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Biofilm Inhibition of Bioactive Versus Conventional Resin Composite Restorative Materials: An in Vitro Comparative Study

Nadia Mohamed Zaghloul[1], Heba Elsayed Eldegla[2], Naglaa Rizk Elkholany[1], Ihsan Sabah Jawad[1*]

[1]Department of Conservative Dentistry Department, Faculty of Dentistry, Mansoura University
[2]Department of Medical Microbiology and Immunology, Faculty of Medicine, Mansoura University

[*]**Corresponding author:** Ihsan Sabah Jawad
E-mail:ihsanmans3@gmail.com

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Abstract
Cariogenic bacteria in tooth and resin restorations lead to caries and secondary caries. Biofilms typically develop on diverse surfaces such as indwelling medical devices and implants, natural and portable pipework for water systems, fermentation containers, food businesses, and living tissues. The effect of the biofilm on the degradation of the interfacial adhesion between the composite and the tooth structure has been demonstrated. Demineralization refers to the process by which organic acids produced by plaque microorganisms destroy the mineral content from the surface of HA crystals. The most stable form of hydroxyapatite exists in an environment with a pH of 7.4. There is a constant chemical equilibrium between the hydroxyapatite in the enamel (Ca10(PO4)6(OH)2) and the dissolved hydroxyapatite in the plaque biofilm. Saliva protects against demineralization due to its cleansing effects and mineral composition, buffering pH changes and promoting remineralization. Dentin recovery is more complex than enamel due to the reconstitution of organic type I collagen and inorganic apatite. Remineralization alone is insufficient for full recovery, as it requires restoring collagen matrix structure and linking both phases.
Keywords: Biofilm Inhibition, Conventional Resin Composite, Restorative Materials

Introduction

Cariogenic bacteria in tooth and resin restorations lead to caries and secondary caries. Biofilms typically develop on diverse surfaces such as indwelling medical devices and implants, natural and portable pipework for water systems, fermentation containers, food businesses, and living tissues. Gram-negative bacteria such as Pseudomonas aeruginosa, Pseudomonas fluorescence, Escherichia coli, and Vibrio cholera are being investigated for their ability to form biofilms. On the other hand, Gram-positive bacteria like Staphylococcus aureus and Staphylococcus epidermis have been extensively studied for their biofilm formation**(1).**

Accumulation in resins is significantly influenced by substratum surface properties such as leaching out of unreacted monomers, composite leachable ions, and/or the physical property of surface roughness. In addition, a salivary agglutinin glycoprotein (gp340) may contribute to the attachment of the biofilm at the vulnerable resin interface between the restoration and the tooth,Streptococcus mutans has the capability to produce extracellular polysaccharides (EPS) from sucrose, which helps in regulating the pH of biofilms and modifying the surface of resin. The most probable cause of this is the action of bacterial enzymes known as microbial glucosyltransferases (Gtfs)**(2).**

This study aimed to compare and evaluate the biofilm inhibition of two contemporary bioactive restorative materials versus a conventional resin composite.

Oral biofilm

The presence of cariogenic bacteria in tooth and resin restorations leads to the development of caries and secondary caries. Biofilms typically develop on diverse surfaces such as indwelling medical devices and implants, natural and portable pipework for water systems, fermentation containers, food businesses, and living tissues.[1] Gram-negative bacteria such as Pseudomonas aeruginosa, Pseudomonas fluorescence, Escherichia coli, and Vibrio cholera are being investigated for their ability to form biofilms. On the other hand, Gram-positive bacteria like Staphylococcus aureus and Staphylococcus epidermis have been extensively studied for their biofilm formation. In addition to these, Streptococcus species such as Group A streptococci, Viridans group streptococci, Haemophillus influenza, and Actinomyces israelli are also extensively studied for their ability to form biofilms in various infectious processes. In contrast, certain bacteria, such as species of Lactobacillus, which are present in the digestive tract flora, create biofilms that serve as a protective barrier, preventing the colonization of hazardous pathogens**(3).**

Accumulation in resins is influenced by surface properties like leaching, composite leachable ions, and surface roughness. Salivary agglutinin glycoprotein (gp340) may attach biofilms at resin interfaces. Streptococcus mutans produces extracellular polysaccharides (EPS) from sucrose, regulating biofilm pH and resin surface modification through microbial glucosyltransferases**(4).**

Secondary caries and Demineralization

The effect of the biofilm on degradation of the interfacial adhesion between the composite and the tooth structure has been demonstrated. [17] Demineralization refers to the process by which organic acids produced by plaque microorganisms destroy the mineral content from the surface of HA crystals. The most stable form of hydroxyapatite exists in an environment with a pH of 7.4. There is a constant chemical equilibrium between the hydroxyapatite in the enamel ($Ca_{10}(PO_4)_6(OH)_2$) and the dissolved hydroxyapatite in the plaque biofilm. Mineral crystal dissolution takes place when the pH level of the plaque falls below**(5).**

Secondary caries can be linked to defective restorations, intact restorations, or primary caries adjacent to existing restorations. These can occur due to gaps between the restoration and the tooth, lower buffering capacity of the restoration, or inadequate patient-level address of the caries process**(6).** The resulting pathogenic pathways may lead to demineralization on the tooth surface, as seen in primary carious lesions, and along the interface with a surface and wall lesion. These pathogenic pathways can result in demineralization on the tooth surface**(7).**

Saliva protects against demineralization due to its cleansing effects and mineral composition, buffering pH changes and promoting remineralization. Dentin recovery is more complex than enamel due to the reconstitution of organic type I collagen and inorganic apatite. Remineralization alone is insufficient for full recovery, as it requires restoring collagen matrix structure and linking both phases**(8).** Biomineralization uses biomimetic analogs of dentin matrix proteins to induce amorphous calcium phosphate nanocrystals within collagen fibers, creating nanocrystals that fit into gap zones, establishing a hierarchical order in mineralized collagen**(9).**

"Bioactive" material

Biomaterials, bioactive substances, bioinductive substances, and biomimetics are terms used to describe substances, surfaces, or designs that interact with biological systems. Biomaterials are substances that elicit specific reactions or impact living organisms, tissues, or cells. Bioactive substances stimulate specific reactions, while bioinductive substances provoke reactions in biological systems. Biomimetics is a synthetic procedure that mimics natural processes of living organisms, aiming to reproduce the creation, arrangement, or operation of biologically derived substances, materials, and mechanisms**(10).** Bioactive materials aid in mineral attachment to dentin, promoting remineralization of demineralized dentin. These materials have sterility, bactericidal or bacteriostatic properties, stimulate reparative dentin production, and preserve pulp vitality. Their surface chemistry, structure, and surrounding liquid microenvironment properties support mineralization**(11).**

Historically, polymeric materials used for restorations have not been bioactive, increasing the risk of secondary caries formation in resin-based composite restorations. To address this, scientists are developing antibacterial properties to decrease bacterial adhesion, inhibit biofilm formation, and impede demineralization**(12).** The incorporation of bioactive compounds into dental polymer formulations is crucial for controlling tooth decay around restorations. Research has explored integrating antibacterial substances or particles and employing remineralization procedures to counteract oral biofilm acidity and hinder biofilm formation**(13).**

Antibacterial Resin Restorative composites

Composite resin materials are typically used to repair decayed areas of enamel and dentin. Since its inception in dentistry, the mechanical characteristics of composites have undergone tremendous enhancements. However, the act of installing these restorations does not effectively target the caries-related bacteria that are present in other areas of the mouth. These bacteria have a high propensity to generate new plaque on the composite surface, frequently resulting in recurrent caries around the repair, as previously explained**(14).**Several researchers have explored composite materials that incorporate antibacterial components released gradually over time**(15).**

Examined multiple publications about these materials, distinguishing three classifications of antibacterial substances: leachable agents such as benzalkonium chloride and chlorhexidine, polymerizable monomers like quaternary ammonium (QA) methacrylate, and filler particles that include Nano silver. From 2012 to 2017, researchers researched many antibacterial chemicals. However, only four of these - benzalkonium chloride, chlorhexidine, glutaraldehyde, and 12-ethacryloyloxydodecylpyridinium bromide - were actually employed in commercial products. Over time, the slow release of these chemicals led to reduced antibacterial effectiveness, deteriorated composite material, and discolouration**(16).** Hence, there is a requirement for composite restorative materials that integrate antibacterial compounds which possess long-lasting antibacterial efficacy without being discharged. The primary disadvantage of releasing agents, such as chlorhexidine and nano or micro-sized silver and zinc particles, is their absence of chemical adhesion to the polymer matrix. This leads to unregulated, fast discharge, possible health hazards, and reduced compatibility with living organisms**(17).**

Resin restorative composites with antibacterial properties that include nonleaching antibacterial components.

Several research groups are investigating composite restorative materials that incorporate antibacterial components which are not released over time**(18).**The objective is for the antibacterial element to retain its effectiveness even when concealed by dental plaque. A research team at the University of Pennsylvania has done laboratory experiments on a novel composite material that incorporates a non-leaching antibacterial component. This composite demonstrates potent antibiofilm properties by combining a polymerizable antibacterial resin containing imidazolium (ABR) with a modified composite based on methacrylate (ABR-MC)(**18**). The addition of the ABR moiety at a concentration of around 2% resulted in the composite material retaining its mechanical strength while exhibiting bioactivity and low cytotoxicity. The antibiofilm capabilities of the composite were evaluated using a Streptococcus mutans biofilm model. The results showed that the composite modified the structure of the biofilm by inhibiting the formation of structured bacterial clusters on its surface. On the other hand, the control composite facilitated the development of a durable biofilm. Crucially, the mechanical qualities of the composite were intact despite the inclusion of the antibacterial component. The laboratory experiments demonstrate the capability of this novel antibacterial composite, which does not leach, to prevent the recurrence of tooth decay surrounding dental restorations**(19).**

However, in vivo and human clinical studies are yet to be reported. In 2021, a comprehensive laboratory study of a novel resin that integrates both antibacterial and remineralizing components has been reported**(20).**The researchers created a nanocomposite that has low shrinkage, antimicrobial properties, and the ability to remineralize. The composition of this resin consists of urethane dimethacrylate, triethylene glycol divinylbenzyl ether, 3% dimethylaminohexadecyl methacrylate, and 20% nanoparticles of amorphous calcium phosphate. Through experimentation using a laboratory model, they proved that the resin's ability to kill bacteria remained effective for several months in an acidic environment that contained a biofilm. Although this substance exhibits potential, currently only data obtained in laboratory settings are accessible**(21).**

Antibacterial resin restorative composites that include QA compounds.

Quaternary ammonium (QA) compounds have been employed for many years as highly effective antibacterial agents for a wide range of uses, such as food packaging and biomedical applications. [37] A significant amount of study has been devoted to discovering compounds with excellent antibacterial properties for specific uses in the field of QA. Research in dental materials indicates that quality assurance compounds with chain lengths ranging from 12 to 16 carbon atoms exhibit the highest level of effectiveness. [38] A comprehensive review conducted by Makvandi et al. (**22**)Addressed were articles pertaining to quality assurance chemicals in dental materials

Two types of materials are QA polyethylenimine (QPEI) and QA Silica (QASI).

A highly promising antibacterial agent is quaternary ammonium polyethylenimine (QPEI), which exists in the form of tiny polymeric particles.' QPEI is formed through the polymerization of a powerful antibacterial quaternary ammonium compound into tiny particles. These particles can be integrated into dental composites, cements, or sealants without losing their antibacterial properties and without releasing any substances over time. For many years, quaternary ammonium compounds have been utilized as antibacterial agents in a wide range of applications. A comprehensive investigation was carried out, wherein QPEI particles were added at a concentration of 1% (weight/weight) to bonding, flowable, and hybrid dental composite resins commonly used in clinical practice. One hundred twenty The antibacterial activity and important mechanical properties of these composite materials were investigated, yielding promising findings**(23).**

Additionally, an exhaustive laboratory study by the same group assessed the effect of incorporated QPEI on bacterially induced changes in composite materials, including surface roughness over time,A specific research group evaluated an orthodontic cement incorporating QPEI particles at concentrations of 0%, 1%, and 1.5% (wt/wt)(**24**).The material containing 1.5% QPEI consistently exhibited antibacterial activity against S. mutans in the direct contact model throughout the duration of the experiment. Similarly, dental materials that include quaternary ammonium silica (QASi) have been subjected to thorough laboratory testing and have demonstrated outstanding antibacterial capabilities in several investigations**(25).**

Featherstone et al. (**25**)study The study determined that dental restorative materials containing QASi (Infinx resin composite) has long-lasting antibacterial qualities, exhibit mechanical properties that are comparable to currently available materials in the market, and have received clearance from the US Food and Drug Administration(**25**).Clinical studies Evidence suggests that composites containing QASi have a notable impact on reducing tooth decay in the area surrounding dental restorations. Restorative materials that have long-lasting antibacterial qualities will greatly reduce the occurrence of secondary caries, considering how common caries is in such places.

Scanning Electron Microscope (SEM)

Scanning electron microscopy (SEM) is a widely used method for assessing minute structural alterations in dental hard tissues. Since 1962, it has been utilized in the field of dentistry. Currently, the majority of research facilities at universities and institutions has their own scanning electron microscope (SEM) facility. SEM is capable of generating three-dimensional images that accurately depict the microscopic structure of the sample. This is generated by directing an electron beam with a precise amount of energy towards a sample using an electric cannon. The impact generates a series of signals that are gathered by a detector to create a highly magnified micrograph(**26**).

The scanning electron microscope (SEM) produces two types of signals for surface detection and picture recording: secondary electrons (SE) and backscattering electrons (BSE). Backscattered electron (BSE) imaging is produced by the elastic collision between electrons and massive atoms Secondary electron (SE) imaging is the result of inelastic interaction between electrons and atoms. Unlike Bovine Spongiform Encephalopathy (BSE), Scrapie Encephalopathy (SE) does not originate from deeper regions of the sample(**27**). Therefore, it is beneficial for assessing the topography of the surface of the sample. Traditional scanning electron microscopy (SEM) necessitates the thorough drying of the sample under high vacuum conditions, and subsequently applying a carbon or gold coating to prevent the accumulation of electric charges. Regrettably, this may result in the deterioration of valuable artifacts. Therefore, the limitations can be partially mitigated by employing environmental scanning electron microscopy (SEM). Environmental scanning electron microscopy (SEM) utilizes a reduced level of vacuum to enable the study of samples in humid settings(**28**). The use of Environmental Scanning Electron Microscopy (ESEM) shows promise as a method that eliminates the need for sample pretreatment. Additionally, it has the capability to conduct several exams on the identical material. In cariology study, SEM examinations have been demonstrated to be efficient in assessing surface alterations caused by mineral dissolution or particle precipitation. It is regarded as an optimal descriptive method for assessing the interaction between the tooth structure and restoration. SEM analysis at the enamel lesion level yielded ample information regarding demineralization and the precipitation of minerals. It provided ample information regarding etching designs**(29)(30).**

Conclusion

We concluded that Contemporary bioactive resin composites, particularly bioactive resin composite (Infinix), demonstrate superior biofilm inhibition and ion release compared to conventional resin composites. The bioactive self-adhesive resin composite (Surefill One) has superior ion release capabilities but limited by poor remineralizing potential due to gap formation, low mineral uptake over time, and inadequate antibacterial properties.

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