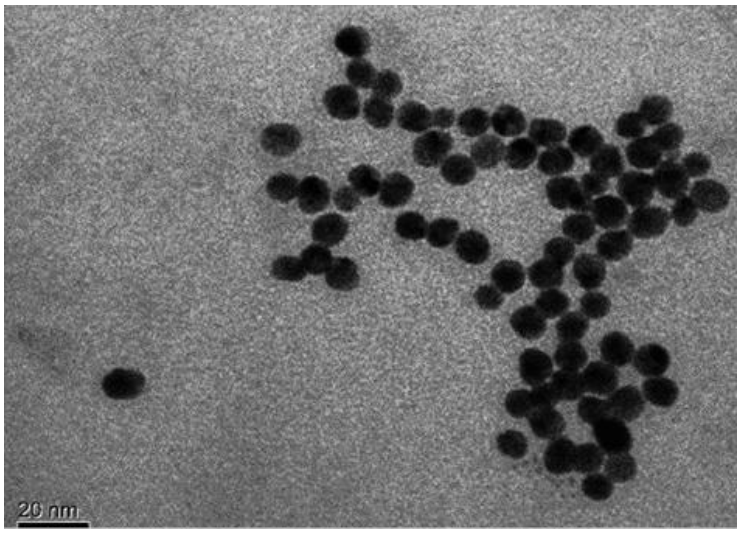

Figure 15: Electron microscopic image of AuNPs (Rieznichenko et al., 2012).

Although the mechanism of action of gold as a molecule is known, its mechanism as a nanoparticle is still unknown. Due to its small size, gold nanoparticles have a high surface area to volume ratio and may have their physical and chemical properties changed (Thakor et al., 2011). These factors can lead to unexpected reactions in terms of their toxicity and their interaction in the human body. According to a study by Rieznichenko et al. (2012), the antimicrobial and biological activity of gold nanoparticles depends on the size of the nanoparticle itself. The size and shape of the nanoparticle can be manipulated in order to achieve biocompatibility in the human body (Park et al., 2014). In similarity to silver nanoparticles, it is believed that gold nanoparticles may also cause oxidative stress due to the release of reactive oxygen species (Thakor et al., 2011; Park et al., 2014).

Gold nanoparticles have shown a bacteriostatic effect against *Streptococcus mutans* (Park et al., 2014). In this study, AuNPs were combined with low-temperature plasma to test their potential antimicrobial activity. The results have shown that, when combined, gold nanoparticles and low-temperature plasma have a higher effectiveness against *Streptococcus mutans* and can inhibit bacteria that accumulate around the tooth structure. Since dental caries are caused by *Streptococcus mutans* (Aas et al., 2008), the authors suggest that this combination could be used to decrease the recurrence of dental caries (Park et al., 2014).

IV.4. Copper and copper oxide nanoparticles

Copper is one of the essential metallic elements to the human health. It has been used for many centuries for the disinfection of biological tissues and liquids (Perelshtein et al., 2009; Ahmad et al., 2012). It has been reported that in 400 B.C. Hippocrates prescribed copper to purify water and to treat pulmonary diseases (Ingle, Duran, & Rai, 2014). Nowadays, it is mostly used to purify water, as an antibacterial, as a fungicide, as a nematocide and as an antifouling agent. In fact, copper is an essential metal required to the human health and can be found in many vitamins and amino and fatty acids required for metabolic processes (Perelshtein et al., 2009).

Copper oxide is very stable in terms of its chemical and physical properties: electron correlation effects; high temperature superconductivity; photoconductivity; and spin dynamics (Ren et al., 2009). It is also cheaper than other metals, which can make it a better cost-effective option (Ahmad et al., 2012; Ingle et al., 2014). Copper oxide can be mixed with various polymers and can be prepared with multiple crystal morphologies. Since the documentation about the antimicrobial effects of copper oxide nanoparticles is limited, these features can influence future studies and production of these nanoparticles (Ren et al., 2009).

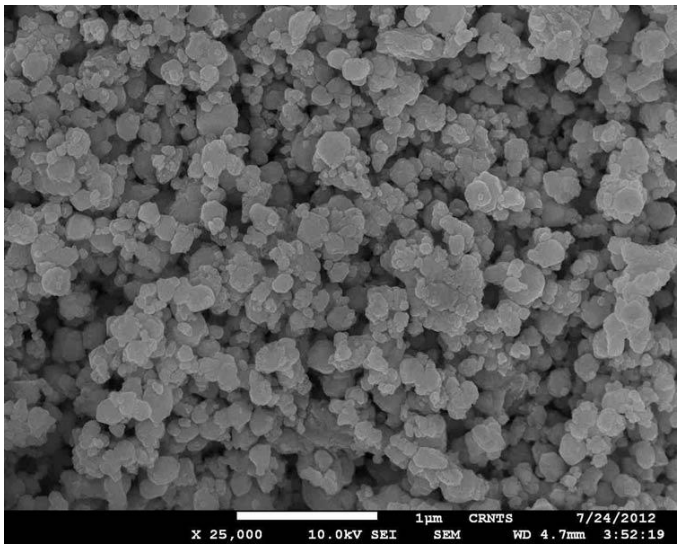

Figure 16: Structure of copper nanoparticles (Nano Labs, 2012).

Despite the lack of information about the antimicrobial activity of copper and copper oxide nanoparticles, some studies report that they share a similar mode of action with silver nanoparticles (Ruparelia, Chatterjee, Duttagupta, & Mukherji, 2008). Copper based nanoparticles have a wide antibacterial range of action due to the nanoparticle binding with thiol groups and the production of reactive oxygen species. This weakens

the bacterial cell wall and enables the entrance of more nanoparticles into the cell. The nanoparticles also bind with DNA and some enzymes, leading to cell death (Kim et al., 2007; Ingle et al., 2014).

Copper nanoparticles have shown antibacterial properties against *Streptococcus mutans*, with a MBC and MIC of 100 µg/mL (Eshed, Lellouche, Matalon, Gedanken, & Banin, 2012).

Copper oxide nanoparticles have shown inhibitory effects against *Porphyromonas gingivalis*, with a MIC of 500 µg/mL and MBC of 2500 µg/mL; *Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia* and *Fusobacterium nucleatum* with a MIC of 250 µg/mL (Table 6). However, when in combination with silver nanoparticles, the MIC needed to have an effect on *Porphyromonas gingivalis* and *Prevotella intermedia* was inferior to 100 µg/mL and for *Fusobacterium nucleatum* was 500 µg/mL (Vargas-Reus et al., 2012). These nanoparticles also show biofilm inhibition when tested against *Streptococcus mutans* (Eshed et al., 2012).

	CuO		Ag + CuO	
	MIC	MBC	MIC	MBC
Aggregatibacter actinomycetemcomitans	250	250	250	250
Fusobaterium nucleatum	250	250	500	500
Prevotella intermedia	250	250	<100	<100
Porphyromonas gingivalis	500	2500	<100	<100

Table 6: Minimum inhibitory concentration and minimum bactericidal concentration values expressed in µg/mL of the tested bacterial species CuONPs and AgNPs in combination with CuONPs, adapted from Vargas-Reus et al. (2012).

Copper dioxide nanoparticles also offer a good antimicrobial activity. It has shown a MBC that was less than 100 µg/mL against *Fusobacterium nucleatum*, *Prevotella intermedia* and *Porphryromonas gingivalis*. Against *Aggregatibacter actinomycetemcomitans*, the results of the MBC obtained were slightly higher than the other bacteria tested, with a value of 1000 µg/mL (Table 7).

	MIC	MBC
Aggregatibacter actinomycetemcomitans	1000	1000
Fusobaterium nucleatum	<100	<100
Prevotella intermedia	<100	<100
Porphyromonas gingivalis	<100	<100

Table 7: Minimum inhibitory concentration and minimum bactericidal concentration values expressed in µg/mL of the tested bacterial species Cu_2ONPs, adapted from Vargas-Reus et al. (2012).

IV.5. Zinc oxide nanoparticles

As it was previously mentioned, zinc has antibacterial and antifungal properties and can be found in ointments to treat skin diseases (Gupta et al., 2014) and in oral products to decrease the formation of dental plaque (Allaker, 2010). It can also be used in the formulation of paints, cosmetics and food products (Shukla et al., 2011).

Like many other nanoparticles being studied for potential use in the human body, the mechanism of action and potential toxicity is not entirely known. Some studies indicate that zinc oxide nanoparticles have a selective toxicity against bacteria, with almost no effects in the human body (Allaker & Douglas, 2015). Other studies report that the nanoparticles may induce genotoxicity and damage the cell membrane by releasing reactive oxygen species (Shukla et al., 2011; Wang, Deng, Zhang, Chen, & Ding, 2014).

Zinc oxide nanoparticles inhibit the growth of *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans* and *Fusobacterium nucleatum* with a MIC of 250 µg/mL; and *Prevotella intermedia* with a MIC of 1000 µg/mL (Table 8). If these bacteria are under anaerobic conditions, the MIC ranges from 250 µg/mL to 2500 µg/mL (Vargas-Reus et al., 2012). These nanoparticles also show antimicrobial activity against *Streptococcus mutans* (with a MBC of 500 µg/mL) and *Rothia dentocariosa* (Eshed et al., 2012; Khan et al., 2014).

Silver nanoparticles can help improve the antimicrobial effect of zinc oxide nanoparticles. When combined, the MIC on *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* is 100 µg/mL and on *Prevotella intermedia* is 250 µg/mL (Vargas-Reus et al., 2012).

	ZnO		Ag + ZnO	
	MIC	MBC	MIC	MBC
Aggregatibacter actinomycetemcomitans	250	250	100	100
Fusobaterium nucleatum	250	500	1000	1000
Prevotella intermedia	1000	1000	250	500
Porphyromonas gingivalis	250	250	<100	500

Table 8: Minimum inhibitory concentration and minimum bactericidal concentration expressed in µg/mL of the tested bacterial species with ZnONPs and AgNPs in combination with ZnONPs, adapted from Vargas-Reus et al. (2012).

IV.6. Titanium dioxide nanoparticles

Titanium dioxide is the most common form of titanium and, in bulk, is used as a whitener in toothpastes (Allaker, 2010); as a pigment in diverse areas such as toothpastes, paints, food industry and plastics; as an antimicrobial agent and as a water purifier (Weir, Westerhoff, Fabricius, & von Goetz, 2013). Titanium is also one of the main components of dental implants used nowadays since it can offer biocompatibility and the ability to be osseointegrated (Wood et al., 2015).

In order to have a considerable antimicrobial effect, titanium dioxide nanoparticles have to be in anatase form and in the presence of UV light, since photoactivation increases their antibacterial properties. These factors will lead to the formation of reactive oxygen species, causing damage in the cell membrane, in the DNA and in the respiratory activity which will evidently lead to cell death. Its action depends on the concentration employed, the intensity of the UV light and on the crystal structure (Hartmann et al., 2010; Foster, Ditta, Varghese, & Steele, 2011).

Titanium dioxide nanoparticles have shown antibacterial activity against *Streptococcus mutans* and *Streptococcus sanguis*, bacteria involved in the formation of dental caries (Ahrari, Eslami, Rajabi, Ghazvini, & Barati, 2015). The minimum inhibitory concentration needed against *Aggregatibacter actinomycetemcomitans* is 250 µg/mL; *Fusobacterium nucleatum* and *Prevotella intermedia* is 1000 µg/mL; and *Porphyromonas gingivalis* is 2500 µg/mL (Table 9). Under anaerobic conditions, the MIC ranges between 250 µg/mL and 2500 µg/mL (Vargas-Reus et al., 2012).

	MIC	MBC
Aggregatibacter actinomycetemcomitans	250	>2500
Fusobaterium nucleatum	1000	>2500
Prevotella intermedia	1000	>2500
Porphyromonas gingivalis	2500	>2500

Table 9: Minimum inhibitory concentration and minimum bactericidal concentration values expressed in µg/mL of the tested bacterial species with TiO_2NPs, adapted from Vargas-Reus et al. (2012).

IV.7. Quaternary ammonium compounds nanoparticles

The frequency of recurrent dental caries creates the necessity for new restorative materials with antibacterial effects (Antonucci et al., 2012; Imazato, Chen, Ma, Izutani, & Li, 2012). Common restorative composites may allow the colonization of bacteria, leading to tooth demineralization and, if not treated, dental caries (Antonucci et al., 2012). In order to prevent this situation, quaternary ammonium monomers have been developed due to their antimicrobial activity. They can be used in healthcare and textile products, food industry and water treatment (Kenawy, Worley, & Broughton, 2007). Compounds such as 12-methacryloyloxydodecylpyridinium bromide, methacryloxy-lethylcetyl dimethyl ammonium chloride, quaternary ammonium dimethacrylate and quaternary ammonium polyethylenimine (Ge et al., 2015) are polymers known for their bactericidal activity against a wide range of pathogenic bacteria found in the oral cavity (Tables 10 and 11). They also have remineralization properties, good mechanical properties and can also promote biofilm growth inhibition (Ge et al., 2015). However, when using a higher concentration of these polymers, their properties and structures might change (Antonucci et al., 2012).

	MIC	MBC		MIC	MBC
Streptococcus mutans	15.6	62.5	***Eubacterium alactolyticum***	31.3	125
Enterococcus faecalis	31.25	62.5	***Bifidobacterium bifidum***	31.3	62.5
Fusobacterium nucleatum	31.25	62.5	***Peptostreptococcus asaccharolyticus***	31.3	31.3
Prevotella nigrescens	1.95	7.81	***Lactobacillus plantarum***	7.8	15.6
Streptococcus sobrinus	7.8	62.5	***Lactobacillus salivarius ssp. Salivarius***	7.8	62.5
Streptococcus oralis	16.7	31.3	***Lactobacillus acidophilus***	15.6	62.5
Streptococcus mitis	25	31.3	***Lactobacillus paracasei spp. Paracasei***	15.6	62.5
Streptococcus sanguis	16.7	31.3	***Lactobacillus brevis***	15.6	31.3
Streptococcus gordonii	16.7	31.3	***Lactobacillus salivalius ssp. Salicinius***	15.6	125
Streptococcus salicarius	15.6	31.3	***Lactobacillus fermentum***	15.6	15.6
Propionibacterium acnes	3.9	62.5			

Table 10: Minimum inhibitory concentration and minimum bactericidal concentration values expressed in µg/mL of the tested bacterial species with MDPB, adapted from Imazato et al. (2012); Ge et al. (2015).

	MBC
Streptococcus mutans	4.9
Streptococcus sobrinus	2.4
Streptococcus sanguinis	4.9
Lactobacillus acidophilus	2.4
Actinomyces viscosus	2.4

Table 11: Minimum bactericidal concentration values expressed in µg/mL of the tested bacterial species with DMAE-CB, adapted from Imazato et al. (2012).

Another way to improve the antimicrobial effects and to use higher amounts of quaternary ammonium compounds is to reduce their size to nano-scale levels. QPEI nanoparticles have been developed (Ira Yudovin-Farber, Beyth, Weiss, & Domb, 2010) and have shown better antibacterial properties when incorporated in resins, cements and root canal sealers (Beyth, Yudovin-Farber, Perez-Davidi, Domb, & Weiss, 2010; Barros et al., 2014). When incorporated in cement, QPEI nanoparticles at 1% w/w have shown an antimicrobial effect after fourteen days against *Streptococcus mutans* and *Enterococcus faecalis* (Beyth et al., 2010; Shvero, Davidi, Weiss, Srerer, & Beyth, 2010) and that could last for at least three months (Iran Yudovin-Farber et al., 2008). At 2% w/w, QPEI nanoparticles could inhibit the growth of *Streptococcus mutans*, *Enterococcus faecalis*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* (Beyth et al., 2008). QPEI nanoparticles have not shown significant toxic effects (Beyth et al., 2008; Iran Yudovin-Farber et al., 2008). However, the mechanical properties have to be more thoroughly studied (Ge et al., 2015).

IV.8. Chlorhexidine nanoparticles

As it was previously mentioned in chapter III.1., chlorhexidine is an antiseptic agent with antifungal and antimicrobial properties. It has a non-specific mode of action that can be applied in nanotechnology, improving the antimicrobial properties of current dental implants and the treatment of peri-implant infections (Varoni et al., 2012; Wood et al., 2015). Chlorhexidine is effective against a wide range of bacteria. When applied as a nanoparticle to filling materials, such as cements and ceramics, it can provide these materials with its antimicrobial and antifungal properties (Hook et al., 2014).

Chlorhexidine-hexametaphosphate nanoparticles have been developed and they can provide a continuous and slow release of chlorhexidine. In a study conducted by Wood, N.J. et al. (Wood et al., 2015), CHX-HMP NPs were applied to titanium dental implants as a coating. The purpose of the study was to evaluate the antimicrobial activity of chlorhexidine as a nanoparticle. The dental implants were coated with CHX-HMP NPs and tested against *Streptococcus gordonii* in the presence and in the absence of human saliva. Results have shown that, after 24 hours, there was significant decrease in the CFU's on the implants coated with CHX-HMP NPs. Differences were observed in the presence of saliva: in its presence, the nanoparticles had a bactericidal effect on the colonies; in its absence, the nanoparticles had a bacteriostatic effect (Wood et al., 2015).

Chlorhexidine nanoparticles have also shown positive results against biofilms. A MIC of 19,5 µg/mL and a MBC of 312,5 µg/mL were observed when these nanoparticles were used against *Streptococcus mutans*. The nanoparticles were also tested in the form of a 24 and a 48-hour treatment against mono-species biofilms and were effective against *Acinetobacter actinomycetemcomitans, Fusobacterium nucleatum* and *Streptococcus mutans* with a MIC with a range of 100 µg/mL. It was also tested against *Streptococcus sobrinus* and MIC of 200 µg/mL was obtained (Seneviratne et al., 2014). When using chlorhexidine nanoparticles against mixed-species biofilms, the results shown in Table 12 were obtained.

	24 h	48 h	72h
S. mutans, F. nucleatum* and *P. gingivalis	12,5	50	50
Streptococcus sobrinus, F. nucleatum* and *P. gingivalis	25	50	100
A. actinomycetemcomitans, S. mutans, F. nucleatum* and *P. gingivalis	25	50	50

Table 12: Minimum inhibitory concentration value expressed in µg/mL of the tested bacterial species with chlorhexidine nanoparticles after 24, 48 and 72 hours of exposure, adapted from Seneviratne et al. (2014).

IV.9. Calcium-phosphate nanoparticles

Calcium and phosphate compounds belong to the amorphous calcium-phosphates and have the potential to remineralize the damaged enamel by providing the needed ions to repair it. These compounds are soluble and react with saliva in order to release calcium and phosphate ions, which are then transformed into apatite, a major component of the teeth's enamel (Moreau et al., 2011). They are also compatible with the bone since they are identical at a biochemical level.

In a study conducted by Kovtun et al., (2012), calcium-phosphate nanoparticles were loaded with chlorhexidine in order to test their mineralizing and antibacterial properties when combined. Extracted human teeth without caries were used in this experiment to test the remineralizing effects. *Lactobacillus casei* and *Escherichia coli* were used to test the antimicrobial effects. Results show that there was a continuous release of chlorhexidine after its application on the teeth, a decrease of the bacterial growth and an increase of the quantity of calcium-phosphate. These results show that calcium-phosphate nanoparticles have potential use in diseases where the bone or tooth structure is affected by an infection, such as dental caries and periodontitis (Kovtun et al., 2012).

Conclusions

The evidence of the connection of bacteria with the development of antibiotic resistance is growing (Laxminarayan, 2014). However, there have been a few scientific advancements that will improve and reduce the dependence of antibiotic therapy to treat bacterial infections.

Studies about nanoparticles are being developed in order to use them in the human body. Gold, titanium dioxide and zinc oxide nanoparticles are already used in areas such as cosmetics and the food industry. The next step would be to apply them to treat specific infections, specifically those in the oral cavity since these nanoparticles already exhibit antimicrobial properties against pathogenic bacteria in this area. Nevertheless, most studies focus only on the antimicrobial aspects of the nanoparticle and neglect their potential toxic effect in the human body. Also, nanoparticles are smaller than the antibiotic molecules, making them susceptible to accumulate in organs, tissues and cells that antibiotics would not. For example, silver nanoparticles have shown good antimicrobial properties but the insufficient studies about their toxic effects may affect their development for future use in the dental area. In order to change this outcome, more studies about the mechanisms of action and toxic effects of metal or other potential nanoparticles are needed to understand their impact on bacteria and oral infections.

With this dissertation, it can be concluded that the choice of antibiotics to treat oral infections has not changed and that the current guidelines for Portugal are up-to-date. However, development of new antibiotics has stagnated along the years. Since chronic diseases are more profitable, fewer pharmaceutical companies are interested in developing new antibiotics.

It can also be concluded that there is a potential and broader use for metal nanoparticles in the oral cavity once their toxic effect and mechanisms of action are thoroughly studied. There is also a need to standardize the tests used since the researchers often use different nanoparticle sizes and methods. Chlorhexidine, as it is currently used or as a nanoparticle, could be a good contender for the development of new antimicrobial products since it is less likely to develop antimicrobial resistance, is already studied, has a broad spectrum of activity and has shown promising results in the nanoparticle field.

References

Aas, J. a., Griffen, A. L., Dardis, S. R., Lee, A. M., Olsen, I., Dewhirst, F. E., … Paster, B. J. (2008). Bacteria of dental caries in primary and permanent teeth in children and young adults. *Journal of Clinical Microbiology*, *46*(4), 1407–1417. http://doi.org/10.1128/JCM.01410-07

Ahmad, Z., Vargas-Reus, M. a., Bakhshi, R., Ryan, F., Ren, G. G., Oktar, F., & Allaker, R. P. (2012). Antimicrobial properties of electrically formed elastomeric polyurethane-copper oxide nanocomposites for medical and dental applications. *Methods in Enzymology*, *509*, 87–99. http://doi.org/10.1016/B978-0-12-391858-1.00005-8

Ahrari, F., Eslami, N., Rajabi, O., Ghazvini, K., & Barati, S. (2015). The antimicrobial sensitivity of Streptococcus mutans and Streptococcus sangius to colloidal solutions of different nanoparticles applied as mouthwashes. *Dental Research Journal*, *12*(1), 44–49.

Allaker, R. P. (2010). The use of nanoparticles to control oral biofilm formation. *Journal of Dental Research*, *89*(11), 1175–1186. http://doi.org/10.1177/0022034510377794

Allaker, R. P., & Douglas, C. W. I. (2015). Non-conventional therapeutics for oral infections. *Virulence*, *6*(3), 196–207. http://doi.org/10.4161/21505594.2014.983783

Aminov, R. I. (2010). A Brief History of the Antibiotic Era: Lessons Learned and Challenges for the Future. *Frontiers in Microbiology*, *1*(December), 1–7. http://doi.org/10.3389/fmicb.2010.00134

Antonucci, J. M., Zeiger, D. N., Tang, K., Lin-gibson, S., Fowler, B. O., & Lin, N. J. (2012). Synthesis and characterization of dimethacrylates containing quaternary ammonium funtionalities for dental applications. *Dent Mater*, *28*(2), 219–228. http://doi.org/10.1016/j.dental.2011.10.004.Synthesis

Anwar, A., Amir, Q., & Khan, M. W. (2014). Chronic Periodontitis , A Silent Hazardous Disease. *Biomedica*, *30*(1), 34–39.

B. Sutariya, V., & Pathak, Y. (2015). *Biointeractions of Nanomaterials*. CRC Press.

Barros, J., Silva, M. G., Rôças, I. N., Gonçalves, L. S., Alves, F. F., Lopes, M. A., Siqueira, J. F. (2014). Antibiofilm effects of endodontic sealers containing

quaternary ammonium polyethylenimine nanoparticles. *Journal of Endodontics*, *40*(8), 1167–1171. http://doi.org/10.1016/j.joen.2013.12.021

Berglundh, T., Krok, L., Liljenberg, B., Westfelt, E., Serino, G., & Lindhe, J. (1998). The use of metronidazole and amoxicillin in the treatment of advanced periodontal disease. A prospective, controlled clinical trial. *Journal of Clinical Periodontology*, *25*(5), 354–362. http://doi.org/10.1111/j.1600-051X.1998.tb02455.x

Besinis, A., De Peralta, T., & Handy, R. D. (2014). The antibacterial effects of Ag, TiO2 and SiO2 nanoparticles compared to the Dental Disinfectant Chlorhexidine on Streptococcus mutans Using a Suite of Bioassays. *Nanotoxicology*, *1*(8), 1–16. http://doi.org/10.3109/17435390.2012.742935

Beyth, N., Houri-Haddad, Y., Baraness-Hadar, L., Yudovin-Farber, I., Domb, A. J., & Weiss, E. I. (2008). Surface antimicrobial activity and biocompatibility of incorporated polyethylenimine nanoparticles. *Biomaterials*, *29*(31), 4157–4163. http://doi.org/10.1016/j.biomaterials.2008.07.003

Beyth, N., Yudovin-Farber, I., Perez-Davidi, M., Domb, A. J., & Weiss, E. I. (2010). Polyethyleneimine nanoparticles incorporated into resin composite cause cell death and trigger biofilm stress in vivo. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(51), 22038–22043. http://doi.org/10.1073/pnas.1010341107

Brook, I., Lewis, M. a O., Sándor, G. K. B., Jeffcoat, M., Samaranayake, L. P., & Rojas, J. V. (2005). Clindamycin in dentistry: More than just effective prophylaxis for endocarditis? *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontology*, *100*(5), 550–558. http://doi.org/10.1016/j.tripleo.2005.02.086

Brown, G. D., Denning, D. W., Gow, N. a. R., Levitz, S. M., Netea, M. G., & White, T. C. (2012). Hidden Killers: Human Fungal Infections. *Science Translational Medicine*, *4*(165), 165rv13. http://doi.org/10.1126/scitranslmed.3004404

Chopra, I. (2007). The increasing use of silver-based products as antimicrobial agents: A useful development or a cause for concern? *Journal of Antimicrobial Chemotherapy*, *59*(4), 587–590. http://doi.org/10.1093/jac/dkm006

Cionca, N., Giannopoulou, C., Ugolotti, G., & Mombelli, A. (2009). Amoxicillin and metronidazole as an adjunct to full-mouth scaling and root planing of chronic periodontitis. *The Journal of Periodontology*, *80*(3), 364–371. http://doi.org/10.1902/jop.2009.080540

Coates, A. R., Halls, G., & Hu, Y. (2011). Novel classes of antibiotics or more of the same? *British Journal of Pharmacology*, *163*(1), 184–194. http://doi.org/10.1111/j.1476-5381.2011.01250.x

Cole, S. T. (2014). Who will develop new antibacterial agents? *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *369*(1645), 20130430. http://doi.org/10.1098/rstb.2013.0430

Cope, G., & Cope, A. (2011). Gingivitis: symptoms, causes and treatment. *Dental Nursing*, *7*(8), 436–439. http://doi.org/10.12968/denn.2011.7.8.436

Corbin, A., Pitts, B., Parker, A., & Stewart, P. S. (2011). Antimicrobial penetration and efficacy in an in vitro oral biofilm model. *Antimicrobial Agents and Chemotherapy*, *55*(7), 3338–3344. http://doi.org/10.1128/AAC.00206-11

Corrêa, J. M., Mori, M., Sanches, H. L., Dibo, A., Jr, E. P., Andréa, I., & Pola, V. (2014). Silver Nanoparticles in Dental Biomaterials. *International Journal of Biomaterials*, *2015*.

Cushnie, T. P. T., Cushnie, B., & Lamb, A. J. (2014). Alkaloids: An overview of their antibacterial, antibiotic-enhancing and antivirulence activities. *International Journal of Antimicrobial Agents*, *44*(5), 377–386. http://doi.org/10.1016/j.ijantimicag.2014.06.001

Dann, A., & Hontela, A. (2011). Triclosan: environmental exposure, toxicity and mechanisms of action. *Journal of Applied Toxicology*, *31*(4), 285–311. http://doi.org/10.1002/jat.1660

Darby, M. L., & Walsh, M. (2014). *Dental Hygiene: Theory and Practice* (4th ed.). Elsevier Health Sciences.

Davison, J., & Maillard, J. (2010). *Opinion on triclosan - Antimicrobial resistance*. *Scientific Committee on Consumer Safety SCCS*.

Dhillon, G. S., Kaur, S., Pulicharla, R., Brar, S. K., Cledón, M., Verma, M., & Surampalli, R. Y. (2015). Triclosan: Current Status, Occurrence, Environmental Risks and Bioaccumulation Potential. *International Journal of Environmental Research and Public Health*, *12*(5), 5657–5684. http://doi.org/10.3390/ijerph120505657

Direcção Geral da Saúde. (2014). *Norma n^{o} 064/2011* (Vol. 064/2011).

Dörr, T., Davis, B. M., & Waldor, M. K. (2015). Endopeptidase-Mediated Beta Lactam Tolerance. *PLOS Pathogens*, *11*(4), e1004850. http://doi.org/10.1371/journal.ppat.1004850

Dykman, L., & Khlebtsov, N. (2012). Gold nanoparticles in biomedical applications: recent advances and perspectives. *Chemical Society Reviews*, *41*(6), 2256. http://doi.org/10.1039/c1cs15166e

Elechiguerra, J. L., Burt, J. L., Morones, J. R., Camacho-Bragado, A., Gao, X., Lara, H. H., & Yacaman, M. J. (2005). Interaction of silver nanoparticles with HIV-1. *Journal of Nanobiotechnology*, *3*, 6. http://doi.org/10.1186/1477-3155-3-6

Eshed, M., Lellouche, J., Matalon, S., Gedanken, A., & Banin, E. (2012). Sonochemical Coatings of ZnO and CuO Nanoparticles Inhibit Streptococcus mutans Biofilm Formation on Teeth Model. *Langmuir*, *28*(33), 12288–12295. http://doi.org/10.1021/la301432a

European Comission. (2014). Commission Regulation (EU) No 344/2013 of 4 April 2013 amending Annexes II, III, V and VI to Regulation (EC) No 1223/2009 of the European Parliament and of the Council on cosmetic products Text with EEA relevance. Retrieved from http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32013R0344

Fernandes, J. M. F. A., Menezes, V. A., Albuquerque, A. J. R., Oliveira, M. A. C., Meira, K. M. S., Menezes Júnior, A. R., & Sampaio, F. C. (2015). Improving Antimicrobial Activity of Dental Restorative Materials. In M. Virdi (Ed.), *Emerging Trends in Oral Health Sciences and Dentistry*. InTech. http://doi.org/http://dx.doi.org/10.5772/59252

Finberg, R., & Guharoy, R. (2012). Metronidazole and Clindamycin. In *Clinical Use of Anti-infective Agents SE - 11* (pp. 67–70). Springer New York. http://doi.org/10.1007/978-1-4614-1068-3_11

Foster, H. a., Ditta, I. B., Varghese, S., & Steele, A. (2011). Photocatalytic disinfection using titanium dioxide: spectrum and mechanism of antimicrobial activity. *Applied Microbiology and Biotechnology*, *90*(6), 1847–1868. http://doi.org/10.1007/s00253-011-3213-7

Garrido-Mesa, N., Zarzuelo, a., & Gálvez, J. (2013). Minocycline: Far beyond an antibiotic. *British Journal of Pharmacology*, *169*(2), 337–352. http://doi.org/10.1111/bph.12139

Ge, Y., Wang, S., Zhou, X., Wang, H., Xu, H., & Cheng, L. (2015). The Use of Quaternary Ammonium to Combat Dental Caries. *Materials*, *8*(6), 3532–3549. http://doi.org/10.3390/ma8063532

Gupta, M., Mahajan, V. K., Mehta, K. S., & Chauhan, P. S. (2014). Zinc Therapy in Dermatology: A Review. *Dermatology Research and Practice*, *2014*, 1–11. http://doi.org/10.1155/2014/709152

Habiboallah, G., Mahdi, Z., Majid, Z., Nasroallah, S., Moeen Taghavi, A., Forouzanfar, A., & Arjmand, N. (2014). Enhancement of Gingival Wound Healing by Local Application of Silver Nanoparticles Periodontal Dressing Following Surgery : A Histological Assessment in Animal Model. *Modern Research in Inflammation*, *3*(August), 128–138.

Haraszthy, V. I., Sreenivasan, P. K., & Zambon, J. J. (2014). Community-level assessment of dental plaque bacteria susceptibility to triclosan over 19 years. *BMC Oral Health*, *14*(1), 61. http://doi.org/10.1186/1472-6831-14-61

Hartmann, N. B., Von der Kammer, F., Hofmann, T., Baalousha, M., Ottofuelling, S., & Baun, a. (2010). Algal testing of titanium dioxide nanoparticles-Testing considerations, inhibitory effects and modification of cadmium bioavailability. *Toxicology*, *269*(2-3), 190–197. http://doi.org/10.1016/j.tox.2009.08.008

Heller, J. L., Zieve, D., & Ogilvie, I. (2014). Tooth anatomy. Retrieved June 1, 2015, from http://www.nlm.nih.gov/medlineplus/ency/imagepages/1121.htm

Heymann, H. O., Swift Jr., E. J., & Ritter, A. V. (2014). *Sturdevant's Art & Science of Operative Dentistry* (6th ed.). Elsevier Health Sciences.

Hook, E. R., Owen, O. J., Bellis, C. a, Holder, J. a, O'Sullivan, D. J., & Barbour, M. E. (2014). Development of a novel antimicrobial-releasing glass ionomer cement functionalized with chlorhexidine hexametaphosphate nanoparticles. *Journal of Nanobiotechnology*, *12*(1), 3. http://doi.org/10.1186/1477-3155-12-3

Imazato, S., Chen, J. H., Ma, S., Izutani, N., & Li, F. (2012). Antibacterial resin monomers based on quaternary ammonium and their benefits in restorative dentistry. *Japanese Dental Science Review*, *48*(2), 115–125. http://doi.org/10.1016/j.jdsr.2012.02.003

Infarmed. (2015). Prontuário Terapêutico. Retrieved August 30, 2015, from https://www.infarmed.pt/prontuario/index.php

Ingle, A. P., Duran, N., & Rai, M. (2014). Bioactivity, mechanism of action, and cytotoxicity of copper-based nanoparticles: A review. *Applied Microbiology and Biotechnology*, *98*(3), 1001–1009. http://doi.org/10.1007/s00253-013-5422-8

Jensen, S. E. (2012). Biosynthesis of clavam metabolites. *Journal of Industrial Microbiology & Biotechnology*, *39*(10), 1407–1419. http://doi.org/10.1007/s10295-012-1191-0

Kenawy, E. R., Worley, S. D., & Broughton, R. (2007). The chemistry and applications of antimicrobial polymers: A state-of-the-art review. *Biomacromolecules*, *8*(5), 1359–1384. http://doi.org/10.1021/bm061150q

Khan, S. T., Ahamed, M., Musarrat, J., & Al-Khedhairy, A. a. (2014). Anti-biofilm and antibacterial activities of zinc oxide nanoparticles against the oral opportunistic pathogens *Rothia dentocariosa* and *Rothia mucilaginosa*. *European Journal of Oral Sciences*, *122*(6), 397–403. http://doi.org/10.1111/eos.12152

Khodaeiani, E., Fouladi, R. F., Yousefi, N., Amirnia, M., Babaeinejad, S., & Shokri, J. (2012). Efficacy of 2% Metronidazole Gel in Moderate Acne Vulgaris. *Indian Journal of Dermatology*, *57*(4), 279–281. http://doi.org/10.4103/0019-5154.97666

Kim, J. S., Kuk, E., Yu, K. N., Kim, J. H., Park, S. J., Lee, H. J., … Cho, M. H. (2007). Antimicrobial effects of silver nanoparticles. *Nanomedicine: Nanotechnology, Biology, and Medicine*, *3*(1), 95–101. http://doi.org/10.1016/j.nano.2006.12.001

Kovtun, A., Kozlova, D., Ganesan, K., Biewald, C., Seipold, N., Gaengler, P., … Epple, M. (2012). Chlorhexidine-loaded calcium phosphatenanoparticles for dental maintenance treatment: combination of mineralising and antibacterial effects. *RSC Adv.*, *2*(3), 870–875. http://doi.org/10.1039/C1RA00955A

Lachenmeier, D. W. (2014). *Side Effects of Drugs Annual*. *Antiseptic Drugs and Disinfectants* (36th ed.). Elsevier B.V. http://doi.org/10.1016/B978-0-444-63407-8.00024-1

Lara, H. H., Garza-Treviño, E. N., Ixtepan-Turrent, L., & Singh, D. K. (2011). Silver nanoparticles are broad-spectrum bactericidal and virucidal compounds. *Journal of Nanobiotechnology*, *9*(1), 30. http://doi.org/10.1186/1477-3155-9-30

Laxminarayan, R. (2014). *The coming crisis in antibiotics*. Retrieved from https://www.ted.com/talks/ramanan_laxminarayan_the_coming_crisis_in_antibiotics

Li, X., Ying, G.-G., Zhao, J.-L., Chen, Z.-F., Lai, H.-J., & Su, H.-C. (2013). 4-Nonylphenol, bisphenol-A and triclosan levels in human urine of children and students in China, and the effects of drinking these bottled materials on the levels. *Environment International*, *52*, 81–86. http://doi.org/10.1016/j.envint.2011.03.026

Ligon, B. L. (2004). Penicillin: its discovery and early development. *Seminars in Pediatric Infectious Diseases*, *15*(1), 52–57. http://doi.org/10.1053/j.spid.2004.02.001

Lôbo, A. L. G., & Martins, G. B. (2009). Consequências da Radioterapia na Região de Cabeça e Pescoço : Uma Revisão da Literatura. *Revista Portuguesa de Estomatologia, Medicina Dentária E Cirurgia Maxilofacia*, *50*(5), 251–255.

Long, T. C., Saleh, N., Tilton, R. D., Lowry, G. V., & Veronesi, B. (2006). Titanium dioxide (P25) produces reactive oxygen species in immortalized brain microglia (BV2): Implications for nanoparticle neurotoxicity. *Environmental Science and Technology*, *40*(14), 4346–4352. http://doi.org/10.1021/es060589n

Lu, Z., Rong, K., Li, J., Yang, H., & Chen, R. (2013). Size-dependent antibacterial activities of silver nanoparticles against oral anaerobic pathogenic bacteria. *Journal of Materials Science: Materials in Medicine*, *24*(6), 1465–1471. http://doi.org/10.1007/s10856-013-4894-5

Mani, A., Dalvi, A., & Mani, S. (2015). To Prescribe or Not To : Systemic Antibiotics in Treatment of Periodontal Infections. *International Journal of Science and Research*, *4*(9), 1902–1904.

Manuel José Guedes da Silva Lda, & Guia de Saúde: Edição e Comunicação Audio-Visual (Eds.). (2014). *Mapa Terapêutico 2014* (11th ed.).

Markowska, K., Grudniak, A. M., & Wolska, K. I. (2013). Silver nanoparticles as an alternative strategy against bacterial biofilms. *Acta Biochimica Polonica*, *60*(4), 523–530.

Mayo Foundation for Education and Research. (n.d.). Gingivitis. Retrieved May 20, 2006, from http://www.mayoclinic.org/diseases-conditions/gingivitis/multimedia/gingivitis/img-20006944

Melo, M. A. S., Guedes, S. F. F., Xu, H. H. K., & Rodrigues, L. K. A. (2013). Nanotechnology-based restorative materials for dental caries management. *Trends Biotechnology*, *31*(8), 997–1003. http://doi.org/10.1016/j.tibtech.2013.05.010

Miljøstyrelsen. (2014, March). Slut med mistænkeligt kemikalie i tøj. Retrieved from http://mst.dk/service/nyheder/nyhedsarkiv/2014/mar/slut-med-mistaenkeligt-kemikalie-i-toej/

Moreau, J. L., Sun, L., Chow, L. C., & Xu, H. H. K. (2011). Mechanical and acid neutralizing properties and bacteria inhibition of amorphous calcium phosphate dental nanocomposite. *Journal of Biomedical Materials Research - Part B Applied Biomaterials*, *98*(1), 80–88. http://doi.org/10.1002/jbm.b.31834

Nano Labs. (2012). Copper Oxide Nanoparticles. Retrieved September 25, 2015, from http://www.nanolabs.co.in/copper-oxide-nanoparticles.htm

National Center for Biotechnology Information. (n.d.-a). PubChem Compound Database; CID=23670298. Retrieved September 5, 2015, from https://pubchem.ncbi.nlm.nih.gov/compounds/23670298

National Center for Biotechnology Information. (n.d.-b). PubChem Compound Database; CID=29029. Retrieved June 5, 2015, from https://pubchem.ncbi.nlm.nih.gov/compound/29029

National Center for Biotechnology Information. (n.d.-c). PubChem Compound Database; CID=33613. Retrieved June 5, 2015, from https://pubchem.ncbi.nlm.nih.gov/compound/33613

National Center for Biotechnology Information. (n.d.-d). PubChem Compound Database; CID=4173. Retrieved June 5, 2015, from https://pubchem.ncbi.nlm.nih.gov/compound/4173

National Center for Biotechnology Information. (n.d.-e). PubChem Compound Database; CID=54675783. Retrieved June 5, 2015, from https://pubchem.ncbi.nlm.nih.gov/compound/54675783

National Center for Biotechnology Information. (n.d.-f). PubChem Compound Database; CID=5564. Retrieved June 5, 2015, from https://pubchem.ncbi.nlm.nih.gov/compound/5564

National Center for Biotechnology Information. (n.d.-g). PubChem Compound Database; CID=9552079. Retrieved June 5, 2015, from https://pubchem.ncbi.nlm.nih.gov/compound/9552079

Nel, A. E., Madler, L., Velegol, D., Xia, T., Hoek, E. M. V, Somasundaran, P., … Thompson, M. (2009). Understanding biophysicochemical interactions at the nano-bio interface. *Nat Mater*, *8*(7), 543–557. Retrieved from http://dx.doi.org/10.1038/nmat2442

Nel, A., Xia, T., Mädler, L., & Li, N. (2006). Toxic potential of materials at the nanolevel. *Science (New York, N.Y.)*, *311*(5761), 622–627. http://doi.org/10.1126/science.1114397

Norrby, R., Powell, M., Aronsson, B., Monnet, D. L., Lutsar, I., Bocsan, I. S., … Gyssens, I. C. (2009). *The bacterial challenge : time to react*. *ECDC/EMEA Joint Working Group* (Vol. 6 July 201). Retrieved from http://ecdc.europa.eu/en/publications/Publications/0909_TER_The_Bacterial_Challenge_Time_to_React.pdf

Nuñez-Anita, R. E., Acosta-Torres, L. S., Vilar-Pineda, J., Martínez-Espinosa, J. C., Fuente-Hernández, J. de la, & Castaño, V. M. (2014). Toxicology of antimicrobial nanoparticles for prosthetic devices. *International Journal of Nanomedicine*, *9*, 3999–4006. http://doi.org/10.2147/IJN.S63064

Ordem dos Médicos Dentistas. (2010). Plano Nacional De Saúde 2011-2016. *Estratégia de Saúde Oral Em Portugal – Um Conceito de Transversalidade Que Urge Implementar*, 28. Retrieved from http://pns.dgs.pt/files/2010/06/omd.pdf

Oyar, P. (2014). DİŞ HEKİMLİĞİNDE NANOPARTİKÜLLERİN KULLANIM ALANLARI (Nanoparticles application areas in Dentistry). *J Dent Ataturk Uni*, 49–55.

Pal, S., Tak, Y. K., & Song, J. M. (2007). Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium Escherichia coli. *Applied and Environmental Microbiology*, *73*(6), 1712–1720. http://doi.org/10.1128/AEM.02218-06

Parashar, A. (2015). Mouthwashes and Their Use in Different Oral Conditions. *Scholars Journal of Dental Sciences (SJDS)*, *2*(2B), 186–191.

Park, S., Lee, H., Hong, J., Lee, H., Kim, J., Choi, B., … Jeon, Y. (2014). Enhancement of the killing effect of low-temperature plasma on Streptococcus mutans by combined treatment with gold nanoparticles. *Journal of Nanobiotechnology*, *12*(1), 29. http://doi.org/10.1186/s12951-014-0029-5

Perelshtein, I., Applerot, G., Perkas, N., Wehrschuetz-Sigl, E., Hasmann, a., Guebitz, G., & Gedanken, a. (2009). CuO-cotton nanocomposite: Formation, morphology, and antibacterial activity. *Surface and Coatings Technology*, *204*(1-2), 54–57. http://doi.org/10.1016/j.surfcoat.2009.06.028

Powell, L. V. (2013). Amoxicillin/metronidazole therapy may improve the effectiveness of scaling and root planing in patients with periodontitis. *Journal of the American Dental Association (1939)*, *144*(6), 640–2. http://doi.org/10.14219/jada.archive.2013.0175

Powers, J. M., & Wataha, J. C. (2014). *Dental Materials: Properties and Manipulation* (10th ed.). Elsevier Health Sciences.

Prabhu, S., & Poulose, E. K. (2012). Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. *International Nano Letters*, *2*(1), 32. http://doi.org/10.1186/2228-5326-2-32

Rai, M., Yadav, A., & Gade, A. (2009). Silver nanoparticles as a new generation of antimicrobials. *Biotechnology Advances*, *27*(1), 76–83. http://doi.org/10.1016/j.biotechadv.2008.09.002

Ren, G., Hu, D., Cheng, E. W. C., Vargas-Reus, M. a., Reip, P., & Allaker, R. P. (2009). Characterisation of copper oxide nanoparticles for antimicrobial applications. *International Journal of Antimicrobial Agents*, *33*(6), 587–590. http://doi.org/10.1016/j.ijantimicag.2008.12.004

Rieznichenko, L. S., Dybkova, S. M., Gruzina, T. G., Ulberg, Z. R., Todor, I. N., Shpyleva, S. I., & Chekhun, V. F. (2012). Gold Nanoparticles Synthesis and Biological Activity, 25–28.

Rizzoto, M. (2012). Metal Complexes as Antimicrobial Agents. In D. V. Bobbarala (Ed.), *A Search for Antibacterial Agents* (pp. 73–88). InTech. http://doi.org/10.5772/45651

Robertson, D., & Smith, A. J. (2009). The microbiology of the acute dental abscess. *Journal of Medical Microbiology*, *58*(2), 155–162. http://doi.org/10.1099/jmm.0.003517-0

Ruparelia, J. P., Chatterjee, A. K., Duttagupta, S. P., & Mukherji, S. (2008). Strain specificity in antimicrobial activity of silver and copper nanoparticles. *Acta Biomaterialia*, *4*(3), 707–716. http://doi.org/10.1016/j.actbio.2007.11.006

Russell, A. D. (2004). Whither triclosan? *Journal of Antimicrobial Chemotherapy* , *53* (5), 693–695. http://doi.org/10.1093/jac/dkh171

Seneviratne, C. J., Leung, K. C.-F., Wong, C.-H., Lee, S.-F., Li, X., Leung, P. C., … Jin, L. (2014). Nanoparticle-encapsulated chlorhexidine against oral bacterial biofilms. *PloS One*, *9*(8), e103234. http://doi.org/10.1371/journal.pone.0103234

Shukla, R. K., Sharma, V., Pandey, A. K., Singh, S., Sultana, S., & Dhawan, A. (2011). ROS-mediated genotoxicity induced by titanium dioxide nanoparticles in human epidermal cells. *Toxicology in Vitro*, *25*(1), 231–241. http://doi.org/10.1016/j.tiv.2010.11.008

Shvero, D. K., Davidi, M. P., Weiss, E. I., Srerer, N., & Beyth, N. (2010). Antibacterial effect of polyethyleneimine nanoparticles incorporated in provisional cements against Streptococcus mutans. *Journal of Biomedical Materials Research - Part B Applied Biomaterials*, *94*(2), 367–371. http://doi.org/10.1002/jbm.b.31662

Sloane, J. (2012). Mercury: Element of the Ancients. Retrieved March 20, 2015, from http://www.dartmouth.edu/~toxmetal/mercury/history.html

Soares, G. M. S., Figueiredo, L. C., Faveri, M., Cortelli, S. C., Duarte, P. M., & Feres, M. (2012). Mechanisms of action of systemic antibiotics used in periodontal treatment and mechanisms of bacterial resistance to these drugs. *Journal of Applied Oral Science*, *20*(3), 295–309. http://doi.org/10.1590/S1678-77572012000300002

Takahashi, N., & Nyvad, B. (2011). The Role of Bacteria in the Caries Process: Ecological Perspectives . *Journal of Dental Research* , *90* (3), 294–303. http://doi.org/10.1177/0022034510379602

Tancawan, A. L., Pato, M. N., Abidin, K. Z., Asari, A. S. M., Thong, T. X., Kochhar, P., … Barker, K. (2015). Amoxicillin / Clavulanic Acid for the Treatment of Odontogenic Infections : A Randomised Study Comparing Efficacy and Tolerability versus Clindamycin. *International Journal of Dentistry*, *2015*. http://doi.org/http://dx.doi.org/10.1155/2015/472470

Thakor, A. S., Jokerst, J., Zavaleta, C., Massoud, T. F., & Gambhir, S. S. (2011). Gold nanoparticles: A revival in pecious metal administration to patients. *Nano Lett.*, *11*, 4029–4036. http://doi.org/10.1021/nl202559p

The Trustees of Indiana University. (2002). Syphilis in History. Retrieved March 20, 2015, from http://poynter.indiana.edu/sas/lb/syphilis.html

Tsaousoglou, P., Nietzsche, S., Cachovan, G., Sculean, A., & Eick, S. (2014). Antibacterial activity of moxifloxacin on bacteria associated with periodontitis within a biofilm. *Journal of Medical Microbiology*, *63*, 284–292. http://doi.org/10.1099/jmm.0.065441-0

Vargas-Reus, M. a., Memarzadeh, K., Huang, J., Ren, G. G., & Allaker, R. P. (2012). Antimicrobial activity of nanoparticulate metal oxides against peri-implantitis pathogens. *International Journal of Antimicrobial Agents*, *40*(2), 135–139. http://doi.org/10.1016/j.ijantimicag.2012.04.012

Varoni, E., Tarce, M., Lodi, G., & Carrassi, A. (2012). Chlorhexidine (CHX) in dentistry: state of the art. *Minerva Stomatologica*, *61*(9), 399–419. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/22976567

Verran, J., Sandoval, G., Allen, N. S., Edge, M., & Stratton, J. (2007). Variables affecting the antibacterial properties of nano and pigmentary titania particles in suspension. *Dyes and Pigments*, *73*(3), 298–304. http://doi.org/10.1016/j.dyepig.2006.01.003

Waltimo, T. M. T., Sirén, E. K., Torkko, H. L. K., Olsen, I., & Haapasalo, M. P. P. (1997). Fungi in therapy-resistant apical periodontitis. *International Endodontic Journal*, *30*(2), 96–101. http://doi.org/10.1111/j.1365-2591.1997.tb00681.x

Wang, J., Deng, X., Zhang, F., Chen, D., & Ding, W. (2014). ZnO nanoparticle-induced oxidative stress triggers apoptosis by activating JNK signaling pathway in cultured primary astrocytes. *Nanoscale Research Letters*, *9*(1), 117. http://doi.org/10.1186/1556-276X-9-117

Weir, A., Westerhoff, P., Fabricius, L., & von Goetz, N. (2013). Titanium Dioxide Nanoparticles in Food and Personal Care Products. *Environmental Science and Technology*, *46*(4), 2242–2250. http://doi.org/10.1021/es204168d

Wood, N. J., Jenkinson, H. F., Davis, S. a., Mann, S., O'Sullivan, D. J., & Barbour, M. E. (2015). Chlorhexidine hexametaphosphate nanoparticles as a novel antimicrobial coating for dental implants. *Journal of Materials Science: Materials in Medicine*, *26*(6), 201. http://doi.org/10.1007/s10856-015-5532-1

Yang, N.-Y., Zhang, Q., Li, J.-L., Yang, S.-H., & Shi, Q. (2014). Progression of periodontal inflammation in adolescents is associated with increased number of Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythensis, and Fusobacterium nucleatum. *International Journal of Paediatric Dentistry*, *24*(3), 226–233. http://doi.org/10.1111/ipd.12065

Yazdankhah, S. P., Scheie, A. A., Høiby, E. A., Lunestad, B.-T., Heir, E., Fotland, T. Ø., … Kruse, H. (2006). Triclosan and Antimicrobial Resistance in Bacteria: An Overview. *Microbial Drug Resistance*, *12*(2), 83–90. http://doi.org/10.1089/mdr.2006.12.83

Yek, E. C., Cintan, S., Topcuoglu, N., Kulekci, G., İssever, H., & Kantarci, A. (2010). Efficacy of Amoxicillin and Metronidazole Combination for the Management of Generalized Aggressive Periodontitis. *Journal of Periodontology*, *81*(7), 964–974. http://doi.org/10.1902/jop.2010.090522

Yudovin-Farber, I., Beyth, N., Nyska, A., Weiss, E. I., Golenser, J., & Domb, A. J. (2008). Surface characterization and biocompatibility of restorative resin containing nanoparticles. *Biomacromolecules*, *9*(11), 3044–3050. http://doi.org/10.1021/bm8004897

Yudovin-Farber, I., Beyth, N., Weiss, E. I., & Domb, A. J. (2010). Antibacterial effect of composite resins containing quaternary ammonium polyethyleneimine nanoparticles. *Journal of Nanoparticle Research*, *12*(2), 591–603. http://doi.org/10.1007/s11051-009-9628-8