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1. Introduction

The oral cavity harbors a great diversity of microbial species that have a strong tendency to colonize dental surfaces, tongue and oral mucosa [1,2]. These accumulations of oral bacteria on dental surfaces are natural forms of biofilm growth in humans. They are also known as dental plaque and in spite of several favorable conditions (e.g. temperature, humidity) these biofilms are constantly challenged by host factors. It is recognized that structural organization of a dental biofilm are influenced by the interplay of many unfavorable and also several favorable ones such as the chemical nature of the substrate and the type of the surface where the biofilm develops [3].

In dentistry, restoration failure is generally attributed to a combination of oral bacteria and inappropriate features of dental materials. Efficient dental restorative materials are important for an adequate recovery of masticatory and esthetic functions. However, these materials are prone to biofilm formation, affecting oral health. It is well accepted that under in vivo conditions, rough surfaces attract more biofilm than smooth ones, but the variables that influence bacterial adhesion to dental materials are still a matter of debate.

Dental caries is the most prevalent disease found in the oral cavity of humans. It is regarded as multifactorial chronic and complex disease which is dependent of a cariogenic biofilm [4,5]. Thus, a carious lesion takes some time to develop. However, initial carious lesions are easily and rapidly formed during a three-day of high sucrose regime and poor oral hygiene conditions. So, as long as there is a cariogenic microbial biofilm attached to a dental surface there is a great chance to find a carious lesion on this tooth spot [6]. Growth of oral
bacteria on dental surfaces requires adhesion strategies because there is a constant flow of host secretions (e.g. saliva) that can interfere on the ability of planktonic cells (non-attached bacteria). As a result, the formation of the oral biofilm is not homogenous and it contains multiple bacterial species [4,7,8].

Oral bacteria can adhere to hydrophobic as well as to hydrophilic surfaces and many explanatory theories are suggested including the influence of complex electrostatic mechanisms such as van der Waals energy. After biofilm establishment on restorations, surface deterioration of materials (e.g.: resin composites and glass-ionomer cements) will take place facilitating the development of a mature biofilm resulting in dental carious lesions. The microflora from these diseased teeth sites is significantly different from healthy sites on a tooth [10]. The frequent changes in environmental conditions can lead to shifts in biofilm microflora and as a result the microbial homeostasis breaks down in dental plaque (e.g. low pH), and disease occurs.

It must be pointed out that the presence of these oral microbes in the mouth is natural, and is also essential for the normal development of the physiology of the oral cavity [9]. Hence, any antimicrobial strategy has to consider the perspective of restoring some microbial equilibrium and not a complete depletion of oral bacterial from the mouth. Many antimicrobial substances, compounds or mixture of antibacterial agents (e.g. bisbiguanides, metal ions, quaternary ammonium compounds, essential oils) have been successfully formulated into home care products to control oral biofilms. Several investigations have proved their efficacy in controlling the development of oral biofilms despite important drawbacks as tooth staining, bad taste, etc. [3,4]. Moreover, at moderate or high concentrations, these antimicrobial mouthwashes and toothpastes can inhibit bacterial growth in many different modes and truly affect biofilm-forming capacity of some pathogenic traits. Hence, to be considered a successful antimicrobial agent a substance, compound or the mixture of both must be able of maintaining the oral biofilm at “normal” cariogenic bacterial levels which are compatible with the individual oral health. Simultaneously, the material must be effective without any interference on the beneficial properties of the resident oral microflora.

Mouthwashes and toothpastes are accepted methods to deliver antimicrobials into the oral mouth. However, they are completely dependent on the discipline and compliance of the patient to the oral treatment. In addition, many of these antimicrobials are prescribed for short periods to avoid any risk of disturbing the resident oral microflora [3,10]. Hence, one strategy is to incorporate antimicrobials into dental materials. The possibility that dental restorative material may release antimicrobial compounds are regarded as an interesting strategy for overcoming the development of cariogenic dental biofilms and the risk for secondary dental caries. In addition, there is a chance that under less biofilm stress dental materials could increase longevity. This strategy is of great importance since dental restorations properties may be improved if an antibiotic-dental material is used.

The aim of the present review is to shed light on the techniques and effectiveness on improving antibacterial activities of dental restorative materials. The main focus is on incorporation and subsequent slow-release of antimicrobial chemical species, molecules, compounds and low molecular weight antibacterial agents such as metal ions, iodine, antibiotics, chlorhexidine and natural products such as essential oils. The in vitro and in vivo techniques used in microbiology
are also explored taking into account that main bacteria involved are Gram-positive cocci shaped bacteria such as *Streptococcus sobrinus*, *Streptococcus mutans* and *Lactobacillus* sp.

2. *In vitro* and *in vivo* techniques for studying biofilms

In 1940’s microbiologists described an interesting phenomenon that occurs when fresh sea water is kept in a glass bottle, the so-called “bottle effect”. It was observed that the number of microorganisms attached a glass surface increase while at the same time there is a reduction in free-living microorganisms [11]. This is a relevant historical landmark because it represents the starting point of a paradigm shift that is still valid these days. In fact, only 30 years later, scientific community understood that the biofilm mode of life is the rule rather the exception when bacteria and fungi species are collected, studied and investigated in nature under real life conditions. Biofilms are defined as complex consortia of microorganisms that are attached to a surface that can be of biotic or abiotic nature [12].

The microbial biofilm formation involves a multi-stage process in which bacterial and fungi adhere to the surface. For more details see figure 1 which is based in several reports [13-16].

Figure 1. The multi-stage process of biofilm formation by oral microorganisms.
At this stage only weak forces are operating. Therefore it is also known as the initial reversible attachment stage. Subsequently, there is a production of an extracellular matrix (containing polysaccharides, proteins and DNA) that results in a stronger attachment which is also known as the irreversible attachment stage [16]. In general, after attachment, biofilm growth follows two other distinct phases or behavior: spreading and dispersal of microorganisms.

Basically, the attachment process involves equilibrium of electrostatic forces. Microbes and tooth surfaces are negatively charged. As they are immersed in a fluid (saliva) system which is rich in calcium and other counterions, these negative charged surfaces attract and mobilize cations. As a result, a double charged layer is formed (electrical double layer) and this overlap causes a repulsive electrostatic force. Simultaneously, as the bacterium approaches the tooth surface, they also experience a repulsive force (van der Waals force). Finally, the combinations of repulsive and attraction forces known as DLVO theory modulate the microorganism adherence to dental surfaces. This is valid for dental restorative materials as well, and one must consider the fact that it can be more favorable if a porous or irregular surface is facilitating bacterial adherence [3,13-16].

The first bacteria to attach to the acquired pellicle (layer of glycoproteins) on the tooth surface are called the pioneer species (Streptococcus oralis, Streptococcus mitis, Streptococcus sanguis). Surprisingly, S. mutans is not a first colonizer despite its high cariogenic nature. In fact, S. mutans is the most studied bacteria in oral microbiology but under clinical environment one must consider that a multispecies biofilm is operating [3,9].

Historically, in Dentistry, the examination of mature oral biofilms started when electron microscope became available for microbiologists [19]. Later, molecular biological tools became popular and new insights about how microbes attach and develop on tooth surfaces were finally confirmed. One "striking" observation was actually a confirmation of an obvious theory that microbes stick to a surface many benefits are obtained: a) selection of sites where they stay in favorable environments, b) these surfaces may have enough substrate or can contribute to diffuse some nutrient and c) the different species often work together and this consortium provides physical support and protection [20-29].

More recently, zeta potential, confocal laser scanning microscopy (CLSM) together with fluorescence techniques received attention and became useful techniques to study bacteria adhesion to surfaces [29]. In spite of the great evolution in techniques, many limitations have to be considered when comes to the evaluation of an antimicrobial substances against biofilms development. First, there is still the gap of in vitro and in vivo environments. In situ studies can overcome some of these limitations but other drawbacks cannot be ruled out. Most studies on bacteria adhesion to surfaces were carried out under in vitro conditions which do not reflect the real life. Secondly, there is the paradox of testing planktonic cells but interpretations are generalized to conditions of biofilm formation. It is well established that biofilms express genes different from those of planktonic cells. Moreover, it has been observed that biofilm cells are generally believed to closely resemble planktonic cells in stationary phase. However, biofilms were found to more closely resemble to planktonic cells at exponentially growing than those of planktonic cells in the stationary phase [19,20]. In addition, it cannot be ruled out the differences between single species biofilm versus multispecies biofilms since under laboratory
conditions monospecies biofilm can survive for only 72 hours in absence of sugar. The duration of survival can be extended with addition of mucin, but how close is this to the real oral mouth of a patient? [21] Another flow systems versus static models, pre-treatment of acquired pellicle or no pre-treatment at all. [22]. Finally, a crucial point is: how to validate microbial growth? BacLight staining techniques only measure the presence of intact membranes and may not correlate with the culturability or viability of bacteria from oral biofilms [23].

After all these relevant methodological points, more questions marks can be attributed on how to evaluate antimicrobial agents against biofilms. In addition to evaluate the effects in biofilms itself, one must consider the understanding of suitable methods related to the incorporation of antimicrobials into these dental restorative materials. For instance, the concentration of the antimicrobial agent, the volume or amount of material to be included and how far these substances can interfere on mechanical and esthetic features of a restoration [21-26].

Considering the presence of a mature biofilm covering a dental restoration, it must be pointed out that a major requirement of the final formulation is to deliver sufficient concentration of the inhibitor in the surroundings. Moreover, the antimicrobial effect must be kept on a prolonged time or at least for a enough period of time that will maintain an effective dose
operating. This point is quite important since oral bacteria do not live as independent entities. So, as highlighted previously, a high resistance to antibiotics is likely to occur [27-30].

Along the last decades biofilms have been studied extensively because they are present in several surfaces, such as all solid surfaces in the oral cavity, in biomaterials implanted in the human body, in catheter surfaces, in water pipes [24]. After the establishment of a biofilm on dental restorations, deterioration of the outer layer surface of these materials will take place and facilitate bacteria adhesion [25]. On the other hand, the possibility that dental restoring materials can deliver antimicrobials may reduce considerably the risk of secondary caries in spite of the limitations of some dental materials.

3. Antimicrobials in dental materials: How much is enough versus how much is safe?

Oral bacteria can attach to many restorative materials like amalgam, gold, ceramics, resin composite, glass ionomer cements. In order to achieve long-term success of dental fillings there are many requirements. Some are related to the professional ability in manipulating and polishing these materials. However, some considerations rely on physical, chemical and biological characteristics of the dental material used. Surface roughness is not the focus of this review, but it may be influenced by the interplay of professionals’ ability as well as dental materials features.

The incorporation of 5, 10, 15 up to 30% of antimicrobial compounds or substances into dental materials have been proposed [24-30]. However, the higher amount of antimicrobial agents, the higher is the risk to lose important features in dental restoration as biocompatibility and resistance. Hence, how much is enough and how much is safe? In the literature, addition of 1.5% can be effective if the antimicrobial is potent enough [27].

Figure 3 presents this dilemma related to the interference of “extra” substances to be incorporated into dental materials and limitations regarding the loss of important features of the material.

An interesting report showed that incorporation of 1% chlorhexidine (CHX) diacetate in GIC (glass ionomer cements) is optimal for clinical use. This is valid in terms of antimicrobial activities, CHX-release pattern, physical properties and bonding ability to tooth surfaces [28]. An additional valuable information was the conclusion that incorporation of CHX diacetate at 2% or greater values of percentage participation significantly decreased compressive strength and adversely affected bond strength to dentin.

It has been observed that some dental materials (e.g. gold and its alloys) are naturally able to kill bacteria in the adhering biofilms [29]. Glass ionomer cements (GIC) are recognized for releasing fluoride ions that can modulate biofilm formation [27,30]. The point is that this is not enough since GIC reduces its ability to release fluoride in short periods. So, it is expected that dental restoration with antimicrobial properties may have extended potential for inhibiting biofilm formation in a long-term basis.
In addition to chemical changes due to incorporation of antimicrobials into dental restorative materials, there is also the problem of chemical changes also interfere in the distribution of masticatory forces applied on a tooth. For instance, the presence of a carious lesion in molar tooth can demand fast treatment protocol for the affected area and depending on the lesion extension, it must receive a temporary filling [31-34]. In general, temporary filling materials are typically made from a combination of zinc oxide and eugenol which has good antimicrobial activity. Eugenol is also important due to its sedative properties. The zinc oxide powder is a very versatile compound that can present different properties when combined with various agents. When mixed together, the material starts off soft and in few minutes it becomes more hard and brittle. However, this mixture is not harder enough to be compared to regular dental fillings and its far from restoring tooth hardness. This material is classified as intermediate restorative material (IRM) and it is a good example that the beneficial aspects of antimicrobial and anti-inflammatory properties are achieved while mechanical properties of resistance become very low. Therefore, this material must be accepted as a temporary and not a definite filling material. Under the influence of masticatory forces, as previously mentioned, there will be a stress in the remaining parts of the dental element that will certainly compromise the longevity of the restoration as well as the whole tooth structure [31-34].

A comparative study analysing deformations done through Finite Element Method (FEM) and applying the software ANSYS shows the differences in compressive loads between sound and restored teeth with intermediate restorative materials (IRM), see figure 4A and 4B. It is shown that the restored tooth IRM (figure 4B) is deformed in a different way when compared to the
sound tooth (4A). Figure 5 shows this simulation evaluating a map of tension for both conditions: A (sound tooth) and B (restored).

As expected, the analysis shows that the distribution of forces in the interior of the teeth flows in different patterns. As a result, the restored tooth experiences a higher stress in some parts. Basically, these maps show compressive forces throughout the whole sound tooth (A) whereas for restored teeth tensile stress forces are observed.

It well is established that development of a numerical model as FEM makes it possible to quantify and evaluate masticatory loads [34]. However, few studies have considered the influence of antimicrobials in dental restorations. One must bear in mind that a good balance has to be achieved between the beneficial aspects of having an “antibacterial restoration”
compared to a regular one. Certainly the size and shape of the restoration are important variables but interesting results can be obtained by these simulations.

It is important to realize that the changes in the map of tensions are directly related to the changes in the physical constitution of the tooth because in this case, the dental enamel was substituted by a restorative material. This change cannot necessarily be attributed to a change of forces because the molar frequently will be constantly submitted to the same masticatory forces it was receiving before the carious lesion. As shown in figure 5, the red colour of the figure 5B indicates a significant higher tension the 5A. The structural fatigue is the main mechanism of collapse of the reminiscent dental tissue and this process can be aggravated when it is submitted to long treatment periods, particularly if IRM is used. In other words, the IRM used as temporary fillings must have a short life because they reduce the mechanical efficiency of the teeth in spite of its beneficial support to control biofilm formation.

According to Noort (2013) [35], there is a subtle distinction between safety and biocompatibility two important features of dental materials. Safety is concerned with the fact that materials when in contact with the human body should not cause any adverse effect, whereas biocompatibility is the quality of being non-destructive in the biological environment maintaining the beneficial effect to the patient. So far, few materials can be regarded as completely safe and fully biocompatible in the oral environment. Most dental materials interact with the oral environment and this interaction might be a release of components with undesirable side effects for oral tissues.

4. Dental restorative materials with antimicrobials

Dental materials must simulate dental structure and have to restore the anatomy and the function of affected dental surfaces due to dental caries or trauma. However, the desirable aesthetics and the concerns with biocompatibility have not been forgotten and this is valid for resin composite, glass ionomer cements and IRM (MJOR et al., 1990). It must be highlighted that bone and dentin can be considered as natural composites, whose main constituents are collagen (polymer) and apatite (a ceramic) [35].

Metals have been used for centuries as antimicrobial agents and they continue to be useful at the present time. Silver, copper, gold, titanium and zinc are the most common examples in Dentistry [36]. Dioxide of titanium has been used as whitening agent. However, silver and copper has been receiving larger attention due to their antimicrobial properties. As a result, these metals are incorporate to several dental products to control halitosis and dental biofilms [5].

As for dental resins, GIC and IRM, these materials are probably the best examples of improvement of restorative materials that has contributed to the recovery of ideal anatomical form and function with less removal of tooth structure. The use of “fluoride-release” materials, “smart-materials” and “bio-active” materials are some desirable features that are becoming necessary in many clinical situations because minimally invasive treatment of carious lesions is much
more acceptable nowadays. Probably, the first experiences to produce a useful “smart-material” were related to the concept that fluoride-releasing materials. Glass ionomer cements do not undergo great dimensional changes in a moist environment and exhibit noticeable shrinkage in a dry environment at temperatures higher than 50°C, which is similar to the behavior of dentin [37]. This is a good example of biocompatibility.

Attempts to improve GIC have been quite successful. There is one report indicating that zinc addition to GIC can decrease microorganisms growth and improve fluoride release, without significantly affecting the materials’ flexural strength and solubility [38]. In another report, conventional glass ionomer cement (GIC) liner was mixed with different antibiotics such as metronidazole, ciprofloxacin and cefaclor to produce an antibacterial GIC. After an in vitro evaluation of infected dentin sealed with this product, there was a 98.6% decrease in microorganisms, bacterial aggregates, and intertubular dentin with exposed collagen fibers and dentinal tubules [41]. When conventional GIC was added with 1.5, 3.0 and 4.5% of ciprofloxacin, metronidazole and minocycline this material was effective for inhibiting S. mutans and L. casei, and the addition of a 1.5% antibiotic mixture was optimal to provide appropriate physical and bonding properties [39]. For more than a decade, several reports in the literature have been demonstrating antibacterial activity against S. mutans, S. oralis, S. salivarius and Streptococcus sp when GIC are reinforced with antimicrobials or due to fluoride or pH equilibrium [7, 40-44]. It has been claimed that GIC has the ability to increase pH and this is likely to be an important mechanism of caries protection under clinical conditions since oral bacteria can produce lactic acid [43].

The resin composites have been used, frequently, as restoring material due to its great aesthetics and physiologic properties [44]. More recently, incorporation of 12-methacryloyloxydodecylpyridinium bromide, a monomer also known as MDPB showed good results for its antibacterial activity when incorporated in bonding agents [28].

However, instead of releasing an antimicrobial substance, the strategy to incorporate them to act as part of its structure is also possible. In this perspective, nanoparticles can provide good optical properties for conventional and hybrid composites [45]. However, there is still a lack of studies in the literature showing the beneficial aspects for placing such material in a dental cavity. For instance, it is still unknown how effective these materials can inhibit caries activity close to restorations when active bioparticles are incorporated into these resins [46-48]. As a general observation, it must be highlighted that there are many in vitro studies but very few clinical trials to support their use under regular clinical activity [46].

Finally, it can be stated that two main approaches can be presented when antimicrobial bioactive materials are prepared. One approach is to prepare a substance-release material (e.g. GIC). Another perspective is to incorporate the antimicrobial to be active being part of its structure without any release of active component. Basically, this latter option is of outmost importance since the release of a substance implies in loss of matter and in theoretical basis this means some loss in mechanical properties. Taking into account that GIC acts as battery charges for fluoride, it must be pointed out that “recovery” of fluoride ions does not reach original levels [47-49]. Hence, other advantages have to be operating to consider this material as a good option for dental restorations.
Another point to be considered is the fact concentrations of substances released from some dental materials such as GIC materials were not different, regardless of the amount of antimicrobial substance incorporated. Thus, as long as the antimicrobial is not interfering in the mechanical properties, an increase in the amount of antimicrobial drug will not provide additional benefits.

5. Dental restorative materials with nanoparticles

Nanoparticles are generally defined as particles that are smaller than 100 nanometers in diameter. So, in order to provide a good perspective, it can be emphasized that nanotechnology deals with structures as small as $10^{-9}$ m while oral bacteria reach a size of $10^{-6}$ m. Although there is a large difference in size, the improvements of many technologies in the 1980s made possible the combination of these two worlds. Many researchers’ points out that nanotechnology has been applied for dental materials as an innovative concept for the development of materials with better properties including the anti-caries effect [5,45,49].

It is recognized that many nanoparticles do have a great antimicrobial activity, particularly if it is a metallic nanoparticles. The antimicrobial activity of many types of nanoparticle is certainly a function of their size but other features are important such as high surface area, unusual crystal morphologies (edges and corners) and reactive sites. There is a great difference of a regular metal and a $10^{-9}$ m particles when incorporated into dental materials. Consequently, their properties can radically change, as hardness, area of active surface, chemical reactivity and biological activity [26].

The inverse relationship between the size of some particles and its antimicrobial activity has been demonstrated for particles of up to 10 nm were tested against *Escherichia coli* [50]. Thus, this might be valid for nanoparticles as well. The main mechanism or mechanisms behind the antimicrobial activity of nanoparticles are not fully elucidated. Hence, several studies focusing on the antimicrobial activity of different metals and metallic nanoparticles against oral microorganisms have to be performed for a clear picture on this matter. Another point to be considered is the effectiveness of these nanoparticles to control the development of a biofilm. Considering that biofilms are rather organized and can avoid the penetration of big molecules (e.g. chlorhexidine) the small size of these particles can be advantageous. However, so far these particles have been introduced into prosthetic devices coatings and oral care products. The strategy for placing them within dental materials is currently being explored in vitro and more research is needed to consider their regular use in the dental clinic.

Basically, the most promising nanoparticles are: silver, zinc oxide, calcium-phosphates [5]. Nevertheless, must be also known that an interesting systematic map demonstrated that there is currently a limited amount of information concerning the release of nanoparticles from polymer-based dental materials. After reviewing 140 full-text articles on this matter, only 3 were regarded as methodological sound. Actually, a passive release of nanoparticles from a polymer-based dental material was not observed by the investigated reports. [51]. Table 1 summarizes some important features of these materials when present within dental materials.
Nanoparticles | Observations | References
--- | --- | ---
Silver | • It may provoke structural changes and damage bacterial membranes, resulting in cell death.  
• Incorporated into dental adhesives could reduce *S. mutans* close to orthodontic brackets.  
• Concentrations of 0.5-1% provided antimicrobial activity with preservation of aesthetic and mechanical properties of dental materials (resin composites).  
• Future research must focus on silver-biofilm interaction and silver-polymerization processes of dental materials. | 5,26,49, 52-59 |
Zinc oxide | • The mechanism of action may be attributed to oxidative stress by H$_2$O$_2$ and structural changes in cell wall.  
• Incorporated into dental materials ZnO may release Zn$^{2+}$ which interferes in sucrose metabolism and magnesium depletion that is important for biofilm equilibrium.  
• Future research must focus on the determination of ideal concentrations of nanoparticles in order to have antimicrobial activity without compromising mechanical properties of the materials. | 5,26,49, 58, 60, 61 |
Quaternary Ammonium | • This compound was selected due to its good antimicrobial activity and because it can be copolymerized with other monomers providing a strong bonding system with the material. However, difficulties in controlling the release of such agents may be a potential drawback.  
• The hydrophobic nature and positive charge of these nanoparticles may enhance the antimicrobial activity.  
• Future research must focus on kinetics to optimize the release characteristics. | 5,26,63-65 |
Calcium-phosphates | • These compounds can interfere on adherence and growth of *Streptococcus mutans*.  
• The resin composites with these nanoparticles can increase up to four times the capacity of remineralization of the enamel in comparison with the composites with fluoride.  
• Hydroxyapatite nanocrystals may interact with bacterial adhesins and can reduce bacterial adherence to dental surfaces.  
• Future research must focus on efficacy of products that are already available in the market such as casein phosphopeptide (CPP)-amorphous and calcium phosphate (ACP) nanocomplex. | 5,26,66-71 |

Table 1. Observations and conclusions related to nanoparticles incorporated into dental materials.

### 6. Final considerations

The oral environment imposes difficulties when it is designed a study for evaluating dental materials [3,9,10,25]. Since 1950’s it is know that microbial microleakage at the cavity wall/
material interface is a problem to restoration survival. The persistence of microorganisms underneath fillings is also recognized as a serious problem in restorative dentistry. The antibacterial properties of restorative materials can substantially influence the success of a dental filling in the oral cavity. The frequent problem is that dental materials “natural” antibacterial properties are not enough to cope with the facility of biofilm formation. Thus, the incorporation of antimicrobials in restorative materials has to take into account the properties of each dental material. For instance, restorations of glass-ionomer cements are based on an acid-base reaction between a polyacrylic acid solution and fluoroamino-silicate glass particles. This reaction yields a structure that is more stable than composites. As a result, by adhering to tooth structure the glass-ionomer cements potentially reduces microleakage. This is an important property since it can enhance fluoride release. So, why not incorporating antibiotics as well? Hence, glass ionomer cements are strong candidates to have antimicrobials incorporated as long as it does not disturb the acid-base reaction. On the other hand, resin composites are much better materials considering aesthetic properties. Finally, coatings killing bacteria upon contact seems to be more promising than antimicrobial-releasing coatings. However, many in vitro studies cannot support the findings that are observed in vivo. This observation suggests that more clinical research is needed to clarify this issue. Hence, clinical research on this topic is of outmost relevance for minimum intervention restorative techniques in dentistry and for promoting oral health. Another point to consider is the challenge for the future dental materials with antimicrobials properties: to develop even more effective materials that are able to improve clinical antimicrobial efficacy while still preserving the benefits of the normal, resident oral microflora.

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