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Literature Review Article

RESIN-BASED BIOMATERIALS WITH ANTIBACTERIAL ACTIVITY

Daniela Soares Pinto

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To Professor Ana Portela,

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To my colleagues and friends,

For all the experiences shared, for the incredible team work, for their constant presence in the best and in the worst moments and, above all, for their friendship.

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ACRONYMS AND ABBREVIATIONS INDEX

BTAM - 2-[3-(2HBenzotriazol-2-yl)-4-hydroxyphenyl]ethyl Methacrylate

CaF_2 - Calcium Fluoride

CFU - Colony-forming Units

CHX - Chlorhexidine

DMADDM - Dimethylaminododecyl Methacrylate

DMAHDM - Dimethylaminohexadecyl Methacrylate

DMAHM – Dimethylaminohexane Methacrylate

DMAODM - Dimethylaminooctadecyl Methacrylate

DMAPM - Dimethylaminopropyl Methacrylate

DOX - Doxycycline

MADQUAT - [2-(Methacryloyloxy)ethyl] Trimethylammonium Chloride

MAE-HB - 2-methacryloxylethyl Hexadecyl Methyl Ammonium Bromide

MDPB - Methacryloyloxydodecylpyridinium Bromide

MIC - Minimum Inhibitory Concentration

MPC - 2-methacryloyloxyethyl Phosphorylcholine

MSNs - Mesoporous Silica Nanoparticles

NACP - Nanoparticles of Amorphous Calcium Phosphate

NAg - Nanoparticles of Silver

NPs - Nanoparticles

PAA-CuI - Polyacrylic Acid Coated Copper Iodide

QACs - Quaternary Ammonium Compounds

QADM - Quaternary Ammonium Dimethacrylate

QAM - Quaternary Ammonium Monomethacrylate

QPEI - Quaternised Polyethyleneimine

TAT - 1,3,5-triacryloylhexahydro-1,3,5-triazine

TiO_2 - Titanium Dioxide

UDMQA-12 - Urethane Dimethacrylate Quaternary Ammonium Compound

WSLs - White Spot Lesions

ABSTRACT

Introduction: Resin-based materials are widely used due to their physical properties and pleasant aesthetics. Nevertheless, the formation of microgaps on the tooth-restoration interface and dental plaque accumulation lead to secondary caries. In addition, the increased accumulation of bacterial plaque around the orthodontic brackets is responsible for enamel demineralization and formation of white spot lesions. Bioactive materials with antibacterial potential have been developed, promising a longer life span of restorations and protection against secondary caries and white spot lesions.

Purpose: The aim of this dissertation is to study and systematize the new resin-based biomaterials with antibacterial activity and to evaluate their efficacy, remineralizing effects and mechanical properties.

Methodology: Literature search was performed in PubMed/MEDLINE and Scopus databases to include articles from the last 5 years. Restrictions included 'human species' and English, Portuguese, Spanish and French languages.

Development: Several antibacterial agents were incorporated in composite resins, adhesive systems and orthodontic cements to cause a significant decrease in biofilm accumulation, metabolic activity and acid production, mostly without compromising their physical properties. Agents with remineralizing and protein-repellent capabilities were also incorporated, increasing pH and reducing bacterial adhesion, respectively. The combination of two or three antibacterial agents results in a synergistic effect.

Conclusion: The incorporation of these agents in resin-based restorative materials is a promising strategy to diminish the risk of secondary caries and white spot lesions. However, more studies are still necessary to ensure their antibacterial effects, safety, biocompatibility and mechanical properties, before their incorporation in commercial materials.

Key-words: antibacterial agent, secondary caries, white spot lesion, composite resin, adhesive.

INTRODUCTION

Resin-based restorative materials are increasingly used due to their physical properties[1, 2], direct-filling capabilities[3, 4] and pleasant aesthetics.[5, 6] However, approximately half of the restorations performed fail within 10 years.[6] One of the main reasons for failure is secondary caries in the tooth-restoration margins[7, 8], which are considered the weakest link of the restorations.[3, 9]

A disadvantage of these materials is polymerization contraction, which can cause microgaps in the tooth-restoration interface[10], promoting marginal infiltration of bacteria.[9, 11, 12] Bacteria penetrating into the bonded interface, produces acids that demineralize the dental tissues and lead to the formation of secondary caries.[3, 6, 13]

Besides dimensional shrinkage, resin-based materials also exhibit higher levels of bacterial plaque accumulation[3] when compared to other restorative materials - such as Amalgam[14, 15] and Resin-modified Glass Ionomer[16] - due to surface roughness and the release of residual monomers after polymerization.[17]

Biofilms are responsible for acid production in the presence of fermentable carbohydrates[9, 18], inducing a decrease in the local pH to a cariogenic level that inevitably leads to teeth demineralization and caries recurrence.[16, 19]

Another common concern is the prevalence of white spot lesions (WSLs) related to the brackets of orthodontic appliances. WSLs correspond to incipient caries on the enamel surfaces[20, 21] that are caused by the higher cariogenic challenge associated with the increased accumulation of bacterial plaque around the brackets.[20, 22, 23] This biofilm accumulation is due to the irregular surfaces of the brackets[20] that provide sites for rapid attachment and growth of bacteria[24] and hinder plaque removal during tooth brushing.[20]

The prolonged accumulation of biofilms around the brackets lowers pH in these sites, inducing demineralization[25, 26] which can occur within a span of four weeks.[27, 28]

Therefore WSLs are responsible for aesthetic impairment at the end of the treatment[20, 29] and must be prevented.

In order to avoid WSLs, secondary caries and to extend the life span of restorations, several studies developed a variety of bioactive materials with: antibacterial potential, by reducing bacterial activity[2, 26, 30] and/or adhesion[4, 31]; and remineralizing potential of the affected dental tissues through pH increase.[16]

Adhesives, composite resins and orthodontic cements are rendered antibacterial by the direct incorporation of agents such as chlorhexidine[32, 33]; glutaraldehyde[32, 33]; nanoparticles of silver

(NAg)[2, 34], copper[35] and copper iodide[1], quaternised polyethyleneimine (QPEI)[14, 36] or titanium dioxide (TiO_2)[28, 37]; 1,3,5-triacryloylhexahydro-1,3,5-triazine (TAT)[22]; calcium fluoride (CaF_2)[17]; doxycycline (DOX)[38]; 2-methacryloyloxyethyl phosphorylcholine (MPC)[31, 39]; arginine[10]; 2-[3-(2HBenzotriazol-2-yl)-4-hydroxyphenyl]ethyl Methacrylate (BTAM)[40]; and quaternary ammonium compounds (QACs) as methacryloyloxydodecyl-pyridinium bromide (MDPB)[7, 12, 21, 29, 41], dimethylaminododecyl methacrylate (DMADDM) [6, 18, 27, 42], dimethylaminohexadecyl methacrylate (DMAHDM)[23, 26], [2-(Methacryloyloxy)ethyl] trimethylammonium chloride (MADQUAT)[20], 2-methacryloxylethyl hexadecyl methyl ammonium bromide (MAE-HB)[24], quaternary ammonium dimethacrylate (QADM)[2, 30], urethane dimethacrylate quaternary ammonium compound (UDMQA-12).[5]

Resin-based materials acquire antibacterial properties through the incorporation of releasing or non-releasing agents.[3, 24] Releasing agents, such as CHX[43] and NAg[34], have their effect attributed to the release of antibacterial products, acting at a distance, and may compromise the physical properties of the material.[11] Non-releasing agents, as QACs[4, 26, 44], copolymerize covalently with the resin matrix[6], being immobilized within that matrix and not lost over time.[27] Therefore they act through direct contact with bacteria.[32]

The ideal resin-based biomaterial would have antibacterial properties[40], the ability to eradicate residual bacteria in the prepared cavity as well as the new invading bacteria at the margins[3, 16, 44], to repel proteins and to neutralize acidic environments.[9] These characteristics, along with the promotion of tertiary dentin formation, would promote dental pulp protection[9] and decrease the risk of secondary caries thus prolonging the longevity of restorations.[32]

The topic of this review was chosen due to its pertinence and interest in modern Dental Medicine. The aim of this dissertation is to study and systematize the new resin-based biomaterials with antibacterial activity developed and investigated in the last 5 years and to evaluate: their composition and antibacterial properties; the effects of the antibacterial agents' addition on the mechanical properties of the materials; and the main results.

METHODOLOGY

Literature search was performed in PubMed/MEDLINE and Scopus databases between 19th November 2018 and 21st March 2019.

Two searches were carried out. The first was done only with MeSH terms (table I) and the second included all terms (table II).

Only the articles published in the last 5 years were accepted. Some other restrictions included 'human species' and English, Portuguese, Spanish and French languages.

The articles were selected accordingly to the aims of this review, that is, resin-based materials such as adhesives, composites and cements that display antibacterial properties in order to prevent tooth demineralization and caries recurrence.

Repeated articles were excluded. Title and abstract were analysed and used as the second step of exclusion, followed by a full text analysis of the remaining articles.

Some references cited in the articles included in this review were also checked.

From all the articles recovered, 73 were chosen for a full analysis, in which 19 were excluded.

The 54 references used in this review were imported to the software *EndnoteX9.*

Table I - Search strategy with MeSH terms

	Terms
#1	Antibacterial Agents AND Composite Resins AND Dentistry
#2	**Antibacterial Agents AND Composite Resins**
#3	Antibacterial Agents AND Composite Resins AND Nanoparticles
#4	**Antibacterial Agents AND Adhesives AND Dentistry**
#5	Antibacterial Agents AND Adhesives
#6	**Antibacterial Agents AND Resin Cements AND Dentistry**
#7	Antibacterial Agents AND Resin Cements
#8	**Antibacterial Agents AND Orthodontics AND Adhesives**
#9	Antibacterial Agents AND Orthodontics AND Resin Cements

Table II - Search strategy with all terms

	Terms
#1	Antibacterial Activity AND Adhesives
#2	**Antibacterial Activity AND Adhesive System**
#3	Antibacterial Activity AND Bonding System
#4	**Antibacterial Activity AND MDPB**
#5	Antibacterial Activity AND Resin Biomaterials
#6	**Antibacterial Activity AND Dental Resin Biomaterials**
#7	Antibacterial Activity AND Composite Resin
#8	**Antibacterial Activity AND Nanoparticle Composite Resin**
#9	Antibacterial Activity AND Resin Cements
#10	**Antibacterial Activity AND Dental Resin Cements**
#11	Antibacterial Capacity AND Adhesive

DEVELOPMENT

Due to minimally invasive approaches, which defend the preservation of tooth structure in order to prevent pulpal damage[11, 30], it is expected that more affected dentin will be preserved and therefore will harbour more residual bacteria.[15]

Secondary caries, one of the principal reasons for failure of resin-based restorations[3, 44], is caused by biofilms' acid production.[31, 39]

Caries recurrence may occur because after caries removal residual bacteria is left in the prepared tooth cavity.[42, 45] Furthermore, new invading bacteria infiltrates the tooth-restoration interface causing marginal leakage and favouring caries development.[30, 42]

The presence of dental plaque around orthodontic brackets is also a responsible factor for enamel demineralization or WSL[35] and dental caries close to the bonding interface, which may compromise the bond strength of the bracket to the tooth.[20]

Of all the bacteria used to study the effects of antibacterial agents, *Streptococcus mutans* is one of the favourites[22, 30, 37, 44, 46] since it is an important etiologic agent in the development of dental caries[33, 47] due to its ability to initiate cariogenic biofilm formation.[33] Other microorganisms commonly studied are *Actinomyces viscosus*[14, 36, 41], *Lactobacilus casei*[14, 32, 33, 43], *Streptococcus gordonii.*[10, 29, 41, 44] Biofilms are present in the oral cavity and are less sensitive to antibacterial agents than planktonic bacteria and thus it may be necessary a higher concentration to eliminate them.[45] In order to better mimic dental plaque in oral conditions, a microcosm biofilm model with an inoculum from human saliva has also been used.[23, 26, 31, 34, 48]

Current resin-based restorative materials are biocompatible but not bioactive.[9] Restorative biomaterials with therapeutic and satisfactory mechanical properties would be highly desirable for reducing bacterial adhesion, eliminating biofilms and neutralizing the cariogenic pH[9, 16] and consequently for the prevention of tooth structure's demineralization and secondary caries.[16, 32]

It is important that this new antibacterial biomaterials retain or improve the mechanical properties, such as bond strength, because a significant decrease in bond strength will possibly increase the risk of microleakage and, as a result, of secondary caries.[10]

An orthodontic cement or adhesive with the ability to prevent the development of WSLs on orthodontic patients without compromising enamel bond strength would also be advantageous since it wouldn't depend on patient's compliance.[26]

Orthodontic treatment last for an average of 2 years and, during that time, the orthodontic cement/adhesive must be capable of bonding to the enamel surface of the teeth with high bond

strength in order to resist masticatory loads. However, this enamel bond strength should not be so high it damages the enamel while removing the bracket.[22]

The excess of resin-based orthodontic cements/adhesives used to bond the brackets also induces the accumulation of dental plaque around those orthodontic appliances due to their irregular surfaces.[35] The biofilm degradation of this resin-based materials, caused by the acidic pH, may contribute to the early debonding of the brackets.[27]

Therefore, it would be imperative the development of an antibacterial orthodontic cement/adhesive that can inhibit bacterial growth without affecting the enamel bond strength.[25]

Several different approaches are being used to develop such a biomaterial including the incorporation of calcium phosphate nanoparticles into adhesives[13, 16] and/or composites[2, 19, 34] to remineralize pre-existing carious lesions[8, 16] and to inhibit future caries.[48] Another approach renders the resins with protein-repellent capability thus hindering bacteria attachment.[4, 16, 19, 31] A third approach is based on the incorporation of antibacterial agents such as QACs[5, 23, 24, 26, 30] that copolymerizes with the resin matrix[2] to supress biofilm growth by causing bacterial lysis[18, 26], silver nanoparticles[46] that releases silver ions and induces the death of bacteria by causing the DNA to lose its replication capability[6, 11] or other substances.[1, 10, 17, 22, 35, 36] Numerous studies combined different approaches to achieve synergistic effects.[2, 13, 16, 34]

The main results of experimental antibacterial adhesives (Attachment 1), composites (Attachment 2) and orthodontic adhesives and cements (Attachment 3) were investigated as well as the tested microorganism and samples used for each antibacterial agent.

1. QACs

Quaternary ammonium compounds are progressively more chosen as the antibacterial agents to be incorporated in resin-based materials due to their ability to be immobilized in the resin[2, 26] by covalent bonding with the polymer matrix[2, 6] and not released over time[2, 27], maintaining the initial concentration and providing durable contact-inhibition against bacteria.[6]

QACs are cationic agents that react with the negatively charged bacterial surfaces[24, 41], disrupting the electric balance of the cell membrane[18, 42] and causing membrane damage[24] and lysis[18] with loss of cytoplasmic constituents.[24] This mechanism is called “contact-killing” effect.[18, 44]

Quaternary Ammonium Monomethacrylate (QAM) is a family of QACs that has only one methacrylate group and hence can only undergo linear polymerization.[47]

While QAM has only one reactive group, QADM is a dimethacrylate and thus both ends of molecule have a reactive group available for covalent bonding with the resin matrix during

polymerization[45], which allows it to be a cross-linking agent.[47] For that reason, it is expected to have minimal monomer leach.[45]

QACs were incorporated in adhesive systems in the form of DMADDM[6, 44], DMAHDM[42], MDPB.[32, 33, 41] All these experimental adhesives resulted in a strong antibacterial activity.[6, 32, 41, 42, 44]. Common results are the decrease in acid production, biofilm growth, CFUs and metabolic activity.[6, 18, 27, 44]

Several studies show that MDPB presents a significant antibacterial activity, despite requiring 30min to destroy most of the microorganisms studied in André C.B. *et al* article.[32] MDPB also showed inhibition halos against the tested bacteria and kept its antibacterial properties after being light-cured.[33] The antibacterial effect of MDPB increased with contact time and was slightly lower on mixed bacterial suspensions than on single species[41], probably because biofilms are less susceptible to antibacterial agents than planktonic bacteria. Sande F.H. *et al* developed an *in situ* investigation with a split-mouth design which reported that adhesive system with MDPB presented lower mineral loss next to the composite than the control after 8 weeks[12], which is in accordance to the previous studies that report antibacterial activity since the reduction of the bacterial load might reduce the pH drop and thus prevent tooth demineralization. On another *in situ* study, MDPB and/or Fluoride displayed no statistical differences in microhardness values from the control groups thus they were not able to inhibit secondary caries.[7] One reason that may justify the different results between both *in situ* studies is the methodology used. While the first study evaluated mineral loss on dentin during 8 weeks and provided cariogenic challenge with 20% sucrose solution 4 times a day[12], the second evaluated enamel demineralization during 2 weeks and provided the same cariogenic challenge 8 times a day.[7]

The incorporation of DMADDM in adhesive systems greatly reduced *S.mutans*[18, 44] and *S.sanguinis* biofilms while the proportions of *S.gordoniis* increased.[44] Therefore, DMADDM was responsible for reducing cariogenic bacteria in biofilms and thereby changing them from caries propensity to health tendency, delaying the pH drop.[44] In another study, Banzi É.C.F. *et al* showed that pre-treatment of the resin surface with salivary pellicles would increase the metabolic activity of biofilms but DMADDM was still capable of displaying antibacterial activity.[6] The incorporation of DMADDM did not significantly affect the dentin microtensile bond strength[18] when added at concentrations up to 10%, even though there was a slight decrease in concentrations higher than 7.5%.[30] Chen C. *et al* compared the antibacterial activity of DMADDM and QADM and concluded that the first one had a stronger antibacterial effect, demonstrating that DMADDM is more efficient in eliminating bacteria impregnated in dentin blocks.[30]

DMAHDM was also added to a bonding system displaying a strong antibacterial activity. Increasing the concentration up to 10% did not affect dentin bond strength.[42]

QACs have also been widely used in experimental orthodontic adhesives and cements[20, 21, 23, 24, 26, 27] and are a promising strategy to prevent WSLs and dental caries.[20, 27]

For instance, MADQUAT was incorporated in an orthodontic adhesive by Nascimento P. *et al* and improved the degree of conversion. Only the adhesive containing 10% MADQUAT was capable of reducing the integrated mineral loss, resulting not only in the lowest demineralization around the brackets but also in the lowest values of bond strength. Despite remaining within the range of acceptable values, the decrease of bond strength may favour the premature debonding of brackets. This study reports that demineralization under the brackets was not affected by adhesive type nor the cariogenic challenge whereas demineralization around the brackets was.[20]

On the other hand, Yu F. *et al* used MAE-HB, which displayed antibacterial activity by contact-inhibition against *S.mutans*, mainly when added at a concentration of 3 and 5wt%. This effect was maintained after 6 months of water ageing. The agar diffusion test resulted in no inhibition zone around the disks with MAE-HB, indicating that this antibacterial agent was entirely immobilized in the adhesive and is not released. It is expected it provides a durable antibacterial capability and minimal cytotoxicity.[24]

Passariello C. *et al* and Oz A.Z. *et al* opted to add another QAM into the orthodontic adhesive, MDPB.[21, 29] While the first one concluded that the group of adhesive containing MDPB, as well as the benzalkonium chloride group, demonstrated higher and longer antibacterial activity than any other group[29], the second one performed a micro-CT study that concluded that all WSLs were located around the brackets and not under them and that the antibacterial adhesive did not have any significant effect on the inhibition of their progress.[21] This result differ from the majority of the literature maybe due to the different methodology applied considering that Oz A.Z. *et al* did not study the antibacterial effect of the orthodontic adhesive but its ability to prevent enamel demineralization around the brackets. Besides that, in this study, the brackets remained in the oral cavity for 8 weeks[21], which represents actual oral biofilms and cariogenic challenges and, therefore, may be more reliable than microcosm biofilm models and single species biofilms.

Incorporation of DMADDM in an orthodontic cement significantly inhibited biofilm viability. Despite the increase of antibacterial potency with the increase of the mass fraction, shear bond strength was reduced when 5% concentration was reached.[27]

More recently, Feng X. *et al* and Wang X. *et al* studies on the incorporation of DMAHDM demonstrated that it exhibited strong antibacterial activity.[23, 26] The enamel bond strength was not

compromised until the mass fraction of DMAHDM reached 7.5%.[26] Shear bond strength was maintained after water-ageing for 30 days[26] and 3 months.[23]

A meta-analysis from 2015 compared four different orthodontic adhesives with and without antibacterial agents (chlorhexidine, silver nanoparticles, MDPB and titanium dioxide) and concluded that beside CHX, that reduces bond strength, there was no significant differences between conventional and antibacterial orthodontic adhesives thereby, the addition of antibacterial agents may not influence in vitro bond strength.[25]

The incorporation of QACs in experimental resin-based materials is becoming very popular. Increasing the mass fraction of these compounds leads to an increase on the antibacterial potential of the adhesive, composite or orthodontic cement[23, 27, 48], because there are more positive charges to interact with bacteria.[42] Therefore, the higher the concentration of QAC, the lowest the values of acid production[18, 44, 48], metabolic activity[18], bacterial colonization and viability[42, 48] and the higher the values of compromised bacteria.[23, 26, 27]

2. Nanoparticles

A relatively recent advance in the area is the use of nanotechnology to render the resin-based materials with antibacterial activity [2, 35-37] and/or remineralizing capability.[2, 13, 19]

The small size of these particles provides a higher surface area-to-volume ratio[1, 6, 34] allowing a greater proportion of material available for reaction.[34] In other words, a low filler level of nanoparticles may impart a strong antibacterial effect.[6, 14] For instance, the incorporation of nanoparticles in resins at a lower concentration than the equivalent agent on the micro-scale may reach the same antibacterial activity.[34]

Besides stronger antibacterial properties, nanoparticles can also provide higher dimensional stability[35], reducing polymerization contraction[47], and decrease the surface roughness and consequently bacterial adhesion.[34, 35]

Another advantage of nanoparticles, when added to adhesives, is that they're able to flow into the dentinal tubules, where they may inhibit residual bacteria and/or remineralize affected dentin.[45]

Besides all the advantages cited, resin-based materials incorporating nanoparticles may act through the release of antibacterial agents, for instance the case of NAg.[6, 34] It remains a challenge to control the release kinetics of these agents, which may cause a decrease of function with time or affect the physical properties of the resin material. Antibacterial agents leaching may also cause tooth discoloration and cytoxicity.[24]

Both Shvero D.K. *et al* and, more recently, Pietrokovski Y. *et al* used QPEI nanoparticles to render composite resins with antibacterial effects causing cell surface changes, cell wall damage and membrane disruption.[14, 36] The incorporation of a small percentage of QPEI nanoparticle in the composite demonstrated a long-lasting and wide spectrum antibacterial effect against oral bacteria without decreasing the degree of conversion. The distribution of QPEI nanoparticle is uneven, being more evident in the inner part of the material.[14]

Mesoporous silica nanoparticles (MSNs) are used as reservoirs which can encapsulate and release molecules. Zhang J.F. *et al* used MSNs to encapsulate CHX and incorporated the resultant agent in a composite resin. Composites with MSNs+CHX presented smooth surfaces, a controlled release of CHX over a long period of time without sacrificing the mechanical properties and a better wear resistance than the control. When CHX was directly mixed with the composite, there was a burst release of CHX in a short time in addition to rougher surfaces and a reduction on the flexural strength and modulus.[43]

Composites with HA–PDA–Ag nanowires displayed a fast release of silver ions initially. Over time, the release rate gradually slowed until it achieved a sustained release behaviour after 14 days. The increase of the mass fraction up to 8wt% improved the antibacterial potency and the flexural properties of the composite. Further increase to 10wt% would decrease both the flexural strength and modulus. The nanowires were well dispersed in the composite, which had a brownish colour.[46]

According to Li F. *et al*, adhesives containing NAg resulted in much more compromised bacteria than the control even when the resin surface was pre-treated with salivary pellicles. The same study reported a well dispersion of nanoparticles throughout the resin matrix.[6]

PAA-CuI nanoparticles-containing adhesive demonstrated significantly lower viable bacteria concentration while keeping the shear bond strength. PAA-CuI nanoparticles were adequately dispersed despite showing some agglomeration.[1]

In another study, the amount of DOX incorporated in the nanotubes and later in the resin was enough to hinder *S.mutans* growth by direct contact without compromising dentin bond strength. Furthermore, DOX did not compromise the degree of conversion even though it led to a slightly darker adhesive. DC could be increased with increasing curing times.[38]

More recently, Florez F.L.E. *et al* incorporated TiO_2 nanoparticles in an adhesive, which displayed a tendency to agglomerate in ethanol. When the mass fraction of TiO_2 nanoparticles was increased, the higher concentration of particles resulted not only on a rougher material surface but also on a stronger antibacterial adhesive. Despite having antibacterial capabilities in both dark and light

irradiated conditions, biofilms cultivated under light irradiation conditions haver lower viability levels.[37]

Nanoparticles can also be incorporated in orthodontic adhesives/cements. One study added copper nanoparticles, achieving significant antibacterial activity and increasing material shear bond strength when used at a concentration of 0.0100 wt%. It was obtained a homogeneous distribution of copper NPs within the matrix with no aggregation observed.[35] Another study opted for the incorporation of TiO2 nanoparticles and concluded that the 2% group maintained enamel microhardness within the range of normal values, displaying an increase in the antibacterial effect when compared to a commercial control. 1% TiO2 nanoparticles resulted in an antibacterial activity inadequate to prevent demineralization.[28]

3. QACs + Nanoparticles

Recently, NAg and QACs, such as DMAHDM, were combined in adhesive, primer, and composite resins[34] in order to achieve greater antibacterial activity against biofilms by acting through direct contact-killing via QAC[32, 34] and through long distance-killing via Ag ions' release.[34]

In Melo M.A.S. *et al* study, NAg and DMAHDM were incorporated in both the bonding system and the composite. These experimental materials revealed that NAg could disperse well in the resin matrix with minimal aggregation. The materials with two agents had smoother surfaces than the control, hindering initial bacterial adhesion, and displayed more areas of compromised bacteria. Besides the strong antibacterial effect, multi-agent material also improved the flexural strength as well as the fatigue resistance of the bonded interface.[34]

The application of NACP into resin-based materials increases the Ca and P ion release when the pH is low and more prone to induce demineralization.[2, 16, 45] Therefore, by promoting pH increase, NACP has acid neutralizing[34] and remineralization capabilities.[8, 16, 48]

One study investigated the effect of increasing the chain length of a QAM incorporated in a NACP composite. The results showed that increasing the chain length from 3 to 16 greatly decreased the metabolic activity, acid production and enhanced the antibacterial activity. Further increasing the chain length to 18 resulted in a decrease in the antibacterial activity and did not improve the acid production or metabolic activity either. QAM+NACP composite had flexural strength and elastic modulus that matched a commercial control.[8]

According to Wu J. *et al,* NACP+DMAHDM composite exhibited a strong antibacterial activity and remineralizing properties to combat biofilms, acid production and to prevent secondary caries,

without compromising the mechanical properties of the composite. The composite also displayed good fracture resistance.[48]

A composite with NACP+QADM+NAg was developed by Cheng L. *et al*. The incorporation of QADM and/or NAg into the NACP composite resulted in a durable antibacterial activity and a flexural strength and elastic modulus comparable to those of a commercial control even after 12months of water-ageing. The combination of two antibacterial agents, NAg and QADM, lead to a lower metabolic activity, acid production and biofilm viability than using each of them alone.[2]

4. MPC

The first step of biofilm formation is bacterial adhesion to the salivary pellicle[13] that coats the tooth surface[9] and is formed by adsorbed salivary proteins.[39] Therefore a substance capable of inhibiting protein adsorption and consequently bacteria attachment would be highly desirable to prevent secondary caries by supressing biofilm formation.[13]

Regarding this situation, several studies incorporated MPC in their experimental adhesives and/or composites.[4, 13, 16, 19, 31, 39] A common characteristic in the results of these studies is that all the experimental adhesives and composites with MPC had significantly less bacterial adhesion[4, 13, 16, 39] showing that MPC does have the ability to reduce protein adsorption[4, 19, 31, 39] and thus to display protein-repellent effects.[4, 16] Since MPC has the ability to repel bacteria and reduce biofilm growth, there is a decrease in acid production and, as a consequence, a higher and less cariogenic pH.[16]

None of these novel biomaterials displayed a negative effect in dentin bond strength.[13, 16, 39] In fact, Zhang N. *et al* reported that the incorporation of 7.5%MPC in the adhesive resulted in a greater dentin bond strength than de commercial control.[31] In another study, the degree of conversion was studied and was not affected.[39] Xie X. *et al* created a composite that while having a flexural strength similar to the control, presented a decrease on the elastic modulus.[19]

The incorporation of 3%MPC[19] and 7.5%MPC[31, 39] in the adhesive resulted in a homogeneous dispersion throughout the volume and not limited to the surface.[19, 31, 39] Therefore, it is expected that the protein-repellent capability won't be lost by wear[19, 39] rendering the adhesive with durable effect.[19, 31]

The 6 months water ageing of the adhesive did not affect the protein-repellent capability, indicating excellent endurance of its resistance to protein adhesion.[31]

5. QACs + MPC

In order to improve the antibacterial effect and further reduce secondary caries risk, MPC was combined with QACs to form an antibacterial adhesive with protein-repellent activity.[39] Protein-repellent capability prevents the formation of the salivary pellicle that coats the antibacterial resin surface and separates it from oral biofilms, reducing the antibacterial effect.[4, 6]

The incorporation of MPC + DMAHDM in both composite[4] and adhesive system[13, 31, 39] resulted in a strong antibacterial and protein-repellent properties[4, 13, 39] since there was much less bacterial adhesion and the bacteria were mostly dead.[4, 31]

Increasing the mass fraction of DMAHDM to 2.25% and 3% in the composite caused a decrease in the mechanical properties[4] while in the adhesive system the bond strength was only reduced when DMAHDM mass fraction reached 7,5%.[39]

When used at lower concentrations than those referred, DMAHDM + MPC resin-based materials displayed mechanical properties similar to the control[4, 31, 39] that were maintained for 6 months of water-ageing[31, 39] as well as the antibacterial efficacy.[31]

The combination of QAC and MPC results in a synergistic effect on anti-biofilm properties[16, 39], in other words, using MPC + DMAHDM together in the adhesive resin resulted in a stronger antibacterial effect than using each of them alone.[4, 31, 39] The probable mechanism that justifies these findings is that MPC, by greatly reducing protein adsorption, would expose the resin surface and favour a more direct-contact of bacteria with DMAHDM, thus enhancing its contact-killing efficacy.[16, 31, 39]

6. QACs + MPC + Nanoparticles

Some authors explored the incorporation of both MPC and QACs into a NACP composite or adhesive system in order to achieve triple benefits of protein-repellent ability, antibacterial activity and remineralization capability, respectively.[9, 13, 16]

MPC, DMAHDM and NACP were incorporated in a composite[19] and in adhesive systems[13, 16] greatly reducing protein adsorption, biofilm growth and viability and also maintaining a high pH[19] due to the high concentration of Ca and P ions, which induced remineralization.[13, 16]

Composite with 3%MPC + 3%DMAHDM + 30%NACP resulted in a flexural strength similar to the control but a lower elastic modulus.[19]

NACP adhesive had dentin bond strength similar to the controls except when the filler level reached 40%.[13, 16] NACP-containing adhesive could be recharged to have durable effect.[16]

7. Other Agents

Different agents were also incorporated in dental resin-based materials to provide antibacterial activity, such as arginine[10], BTAM[40], TAT[22], CaF_2.[17]

Arginine, a releasing-agent, was incorporated in an experimental adhesive. Secreted in saliva, arginine is an amino acid that is metabolized by some oral bacteria to produce ammonia and thereby to neutralize acids and increase pH of biofilms, inhibiting tooth demineralization. Results show that there was a high rate of release in the first 2h due to the rapid dissolution and depletion of arginine, and that it decreased after 24h, settling after 3 days. This experimental adhesive showed no significant effects on the degree of conversion and on dentin microtensile bond strength compared to the control group. Adhesive system containing 7% arginine had minimal or no bacterial growth while showing release and moderate recharge of arginine over 30 days[10] which may result in a durable antibacterial effect.

In another study, Centenaro C.C. *et al* added BTAM, a compound with a triazole group, to an adhesive, which displayed antibacterial activity compared to the negative control but also lower values on the degree of conversion. The copolymerization with the adhesive prevents leaching and prolongs the antibacterial effect over time. After 2 hours of immersion on ethanol, all groups studied showed a reduction in microhardness values which may negatively affect the mechanical properties.[40]

TAT-containing orthodontic adhesive significantly reduced bacterial growth. This agent copolymerized with the adhesive matrix and when added at a concentration of 15% or 20% also increased the degree of conversion and shear bond strength.[22]

According to Lukomska-Szymanska M. *et al*, composite materials modified with CaF_2 reduced biofilm growth. Furthermore, in the presence of calcium fluoride, bacteria showed greater sensitivity to the acidic environment.[17]

8. Cavity Disinfectants

Another strategy used to reduce the risk of secondary caries and post-operative sensitivity consists in creating a more aseptic environment[49] through the elimination of residual bacteria[49, 50] before the application of resin-based restorative materials.[49] Several studies investigated the pretreatment of cavities with disinfectants such as CHX[49-52], MDPB[49, 51, 52], LASER[49], ozone[49], urushiol[50], NaOCL[50] and Nag.[53]) Most displayed strong antibacterial activity[49-51, 53] while influencing the mechanical properties of the restorative materials, either improving the bond strength[53] or decreasing it[50, 51], which can affect the longevity of the restorations.[52]

9. Resistance

Resistance to new antibacterial resin-based materials may prove to be a negative aspect to consider. However, Wang S. *et al* showed that both MDAHDM and CHX induced persistence in *S.mutans* biofilms but not resistance since there was no increase in the minimum inhibitory concentration (MIC) values of persisters when compared to parental strains. Furthermore, higher concentrations of these antibacterial agents could completely eradicate persister biofilms.(54)

10. Limitations and Future Prospects

Due to the fact that current studies and investigations adopt different methodologies and variables, testing a great variety of antibacterial agents on different microorganisms, and presenting an inconstant sample size, it is difficult to compare the antibacterial effects of the experimental biomaterials. For that, it would be necessary to standardize the investigation protocols.

Microcosm biofilm model using an inoculum from human saliva is a more reliable methodology than studying single species biofilms because it better resembles the oral microbiota. Nevertheless, there are still few studies using this approach. *In situ* studies would also be desirable since they duplicate no only the oral biofilms, but also the cariogenic challenges.

More studies are necessary to ensure the antibacterial effects of the tested materials as well as their safety, biocompatibility and mechanical properties, before their incorporation in commercial materials.

CONCLUSION

The incorporation of antibacterial agents in resin-based restorative materials is a promising strategy to diminish the risk of secondary caries and white spot lesions associated to orthodontic brackets since both, the reduction of the bacterial load and the increase in pH, influence the dynamic process of remineralization/demineralization favouring dental remineralization. This permits a superior preservation of dental tissue, reducing the risk of pulpal exposure and post-operative sensitivity, which follows the ideals of Minimally Invasive Dentistry.

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ATTACHMENTS

Attachment 1

Table III – Studies that incorporated antibacterial agents in adhesive systems

Publication Year	Authors	Tested Microorganisms	Active Principle	Incorporation in the Adhesive and/or Primer	Sample	Main Results
2014	Li F. *et al*	*Streptococcus mutans*	DMADDM 2.5%, 5%, 7.5% and 10%	Adhesive and Primer	Human third molars (dentin) + Resin disks	Increasing the charge density of a bonding system greatly reduced *S. mutans* adhesion and decreased biofilm CFU without negatively affecting dentin bond strength.
2014	Li F. *et al*	Microcosm biofilm model (inoculum from human saliva)	DMADDM 5%; NAg 0.1%	Adhesive and Primer	Resin disks	Bonding agents containing DMADDM or NAg greatly inhibited biofilm activity, CFU counts and lactic acid production, even when salivary pellicles were present.
2014	Banzi É.C.F. *et al*	*Streptococcus mutans* *Streptococcus sobrinus* *Streptococcus gordonii* *Actinomyces viscosus* *Lactobacillus lactis* (single species and mixed bacterial suspensions)	MDPB - Clearfil SE Protect	Primer	Dentin bonding system disks	MDPB significantly reduced the viability and metabolic activity of the tested bacteria both on single and multispecies bacterial cultures.
2014	Sande F.H. *et al*	–	MDPB - Clearfil SE Protect	Primer	Lower prosthesis with samples of human molars (dentin)	Adhesive system with MDPB displayed significantly lower mineral loss next to dentin-composite interface than the control.
2014	Vasconcelos S.M.L.C. *et al*	Mutans streptococci Lactobacilli	MDPB - Clearfil SE Protect + Fluoride	Primer (MDPB)	Intraoral palatal appliances with two slabs of human third molars (enamel)	The tested adhesive systems containing or not fluoride and/or MDPB did not inhibit caries recurrence in the *in situ* model used in this research.
2014	Wang S. *et al*	*Streptococcus mutans*	DMADDM 2.5% and 5%	Adhesive	Adhesive-coated composite disks + Human third molars (dentin)	Adhesives containing DMADDM reduced *S. mutans* biofilm growth, lactic acid production and EPS metabolism with no adverse effect on dentin bond strengh.
2014	Feitosa S.A. *et al*	*Streptococcus mutans*	HNT 15% + DOX 10%	Adhesive	Adhesive disks + Human third molars (dentin)	The incorporation of DOX resulted in the ability to inhibit MMPs and *S. mutans* growth by direct contact, without affecting microtensile bond strength.
2015	Zhang K. *et al*	*Streptococcus mutans* *Streptococcus gordonii* *Streptococcus sanguinis*	DMADDM 2.5% and 5%	Adhesive	Adhesive disks	Adhesives containing DMADDM significantly inhibited multispecies biofilm growth, acid production, and EPS synthesis, mainly when added at a concentration of 5%.
2015	André C.B. *et al*	*Staphylococcus aureus* *Enterococcus faecalis* *Lactobacillus casei* *Streptococcus mutans* *Porphyromonas gingivalis* *Prevotella intermedia* *Prevotella nigrescens* *Fusobacterium nucleatum*	MDPB - Clearfil SE Protect; Glutaraldehyde 5%; CHX 0.2% and 2%	Adhesive (Glutaraldehyde and CHX) Primer (MDPB)	Human third molars (dentin) + Direct contact of the tested materials	All four adhesives presented antibacterial activity and did not suffer significant variations on bond strength after storage in artificial saliva. Glutaraldehyde and CHX eliminated all tested microorganisms in 5min while MDPB needed 30min to destroy most of them.

Table III – Studies that incorporated antibacterial agents in adhesive systems (cont.)

Publication Year	Authors	Tested Microorganisms	Active Principle	Incorporation in the Adhesive and/or Primer	Sample	Main Results
2015	Centenaro C.C. *et al*	*Streptococcus mutans*	BTAM 1%, 2.5% and 5%	Adhesive	Direct contact of the tested materials + Adhesive specimens + Bovine lower incisors (dentin)	Adhesive containg 5% BTAM showed the highest antibacterial activity against *S. mutans* and the lowest values of Knoop microhardness after ethanol immersion.
2015	Sabatini C. *et al*	*Streptococcus mutans*	PAA-CuI nanoparticles 0.5mg/ml and 1.0mg/ml	Adhesive (etch-and-rinse system) Adhesive and Primer (self-etch system)	Adhesive-coated composite disks + Adhesive disks + Human molars (dentin)	PAA-CuI containing adhesives demonstrated strong antibacterial properties with no significant variations in shear bond strength or cytotoxicity.
2015	Zhang N. *et al*	Microcosm biofilm model (inoculum from human saliva)	MPC 7.5% + DMAHDM 5% + NACP 20%, 30% and 40%	Adhesive and Primer (DMAHDM and MPC) Adhesive (NACP)	Human Molars (dentin) + Bovine incisors (root dentin) + Resin disks	7.5%MPC + 5%DMAHDM + 30%NACP group greatly reduced protein adsorption, bacteria attachment, metabolic activity, biofilm growth, CFU counts and lactic acid production, without compromising the dentin shear bond strength.
2015	Zhang N. *et al*	Microcosm biofilm model (inoculum from human saliva)	MPC 7.5% + DMAHDM 5%, 7.5% and 10%	Adhesive and Primer	Human molars (dentin) + Resin disks	7.5%MPC + 5%DMAHDM containing adhesive greatly reduced bacterial adhesion, CFU counts, metabolic activity and lactic acid production, displaying a synergistic effect without adversely affecting dentin shear bond strength.
2016	Melo M.A.S. *et al*	Microcosm biofilm model (inoculum from human saliva)	NAg 0.1% + DMAHDM 5%	Adhesive and Primer	Human third molars (dentin)	Multi-agent resin-based materials reduced the cariogenic impact of the biofilm, improving the strength and fatigue resistance of the bonded interface.
2016	Chen C. *et al*	*Streptococcus mutans*	DMADDM 2.5%, 5%, 7.5% and 10%; QADM 10%	Primer	Human molars (dentin blocks) + Paper disks	DMADDM-containing primer had a much stronger antibacterial effect than QADM without negatively affecting the dentin bond strength.
2016	André C.B. *et al*	*Staphylococcus aureus Enterococcus faecalis Lactobacillus casei Streptococcus mutans Porphyromonas gingivalis Prevotella intermedia Prevotella nigrescens Fusobacterium nucleatum*	MDPB - Clearfil Protect Bond; CHX 0.2% and 2%; Glutaraldehyde 5%	Adhesive (glutaraldehyde and CHX); Primer (MDPB)	Transparent plastic cylinders filled with adhesive + Adhesive-coated hydroxyapatite disks + Human third molars (dentin)	Bonding agent containing MDPB showed a decrease on *S. mutans* biofilm's viability and greater inhibition for all oral pathogens tested than glutaraldehyde and CHX.
2017	Xie X. *et al*	Microcosm biofilm model (inoculum from human saliva)	MPC 5% + DMAHDM 5% + NACP 20%, 30% and 40%	Adhesive and Primer (DMAHDM and MPC); Adhesive (NACP)	Human third molars (dentin) + Resin disks	Adhesive containing NACP+MPC+DMAHDM showed a faster pH increase, a strong protein-repellent capability and a decrease in bacteria attachment and viability, reducing the biofilm CFU.

Table III – Studies that incorporated antibacterial agents in adhesive systems (cont.)

Publication Year	Authors	Tested Microorganisms	Active Principle	Incorporation in the Adhesive and/or Primer	Sample	Main Results
2017	Geraldeli S. *et al*	*Streptococcus mutans* *Streptococcus gordonii*	Arginine 5%, 7% and 10%	Adhesive	Adhesive specimens+ Human third molars (dentin)	7% arginine containing adhesive was released at a rate and concentration that exhibited antibacterial effects without compromising physical and mechanical properties.
2018	Zhang N. *et al*	Microcosm biofilm model (inoculum from human saliva)	MPC 7.5% + DMAHDM 5%	Adhesive and Primer	Resin disks + Human molars (dentin)	Bonding agent containing 7,5%MPC + 5%DMAHDM had significantly greater dentin bond strength than a commercial control after water-ageing for 6 months, without losing its protein-repellent and anti-biofilm effects.
2018	Florez F.L.E. *et al*	*Streptococcus mutans*	TiO2 nanoparticles 50%, 67% and 80% (v/v)	Adhesive	Adhesive specimens	Adhesives containing TiO2 nanoparticles demonstrated strong antibacterial behaviour in dark and light irradiated conditions.

Attachment 2

Table IV – Studies that incorporated antibacterial agents in composite resins

Publication Year	Authors	Tested Microorganisms	Active Principle	Sample	Main Results
2014	Shvero D.K. *et al*	*Enterococcus faecalis* *Streptococcus mutans* *Actinomyces viscosus* *Lactobacillus casei* Whole saliva bacteria from clinical isolate	QPEI nanoparticles 1wt% and 2wt%	Direct contact of the tested materials + Composite disks	The incorporation of a small percentage of QPEI nanoparticles achieved a wide range antibacterial effect without affecting the degree of conversion.
2014	Zhang J.F. *et al*	*Streptococcus mutans* *Lactobacillus casei*	MSN + CHX 3%, 5%, and 6.3%; CHX 3%, 5%, and 6.3%	Composite specimens	Composite containing CHX+MSN significantly reduced bacterial growth through the recharge and release of CHX, without compromising mechanical strength, aesthetics and surface integrity.
2015	Zhang K. *et al*	Microcosm biofilm model (inoculum from human saliva)	NACP 20% + QAMs 3% - DMAPM (CL3), DMAHM (CL6), DMADDM (CL12), DMAHDM (CL16) and DMAODM (CL18).	Composite specimens/ disks	Composite containing NACP + DMAHDM (CL16) significantly reduced biofilm CFU, metabolic activity and acid production without negatively affecting the composite mechanical properties.
2015	Wu J. *et al*	Microcosm biofilm model (inoculum from human saliva)	NACP 20% + DMAHDM 0.75%, 1.5%, 2.25% and 3%	Composite specimens/ disks	Increasing the DMAHDM mass fraction in NACP nanocomposite showed stronger antibacterial activity, reducing biofilm growth and acid production without adversely affecting the fracture toughness.
2015	Zhang N. *et al*	Microcosm biofilm model (inoculum from human saliva)	MPC 3% + DMAHDM 1.5%, 2.25% and 3%	Composite specimens	The composite with 3%MPC + 1.5%DMAHDM achieved great protein-repellent and antibacterial capabilities, reducing bacteria attachment, biofilm growth, metabolic activity and lactic acid production, without compromising the mechanical properties.
2016	Melo M.A.S. *et al*	Microcosm biofilm model (inoculum from human saliva)	NAg 0.1% + DMAHDM 5% + NACP 30%	Human third molars (dentin)	Multi-agent resin-based materials reduced the cariogenic impact of the biofilm, improving the strength and fatigue resistance of the bonded interface.
2016	Cheng L. *et al*	Microcosm biofilm model (inoculum from human saliva)	NACP 20% + QADM 6% + NAg 0.1%	Composite disks	Incorporation of QADM and/or NAg into the NACP composite showed great antibacterial activity, reducing biofilm viability, metabolic activity, CFU counts and lactic acid production without negatively affecting the composite mechanical properties. The antibacterial potency was maintained after 12 months of water-ageing.
2016	Lukomska-Szymanska M. *et al*	*Streptococcus mutans* *Lactobacillus acidophilus*	CaF2 1.5wt%, 2.5wt% and 5wt%	Composite specimens	Composite materials modified with CaF2 reduced biofilm growth especially when used at a concentration of 1.5wt%.
2016	Ai M. *et al*	*Streptococcus mutans*	HA–PDA–Ag nanowires 4wt%, 6wt%, 8wt% and 10 wt%	Composite specimens	Composites with HA–PDA–Ag nanowires demonstrated high antibacterial activity and significantly improved flexural properties when added at a concentration of 8wt%.
2016	Xie X. *et al*	Microcosm biofilm model (inoculum from human saliva)	NACP 30% + DMAHDM 1.5% and 3% + MPC 3%	Composite specimens/ disks	3%MPC + 3%DMAHDM group inhibited biofilm growth and viability, maintaining a flexural strength similar to the control. There was a decrease in the elastic modulus.

Table IV – Studies that incorporated antibacterial agents in composite resins (cont.)

Publication Year	Authors	Tested Microorganisms	Active Principle	Sample	Main Results
2016	Pietrokovski Y. *et al*	*Streptococcus mutans* *Actinomyces viscosus*	QPEI nanoparticles 1wt%	Direct contact of the tested materials + Composite disks	Resin-based foundation material incorporating 1wt% QPEI nanoparticles exhibited strong antibacterial activity.
2018	Huang Q. *et al*	*Streptococcus mutans* Human saliva	UDMQA-12 9%	Direct contact of the tested materials + Composite specimens/ disks	Composite with UDMQA-12 inhibited *S. mutans* growth, showing significant antibacterial effects while maintaining the mechanical properties and biocompatibility.

Attachment 3

Table V – Studies that incorporated antibacterial agents in orthodontic adhesives and cements

Publication Year	Authors	Tested Microorganisms	Active Principle	Sample	Main Results
2014	Passariello C. *et al*	*Streptococcus gordonii* *Streptococcus sanguinis* *Streptococcus mutans* *Lactobacillus acidophilus*	MDPB - Clearfil Protect Bond; benzalkonium chloride 2.5% and 5%	Adhesive disks	The addition of benzalkonium chloride or MDPB to a resin adhesive showed a strong antibacterial activity which was maintained after 180 days.
2014	Melo M.A.S. *et al*	Microcosm biofilm model (inoculum from human saliva)	DMADDM 1.5%, 3% and 5%	Orthodontic cement disks + Human first premolars (enamel)	3% DMADDM group inhibited oral biofilms, greatly reducing bacterial viability, metabolic activity and lactic acid production, without compromising the enamel bond strength.
2015	Altmann A. *et al*	*Streptococcus mutans*	TAT 10%, 15% and 20%	Adhesive specimens + Bovine Incisors (enamel)	TAT reduced *S. mutans* growth by direct contact and increased the shear bond strength with no enamel fracture observed.
2015	Figueroa L. *et al*	*Staphylococcus aureus* *Escherichia coli* *Streptococcus mutans*	copper nanoparticles 0.0100wt%, 0.0075wt% and 0.0050wt%	Paper disks + Human premolars (enamel)	Only the addition of 0.0100 wt% copper NPs expressed antibacterial activity and increased material shear bond strength without negatively affecting colour and appearance.
2017	Nascimento P. *et al*	Microcosm biofilm model (inoculum from human saliva)	MADQUAT 5% and 10%	Human premolars (enamel)	Adhesive containing MADQUAT reduced the integrated mineral loss only when added at a concentration of 10% but also resulted in the lowest values of bond strength.
2017	Yu F. *et al*	*Streptococcus mutans*	MAE-HB 1wt%, 3wt% and 5wt%	Adhesive specimens + Human premolars (enamel)	MAE-HB-incorporated adhesive exhibited a strong contact inhibitory effect on the growth of *S. mutans* without compromising the shear bond strength.
2017	Oz A.Z. *et al*	–	MDPB - Clearfil Protect Bond	Human first premolars (enamel)	There was no significant difference between white spot lesion (WSL) rates of the adhesives.
2017	Feng X. *et al*	Microcosm biofilm model (inoculum from human saliva)	DMAHDM 1.5% and 3%	Orthodontic cement disks + Human first premolars (enamel)	3% DMAHDM group obtained a strong antibacterial effect, reducing biofilm metabolic activity, lactic acid production and CFU counts, without compromising the enamel bond strength.
2017	Andriani A. *et al*	–	TiO2 nanoparticles 1% and 2%	Human upper first premolars (enamel)	2% TiO2 nanocomposites have the ability to maintain enamel microhardness within the range of normal values indicating the presence of antibacterial effect.
2018	Wang X. *et al*	Microcosm biofilm model (inoculum from human saliva)	DMAHDM 1.5%, 3%, 5% and 7.5%	Adhesive Disks + Human premolars (enamel)	5% DMAHDM group displayed the strongest antibacterial function without compromising the enamel bond strength.

Attachment 4

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DECLARAÇÃO DE AUTORIA DO TRABALHO APRESENTADO

Declaro que o presente trabalho, com o título: "RESIN-BASED BIOMATERIALS WITH ANTIBACTERIAL ACTIVITY", no âmbito da Monografia de Investigação, integrado no MIMD da FMDUP, é da minha autoria e todas as fontes foram devidamente citadas

Porto, 16 de maio de 2019

A investigadora,

Daniela Soares Pinto

(Daniela Soares Pinto)

Attachment 5

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PARECER DO ORIENTADOR PARA ENTREGA DEFINITIVA DO TRABALHO

Informo que o Trabalho de Monografia desenvolvido pela Estudante Daniela Soares Pinto com o título: "RESIN-BASED BIOMATERIALS WITH ANTIBACTERIAL ACTIVITY" está de acordo com as regras estipuladas na FMDUP, foi por mim conferido e encontra-se em condições de ser apresentado em provas públicas.

Porto, 16 de maio de 2019

A Orientadora,

(Professora Doutora Ana Isabel Pereira Portela)