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Antibacterial effect of bioactive glass incorporated in acrylic resins against *Streptococcus mutans* and *Lactobacillus acidophilus* activity in biofilm

Efeito antibacteriano do vidro bioativo incorporado em resinas acrílicas sobre a atividade de *Streptococcus mutans* e *Lactobacillus acidophilus* em biofilme

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ABSTRACT

Objective: The rough surfaces of removable appliances used in pediatric dentistry or orthodontics, may result in an environment for biofilm accumulation, yielding to enamel demineralization. This study aimed to assess the effects of adding nanoparticles of bioactive glass to polymethylmethacrylate to promote the antibacterial activity in acrylic resins. Material and Methods: Acrylic resin specimens (20x20x1mm3) were prepared by adding 2% or 5% bioactive glass. The specimens in the control group without bioactive glass were prepared from the mixture of acrylic powder containing nanoparticles and liquid monomer (n=10 per group). The antibacterial activity of the specimens against *Streptococcus mutans* and *Lactobacillus acidophilus* activity in biofilm was investigated through counting colony forming units (CFU). Data were analyzed using a one-way analysis of variance and Tukey's post hoc tests at the significance level of 0.05. Results: The incorporation of 2% ($p=0.001$) and 5% ($p<0.001$) bioactive glass in acrylic resin reduced the metabolic activity and CFU of *L. acidophilus*. For S.mutans, antimicrobial activity was observed only with the 5% concentration of bioactive glass, and this group was statistically different from the control ($p<0.001$). When *L. acidophilus* was exposed to polymethyl methacrylate with 5% bioactive glass, significant decrease was observed compared to the control group ($p<0.05$). Conclusion: Adding bioactive glass nanoparticles into the acrylic resins used for fabricating removable appliances revealed a greater antibacterial effect against cariogenic bacteria tested.

KEYWORDS

Acrylic resins; Anti-bacterial agents; Bioactive glass; Dental materials; Pediatric dentistry.

RESUMO

Objetivo: As superfícies rugosas dos aparelhos removíveis utilizados em Odontopediatria ou Ortodontia, podem resultar em um ambiente para acúmulo de biofilme, cedendo à desmineralização do esmalte. Este estudo teve como objetivo avaliar os efeitos da adição de nanopartículas de vidro bioativo ao polimetilmetacrilato para promover a atividade antibacteriana em resinas acrílicas. Material e Métodos: Amostras de resina acrílica (20x20x1 mm3) foram preparadas pela adição de 2% ou 5% de vidro bioativo. Os corpos de prova do grupo controle sem vidro bioativo foram preparados a partir da mistura de pó acrílico contendo nanopartículas e monômero líquido (n=10 por grupo). A atividade antibacteriana dos espécimes sobre a atividade de *Streptococcus mutans* e *Lactobacillus acidophilus* em biofilme foi investigada através da contagem de unidades formadoras de colônias (UFC). Os dados foram analisados por meio de análise de variância unidirecional e testes post hoc de Tukey com nível de significância de 0,05. Resultados: A incorporação de 2% ($p=0,001$) e 5% ($p<0,001$) de vidro bioativo em resina acrílica reduziu a atividade metabólica e UFC de *L. acidophilus*. Para *S. mutans*, a atividade antimicrobiana foi observada apenas com a concentração de 5% de vidro bioativo, sendo este grupo estatisticamente diferente do

controle (p<0,001). Quando *L. acidophilus* foi exposto ao polimetilmetacrilato com 5% de vidro bioativo, foi observada diminuição significativa em relação ao grupo controle (p<0,05). Conclusão: A adição de nanopartículas de vidro bioativo nas resinas acrílicas utilizadas na fabricação de aparelhos removíveis revelou um maior efeito antibacteriano contra as bactérias cariogênicas testadas.

PALAVRAS-CHAVE

Resinas acrílicas; Agentes antibacterianos; Vidro bioativo; Materiais dentários; Odontopediatra.

INTRODUCTION

Removable appliances used in pediatric dentistry or orthodontic treatments are often fabricated from polymethyl methacrylate (PMMA) [1,2]. Removable space holders or orthodontic appliances often yield to plaque retention, resulting in bacterial accumulation and thereby, dental and periodontal problems [3,4].

Dental biofilm is a complex structure consisting of bacteria with its own unique nutrition system [5]. *Streptococcus mutans* and *Lactobacillus acidophilus* are in particular responsible for caries lesions. The roles of *S. mutans* at the onset of caries and *L. acidophilus* in caries progression have been well-established [6]. Hence, the fraction of these two bacteria in the saliva is considered as an important parameter in caries susceptibility in the oral cavity [6].

Lately, plaque retention and increased caries incidence during the use of removable appliances in children prompted exploration for antibacterial and biocompatible materials one of which is bioactive glass (BAG) [7]. BAG has been classified as bioactive ceramics in the field of biomaterials research and have gained recognition in dentistry in recent years [8]. BAG consists of a bioactive hydroxycarbonapatite layer, which allows the material surface to bind to the tissues. Owing to this feature, BAG can chemically bound to hard tissues and even in some cases to the soft tissues [8].

The antibacterial and remineralizing effect of BAG has been linked to the release of ions such as calcium and phosphate, increasing the pH and eventually neutralizing the acidic medium required for demineralization. Neutral pH on the other hand decreases cariogenic potential of bacteria [9-11].

The composition of BAG has a significant effect on caries producing pathogens such as *Streptococcus sangius*, *S. mutans*, *Actinomyces viscosus*, *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Prevotella intermedia*, *Actinobacillus actinomycetemcomitans*, *L. acidophilus*. Several previous studies showed that addition of BAG to experimental materials could increase the antibacterial activity [8,12-15].

The objective of this study therefore was to evaluate the effects of adding BAG nanoparticles to PMMA on the antibacterial effectiveness of the material. The hypothesis tested in this study was that adding BAG to PMMA would promote the antibacterial activity of PMMA used in removable appliances.

MATERIALS AND METHODS

Specimen preparation

This study was carried out with the approval of the Necmettin Erbakan University Faculty of Dentistry, Ethics Committee for Pharmaceutical and Non-Medical Research (2019/002). The procedures used in this study complied with the principles of the Helsinki Declaration.

Experimental materials with different concentration were prepared by adding 2% (BAG 2) and 5% (BAG 5) BAG powder to the powdered portion of the auto-polymerizing acrylic resins (Tables I and II). The acrylic resin group without BAG served as the control group (BAG 0). For each group, powder mixtures were mixed with acrylic resin liquid monomer according to the manufacturer's instructions. The prepared mixtures were placed in teflon molds to form cylindrical specimens (width: 20 mm x length: 20 mm x thickness: 1 mm) (n=10). After placing the mixtures in the mold, the excess material was removed by applying finger pressure between the two glasses. The specimens were removed from the mold 1h later and stored in deionized water at 37°C for the entire duration of the experiment.

Table I - Types, manufacturers and chemical composition of the materials used in the study

Material Type	Manufacturer	Chemical Composition
Polymethyl methacrylate	O80, Imıcryl Inc., Konya, Turkey	Powder: Polymethylmethacrylate, benzoyl peroxide, di-butyl phthalate, opacifier and pigments Liquid: Methylmethacrylate (MMA) monomer, ethylene glycol dimethacrylate, and hydroquinone
Bioactive glass particles	BonAlive Biomaterials, Turku, Finland	S53P4: SiO_2, Na_2O, CaO, and P_2O_5

Table II - Group names and chemical composition of the control group and experimental groups with 2 and 5% mixtures of bioactive glass

Experimental Groups	Group name	Chemical Composition
Control group	BAG 0	Polymethyl methacrylate
Experimental group 1	BAG 2	Polymethyl methacrylate + 2% bioactive glass particles
Experimental Group 2	BAG 5	Polymethyl methacrylate + 5% bioactive glass particles

In order to study the antibacterial effects, the surface roughness of all specimens was standardized at 0.2μm after measuring in a surface roughness measurement device (Surftest SJ-201P, Mitutoyo Corp, Kawasaki, Japan) [16].

Antibacterial activity in biofilm

The test strain of *S. mutans* (ATCC 29212) and *L. acidophilus* (ATCC 43121) were used to create biofilms in the prepared disks. The stock cultures of the test strains were transferred to Tryptic Soy Broth (TSB) and incubated overnight at 37°C. The resulting culture was diluted 10 times in fresh TSB and used as a vaccine for biofilm formation adjusted to the 0.5 McFarland density standard.

Ten disks for each test group were placed in the wells of a 24-well tissue culture plate (Corning, MA, USA). Two milliliters of bacterial vaccines were added to each well, and the plate was incubated at 37°C. During the incubation period, 1mL of TSB was taken from the wells with a micropipette every 12h, and an equal volume of fresh TSB was added. At the end of the 48h incubation, the disks were transferred to the corresponding wells of a new plate and washed three times with sterile phosphate-buffered saline (PBS) to remove non-adherent cells. The disks were then placed in glass tubes containing 2mL of PBS, and the tubes were sonicated four times at 35kHz (Bandelin GmbH, Berlin, Germany) for 1min to separate the bacteria from the biofilm matrix. The suspensions with bacteria for each were then added to the Nutrient Agar (NA) plates. After 48h of incubation at 37°C, colonies grown on plates were counted and the average microorganism count was calculated as log colony forming unit (CFU)/disk for each specimen. The analysis performed triplicate on each disk at a single moment [8].

Statistical analysis

Statistical analysis was performed using a statistical software (SPSS Inc., IL, USA). Kolmogorov Smirnov test was performed for normal distribution of the data. The data obtained were then analyzed using a one-way analysis of variance (ANOVA). Mean values and between group analyses were evaluated using Tukey`s post-hoc test. The confidence interval of the study was kept at 95%. Therefore, p value less than 0.05 was considered statistically significant.

RESULTS

The CFU log values of experimental groups are presented in Table III.

CFU values were observed in the range of 2.85–6.21 log CFU/disk. The addition of 5% BAG significantly reduced the metabolic activity of *S. mutans* and *L. acidophilus* and decreased CFU values (Table III).

A statistically significant decrease was observed between the level of *L. acidophilus* in BAG 0 ($p=0.001$) and BAG 2 groups ($p<0.05$,) and BAG 0 ($p<0.001$) and BAG 5 ($p<0.05$) groups. In the BAG 2 ($p=0.121$) and BAG 5 ($p>0.05$) groups, no statistically significant difference was observed.

For *S. mutans* CFU values, in the BAG 0 ($p=0.115$) and BAG 2 ($p>0.05$) groups, no

Table III - Colony forming unit (CFU) log values of experimental groups for two S. mutans and L. acidophilus

	CFU log	
Groups	*S. mutans*	*L. acidophilus*
BAG 0	6.21 ± 0.66[a]	5.43 ± 0.79[a]
BAG 2	5.46 ± 0.63[a]	3.71 ± 0.54[b]
BAG 5	3.65 ± 0.69[b]	2.85 ± 0.76[b]

Different letters in each column indicate statistical difference between groups for the same microorganism ($p<0.05$).

statistically significant difference was observed. Between the BAG 0 and BAG 5 groups ($p<0.001$) and BAG 2 and BAG 5 groups ($p<0.001$) significant differences were observed.

DISCUSSION

The results of this study evaluating the effects of adding BAG to PMMA on the antibacterial effectiveness of the material suggested that PMMA containing BAG might have potential broad-spectrum antibacterial activity on *S. mutans* and *L. acidophilus*.

The oral biofilm on removable appliances increases the incidence of caries [2,6,7,17]. In oral biofilm formation, the materials contained in the appliances and the surface properties of these materials are of great importance. The first affinity of bacteria to solid surfaces when forming biofilms is due to electrostatic and hydrophobic interactions. In addition, the physicochemical properties and saliva composition of hard surfaces are important as mediators in the attachment of bacteria to the surface [18]. Oral biofilm formation depends on not only the surface properties but also the extent of surface opening into the mouth environment. The irregularity observed on the material surfaces contributes to the protection of microorganisms and biofilm formation [19]. Many studies reported an increase in clinical periodontal indices such as plaque index, gingival index, and pocket depth after the use of fixed or removable appliances [20-22]. The reason for the increase in plaque formation in the apparatuses is that it contacts the gingival margins, applies pressure to the soft tissue, and makes it difficult to apply oral hygiene measures [20].

Studies investigating the cleaning of acrylic appliances used by pediatric patients reported that polished surfaces were cleaned better by patients, and more biofilm formation was observed due to less cleaning of surfaces of acrylic prostheses that contacted tissues [20]. An increase in the gingiva and pocket depth was reported after the use of the apparatus in pediatric patients. The change in microflora seen in children under treatment was similar to the change seen in periodontal diseases [21].

The surface energy, wettability, and microhardness properties of the materials affect biofilm formation. Many studies emphasized that the most important reason for the increase in the carcinogenicity of patients using such appliances was the surface properties of the materials from which the appliances were made [22,23]. Previous studies examining the acrylic base prosthesis used by adult patients demonstrated that the rough surfaces of the acrylic surfaces have a more favorable environment for the settlement of *Candida albicans* compared with the other components of the prosthesis and the tooth surface [24,25]. In order to address these problems, various antibacterial agents have been added to the prosthetic and orthodontic appliance materials used by adults and children for rehabilitation. Casemiro et al. added 2.5%, 5.0%, 7.5%, and 10% silver-zinc zeolite to prosthetic resins. This treatment provided good antibacterial efficacy but had a negative effect on mechanical properties, depending on the percentage of zeolite and therefore they suggested adding a low rate of silver-zinc antibacterial zeolites to PMMA [26]. Nam et al. reported that the 20% by weight and 30% by weight experimental formulation of AgNPs with slow-release silver ions behaved like a latent antifungal material [27]. AgNPs also showed higher toxicity to the oral pathogenic species of *S. mutans* [28]. Based on these findings, Wady et al. reported that AgNPs reduced the hydrophobicity of the resin but had no effect on the attachment of organisms and biological film formation after being added to the prosthetic resin [29]. As an alternative to these formulations, also TiO2NP was experimentally formulated and tested by mixing in PMMA before polymerization. Shibata et al. reported that acrylic resin containing 5% by weight apatite coated TiO2 photocatalyst had an antifungal effect [30]. However, a recent study reported significant cytotoxicity with reduced cell viability and decreased induction of apoptosis in murine microglia N9 cells [31].

In 2018, Funk et al. reported that the addition of borate bioactive glass to commercial PMMA

bone cement has been shown to be an effective bioactive filler to increase vancomycin from cement [32]. Although materials may have a cytotoxic effect such as silver and titanium show distinct antibacterial efficacy, BAG was considered as a more favorable material due to its long-term antibacterial effect with some concerns on their biocompatibility [33]. BAG has been proposed as a promising material for dental and medical applications, as it has anti-adhesion properties and inhibitory effects on bacterial biofilms [34-36]. Korkut et al. investigated the effects of adding BAG to experimentally produced composite resins on the antibacterial and physical properties of the composite. However, they reported that the addition of BAG increased the antibacterial properties of composite resins and decreased their physical properties [8]. Allan et al. (2002) evaluated the antibiofilm effects of BAG on subgingivally modeled mixed-type biofilms and showed that BAG had the potential to reduce bacterial colonization in the long term [34]. The results of the present study also showed that the addition of BAG increased the antibacterial effectiveness of the experimental material.

The antibacterial effect of BAG in an aqueous medium is explained by two mechanisms [37-39]. The first mechanism is an increase in pH and, consequently, a change in osmotic pressure due to the release of sodium, calcium, phosphate, and silicate. These changes can lead to cellular injury in bacteria and inactivation in enzymes. Alkalization of the medium can also prevent the formation of proton motive force for the synthesis of adenosine triphosphate (ATP). The second mechanism is that debris from BAG particles can react with the cell membrane, causing the leakage of cell contents and eventually microbial lysis. *Escherichia coli* cells were observed under a transmission electron microscope and found that the cell walls were severely damaged by needle-like BAG residues on bacterial surfaces [39]. The inclusion of antibacterial agents in dental materials should not lead to the disruption of the ecological homeostasis of oral microflora. Also, the materials should not support the development of bacterial resistance, which can lead to serious therapeutic failures in infectious diseases, to avoid the development of super-pathogens. The BAG kills the microorganisms through inducing cellular damage, and therefore it cannot lead to bacterial resistance [40].

The effects of antibacterial macromolecules on health and environment must be fully evaluated before the materials are placed on the market. Problems such as poor physical properties, unacceptable degree of polymerization, and effect of their release products on cells and tissues should be tackled when determining the appropriate antibacterial macromolecule concentration in the PMMA prosthetic material. The antibacterial effect of BAG addition at varying concentrations with new methodologies and its effects on the physical properties of acrylic resin needs further investigations.

CONCLUSION

From this study, it can be concluded that the addition of bioactive glass nanoparticles to acrylic resins promoted the antibacterial effect against cariogenic bacteria.

Clinical relevance

Considering the promising physical properties reported in previous studies along with the investigated antibacterial reinforcement in this study, polymethylmethacrylate mixed with bioactive glass may be envisaged for the fabrication of pediatric, orthodontic or prosthetic appliances.

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Author's Contributions

HO: Study conception and design, drafting of manuscript, acquisition of data. MO: Design, critical revision, writing the manuscript.

Conflict of Interest

The authors declare no conflicts of interest in any of the materials used in this study.

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Regulatory Statement

This study was carried out with the approval of the Necmettin Erbakan University Faculty of Dentistry, Ethics Committee for Pharmaceutical and Non-Medical Research (2019/002). The procedures used in this study complied with the principles of the Helsinki Declaration. There is no conflict with ethical considerations.

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