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Microleakage of Class I cavities restored with hydroxyapatite and glass ionomer cement

Fernanda Caetano[1], Julia Steck[2], Maria Luiza Q Mohieddine[3], Mariana R Coelho[4], Natalia C Massat[5], Diana Roberta P Grandizoli[6], Carlos Eduardo Fontana[7], Sérgio Luiz Pinheiro[8]

[1,2,3,4,5]Department of Restorative Dentistry, Pontifícia Universidade Católica de Campinas (PUC-Campinas), Campinas, SP, Brazil
[6,7,8]Center for Health Sciences, Graduate Program in Health Sciences, PUC-Campinas, Campinas, SP, Brazil

Abstract*— *Purpose: The aim was to evaluate the use of hydroxyapatite powder (HDX) obtained from human teeth as a material to filled occlusal cavities compared with glass ionomer cement (GIC) through the microleakage analysis. Methods: Sixty-one permanent teeth were selected. Thirty-nine samples were used to obtain the hydroxyapatite powder and 22 used to obtain specimens for microleakage analysis. The teeth were sterilized. Two standardized Class I cavities were performed on the occlusal surface of each tooth. The specimens were randomly distributed in two groups (n = 11) GIC and HDX. The same sample received in occlusal cavities GIC and HDX restorations. After 24 hours 11 samples were immersed in broth containing S.mutans (ATCC 25175) and 5% methylene blue dye (MB + S. mutans) and the remaining 11 samples were immersed in 5% MB. Samples were sectioned mid-distally and dye leakage was assessed by three calibrated examiners. Results: The data were analyzed with Kruskall-Wallis test. There was no difference between GIC and HDX (P>0.05) and between the MB and MB with S. mutans (P>0.05). Conclusion: Within the limitations of this in vitro study it can be concluded that hydroxyapatite may be an alternative as a restorative material.

Keywords*— *durapatite, dental leakage, methylene blue, glass-ionomer cement.

I. INTRODUCTION

Restorative dentistry over the last few years improve the development of techniques to solve recurrent caries at restoration interfaces. This approach remains as one of the principal reasons for replacement of restorations [1]. Consequently, on restorative dentistry one of the challenges involves the development of biomaterials that promoting the release of high levels of Ca2+, PO43- and F- on enamel and dentin tissues [2]. Therefore, dentistry search materials with biocompatibility, good cavity Glass ionomer cements have biocompatibility, fluoride release and good adhesion to the dental structure. There are still speculations about the ability to inhibit secondary caries at restoration margins of teeth making them an alternative in pacients with increased caries risk such as in pediatric and geriatric dentistry [3,4]. In addition, the coefficient of thermal expansion of the glass ionomer is low and close to the dentin. However, despite its advantages, ionomeric cements have some disadvantages that limit their physical properties and their mechanical resistance such as syneresis, low tensile strength and inferior flexural fatigue characteristics for the conventional ones [5,6].

Hydroxyapatite Ca10(PO4)6(OH)2, can be found in teeth and bone and is widely used in dentistry applications as a promising material, due to its inorganic composition similar to bone and its biocompatibility and bioactivity [7,8]. In the last years, researchers have synthesized hydroxyapatite by chemical methods. There are many methods available, such as dry methods, wet methods, high temperature processes, synthesis from biogenic sources and combination procedures [9-11]. Nevertheless, hydroxyapatite is brittle, and researchers are still developing a method that prepares the bioactive hydroxyapatite with excellent mechanical properties.

Clinical trials are the greatest to evaluate the biological behavior in the oral cavity, when it is intended to test biomaterials. However, ethical issues with experimental clinical studies constantly happen, so in vitro models, less expensive, without patient dependency, can answer fundamental questions by simulating the human oral

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environment [12-14]. Also, clinical studies are lengthy and demand bigger investments [15,16].

The aim of this study was to evaluate hydroxyapatite as a restorative material of Class I cavities by means of the *in vitro* microleakage analysis. The null hypothesis was: There was no difference between HDX and GIC on microleakage.

II. MATERIAL AND METHODS

Experimental Design

This study followed a completely randomized design with two experimental factors: Restorative treatment was two levels: GIC, HDX and, Infiltration solution was two levels for microleakage analysis (MB + S. mutans and MB). The factors were tested in an microleakage analysis model using human teeth specimens (n=11). The response variable was the microleakage on the cavity wall (assessed through the leakage score).

Ethical aspects

In the present study, human permanent teeth were used, so an approval from the local Ethics Committee on Research with Human Beings was necessary. Accordingly, this study was performed after approval was obtained (process number: 3.662.955).

Specimen preparation

Sixty-one sound human permanent teeth previously stored into 0.1% of thymol solution (Sigma-Aldrich, St Louis, MO, USA), under refrigeration at 4 oC, until the beginning of the experiment, were selected. Two standardized class I cavities were performed on the occlusal surface of each tooth, under constant cooling, with enamel termination on the cavity surface and dentin on the back wall (The cavities were prepared directly on the occlusal surface, without polishing). Two different materials were evaluated on the same tooth: GIC and HDX. The cavities were standardized with the following measures (3 mm x 4 mm x 3 mm). The cavity measurements were checked with millimeter probe.

Synthesis of hydroxyapatite powder from biogenic source (human teeth)

Extraction of HAp

According with was described by Aarthy et al. [11], thirty-nine teeth were dehydrated in 100 mL of 99.8% absolute ethyl alcohol (NEON, SP, Brazil) with 100 mL of distilled water forming a hydro alcoholic solution. The teeth were placed in a distillation flask (Laborglass, SP, Brazil) that was coupled to a ball condenser through a universal support (Laborglass, SP, Brazil) using a metallic claw (Laborglass, SP, Brazil) and muffle (Laborglass, SP, Brazil). The reflux process was started by pouring the hydro alcoholic solution into the ball condenser until it reached the distillation flask (Prolab, SP, Brazil). During the heating process, with an ISO 9002 mat (QUIMIS, SP, Brazil) the solution boiled inside the ball condenser (Laborglass, SP, Brazil). On the outside, running water was constantly introduced through a hose connected to the upper end of the ball condenser (Laborglass, SP, Brazil) and dispensed by another hose on the lower end. The running water cooled the ball condenser (Laborglass, SP, Brazil) and the vapor from the solution that was inside came into contact with the cold surface and condensed, remaining constantly in this process of boiling and condensation called the reflux process for four hours thus reaching the temperature of 110 ° C.

Sintering of HAp

After the reflux process, human teeth were wrapped in sterile gauze (Medical textil, SP, Brazil) and pressed with a force of approximately 5 tons in a hydraulic press (ACS Group, Madrid, Spain). To separate the larger and smaller granules, a sieve was used, where the larger granules were taken back to the press until uniform granules were obtained. The granules remained in the mortar jar (ABB, Quebec, Canada) and were moistened with running water to be introduced into a muffle (QUIMIS, SP, Brazil) without thermal shock. The moistened granules were separated into small portions and accommodated in porcelain crucibles model A-48 (Chiarotti, SP, Brazil) so that they can be taken to the muffle (QUIMIS, SP, Brazil) remaining three hours until reaching 900 ° C and three more hours at a constant temperature of 900 ° C. The sample obtained was ground in the mortar jar (ABB, Quebec, Canada). This sample was placed in 150 mL of deionized water Chemistry laboratory (PUC-Campinas, SP, Brazil). The sample remained for two and a half hours in the reflux process in order to dissolve the granules of the powder sample. Soon after, the hydroxyapatite powder was taken to the oven for drying.

Characterization

Infrared spectroscopy (FTIR) of the HA powder calcined

IR spectroscopy was determined based on a method described elsewhere [17] carried out in range of 4000–400 cm−1 at a MB3000-DTGS spectrophotometer (ABB, Quebec, Canada) and analyzed using infrared light using the Horizon MB software for reading. Before measurements, the powdered samples were mixed with a powder of potassium bromide in the ratio of (0.0020 g) of

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the sample to (0.1980 g) of potassium bromide and pressed into pills.

Microleakage analysis

The samples were randomly assigned to two groups (n = 11): GIC and HDX (ANOVA), with a minimum difference between the media of 0.27, the SD of 0.18, the number of definitions 4, the test of 0.80 and the alpha 0.05.

The samples were immersed in 2% chlorhexidine solution (Dental Cremer, Campinas, SP, Brazil) for 24 hours. Then, were stored in 0.9% sodium chloride (Ultrafarma, SP, Brazil) before preparation.

Restorative procedure

In this phase, the specimens were randomly allocated into 2 experimental groups (n = 11), according to the treatments described in Table 1.

Table 1. Details of the groups; names, manufacturer, restorative protocol.

Groups	Product/ Manufacturer	Restorative protocol
GIC	Ketac Molar, 3M Deutschland GmbH, Seefeld, Germany	1. *Ketac conditioner* on the prepared surface for 10 seconds; 2. Wash with water and air dry for 2-3 minutes; 3. Mixing: powder / liquid (1: 1) spatula (nº 24) (Golgran, SP, Brazil); 4. Mixing was done on a mixing block in which the powder was introduced into the liquid in a maximum of two portions, until a homogeneous consistency was obtained (3 min); 5. The CIV application was carried out with an insertion spatula (Golgran, SP, Brazil) with vibrating movements; 6. Surface protection of the CIV with the adhesive system Primer and Bond 2.1 (Dentsply, RJ, Brazil); 7. 24 hours after the insertion of the CIV, the finishing was done with an excess remover (Golgran, SP, Brazil).
HDX	-	1. *Ketac conditioner* on the prepared surface for 10 seconds; 2. Wash with water and air dry for 2-3 minutes; 3. Mixing: powder / liquid (1: 2) (hydroxyapatite powder) using the GIC Ketac Molar Easymix powder dispenser and two drops of Ketac Molar Easymix polyacrylic acid; 4. Mixing (1 min) spatula (nº 24) on a mixing block, until a homogeneous consistency was obtained; 5. HDX application was carried out with an insertion spatula (Golgran, SP, Brazil) with vibrating movements; 6. Surface protection of the HDX with the adhesive system Primer and Bond 2.1; 7. After 24 hours - finishing with excess remover.

All samples were waterproofed with epoxy resin (Araldite, SP, Brazil) and nail varnish (Colorama, SP, Brazil), except 1 mm from the restoration margins.

Microleakage

The samples were randomly distributed in two different methodologies:

a) Bacterial infiltration (MB + S. mutans): The specimens were immersed in a broth containing S. mutans (ATCC 25175) (André Tosello Foundation, Campinas, SP, Brazil) (0.5 MacFarland) and 0.5% methylene blue dye and incubated at 37 °C for 4 h in zero humidity.

b) Methylene blue staining (MB): The specimens were immersed in 5% methylene blue dye incubated at 37 °C for 4 h in zero humidity.

The teeth were then thoroughly rinsed with water to eliminate excess of dye solution and dried with air spray. They were then bucco lingually sectioned through the center of restoration using a cutting machine (KG Sorensen, Cotia, SP, Brazil) with a double-sided diamond blade with 0.3 mm thickness under water coolant (to prevent over-heating).

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Both sections were photographed under a stereo microscope (Stemi DV4, Carl Zeiss, SP, Brazil) at 32x magnification. Three examiners blinded (P < 0.0001) to the group allocation of teeth inspected both the enamel and dentin margins in terms of dye penetration.

Microleakage was scored as follows:

Score 0: No dye penetration; Score 1: Dye penetration to half the depth of the cavity wall; Score 2: Dye penetration exceeding half the depth of the cavity wall; Score 3: Dye penetration reaching the axial wall (Fig. 1). These tests were performed according to the ISO/TS 11405:2003 [18].

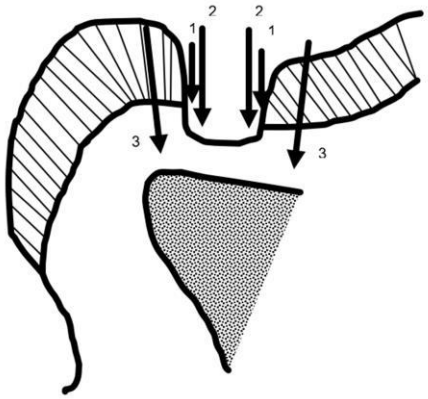

Fig. 1: Schematic picture of microleakage score used for analyzing leakage.

Statistical analysis

To assess the calibration among evaluators Pearson's test was performed. Normality and/or homogeneity were checked with Shapiro-wilk test. Since data did not follow a normal distribution, then Kruskall-Wallis test were performed, considering a significance of 5%. The analyses were performed with the software Biostat 5.3.

III. INDENTATIONS AND EQUATIONS

The evaluators were calibrated as shown in Table 2.

Table 2. Pearson's test of calibration between the examiners.

n (pairs)	R (Pearson)	IC 95%	IC 99%	(*P*)
44	0.8832	0.79 to 0.93	0.75 to 0.95	<0.0001

There was no statistically significant difference between marginal microleakage comparing GIC and HDX (P>0.05). The methods of microleakage evaluation, methylene blue dye or methylene blue dye associated with S. mutans showed no significant difference (P>0.05, Tables 3 and 4).

Table 3. Median and interquartile intervals for the microleakage scores for all groups. Lower case letters show no significant differences between the experimental groups or methodologies.

	GIC	HDX	(*P*)
Methylene blue (MB)	0.00 (0.00)[a]	0.00 (0.50)[a]	0.7366
MB + *S. mutans*	0.00 (0.00)[a]	0.00 (0.00)[a]	

Table 4. Frequency of different microleakage scores in the cavity wall of the study groups.

Groups	Microleakage score			
	Score 0	Score 1	Score 2	Score 3
MB/GIC	11	0	0	0
% within group	100%	0%	0%	0%
MB/HDX	8	3	0	0
% within group	72.7%	27.2%	0%	0%
MB+*S.mutans*/GIC	10	1	0	0
% within group	90.9%	9.09%	0%	0%
MB+*S.mutans*/HDX	10	1	0	0
% within group	90.9%	9.09%	0%	0%

GIC and HDX scores were 0 and 1. In the evaluation with MB, all GIC samples had no microleakage (score 0). HDX samples and MB had 8 specimens with no microleakage (score 0) and 3 specimens with score 1. In the evaluation with MB + S. mutans, 10 GIC samples and 10 HDX samples showed no microleakage (score 0). Only 1 specimen restored with GIC or HDX showed leakage (score 1) (Table 4).

IV. DISCUSSION

This study assessed the microleakage of class I restorations with GIC and HDX using dye penetration test. Microleakage is a passage of ions, molecules, bacteria and liquids through the restorations interface, which is not always clinically detectable [19]. This can lead to the loss

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of marginal seal and an emergence of dentin hypersensitivity, recurrent caries, aesthetic issues, pulp damage and failure of the restoration treatment [20,21]. The assessment of microleakage is important to evaluate the bonding of dental materials and tooth structure [20]. Dye penetration test was performed in this study, which is a reliable test for assessment of microleakage and the marginal seal [22].

Most of the currently used dental biomaterials have a lack on completely seal the cavity margins over the time. Then, microorganisms can penetrate into the tooth tissue structure spaces as deep as 1100 μm [23]. Among of the solutions used to evaluate biomaterials adaptation, methylene blue is the most used in microleakage studies, because it has a high leakage capacity, low molecular weight, is similar to the molecules of microorganisms, in addition has low cost and the manipulation is easy [24-26]. In order to provide more similar condition to that found at the oral environment, this study evaluated microleakage using methylene blue dye and S. mutans, in the same solution [25,27]. Thereby, it is possible to compare whether there are differences between dye associated with microorganisms and only the penetration of the dye alone, an issue very little discussed in the literature. As results methylene blue dye alone or associated with S. mutans did not show difference. One possible explanation for these results could be that the dye can present greater microleakage results than the penetration of bacteria alone that are larger than the dye molecules evaluated [27]. However, the aim of this study was not to evaluate the presence or characterization of microorganisms at the tooth restoration interface. There was no published study evaluating the dye penetration results of dye associated with a bacterium compared with dye alone during GIC or HDX application. So, further microbiological availability analysis and SEM/TEM studies are recommended to analyze the tooth interfaces when using different types of biomaterials.

This study used glass ionomer cement Ketac Molar Easymix (GIC) and hydroxyapatite powder from human teeth (HDX) associated with glass ionomer liquid, there were no differences between the evaluated materials so, the null hypothesis was accepted. Ketac Molar was effective in preventing microleakage [28-30]. One probable reason is due the chemical interactions of polyalkenoic acids and hydroxyapatite's calcium which produce adequate marginal sealing and form a strong chelation reaction with calcium on the tooth surface. In this study we used specimens with cavity preparations on occlusal region with enamel and according another studies cavity margins with this substrate results in stronger bonds once the inorganic structure is higher in enamel than dentin [29,31]. Another feature of the product to be considered is that Ketac Molar conditioner is an acidic primer (pH 0.7±1.2) that partially removes the smear layer, improves the wettability of tooth, and increases the monomer penetration into the underlying surface [32].

HDX restorations presented very low microleakage, this result can be justified by the fact that hydroxyapatite is a material capable of establishing a good interaction with the dentinal structures, allowing a good cavity seal [33,34]. Different kinds of materials are applied in restorative dentistry, of special importance being hydroxyapatite, which substituted the hard tissues. It is possible due to particular properties of hydroxyapatite, like microstructure, high biocompatibility, non-toxic properties, bioactivity and bioconductivity [10,35]. A bioactive material creates an environment compatible with osteogenesis and dentinogenesis with the mineralizing interface developing bonding between biomaterials due a physicochemical process [35]. Therefore, the results suggest that HDX may be an alternative for restorative material. However, further studies should be carried out with a focus on assessing the applicability and properties of HDX compared to another materials.

The results of the present study showed that HDX is not more advantageous than Ketac Molar glass ionomer cement, were statistical equal, from the perspective of effective marginal sealing in class I cavities. Further studies and long-term clinical data are required to confirm our findings.

V. CONCLUSION

Within the limitations of this in vitro study and regarding the results, it can be concluded that hydroxyapatite may be an alternative as a restorative material.

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