Volume 6, Issue 3, 2016,1303-1307 **ISSN 2069-5837**

Biointerface Research in Applied Chemistry

www.BiointerfaceResearch.com

Original Research Article **Open Access Journal**

Received: 10.02.2016 / Revised: 15.05.2016 / Accepted: 14.06.2016 / Published on-line: 15.06.2016

Chitosan bio-active designer materials and orthodontics: development and evaluation of novel materials as enamel protective agents.

V. Tamara Perchyonok [1,*], Rafael Felitti [2], Shengmiao Zhang [3]

[1]VTPCHEM PTY LTD, Glenhuntly, 3163, Victoria, Australia.
[2]Faculty of Dentistry, Catholic University, Montevideo, Uruguay.
[3] School of Material Science and Engineering, East China University of Science and Technology, 130 Meilong Road, Shanghai, 200237, China.
*corresponding author e-mail address:tamaraperchyonok@gmail.com

ABSTRACT

The aim of the investigation is to further develop and evaluate a versatile designed chitosan based bio-active materials for the prevention of demineralization around the orthodontic brackets, while maintaining acceptable shear bond strength and not leave any residual adhesive on the tooth surface after removal of the brackets from the enamel surface and compare the performance of the Clinpro and Tooth Mousse as commercially available protective agents *in vitro*. The bio-active chitosan containing were prepared by dispersion of the corresponding component in glycerol and acetic acid with the addition of chitosan gelling agent. Mechanical performance such as shear bond strength of attachment between the orthodontic bracket and enamel on the extracted premolar teeth were measured, as well as bio-adhesive studies were investigated in order to assess the suitability of these designer materials. The adhesive remnant index (ARI) and failure site assessment was completed immediately after each shear bond strength de-bonding under ×20 magnification. The bioactive modified hydrogels showed a high adhesive force and significantly lower shear bond strength was recorded when Clinpro was used before bonding using the self-etching adhesive system. The bioactive chitosan containing group showed the lower ARI scores to be more frequent, while Clinpro, Tooth Mousse and control groups showed higher ARI scores more frequently. The adhesive remnant was not different between the self-etching and the conventional etching subgroups. In this study we demonstrated that the newly prepared bio-active modified hydrogels which demonstrate the beneficial for the prevention of demineralization around the orthodontic brackets, while maintaining the appropriate shear bond strength and inhibition as alternative materials and compare the performance of the Clinpro and Tooth Mousse as commercially available protective agents.

Keywords: *chitosan, bio-active, orthodontic brackets, shear bond strength.*

1. INTRODUCTION

Despite the advances in orthodontic materials and techniques, the development of cavitations and significant enamel demineralization around the brackets during treatment continues to be a problem [1]. Fixed appliances make it difficult for young patients to maintain adequate oral hygiene during orthodontic treatment [2]. These appliances are linked to a high risk of developing white-spot lesions [3]. The prevalence of new decalcifications among orthodontic patients with fixed appliances is reported to range from 13% to 75% [3, 4].

Patients with orthodontic brackets have an elevated risk of caries, and enamel lesions can occur within a month, irrespective of mechanical plaque control and whether fluoridated dentifrice is used [3, 5, 6].

Several methods have been used to prevent or reduce enamel demineralization during orthodontic treatment, including fluoride application in various forms, enamel sealants, rigorous oral-hygiene regimens using glass-ionomer cement for bonding bracket and modified appliance designs[7–9].

Chitosan and modified chitosan are interesting candidates in this respect. Chitosan, a natural linear bio-poly(aminosaccharide) is obtained by alkaline deacetylation of chitin [10–12]. This material is also biocompatible and biodegradable. It is positively charged and combines with the bacterial cell wall and membrane, with bacteriostatic and bactericidal results [15,16].

Muzzarelli et al., [17] demonstrated that chitosan exhibit bactericidal action against several pathogens, including Streptococcus mutans. This is especially important since S. mutans is known to be the principal etiological factor of dental caries [18-20].

The aim of the investigation is to further develop and evaluate a versatile designed chitosan based bio-active materials for the prevention of demineralization around the orthodontic brackets, chitosan mouth gels as alternative materials and compare the performance of the Clinpro and Tooth Mousse as commercially available protective agents via the adhesive remnant index (ARI) and failure site assessment was completed immediately after each shear bond strength de-bonding under ×20 magnification.

2. EXPERIMENTAL SECTION

2.1. Preparation of bracket cements modified with chitosan hydrogels: general protocol.

The bio-active containing gel was prepared by dispersion of 0.2 gm in glycerol (5% w/w) (1 ml) using a mortar and a pestle following the earlier reported generic protocol[6].10 ml of glacial acetic acid (2% w/w) was afterwards added with continuous mixing and, finally, chitosan (10% chitosan w/w) was spread on the surface of the dispersion and mixed well to form the required gel. Then, the gel was mixed into a commercial bracket bonding cement.

2.2. Shear bond strength tests for bracket bonding.

Extracted non-carious, intact, human premolars stored in water containing a few crystals of thymol at 4°C were used within two months. Samples were checked before use for any damage caused by their removal. The teeth were fixed in self-curing acrylic resin placed in flexible molds with the roots embedded in the acrylic and the crown exposed and oriented perpendicularly to the bottom of the mold.

Premolar stainless steel brackets (Equilibrium 2 Roth prescription, 0.022 in. slot size, Dentaurum Orthodontics, Ispringen, Germany) were used. The buccal surface of each tooth was cleaned with non-fluoride oil-free pumice paste using a nylon brush attached to a slow-speed hand piece for 5 s, and then the tooth was rinsed with water for 10 s and dried with an oil-free air spray. Brackets were bonded to the teeth according to the manufacturers'instructions for the adhesive system and stored in distilled water at 37°C until testing.

Bracket debonding was performed 72 h after bonding in a material testing unit (Instron 5960) with an occluso-gingival load applied to the bracket base. The shearing rod was adjusted each time, so the shearing blade is parallel to the base of the bracket contacting it in a reproducible way in each test. The crosshead speed was 2.0 mm/min and the failure load in Newton was divided by the bracket base bonding area of 10.90 mm^2 to calculate the shear bonding strength in MPa.

The adhesive remnant index (ARI) and failure site assessment was completed immediately after each shear bond strength de-bonding under ×20 magnification [26]. The ARI evaluation used the 4-point scale of Årtun and the resin/enamel interface; 1 indicates less than half the resin left on the tooth surface, implying that bond fracture occurred predominantly at the resin/enamel interface; 2 indicates more than half the resin left on the tooth surface, implying that bond fracture occurred predominantly at the bracket/resin interface; and 3 indicates all resin left on the tooth surface, with a distinct impression of the bracket base, implying that bond fracture occurred at the bracket/resin interface [26].

Descriptive statistics, including mean, standard deviation, and minimum and maximum values of the shear bond strength, were calculated for each of the adhesive systems tested. A Kruskal-Wallis test was used in conjunction with a Bonferonni test to compare the differences in the ARI scores between the groups. Significance for all statistical tests was at $P \leq .05$. Statistics were carried out using Prism 6 Statistical Package.

Two types of commercially available enamel protective agents were used in the current study: Tooth mousse (GC Corp., Tokyo,Japan) and Clinpro (3M Unitek, Monrovia, CA, USA) and three chitosan containing hydrogels. The two adhesive systems used in this study were Transbond XT light cure adhesive and Transbond Plus Self Etching Primer (3M Unitek, Monrovia, CA, USA), and Transbond XT light cure adhesive, Transbond XT primer and 37%phosphoric acid (3M Unitek, Monrovia, CA, USA). All materials were used according to the manufacturers' instructions. Each group was divided into two subgroups; in the first one, orthodontic brackets were bonded with self-etching adhesive system and in the second one, a conventional adhesive system was used.

3. RESULTS SECTION

3.1. The characterization of bioactive containing chitosan gels (Gel 1-3).

The SEM images were obtained to characterize the microstructure of the freeze-dried propolis containing bioactive:chitosan gels and are presented in Figure 1. It can be seen that the gels displayed a homogeneously pore structure. It was thought that the micro-porous structure of the gels could lead to high internal surface areas with low diffusional resistance in the gels. The surfaces of the gels were also presented (Figure 1).

Figure 2 gives the shear bond strength values (MPa) after 72 hours of storage of samples in artificial saliva using conventional orthodontic dental adhesive systems such as 3 bottle etch/prime/bond system (standard 1) and 2 bottle self etch/prime and bond system (standard 2) without applying a protective agent.
In general there was an increase in bond strength of the enamel treated with the chitosan containing hydrogels containing bio-actives compared to the bond strength of the conventionally bonded teeth. An increase in the shear bond strength was also previously reported [19] for chitosan containing hydrogels[20].

The additional advantage of the system may suggest that, chitosan: bioactive interaction with crystalline hydroxyapatite structure of the enamel layer increases the enamel bond strength observed especially in the case of the direct bonding between the hydrogel and the enamel surface interface.

The increase of the bond strength is also attributed to the unique capacity of chitosan, as well as biological properties of bio-actives, such as propolis, copaiba oil and oblepicha oil, to influence bio-adhesion of the hydrogels and promote the formation of the reparative hydroxyapatite interface; therefore, starting all the important hydroxyapatite regeneration processes, making these type of designer bioactive hydrogels important prototypes in the development of bioactive restorative materials.

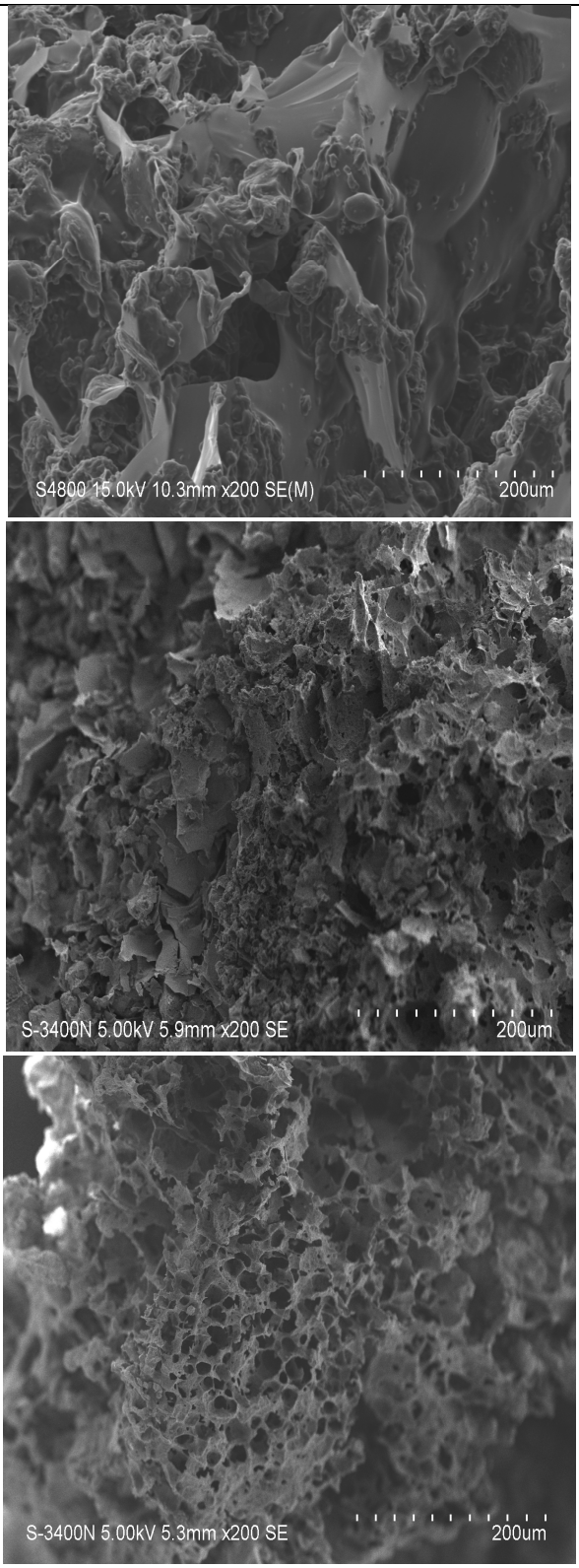

Figure 1. SEM photographs of interior morphology of the selected gels under investigation for (a) Chitosan/Oblepicha, (b) Chitosan/Copaibaand (c) Chitosan/Propolis Brazilian.

The additional benefit of using the chitosan:antioxidant system as bonding/pre-bonding to enamel system lies in its ability to show favorable immediate results in terms of bonding effectiveness as well as the durability of resin-dentin bonds for a prolonged time (up to 12 months) [21].

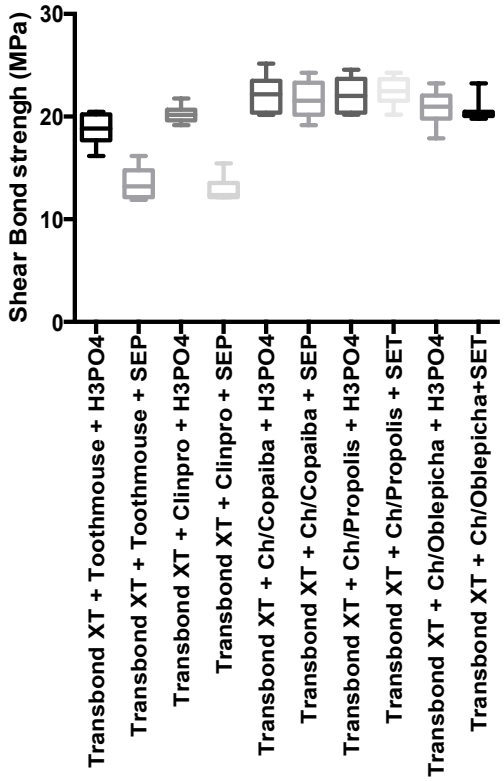

Figure 2. Shear bond strength of hydrogels after 72 hours of bonding to orthodontic brackets to enamel.

The results of the Kruskal-Wallis test showed that the ARI scores (Figure 3) were significantly different between the groups. Results of one-way ANOVA also showed that the type of preventive agent used on the enamel significantly influenced the ARI scores distribution; there was a significant difference depending on whether it was chitosan:bioactive hydrogels (containing propolis, copaiba oil or oblepicha oil) or Clinpro that was used before bonding with self etching primer or with phosphoric acid etching.

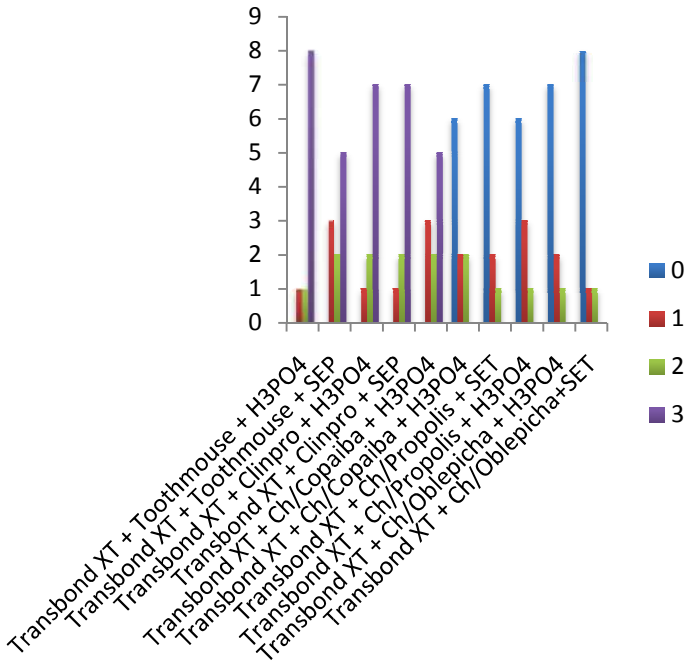

Figure 3. Frequencies of the ARI scores for the groups of interest.

4. DISCUSSION

The lowest shear bond strength was recorded with the samples treated with Clinpro or Tooth Mouse before bonding the orthodontic brackets especially using self etching adhesive system; the shear bond strength in this group was significantly lower than the shear bond strength in the other groups.

In case of Clipro as a protective agent, the loss in shear bond strength could be attributed to the resistance effect that the outer enamel layer acquires from the fluoride content of the Clinpro which may be of significant effect especially when using self-etching primers in bonding due to their more superficial etching effect compared with the etching of the conventionally used phosphoric acid.

Previous studies [22-25] with scanning electron microscope (SEM) indicated that although self-etch priming agents have the potential to etch the enamel surface, the etching pattern is less deep compared to the etching pattern of phosphoric acid. A chemical bonding capacity through the interaction between some functional monomers and the calcium of residual hydroxyapatite may contribute favorably to the bonding effectiveness [26-29], but fluoride affects the enamel surface rendering it moreresistant to demineralization. Fluoride in low concentrations favors the formation of fluoro-hydroxyapatite, which is less susceptible to acidic solubility than hydroxyapatite [34, 35]. Therefore, it is recommended to use these preventive agents after bonding the brackets when self-etch adhesive systems are used.

The shear bond strengths recorded in this study were sufficient for clinical use in all the groups presenting different combinations of adhesive systems and enamel protective agents as well as control groups. The average range of bond strength was suggested by Reynolds [35] to be 5.9 to 7.8 MPa for clinical and 4.9 MPa for laboratory performances. *In vitro* and *in vivo* studies of SBS are both needed; *in vitro* measurements of shear bond strength provide useful information about the bonding efficiency of different types of materials, but the actual performance of these materials can only be evaluated in the environment where they were intended to function [39].

In the case of lower shear bond strength, upon pretreatment of the enamel surface with the Tooth Mousse especially upon using self etching adhesive system, the results are in line with a previously reported study in which calcium phosphate in the form of CPP–ACP added to a Tooth Mousse can enhance the level of re-mineralizationof enamel subsurface lesions when the product is used *in situ* [40].

The distribution of the ARI scores was assessed in this study under ×20 magnifications [36]. Although different quantitative and qualitative methods have been used to assess the ARI scores after orthodontic bracket debonding; the quantitative methods were found preferable, if accurate evaluation of the adhesive remnant is required [37]. ARI score evaluation system has proved to be of value in the studies of orthodontic adhesive systems. ARI score system is a quick and simple method that needs no special equipment. Although SEM evaluation might be more accurate than evaluation under ×10 or ×20 magnification, it is harder to be reflected in clinical applications [38].

The distribution of the ARI scores was found to bedifferent between the major groups under investigation such as Clinpro, Tooth Mousse and Bio-active hydrogels containing chitosan derivatives. In the Clinpro and control groups, both self-etching and conventional etching subgroups, less adhesive remnant tended to be seen left on the enamel surface after debonding. This could be attributed to the chemical bond between the resin infiltration and the adhesive resin.

5.CONCLUSIONS

Based on the above findings, we conclude the following:

Significantly lower shear bond strength was recorded when Clinprowas used before bonding, using the self-etching adhesive system. The bioactive:chitosan containing group showed the lower ARI scores to be more frequent, while Clinpro, Tooth Mousse and control groups showed higher ARI scores more frequently. The adhesive remnant was not different between the self-etching and the conventional etching subgroups.

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