Oral Streptococci Growth on Aging and Non-Aging Esthetic Restorations after Radiotherapy

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The aim of this study was to examine *Streptococcus mutans* biofilm growth on both aged and non-aged restorative dental resins, which were submitted to therapeutic irradiation. Sixty-four disks of an esthetic restorative material (Filtek Supreme) were divided into 2 groups: aged group (AG) and a non-aged group (NAG). Each group was subdivided into 4 subgroups: non-irradiated and irradiated with 10Gy, 35Gy, and 70Gy. The biofilms were produced by *Streptococcus mutans* UA159 growing on both AG and NAG surfaces. The colony-forming units *per* mL (CFU/mL) were evaluated by the ANOVA and the Tukey LSD tests (α=0.05). AG presented smaller amounts of CFU/mL than the NAG before irradiation and after 10Gy of irradiation ($p<0.05$). AG irradiated with 35 and 70Gy showed increased amount of bacterial biofilm when compared to non-irradiated and 10Gy-irradiated disks ($p<0.05$). The exposure to ionizing radiation at therapeutic doses promoted changes in bacterial adherence of aged dental restorative material.

Key Words: Bacterial biofilm, dental restorative material, radiotherapy, radiation.

INTRODUCTION

Photoactived composite resins are widely used in the dental clinic for replacement of hard tissues. Although the mechanical properties of these materials have been improved substantially, their antibacterial properties are still limited (1,2). The bacterial accumulation on the surfaces of restorative materials can provide the bacterial source leading to the development of secondary caries and periodontal diseases. The formation of biofilm on a dental tissues or restoration surface is a complex phenomenon, and different key factors are involved (2).

The adherence of bacteria to solid surfaces is thought to involve non-specific processes mediated by physicochemical interactions, such as hydrophobic interaction, electrostatic interaction and hydrodynamic forces (3). Bacterial accumulation in dental biofilms is highly dependent on the characteristics of the material surface. Composite restorative materials do not show a static state in the biological oral environment (4), being constantly modified by environmental influences that could change the profile of bacterial accumulation.

The oral cavity can suffer severe changes due to the effects of radiotherapy of the head and neck region since the salivary glands, oral mucosa, and jaws are usually included in the radiotherapy portals depending on the location of the tumor (primary tumor, lymph-node metastases) (5,6).

Besides the undisputed anticancer effects, the ionizing irradiation causes damage in healthy tissues located in the field of radiation, resulting in complex oral complications, which affect the salivary glands, oral mucosa, bone, masticatory musculature, and dentition. The clinical consequences include hyposalivation,

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mucositis, taste loss, trismus, and osteoradionecrosis as the most common side-effects. Mucositis and taste loss are reversible consequences that usually diminish after the end of the irradiation treatment, whereas hyposalivation is commonly irreversible, changing the behavior of bacterial accumulation on the restorative composite (5).

In addition, head and neck radiotherapy can also change the composite resin restorations in oral cavity, due to the radiation interaction with any atoms and molecules in the way of the radiation source (6); i.e., tissue, vital organs or biomaterials. Few studies have reported the direct effects of the ionizing radiation on dental materials (7-9) and dental tissues (10-13), and the results of these studies are still unclear. There are no reports regarding to the profile of bacterial accumulation on dental restorative materials exposed to therapeutic dose x-ray radiation. Thus, the aim of this study was to examine the effect of therapeutic irradiation on *Streptococcus mutans* adherence on aged and non-aged restorative material. The null hypothesis tested was that the therapeutic irradiation in different doses does not affect the *S. mutans* biofilm growth on AG and NAG restorative material.

MATERIAL AND METHODS

Restorative Material Disc and Irradiation

Disc-shaped specimens prepared as previously described by Montanaro et al. (1). Briefly, 64 discs (4 mm in diameter, 1 mm thick) were made by placing Filtek Supreme composite resin (3M/ESPE, St. Paul, MN, USA) increments into stainless-steel rings molds, which were placed between two glass slides in order to achieve uniform smooth surfaces. The molds were submitted to 1 kg/cm^2 pressure to remove excess material. The discs were light-cured using the Curing Light 2500 unit (3M/ESPE) according to the manufacturers' instructions.

The discs were randomly divided into two groups (n=32): an aged (AG) group and a non-aged (NAG) group. The AG discs were placed into a box with relative humidity of 50% (±5), illuminated by a mercury light HN ZN 15W (Huaning Corp, Nanjing, China; 253.7 nm, 15 W), for 48 h, resulting in 1157.76 J/cm^2, to induce aging, according to previous studies (18-20). The NAG discs were stored at 37°C (±1), without light and 95% (±5) of relative humidity for the same time.

After 48 h, both AG and NAG groups were divided into 4 subgroups (n=8) according to the radiation exposition: non-irradiated (control group) and irradiated with 10Gy, 35Gy and 70Gy, which are compatible with those doses used in radiotherapy treatment in the head region, considering different treatment protocols. The irradiation was performed in a Clinac® 600 Linear Accelerator (Varian Medical Systems, Palo Alto, CA, USA), with a 6 MV beam irradiation at 1 m target-to-source distance. The discs were kept in water during irradiation in order to obtain the correct radiation doses.

Bacterial Strain and Biofilm Growth Conditions

S. mutans UA159 was used to produce biofilms. The microorganism was grown in a brain-heart infusion broth - BHI (Difco Laboratories, Detroit, MI, USA), under microaerophilic conditions (10% CO_2 - IG150, Jouan, St-Herblain, France) at 37°C.

The specimens were previously placed in a vertical position into 24 polystyrene culture plates containing BHI and 1% (w/v) sucrose. The initial inoculum was spectrophotometrically standardized to 10^8 CFU/mL. The plates were incubated for 24 h at 37°C in 10% CO_2. During 5 days, the medium were daily replaced with a new fresh medium. The Gram's stain was used to check culture purity.

The discs containing the 5-day-old biofilm were removed from the plates and rinsed twice in 7.5 mL of sterile saline for 10 s. Each specimen was transferred to a tube containing sterile saline, and the biofilm was dispersed by sonication (Vibra Cell 400w, Sonics & Materials Inc, Newtown, CT, USA) at 4°C, with 5% amplitude, and 6 pulses (9.9 s each pulse and a 5-s interval). Biofilm suspensions were plated on brain heart infusion agar. Plates were incubated at 37°C, during 48 h under microaerophilic condition. After incubation period, the number of colony-forming units *per* milliliter of suspension (CFU/mL) was determined.

Statistical Analysis

Triplicates of experiments were conducted in each of the assays, and the mean values were considered for analysis. The normality of the data distribution was observed by the Lilliefors test. The colony forming units were logarithmic transformed. The data were submitted to a two-way ANOVA and Tukey's test using Systat 12.0 (Systat Software Inc., London, UK). The regression analysis was used to evaluate the changes in logarithmic values of the CFU/mL at the several radiations doses.

The level of significance was set at 5%.

RESULTS

Figure 1 shows the bacterial growth profile of AG and NAG subgroups. There were statistically significant differences ($p<0.05$) in the bacterial biofilm growth due to both irradiation and aging.

The AG subgroups (non-irradiated and irradiated at 10Gy) presented less CFU/mL than NAG subgroups ($p<0.05$). AG subgroups (irradiated at 35 and 70Gy) showed no significant difference ($p>0.05$) in the amounts of CFU/mL in comparison to the NAG subgroups. In the AG group, there was a significant decrease ($p<0.05$) in the amount of CFU/mL in the dose of 70Gy compared to non-irradiated and irradiated groups at 10 and 35Gy. In the NAG, there was a significant ($p<0.05$) increase in the amount of bacterial biofilm at 35 and 70Gy compared to the non-irradiated and 10 Gy groups.

DISCUSSION

In the present study, photoactivated-composite resin specimens were submitted or not to the aging process and different therapeutic radiation doses, in order to evaluate the influence of material aging on the antibacterial properties of radiation. The results showed that both radiation and aging process caused changes in the profile of bacterial biofilm accumulation, rejecting the null hypothesis of the study.

The increase of the radiation dose caused reduction in the biofilm growth in non-aged samples. This result could indicate an improvement of the material promoted by the irradiation, which corroborated with previous studies (7,8). These findings indicated that the therapeutic ionizing radiation dose does not cause detrimental effects on photoactivated composite dental materials, but it can improve them. However, when radiation and aging were associated, an increasing biofilm growth was verified. This phenomenon may be a possible competition between cross-linking and cross-breaking processes in chemical chains of the material (17-20), which can result in degradation of the material.

The use of the ionizing radiation to improve dental materials was previously studied (9,17,18). However, the radiation doses used in these cases are much higher

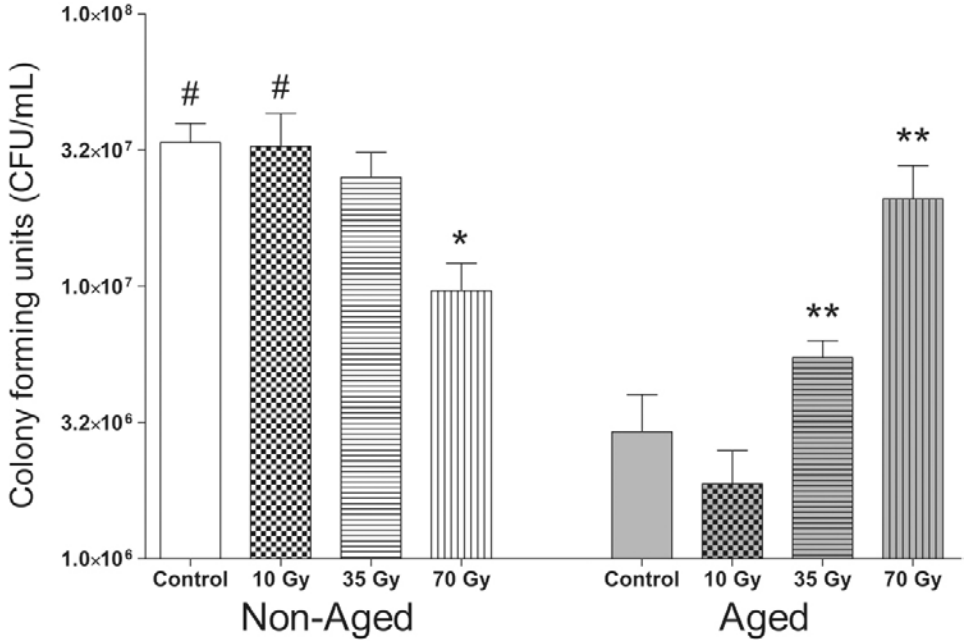

Figure 1. Means (standard deviation) of colony-forming units *per* milliliter (CFU/mL) in the different groups and subgroups. *Statistically significant difference in comparison to the other non-aged specimens; **Statistically significant difference in comparison to the other aged specimens; #Statistically significant difference between aged and non-aged specimens.

than in the clinical situation. These studies evaluated only mechanical properties such as microhardness, diametral tensile strength, water sorption and solubility, abrasion, among others. The present study is the first one to describe the effect of radiation on bacterial biofilm growth on dental materials.

Von Fraunhofer et al. (7) verified that gamma radiation improved some mechanical properties, such as hardness, of composite dental resins, being the effect proportional to the radiation dose. They observed that radiation increased the material hardness due to reactivation and reorganization of residual chemical groups of low molecular weight coupled to the organic matrix of the dental materials. In addition, Haque et al. (9) also observed similar findings showing changes in the degree of conversion caused by radiation.

Other studies (14-16) indicated that UV radiation also was able to cause detrimental effects on dental materials, changing their color, microhardness and the degree of conversion. However, improvements in the bond strength between adhesive and dental tissues were not observed when radiation was applied on dental tissue during radiotherapy simulation. There is no consensus in the literature since there are reports indicating that the radiotherapy alters the bond strength (10,12) and others indicating no alterations (11,13).

The absorption process of radiation by the dental resin composite promotes the excitation and the ionization in its organic matrixes and creates reactive molecules (17-20). These coupled reactive molecules (7,17) inside the matrix are the initial chemical reagents starting the cure process. In contrast, in cured material, which has little molecular mobility inside the matrix when irradiated, the created chemical reagents are linked among themselves or with closer chemical groups until they stabilize. These links occur until exhaustion of the chemical reagents. However, the excess of radiation could promote the breaking of established links causing degradation of material (17).

Radiation is used in biomaterials science for surface modification. There are many radiation sources: high-energy electrons, gamma radiation, ultraviolet (UV) and visible light, which are used to manipulate chemical structures of materials in order to improve their biocompatibility (17,18). These reactions are efficient and fast. They are limited to very thin surface layers of the polymers without affecting bulk properties. The radiation is able to increase surface hydrophilicity to improve material's cytocompatibility (17,18). The interactions between cells and their environment are mediated by a "bio-recognition process", the specific binding of the receptors on cell surface with their corresponding ligants (18).

The same mechanisms apply to the biomaterial surfaces *in vitro*. When foreign materials come into in contact with body fluid or cell culture medium, the initial response is protein adsorption on the surface of the materials. Thus, the materials interact with the cells through the absorbed protein layer. The composition and structure of this protein layer plays a critical role in determining subsequent cell behavior (2,3,18).

In the present study, the radiation probably modified the surface of the material making it more hydrophilic. This change on the hydrophilic properties could be responsible for the bacterial biofilm increased adherence after irradiation and aging. Organic polymers generally are hydrophobic with low surface energy (3). Inorganic materials, such as glass and metals, have higher surface energy and are naturally more hydrophilic (3), because their surface has a smaller amount of open chemical chains (15).

Furthermore, it must be emphasized that the results of an *in vitro* study are not directly applicable to bacterial adherence of dental materials in the oral environment, which was a limitation of this study. In the oral cavity, bacterial adhesion takes place under competition among different microbial species, and the pellicle formed on these materials may be more complex than the experimental ones used in this study.

In conclusion, different doses of therapeutic irradiation can affect the biofilm growth on aged and non-aged dental restorative materials. Under the conditions of present study, the increase of radiation doses on aged materials promoted an increase of *S. mutans* biofilm growth.

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RESUMO

O objetivo deste estudo foi avaliar a formação do biofilme de *Streptococcus mutans* crescido em resina restauradora envelhecida e não-envelhecida, submetidas à radiação terapêutica. Sessenta

e quatro discos do material restaurador Filtek Supreme foram divididos em 2 grupos: grupo envelhecido (AG) e grupo não-envelhecido (NAG) e cada grupo foi dividido em 4 sub-grupos: não-irradiado e irradiado com 10Gy, 35Gy e 70Gy. O biofilme de *S. mutans* UA159 foi produzido na superfície de ambos os discos AG e NAG. As unidades formadoras de colônia/mL (UFC/mL) foram avaliadas por ANOVA e teste de Tukey (α=0,05). O grupo AG demonstrou menores quantidades de UFC/mL que o grupo NAG antes da radiação e após a radiação de 10Gy ($p<0,05$). Os sub-grupos AG irradiados com 35 e 70Gy demonstraram aumento na quantidade de biofilme quando comparado aos não irradiados e irradiados com 10Gy ($p<0,05$). A exposição à radiação ionizante nas doses terapêuticas promoveu mudanças na aderência bacteriana no material restaurador.

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