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Antibacterial agents and Coatings: challenges, perspectives and opportunities

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Abstract: Common reason for restorations failure is secondary caries, which are mainly caused by oral bacteria. In recent years, numerous research studies have been conducted with the common goal of developing a dental restorative material to be used to eradicate the microbial infection and the cause of dental caries. Today, the manufacture and use of biomaterials with antimicrobial effects in medical and dental treatment plans is rapidly progressing. Therefore, manufacturing products containing antimicrobial agents or applying antimicrobial coatings on different material has become an interesting topic for research in the dental field.

Keywords: *antimicrobial, bacteria, antibiotics, herbal, smart materials, implants.*

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Introduction

Antibacterial agents are a class of materials that act against pathogenic bacteria and microorganisms either by killing or reducing their metabolic activity. A therapy reducing their pathogenic effect in the biological environments.(1) Antibacterial agents can be classified according to their mode of action into:

1. Inhibition of peptidoglycan biosynthesis.
2. Selective disruption of cytoplasmic membrane.
3. Interfere with DNA replication, transcription, or translation.
4. Positively charged molecules.
5. Reactive oxygen species (ROS) induced oxidative stress.
6. Interfere with bacterial enzymes and enzymatic activity.
7. Increasing tooth mineralization.
8. Containing natural constituents.
9. Combined mechanisms.

1. Inhibition of peptidoglycan biosynthesis:

Peptidoglycan is a vital component of all bacterial cell walls. The peptidoglycan polymer is responsible for the shape, mechanical strength, and integrity of bacterial cells. If the synthesis of peptidoglycan is selectively blocked, bacteria undergo a number of changes in shape, the cell wall is greatly weakened and they ultimately die as the result of cell lysis. Mammalian cells do not possess a cell wall or macromolecular structures that resemble peptidoglycan. Consequently, antibacterial agents that interfere with peptidoglycan synthesis have excellent selective toxicity and can be used systemically.(2)

1.a. Antibiotics (ß-Lactams):

- **Penicillin:**

Is the most widely used β-lactam antibiotic, it can inhibit the synthesis of the peptidoglycan layer of the bacterial cell walls.

Uses in dentistry:

- Caries prevention: The first use of penicillin to treat dental caries dated from 1946, when McClure and Hewitt reported that penicillin inhibited caries in rats. Four years later, Zander reported that penicillin showed caries inhibition in children.

Limitations:

However, the use of penicillin can cause some side effects, such as diarrhoea, hypersensitivity, nausea, rash, neurotoxicity, and urticaria. Another major problem is the bacterial resistance.(3)

2. Selective disruption of cytoplasmic membrane:

The integrity of the cytoplasmic membrane is vital for normal cell function, but it is a much more difficult structure to target by antibiotics because it is chemically similar in all cell types with few differences to exploit.

Gram-negative bacteria contain an additional outer-membrane structure which provides a protective penetration barrier to potentially harmful substances. The stability of all membranes is maintained by a combination of non-covalent interactions between the constituents involving ionic, hydrophobic and hydrogen bonding. The balance of these interactions can be disturbed by the intrusion of membrane-active agents, which destroy the integrity of the membrane, thereby causing leakage of cytoplasmic contents or impairment of metabolic functions associated with the membrane. Most membrane-active agents which function in this way have very poor selectivity. They cannot be used systemically because of their damaging effects on mammalian cells; instead they are used as skin antiseptics, disinfectants and preservatives.(2)

2.a. Chlorhexidine (CHX):

Chlorhexidine is active against gram-positive and gram-negative bacteria, facultative anaerobes and aerobes.

Uses in dentistry:

- Caries prevention: It has proved to be the most effective for dental caries. Combining CHX in polymeric matrices have been attempted for sustained delivery specifically in glass ionomer cements (GIC). Although incorporation of CHX in GIC enables better protection against caries, it results in cements with inferior mechanical properties that exhibit a less than optimal period of sustained release.(3)

Also, chlorhexidine incorporated in composite resin was released faster in media of lower pH values due to its higher solubility at lower pH. Release rate also was affected by hydrophilicity of resin. Composites with hydrophilic resin tended to release chlorhexidine faster and the chlorhexidine-containing resin lost its antibacterial activities after storage in water for 2 weeks.(1)

- Plaque and gingivitis control: To date, chlorhexidine remains the “gold standard” of antiplaque agents. Chlorhexidine can reduce the binding of bacteria to tooth surfaces adsorbing chlorhexidine. It is a key component in oral formulations such as mouthwashes and oral gels that can be used topically in the management of oral biofilms.

- Endodontic irrigant: 2% is the concentration of root canal irrigating solutions usually found in the endodontic literature. Despite its usefulness as an endodontic irrigants , it cannot be advocated as the main irrigant in standard endodontic cases, because it lacks a tissue dissolving capacity. (4)

Limitations:

Chlorhexidine causes genotoxicity by inducing DNA damage in leukocytes, kidney cells and oral mucosal cells. When in contact with tissues, CHX is irritant and can delay healing, thus care must be taken to prevent its accidental extrusion into soft tissues.(3)

2.b. Alcohols:

The mechanism of action of alcohols depends on coagulation of protein, dehydration of cells and disruption of bacterial membranes.

Uses in dentistry:

- Disinfectant: Disinfectants are substances that are applied to non-living objects to destroy microorganisms that are living on the objects. The efficacy of these disinfectants depends on contact time, temperature, type and concentration of the active ingredient. A 70% aqueous solution of alcohols (usually ethanol or isopropanol) is more effective at killing microbes than absolute alcohols because water facilitates diffusion through the cell membrane. Additionally, 70% ethyl alcohol can also be safely used as antiseptic on skin.(5)

3. Interfere with DNA synthesis, replication, transcription or translation:

Living cellular systems store the genetic information associated with their growth, division, and survival as a sequence of bases within their DNA. Thus, the DNA must be capable not only of accurate self-replication and segregation into daughter cells, but also of accurate transcription and eventual translation into protein. Double-stranded DNA is not only replicated for cell division, but also transcribed into single-stranded RNA. This RNA provide templates for the biosynthesis of protein (mRNA).(2)

3.a. Antibiotics:

- **Metronidazole:**

Can inhibit nucleic acid synthesis and disrupt DNA through DNA strand breakage. The metronidazole is more effective against anaerobic organisms.

Uses in dentistry:

- Caries prevention: More than 99% of the bacteria present in carious lesions and infected root dentin were not recovered in the presence of metronidazole in in vitro experiments.

- Oral infections and lesions: Metronidazole is available as a topical cream for the mouth and has a wide spectrum of bactericidal action against oral obligate anaerobes.

Limitations:

Side effects of metronidazole, include nausea, a metallic taste, headaches, flushing of the skin, tachycardia, loss of appetite, and shortness of breath.(3)

- **Tetracycline:**

Are a group of broad-spectrum antibiotics with the ability to inhibit protein synthesis in bacteria through blocking 30S ribosomal subunit.

Uses in dentistry:

- Caries prevention: Effective against S.mutans and Lactobacilli.

Limitations:

However, tetracycline appears to become incorporated into human teeth, causing discoloration. In 1963, the United States Food and Drug Administration issued a warning regarding the use of such antibiotics for pregnant women and young children since teeth are most susceptible to tetracycline discoloration during their formation.

The side effects of tetracycline include cramps or burning of the stomach, diarrhoea, sore mouth or tongue, skin photosensitivity, headache rarely, and vision problems, with damage to the kidneys also having been reported.(3)

3.b. Aldehydes:

The aldehydes that act through alkylation of amino, carboxyl-or hydroxyl group, and finally damage nucleic acids.

Uses in dentistry:

- Sterilization an disinfectant: They have a wide microbiocidal activity and are sporocidal and fungicidal. The most popular of this subgroup are 40% formaldehyde (used for surface disinfection) and 2% gluteraldehyde (exposure of at least 3 hours can be used to sterilize anasethetic equipments and other medical equipments such as thermometers and cystoscopes). (5)

3.c. Ethylene oxide:

Ethylene oxide is an alkylating agent. It causes alkylation of proteins, DNA, and RNA in microorganisms, which prevents normal cellular metabolism and replication and thus renders affected microbes nonviable.

Uses in dentistry:

- Sterilization: It is a highly effective agent capable of killing spores rapidly. It can be used for sterilization of complex instruments, delicate materials, dental equipments, heat labile materials as rubber and plastics.

Limitations:

Ethylene oxide should be used with high care as it is highly flammable, highly toxic, irritating to eyes and skin, mutagenic and carcinogenic.(5)

4. Positively charged molecules:

Bacterial cells have a negative surface charge cell membrane phosphate that attracts the positively charge molecules. Attachment to these molecules disturb the electric balance, disrupt the membrane functions and the bacterial cell bursts under osmotic pressure. (6)

4.a. Quaternary Ammonium compounds (QACs):

The positive charge of QACs is due to the presence of N+ (nitrogen) in quaternary ammines.

Uses in dentistry:

- Caries prevention: Incorporation of Benzalkonium chloride (BAC) in etchants and dental adhesives owing to its antibacterial activity. BAC is a positively-charged quaternary ammonium compound (QAC). The antibacterial activity of BAC results from its amphiphilicity as it bears both hydrophobic (long alkyl carbon chain) and hydrophilic (cationic ammonium group).

BAC's hydrophilic cationic region destabilizes the pathogen's surface by interacting with negatively charged components, which is followed by penetration of the hydrophobic long alky group into the bacteria leading to cell leakage and lysis.

BAC is stable in acidic media and has been added into commercial phosphoric acid etchants to a final concentration of 1%. Examples of such products include ETCH-37a or UNI-ETCHa. (1)

Sabatini et al., added BAC into All-Bond Universal, a universal dental adhesive with final BAC concentrations of 0.5%, 1%, and 2% (wt/ wt). These BAC-containing adhesives delivered higher bond strength than did the control after 1-year storage in artificial saliva, probably because of their ability to inhibit MMPs. (7)

Methacryloylox-dodecyl-pyridinium (MDPB) is a derivative og QACs and has been added to composite resin restorations owing to its antibacterial effect. MDPB is composed of a compound of dodecyl-pyridinium which is an antibacterial agent, and a methacryloyl group which is able to copolymerize with other dental monomers. MDPB-containing composites demonstrated significant antibacterial effects even after 90 days of immersion in water. Also, MDPB was included in self-etching adhesive systems include (MDPB) present in commercially available Clearfil Protect Bond®. (6)

- Plaque and gingivitis control: In the 1970s, quaternary ammonium salts were first administered to inhibit oral plaque by being incorporated into mouth rinses.

Limitations:

However, administration of Quaternary ammonium salts may have many side effects as gastrointestinal symptoms, coma, convulsions and hypotension.(3)

4.b. Triclosan

Triclosan is a synthetic phenol derivative used as a topical antimicrobial agent. It has a broad spectrum of action including both gram positive and gram negative bacteria.

Uses in dentistry:

- Plaque and gingivitis control: Triclosan is included in tooth paste and mouth washes to reduce plaque formation. In addition to its antimicrobial action, triclosan possess an anti-inflammatory action by inhibition of formation of prostaglandins and leukotrienes.(8)

4.c. Essential oil mouth washes:

Uses in dentistry:

- Plaque and gingivitis control: Over-the-counter mouth rinses consisting of thymol, menthol, and eucalyptol with methyl salicylate can exert an antimicrobial activity and antioxidant activity.

Limitations:

The main concern related to the use of essential oils mouth rinses is their alcoholic content that causes dry mouth and their burning sensation.(8)

5. Reactive oxygen species (ROS) induced oxidative stress:

Reactive oxygen species (ROS) induce oxidative stress. The four ROS types are the superoxide radical (O2−), the hydroxyl radical (-OH), hydrogen peroxide (H2O2), and singlet oxygen (O2). Under normal circumstances, the production and clearance of ROS in bacterial cells are balanced. However, with excessive production of ROS, this unbalanced state produces oxidative stress, which damages the individual components of bacterial protein and DNA. Also, Oxidative stress is a key contributor to changing the permeability of the cell membrane which can result in bacterial cell membrane damage and subsequently bacterial cell apoptosis.(9)

5.a. Hydrogen peroxide:

Uses in dentisrty:

- Root canal disinfection: H2O2 is a commonly used for canal disinfectant at a concentration of 3–6% as it is the strongest oxidizer.

In addition, the elevated oxygen concentration is un-favorable for the growth of strict anaerobes in root canals. Moreover, H2O2 generates effervescence which provides physical clearance of microbial deposits.

- Sterilization and disinfection: Hydrogen peroxide is used in hospitals to disinfect surfaces and it is used in solution alone or in combination with other chemicals as a high level disinfectant. It is used at 6% concentration to decontaminate the instruments, equipments such as ventilators. 3% Hydrogen Peroxide Solution is used for skin disinfection.

- Plaque and gingivitis control: 1.5-2 % Hydrogen peroxide is used as mouthwashes. It is often preferred because it causes far fewer allergic reactions than alternative disinfectants.(10)

Limitations:

The limitation of its use is that the produced hydroxides are inherently caustic.(11)

6. Interfere with bacterial enzymes and enzymatic activity:

6.a. Iodine potassium iodine:

Iodine potassium iodine has a wide-spectrum antimicrobial activity. It is The iodine is the oxidizing agent of this substance, it reacts with free sulfhydryl groups of bacterial enzymes cleaving the disulfide bonds.

Uses in dentistry:

- Endodontic irrigant: It is a traditional root canal disinfectant used in concentrations ranging from 2% to 5%.

Limitations:

A mojor disadvantage of iodine is a possible allergic reaction in some patients.(11)

7. Increasing tooth mineralization:

7.a. b.1. Fluoride (inc mineralization)

The popular mechanism in the antimicrobial action of fluoride is that fluoride ions contact the mineral of the tooth surface and increase remineralization to prevent the acid-induced demineralization caused by cariogenic bacteria. Another proposed mechanism is its ability to inhibit enolase enzyme. Inhibition of enolase results in growth inhibition and reduced acid production of oral streptococci, such as S. mutans.

Uses in dentistry:

- Caries prevention: Fluoride is the simplest anion of fluorine but is one of the most successful cavity prevention agents especially for preventing dental caries. Fluoride is typically supplemented in small quantities to drinking water or products such as mouthwashes, toothpastes, and oral supplement.

Examples for restorations containing fluoride for caries prevention, Glass ionomer cements' (GIC) antibacterial activity is due to its fluoride release. In addition, there is a claim that the low pH of GICs during setting may contribute more than the fluoride leached to their antibacterial properties.

Similarly, resin modified glass ionomer cements (RMGIC) have also been shown to exhibit antimicrobial activity. This antimicrobial activity is due to the release of fluoride and the low initial pH. Also, the release of strontium ions may also produce a synergistic antibacterial effect with fluoride.

Also, fluoride was incorporated in composite resin resulting in fluoride-releasing dental composites. Despite some success in the development of dental composites with sustained fluoride release, the levels achieved are generally very much lower compared to those gained with GICs and compomers.(12)

Limitations:

However, there is a concern regarding dental and skeletal fluorosis, as well as the development of fluoride resistant oral bacteria.(6)

8. Containing natural constituents:

The search for more biocompatible and dentin friendly agents that can overcome the limitations of the chemical antimicrobial agents is the current trend. Herbal products are gaining popularity in every field of medicine, mainly due to their biocompatibility and not likely to cause the severe injuries to patients that might occur. The herbal extracts also possess high medicinal properties such as anti-oxidant, antimicrobial, and anti-inflammatory properties due to their saponins, flavonoids, iso-flavonoids, chalcones, coumarins, aurones, benzofurans, phenols, pterocarpans, and stilbenes content which contribute to its pharmacological properties.(13)

8.a. Tea tree oil:

Tea tree oil's is a native Australian plant with many properties such as being an antiseptic, antibacterial and an antifungal agent.

Uses in dentistry:

Cavity disinfection: The use of tea tree oil and many natural products have shown anti-microbial properties which can be used as cavity disinfectant in the field of dentistry. A study compared the efficacy of herbal antibacterial agents (Tea Tree Oil (TTO)) with commercially available 2% chlorhexidine (CHX) as cavity disinfectant for use in minimally invasive dentistry. Post-disinfection, 2% chlorhexidine showed highest reduction in bacterial count followed by 1% tea tree oil. Therefore, natural antibacterial agents like tea tree oil could be effectively used as cavity disinfectants which will help in minimizing secondary caries and rendering a long-term restorative success.(10)

8.b. Liquorice:

It has an anti-inflammatory, antibacterial, antifungal, antiviral and anticarcinogenic.

Uses in dentistry:

- Caries prevention: Recently, liquorice has been studied extensively for its anticaries properties. He et al.,(14) concluded that liquorice had the highest antimicrobial activity against S. mutans bacteria and therefore has an anti-cariogenic effect.

- Based on this observations, Hu et al.,(15) developed a sugar-free orange flavoured liquorice lollipop for caries prevention. They found that liquorice lollipops are safe and effective against S. mutans when consumed for 10 days (twice daily) by pre-school children.

Limitations:

But, due to the anticoagulant and antiplatelet effects of liquorice there is a potential risk of increased bleeding for patients taking conventional anti-clotting medications for cardiovascular or cerebrovascular diseases.(16)

8.c. Propolis:

Propolis is a resinous hive product collected by bees. Different components of propolis contribute to its antimicrobial acions. Caffeic acid phenethyl ester (CAPE) have anticarcinogenic, antimitogenic and immunomodulatory properties.

Uses in dentistry:

- Caries prevention: Propolis is superior in reducing the salivary levels of S. mutans and Lactobacilli spp. compared to that of chlorhexidine (CHX) mouthwash. The residual beneficial effect of propolis could be observed for a further 45 days. Propolis containing chewing gum also reduced bacterial counts compared to xylitol chewing gum.(17)

- Plaque and gingivitis control: Propolis-containing mouth rinse reduces supragingival plaque and insoluble polysaccharides. Moreover, propolis in toothpaste improves oral health and exhibits inhibitory effect on dental plaque formation. Topical application of propolis improves periodontal health due to reduced P. gingivalis in the gingival crevicular fluid (GCF), probing pocket depth (PPD), and clinical attachment level (CAL) in periodontitis patients.

- Endodontic irrigant.

8.d. Siwak powder:

Uses in dentistry:

- Siwak powder containing denture base material: The effect of adding siwak powder with average particle size of (75 μm) in different concentrations by weight to PMMA on certain mechanical properties was evaluated. The results showed that addition of low concentrations (3%, 5%) Siwak to the heat polymerizing acrylic resin imparted antimicrobial properties to the acrylic resin denture base material and did not affect significantly the tensile, compressive, impact strength or surface roughness. While the addition of (7 %) Siwak powder revealed a significant decrease in tensile, impact and compressive strength.(18)

9. Combined mechanisms:

Some antibacterial agnents relie on the concept of having a combination of more than one mechanism of antibacterial actions together for additional therapeutic effect.

9.a. Nanoparticles incorporation in dental materials:

Nanoparticles (NPs) with their enhanced and unique physicochemical properties, such as ultrasmall sizes, large surface area/mass ratio, and increased chemical reactivity, have led research toward new prospects of treating and preventing dental infections.(19)

Mechanism of action of nanoparticles:

i. Interacting with peptidoglycan cell membrane content of bacteria causing increased permeability and cell lysis.

ii. Interacting with bacterial proteins and disrupting protein synthesis.

iii. Interacting with bacterial DNA causing DNA & RNA damage and preventing DNA replication.

iv. Increase the production of reactive oxygen species (ROS) resulting in increased respiratory stresses.(20)

Uses in dentistry (12, 21):

- **Quaternary ammonium polyethylene-imine (QPEI):**

- Nano-particles containing dental composite: QPEI mechanism of action is a combined mechanism. Being a nano-particle sized and containing positively charged N+ (nitrogen) in quaternary ammonium compounds that attack the negative surface charge in bacteria. Antibacterial activity of quaternary ammonium polyethylene-imine (QPEI) nanoparticles embedded in composite resins and restorative materials has been proven with effects lasting for a month. Moreover, the QPEI nanoparticles did not affect the material's mechanical properties and no leaching of the nanoparticles was detected.

- **Chitosan nanoparticles:**

- Nano-particles containing dental composite: Similarly, chitosan has a positive surface charge of chitosan. The nontoxic and antibacterial effects of chitosan have rendered this material to have multiple uses in dentistal restorations. Chitosan has been analyzed in resin-based composites and results showed that it promoted the antibacterial effects without deteriorating the mechanical properties such as surface hardness.

- Nano-particles containing intracanal medications: Incorporating chitosan NPs into a Ca(OH)2 based paste and zinc-oxide eugenol based sealer had the potential of increasing its antibacterial effect against E. faecalis . The chitosan NPs showed superior penetration into the dentinal tubules and proven antibacterial efficacy.(19)

- **Zinc oxide nanoparticles:**

- Nano-particles containing dental composite: ZnO is able to react with the moisture available in the oral cavity and creates active oxygen species such as H2O2, causing bacterial inhibition in addition to its nanosized properties. The addition of zinc oxide nanoparticles also resulted in antibacterial activity against S. mutans without compromising mechanical properties, on the contrary its addition increased compressive and flexural strength.

- Nano-particles containing intracanal medications: The bactericidal effect of zinc oxide nanoparticles was shown to be related to size and concentration. The smaller the size, the higher the antibacterial effect and the production of reactive oxygen species such as hydrogen peroxide when in contact with an aqueous medium. Also, the higher the concentration, the maximum antibacterial effect is obtained. Varying degrees of antibacterial effects against P. aeruginosa, E. faecalis, C. albicans and S.aureus were shown when zinc oxide nanoparticles were incorporated into polyethylene glycol to form a creamy mix and used as an intra-canal medicament.(22)

- **Silver nanoparticles:**

- Nano-particles containing dental composite: Silver has been used as a broad-spectrum antibacterial agent for centuries and is still one of the most frequently used antibacterial fillers for dental materials.

Silver interacts with thiol group compounds found in the bacterial cell wall, resulting in the inhibition of the respiration process. Also, Silver interferes with bacterial enzymatic activity. Silver nanoparticles have been incorporated in the formulations of dental composites and adhesives and were reported to inhibit S. mutans. Using particles on a nanoscale reduces the amount of silver compounds needed to achieve antibacterial properties, which eliminates the discoloration caused by large amounts of these compounds, and better aesthetics are acquired.

- Nano-particles containing intracanal medications: Nanonsilver root canal irrigation solution has showed high efficiency against E. faecalis. Also, incorporation of silver nanoparticles in Calcium silicate cement such as MTA showed a significant increase in their antimicrobial effects.(19)

- **Titanium dioxide nanoparticles:**

- Nano-particles containing dental composite: Titanium dioxide nanoparticles have been added to composite resin and glass ionomer cements and resulted in improved mechanical and antibacterial properties. But, even small amounts of titanium oxide (0.1–0.25 (wt.%)) can lower aesthetic properties such as translucency and discoloration of resin-based composites, which could be one of the reasons they are not added to these materials.

9.b. Photodynamic therapy (PDT):

PDT is a new antimicrobial strategy that has a combined antibacterial mode of action. The first mechanism involves the combination of a nontoxic photosensitizer (PS) and a light source. The excited PS reacts with molecular oxygen to produce highly reactive oxygen species, which induces injury and death of bacteria. The second mechanism relies on the positive charge of PDT can rapidly bind and penetrate the bacterial cells and therefore, shows a high degree of selectivity for killing microorganisms.

Uses in dentistry:

- Recent advance in root canal disinfection: When conventional endodontic therapy was followed by PDT, there was significantly more bacterial killing and less bacterial growth than endodontic therapy alone.(11)

9.c. Probiotics:

Currently, the known mechanisms of probiotic activity can include the following:

i. Competing for binding sites on the tooth surface;

ii. Competing for nutrients;

iii. Producing antimicrobial compounds to inhibit other oral bacteria, such as hydrogen peroxide.(3)

Uses in dentistry:

Probiotics are available in medicinal preparations as mouth rinses, tablets, capsules, powder, and lozenges or in the form of functional foods, in the form of culture concentrations added to beverages, dairy products such as yogurt.

The dietary probiotics are intolerant to low pH as it decreases their survival. Therefore, the commercial probiotics are needed. The commercially available probiotics are in the form of tablets or capsules which are used as a drug carrier.(23)

9.d. MTAD (Mixture of Tetracyclin Acid Detergent):

Uses in dentistry:

MTAD is an antibacterial commercially available root canal disinfectant. MTAD is a mixture of 3% tetracycline isomer (doxycycline), and 4.25% acid (citric acid), and 0.5% detergent.

One of the characteristic of this solution is a high binding affinity of the doxycycline to dentin which results in sustained antimicrobial activity. Moreover, it was reported to remove the smear layer due to its citric acid content.(11)

9.e. Sodium hypochlorite (NAOCL):

NaOCl possesses a broad spectrum antimicrobial activity against microorganisms and biofilms difficult to eradicate from root canals such as Enterococcus faecalis , Actinomyces and Candida species. Sodium hypochlorite mediate its antimicrobial action by reacting with fatty acids and amino acids. It acts as an organic and fat solvent, degrading fatty acids. Also, it acts by denaturing proteins in the biofilm matrix and inhibiting major enzymatic functions in bacterial cells. The antimicrobial effectiveness of sodium hypochlorite is also based on its high pH (pH>11). Such high pH results in an irreversible enzymatic inhibition and an alteration in cytoplasmic membrane integrity. (4, 24)

Uses in dentistry:

- Endodontic irrigant: Sodium hypochlorite (NaOCl) has been the most widely used root canal irrigant. This is due to its excellent antimicrobial activity and its ability to dissolve pulpal remnants and organic components of dentin. The concentration of NaOCl that is considered both safe and effective is still controversial. Concentrations of NaOCl ranging from 0.5% to 5.25% have been evaluated. Studies reported that higher concentrations of NaOCl resulted in a more rapid and greater bactericidal effect, but unfortunately, more cytotoxic effects were evident.(4, 24)

9.f. Calcium hydroxide:

Antimicrobial activity of calcium hydroxide is related to the release of hydroxyl ions (OH-) in an aqueous environment causing damage to the bacterial cytoplasmic membrane, protein denaturation and damage to their DNA content. The high pH of calcium hydroxide alters the biologic properties of bacterial lipopolysacharide present in the cell walls of gram-negative species.

Uses in dentistry:

- Pulp capping material: Calcium hydroxide is a white odorless powder that was originally introduced to the field of endodontics as a direct pulp-capping agent. It is the most commonly used inter-appointment dressing. (11)

Recent advances and future perspectives in dental restorations with antibacterial properties

Smart dental materials for antibacterial applications (Bioresponsive antimicrobial restorations)

The last 20 years have witnessed a transformative evolution of antimicrobial dental materials. The field is switching from offering "passive" treatments to "smart" antimicrobial biomaterials that are triggered by different internal and external stimuli to deliver "on-demand" therapies with improved control of dosage, location, duration, and efficacy. Smart biomaterials can sense and react to physiological or external environmental stimuli (e.g., mechanical, chemical, electrical, or magnetic signals).

The last decades have seen exponential growth in the use and development of smart dental biomaterials for antimicrobial applications in dentistry. Recent advances in technology and manufacturing tools are enabling the development of "smart" dental materials that offer multiple functionalities for different therapies.(25)

Classification of smart biomaterials according to its smartness:

Four levels of smart biomaterials were defined, including

- **Bioinert:** Bioinert biomaterials cause minimal interaction with surrounding tissues and have minimal harm or toxicity to the surrounding tissues after implantation. For example, polymethyl methacrylate (PMMA).

- **Bioactive**: Active or bioactive materials induce a specific biological response at the material-tissue interface after implantation or contact with tissues, cells, or body fluids. These biomaterials release the therapy "uncontrollably" after being installed in the body. For example,"fluoride-releasing compounds".

- **Bio responsive**: Responsive, bioresponsive, or stimuli-responsive biomaterials can "sense" a specific stimulus (e.g., light, temperature, pH changes, enzymes) and then "respond" by releasing a pre-programmed therapy. For example, a dental composite is fabricated with pH-sensitive NPs that deliver antimicrobial agents under certain pH levels (acidic) to treat caries.

- **Autonomous:** autonomous biomaterials can sense, react and adapt to multiple stimuli. They adjust their response accordingly to offer an appropriate response for each need at different time points. This class of biomaterials is the smartest.(25)

Bio-responsive (smart) antimicrobial therapies

Generally, the antimicrobial agent is incorporated into a carrier/ vehicle (a biomaterial), designed to respond to the specific stimulus by changing its properties (e.g., degradation). To release the antimicrobial agent, some carriers may vary their structure or properties after responding to the stimulus.

a. pH-responsive biomaterials:

They respond to the changes in the pH level of the surrounding medium or microenvironment. Depending on their design, biomaterials may expand, collapse, or change a specific property. For example, in an acidic environment, some hydrogels may expand (structural change) to release the drugs, while basic pH levels force the hydrogel's collapse and the drugs remain protected and unreleased.For example, the pH range of the microenvironment of active dental caries is 4.5–5.5, while in physiological conditions, saliva has a normal pH range of 6.2–7.6. As a result, pH-responsive biomaterials have become an attractive choice to be used in the treatment of caries, periodontitis, and peri-implantitis.

A recent work designed a N-dimethylaminoethylmethacrylate (DMAEMA)-co-2-hydroxyethyl methacrylate (HEMA) (poly(DMAEMA-co-HEMA) hydrogel capable of releasing CHX in response to pH levels to prevent and treat dental caries. This bioresponsive biomaterial inhibited the development of S. mutans biofilm and regulated the oral microecosystem.(25)

b. Enzyme responsive biomaterials:

Salivary and bacterial enzymes act as trigger (or signal) to release antimicrobial agents (as antibiotics or NPs) for treatments. Bacteria and fungi secrete various enzymes, including lipase, esterase, phosphatase, urease, gelatinase, and many more. Some of these enzymes have been established as the marker to indicate active stage of disease. For example, a bacterial by-product in chronic periodontitis is the enzyme matrix metalloproteinase-8 (MMP-8).

This enzyme has been used as a stimulus in bioresponsive delivery systems for managing periodontitis. Activation using MMP-8 has been used in a hydrogel made of gelatin methacrylate (GelMA) loaded with CHX and aluminosilicate nanotubes. The presence of MMP-8 degrades the hydrogel in 20 days and provides a sustained release of CHX for dental infection treatment.(25)

c. Electrically responsive biomaterials:

The effect of electrical currents on microbial biofilms has been studied for several years as an alternative to chemical therapy without leading to antibiotic resistance. The capacity of electrical charges to destroy pathogens depends on the the electrical charge magnitude, density, and polarity. Several mechanisms are proposed to explain the killing ability of electrical charges. These mechanisms include the direct contact theory, in which the electric current directly results in bacterial death by disrupting the integrity of the cell membrane.

For example, The concept of using electrical current as an antibacterial mechanism has been mostly tested in metallic implants. Electrically polarized materials possess electrical charges at the surface due to polar or electric properties. The use of polarized substrates such as HAp has antibacterial activity against gram-positive and gram-negative bacterial strains.(25)

d. Magnetic responsive biomaterials:

In antimicrobial applications, magnetic NPs have been used mainly for positioning or moving the antibacterial agent closer to the infection site. This is highly attractive in dentistry since infected sites are usually deep within tissues and inaccessible to treatment. For example, a urethane dimethacrylate (UDMA)-HEMA system filled with CHX loaded with magnetite Fe_3O_4 NPs showed a significant antimicrobial effect against P. gingivalis for periodontal disease treatment. Under a magnetic field, the CHX/Fe_3O_4 compounds not only can move to the site of infection, but also the movement releases the CHX to the targeted place.(25)

Antibacterial coatings

Titanium and titanium alloys are widely used in orthopedic and dental implants, but infection associated with these implants still poses serious threat leading to possible complications. The implant surface is susceptible to infection because of two main reasons: formation of a surface biofilm and compromised immune ability at the implant/tissue interface. A surface protein layer is formed under physiological conditions on the implant surface. This protein layer actually makes the surface suitable for bacterial colonization and biofilm formation.

Biofilms are defined as a microbially derived sessile community characterized by cells irreversibly attached to a substratum. The biofilm protects adherent bacteria from the host defense system and bactericidal agents. However, the low sensitivity of bacteria to antibiotics induced by the biofilm growth, together with the increasing number of resistant strains, makes the use of antibiotics currently less effective than it has ever been. To prevent such infections, one approach is to improve the antibacterial ability of the materials. Therefore, as an alternative to the use of antibiotics to prevent bacterial infection or to treat established biofilms, surface coatings that prevent viable bacteria from adhering has been developed.(26)

Many different chemical strategies and technologies for antibacterial coatings are described in the literature. For example, antibacterial coatings may contain active eluting agents (e.g. ions or nanoparticles of silver, copper, zinc, or antibiotics, chloride, iodine, etc.), immobilized molecules that become active upon contact (e.g. quaternary ammonium polymers or peptides). In addition to surface and chemical modifications.(26)

Strategies to achieve antimicrobial coatings can be classified as:

1. Safe-by-design strategy (passive).

2. Toxic by design strategy (active).

1. Safe-by-design strategy (passive):

The topography of a surface can by itself significantly affect its hygienic status in a beneficial manner (reducing microbial retention). This is known as the "Anti-adhesive strategy". Anti-adhesive surfaces "Repellant surfaces" or "Antifouling surfaces" can reduce the adhesion force between bacteria and a solid surface to enable the easy removal of bacteria before a biofilm layer is formed on the surface (but they will not reduce the number of germs by killing them).

Attachment of bacteria starts with an initial adsorption of proteins onto the material surface. Hence, elimination of bacterial attachment is achieved by prevention of protein adsorption through; superhydrophobicity and nanostructures or repellant action induced by electrostatic repulsion. (26, 27) Therefore, anti-adhesive/passive strategy is based on the prevention of bacterial adhesion on the implant surface and can be classified into:

1.a. Superhydrophobicity and nanostructures.

1.b. Electrostatic repulsion.

1.a. Superhydrophobicity and nanostructures:

Superhydrophobic surfaces are characterized by a water contact angle of over 150° and they are inspired by natural mimimcingof the Lotus leaf in nature and animals skin architecture as sharks. It was further revealed that the Lotus leaf has a hierarchical micro/nanostructure surface. In general, topographies at the micro-scale do not have bactericidal effects but may limit bacterial adhesion. While in contrast, nano-topographic features cause high deformational stresses on the bacterial membrane leading to their rupture.

Also, effective air entrapment in the three-dimensional nanomorphology in nanostructures renders them superhydrophobic and slippery enables bacteria to "roll off" the surface. Immobilization of superhydrophobic molecules that can resist protein adsorption such as; Poly Ethylene Glycol (PEG), have demonstrated great anti-adhesion properties in vitro and considered as the standard approach for antiadhesive coatings. (25, 26, 27)

1.b. Electrostatic repulsion:

Electrostatic repulsion is a mechanism used in some implant coatings to prevent bacterial adhesion. The electrostatic repulsion mechanism is based on the principle that like charges repel each other. When the surface charge of the implant coating is negative, it repels negatively charged bacteria and prevents them from attaching to the surface. This mechanism can be effective in preventing bacterial colonization and biofilm formation, which are key steps in the development of implant-associated infections.

Coating titanium surfaces with a negatively charged polymer and then exposing them to Staphylococcus aureus, a common bacterial pathogen. The negatively charged surface repelled the bacteria and prevented them from adhering to the surface. (28)

2. Toxic by design strategy (active):

This strategy aims to kill bacteria that exist on the material surface through the release of a toxic substance. It is known as "Bactericidal/Biocide release strategy" and can be classified into:

2.a. Release of antibacterial substances from the materials.

2.b. Contact killing.

2.a. Release of antibacterial substances from the materials:

These coatings typically incorporate antibacterial agents such as antibiotics, antimicrobial peptides, or metal ions that are released from the coating and diffuse into the surrounding tissue to kill bacteria. The bactericidal mechanism of these coatings is based on the ability of the antibacterial agent to disrupt bacterial cell membranes or interfere with essential cellular processes, leading to bacterial death. When incorporated into implant coatings, these agents can prevent bacterial colonization and biofilm formation, reducing the risk of implant-associated infections.

However, one of the challenges of using bactericidal coatings is the potential for the development of bacterial resistance to the antibacterial agent over time. This can limit the long-term effectiveness of the coating and increase the risk of infections. Additionally, there are concerns about the safety and biocompatibility of some antibacterial agents, particularly those that are highly toxic or have off-target effects. Also, they will gradually become inactive (26, 27)

2.a.1. Hydroxyapatite (HA) and Calcium Phosphate (CP) coatings:

Dental implant coatings incorporating hydroxyapatite (HA) and calcium phosphate (CaP) have been studied for their antibacterial properties due to their biocompatibility and osteoconductive properties. The antibacterial activity of these coatings is attributed to their ability to release ions, such as calcium and phosphate, which can interfere with bacterial metabolism and growth.

It was concluded that, amorphous CP and HA nanoparticles displayed antibacterial effect against a number of bacterial species including; S. aureus, S. epidermis, E. coli and P. aeruginosa. Also they have similar levels of activity against Gram-negative bacteria, however, ACP was more effective against the Gram-positive ones. (29, 30)

2.a.2. Chlorhexidine based coating:

Chlorhexidine-based coatings have been studied for their antibacterial properties in dental implant applications. Several studies investigated the use of a chlorhexidine coating on titanium implants. The chlorhexidine coating was found to significantly reduce bacterial adhesion and biofilm formation on the implant surface, while also promoting osteoblast activity and bone formation. (31)

2.a.3. Carbon nanotubes, Graphene oxide or Diamond-like carbons coatings:

Surface coating with carbon nanotubes (CNTs), graphene oxide (GO) or diamond-like carbons (DLCs) showed interesting antibacterial activity since these materials show relatively low cytotoxicity towards mammalian cells. It seems that GO based materials show higher antibacterial activity. The most commonly mechanisms of action are: oxidative stress induction, protein dysfunction, transcription arrest and membrane damage.(27)

2.a.4. Metal nanoparticles based coatings:

Metallic coatings based on metal nanoparticles as silver, copper and golsd has been successful because of their extensive antimicrobial activity. The main disadvantage of metallic nanoparticles coatings are cytotoxicity and resultant decreased biocompatibility.(32)

Examples:

- Silver nanoparticles (AgNPs) coating: A study compared the bactericidal effects of Ag NPs-coated polyethylene terephthalate (PET) mesh against S. aureus and E. coli, with an uncoated one. It was found that Ag NPs-coated PET mesh displayed excellent antibacterial property against Staph. aureus and Escherichia coli with 99% bacterial reduction rate compared to the untreated PET mesh.(33)

- Copper nanoparticles (CuNPs) coating: Possess a lower antibacterial efficacy than that of AgNPs and hence a higher doping of CuNPs is always desired to achieve the similar effect. (34)

- Gold nanoparticles (AuNPs) coating: Biocompatible, exhibited low toxicity to mammalian cells. Proved to be effective against both Gram-negative and positive pathogens and the development of resistance to these NPs was very low. Only AuNPs with size below 3nm are cytotoxic.(35)

2.b. Contact killing:

In contact-active surfaces, the biocidal group is attached to the surface through a polymer chain allowing the biocide to reach the cytoplasmic membrane of the bacteria and to perforate them. Bacteria secrete signaling molecules enabling cell to cell communication and regulation of several bacterial processes (quorum-sensing, QS). Consequently, molecules that target and disrupt QS have been considered antibacterial agents. QS-inhibiting molecules demonstrated excellent in vitro antibacterial properties. Moreover, they are less likely to induce the development of resistance. The most effective compounds for contact-killing coatings are quaternary ammonium compounds (QACs), antimicrobial enzymes (AMEs), chitosan and antimicrobial proteins (AMPs) derived from human salivary proteins.(36)

Recent advances and future perspectives in antibacterial implant coatings

Recent developments in antibacterial implant coatings have focused on improving their effectiveness, biocompatibility and longevity. They include:

1. Hybrid coatings/Combination coatings:

Sometimes two functional strategies are combined to achieve synergistic effects, e.g. by embedding antibacterial substances into antiadhesive surfaces. This is known as "Hybrid coating". Combining different types of antibacterial coatings can improve their overall effectiveness and reduce the risk of bacterial resistance. For example, nanostructured hydroxyapatite (nHA) coating was prepared on titanium (Ti) surface and then the antibacterial agent of chitosan was loaded on the HA surface. The results showed that the hybride nHA/chitosan coating against E. coli inhibited the bacterial growth, and improved biological and antibacterial properties.(37)

2. Smart antibacterial coatings:

Smart implant coatings are coatings that can respond to external stimuli, such as changes in temperature, pH, or chemical composition. These coatings are designed to provide a controlled release of antibacterial agents in response to specific conditions, allowing for targeted and efficient antibacterial activity.

- **pH-responsive coating** can release antibacterial agents in response to acidic conditions. This type of coating can be particularly useful in preventing bacterial infections associated with acidic conditions, such as those that occur in the mouth. For example, coating made from chitosan which has amino groups that can be protonated or deprotonated depending on the pH of the surrounding environment. At acidic pH, the amino groups on chitosan are protonated, giving the coating a positive charge. At neutral or basic pH, the amino groups are deprotonated and the coating becomes neutral or negatively charged. This pH-dependent charge switch can be used to control the release of drugs or other molecules from the coating.(38)

- **Temperature-responsive coating** can release antibacterial agents in response to changes in temperature. This type of coating can be used to prevent bacterial infections associated with inflammation and fever, which can increase the temperature of the implant site.

- **Enzyme-responsive coating** can release antibacterial agents in response to specific enzymes produced by bacteria as phospholipase A2 (PLA2). This type of coating can be used to target specific types of bacteria and prevent biofilm formation, which can be particularly difficult to treat with traditional antibiotics.(39)

- **Photosensitizer implant surface coatings** are a type of antibacterial coating. It is a polymeri containing photosensitizers, such as toluidine blue, immobilized within a polymer matrix. The phoosensitizer is a molecule that can absorb light energy and become excited to generate ROS, which can then damage bacterial cells and lead to bacterial death. These coatings have several advantages, including their ability to selectively target and kill bacteria without damaging host cells and their potential for reducing the development of antibiotic-resistant bacteria. However, there are also some challenges associated with these coatings. One major challenge is ensuring that the photosensitizer remains stable and effective over time, as it can degrade and lose its antibacterial activity if not properly stored or handled. Additionally, the use of light as an activation mechanism may limit the applicability of these coatings in certain clinical settings.(40)

N.B: Stimuli-responsive materials are considered controlled release coatings that release therapeutic agents in a controlled and sustained manner, which can enhance the effectiveness of these agents and reduce the risk of systemic side effects. Controlled release coatings can also minimize the risk of toxicity to surrounding tissues.

3. Zwitterionic coatings:

Zwitterionic dental implant coatings refer to a type of coating composed of molecules that contain both positive and negative charges, which allows them to form a stable layer on the surface of the implant. The term "zwitterionic" comes from the German word "zwitter," which means hermaphrodite or hybrid. In the case of zwitterionic coatings, the molecules have both positive and negative charges, which makes them highly resistant to protein adsorption and bacterial attachment. Zwitterionic coatings can also enhance the biocompatibility of dental implants by reducing inflammation and promoting tissue integration. They can also improve the mechanical properties of the implant surface by increasing its hardness and wear resistance.

The coatings work by creating a repulsive force that prevents bacterial adhesion to the implant surface, as well as promoting the adsorption of proteins that enhance tissue integration. One proposed mechanism is the hydration layer model, which suggests that zwitterionic coatings create a hydrated layer on the implant surface that repels proteins and bacteria. This layer consists of water molecules that are attracted to the positive and negative charges on the zwitterionic molecules, creating a barrier that prevents protein and bacterial adhesion. One example of a zwitterionic coating for dental implants is poly(sulfobetaine methacrylate) (PSBMA). (41)

Challenges of antibacterial coatings (25):

Despite these recent advances, there are still several challenges that need to be addressed in the development of antibacterial implant coatings before they can be widely used in clinical practice. More research is needed to understand their long-term effectiveness and safety, as well as to develop coatings that are effective against multiple bacterial mechanisms and have good biocompatibility.

1. Long-term effectiveness: Typically, the antimicrobial effect of a leachable agent is less than 1 year. Once depleted, the agent could not be recharged. Uncontrolled release of antimicrobial agents challenges the delivery of an appropriate dose. This uncontrolled release can speed up the agent depletion or provide insufficient amount of agent for therapy. This has been avoided to some degree by using nano-carriers and stimuli responsive smart coatings.

2. Bacterial resistance: Antibacterial coatings can also lead to the development of bacterial resistance, which can reduce their effectiveness over time. The lack of bacterial targeting can cause collateral damage (killing of commensal species) and a potential imbalance in the oral microbiota since the therapy will “attack” everything it encounters in the microenvironment. To address this challenge, coatings that target multiple bacterial mechanisms and combinations of coatings should be investigated.

3. Biocompatibility: Some antibacterial coatings (bactericidal release strategy) that leach and release antibacterial agents can be toxic to surrounding tissues, leading to inflammation and other complications. Therefore, Biocompatibility of coatings should be carefully studied to avoid adverse reactions.

Although several clinical trials have successfully evaluated the use of NPs in different dental materials as antimicrobial agents , the wide use of NPs in clinical practice is limited due to concerns regarding the release of toxic ions that could cause inflammation, immunotoxicity, cytotoxicity, and genotoxicity in healthy cells.

4. In the case of safe by design strategy: Antimicrobial polymers have significant limitations regarding the high selectivity against gram-positive strains, the lack of standardized methods for testing the performance of the antibacterial coatings under representative environmental conditions and high cost of manufacturing.

(Although they overcome some drawbacks of the leachable chemical therapies such as long-term activity, no leaching or exhaustion of the antimicrobial compound, limited toxicity against mammalian cells, reduced antimicrobial resistance, and increased chemical stability)

5. Regulatory approval: To be used in clinical settings, antibacterial implant coatings must meet regulatory approval. It can be a long and expensive process, which can delay the development and implementation of new coatings.

Finally, many antibacterial coatings have been shown to be effective in vitro or in animal models, but their long-term effectiveness in humans is still unclear. More research is needed to understand the long-term outcomes of these coatings in clinical settings.

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