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Degradation of dentin-bonded interfaces treated with collagen cross-linking agents in a cariogenic oral environment: An *in situ* study

Viviane Hass[a], Alexandra Mara de Paula[b], Sibelli Parreiras[b], Mário Felipe Gutiérrez[b,c], Issis Luque-Martinez[d], Thalita de Paris Matos[b], Matheus Coelho Bandeca[a], Alessandro D. Loguercio[b,*], Xiaomei Yao[e], Yong Wang[e], Alessandra Reis[b]

[a] Department of Postgraduate Program in Dentistry, CEUMA University, São Luis, MA, Brazil
[b] Department of Restorative Dentistry, School of Dentistry, State University of Ponta Grossa, PR, Brazil
[c] Institute for Research in Dental Sciences, Faculty of Dentistry, University of Chile, Chile
[d] Dentistry Academic Unit, Faculty of Medicine, Pontificia Universidad Católica de Chile, Chile
[e] Department of Oral & Craniofacial Sciences, University of Missouri–Kansas City, Kansas City, MO, USA

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ABSTRACT

Objectives: To evaluate the effect of treatment using collagen cross-linking agents as primer on resin–dentin bond interfaces subjected to cariogenic oral environment (COE).
Methods: Each of forty human teeth had two cavities (4 × 4 × 1.5 mm) prepared within enamel margins. These cavities were acid-etched and treated by the primers containing one of the following treatment agents (6.5% proanthocyanidins, 0.1% riboflavin-UVA activated light, 5% glutaraldehyde or distilled water as a control group). After that the cavities were bonded and restored with resin composite. One restoration for each tooth was tested immediately (IM) and another was included in an intra-oral palatal device that was placed in each mouth of ten adult volunteers for 14 days in COE. After 14 days, the teeth were removed and each restoration was sectioned to obtain a slice for Knoop microhardness (KHN) and resin–dentin bonded sticks for microtensile bond strength (μTBS) and nanoleakage (NL) evaluation. Data were evaluated by two-way ANOVA and Tukey's tests ($\alpha = 0.05$).
Results: After 14 days in a COE, the KHN was reduced for all groups, except for the glutaraldehyde group; however, the proanthocyanidins group retained the highest KHN in IM and after COE ($p < 0.05$). The μTBS was not reduced after COE for the proanthocyanidins and glutaraldehyde groups, however only the proanthocyanidins treatment did not increase the NL after COE ($p > 0.05$).
Conclusion: The *in situ* study model seems to be a suitable short-term methodology to investigate the degradation of the bonding interfaces under a more realistic condition. Under COE, the proanthocyanidins and glutaraldehyde treatments produced stable interfaces that are worth further clinical investigation.

1. Introduction

The hybrid structure formed during the dental bonding procedure occurs by demineralization of the dentin surface, followed by infiltration and subsequent polymerization of monomers around the collagen fibrils [1]. Therefore, to achieve effective and stable bonding, the preservation of dentin collagen is critical, since collagen represents the major organic component of the dentin matrix.

Unfortunately this is not an easy task. During bonding procedures, the demineralized collagen fibrils are not completely infiltrated by resin monomers, [2,3] and these denuded collagen fibrils are more prone to degradation. Fluctuations of acidity produced by different pHs of foods and drinks as well as that induced by bacterial acids may increase the amount of exposed organic matrix to be broken-down by bacterially derived enzymes. Additionally, host-derived enzymes [4] such as matrix metalloproteinases (MMPs) and cysteine cathepsins [5–8] present in the dentin matrix and in the gingival crevicular fluid, also play a role on resin–dentin bond degradation.

* Corresponding author at: Universidade Estadual de Ponta Grossa. Departamento de Odontologia. Avenida Carlos Cavalcanti, 4748—Uvaranas, Ponta Grossa, Paraná, CEP: 84030-900, Brazil. Fax: +55 42 3220 3741.
E-mail addresses: aloguercio@hotmail.com, alelog@uepg.br (A.D. Loguercio).

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The role of these host-derived proteases in the breakdown of the collagen matrices during the pathogenesis of dentin caries [9,10], periodontal disease [11] and degradation of resin–dentin bonded interfaces [12] has already been demonstrated [8,9]. Measures that enhance dentin resistance against collagenolytic activities have great potential for improving the longevity of the dentin bonding. In this context, collagen cross-linking agents have been investigated as dentin biomodifiers.

These crosslinking agents can interact with various extracellular matrix components inducing increases in the mechanical properties of the tissue, decreasing the biodegradation rates and possibly inducing mineral nucleation [13–15], which make them a promising solution for preservation of resin–dentin bonded interfaces [16,17].

However, most of the studies that support the benefits of cross-linking agents are performed in laboratories, where the challenging conditions of the oral environment are barely reproduced. Although randomized clinical studies are the best study design to evaluate both the performance and longevity of restorative materials, they are time demanding, costly and dependent on the approval by a local Ethics Committee. Under this scenario, the conduction of *in situ* studies may gather important information to the field, as it resembles the challenging clinical conditions that resin–dentin interfaces are prone better than *in vitro* studies. *In situ* studies may be considered as an intermediate stage between *in vitro* and clinical studies. Therefore, the aim of this study was to investigate the degradation of resin–dentin interfaces treated by different collagen cross-linking agents after *in situ* cariogenic challenge, using microhardness, microtensile bond strength and nanoleakage. The test null hypotheses were that after 14 days of exposure to an intra oral cariogenic environment, the Knoop hardness, μTBS and nanoleakage of resin–dentin interfaces treated by different collagen cross-linking agents did not change.

2. Material and methods

The study protocol was approved by the Local Ethics Committee Review Board under protocol number 314.563. Ten healthy adult volunteers (aged 21–30 years, female and male) were selected according to the following inclusion criteria: good general and oral health and normal salivary flow rate. Participants that took antibiotics for the last 2 months before the experiment or wearing prosthesis or orthodontic devices were not included in this study. All volunteers agreed to participate and signed an informed written consent.

A total of forty extracted, non-erupted human third molars were used. The teeth were collected after obtaining the patients' informed consent under a protocol approved by the previously described Ethics Committee previously described. Teeth free from cracks or any other kinds of structural defects were selected. The teeth were disinfected by storage in 10% buffered formalin solution, pH 7, for 7 days [18] and stored in distilled water for up to 2 months after extraction.

2.1. Experimental design

This *in situ*, split-mouth study was designed for accumulation of a plaque-like biofilm on the restorations in a high cariogenic challenge promoted by sucrose exposure. This protocol was performed for 14 days. The factors under evaluation were: (1) three different collagen cross-linking agents (proanthocyanidins from grape seed extract, UVA-activated riboflavin, glutaraldehyde and distilled water as control); and (2) evaluation time—2 levels (immediate and 14 days after degradation in a cariogenic oral environment). Then, a total of eight experimental conditions were tested.

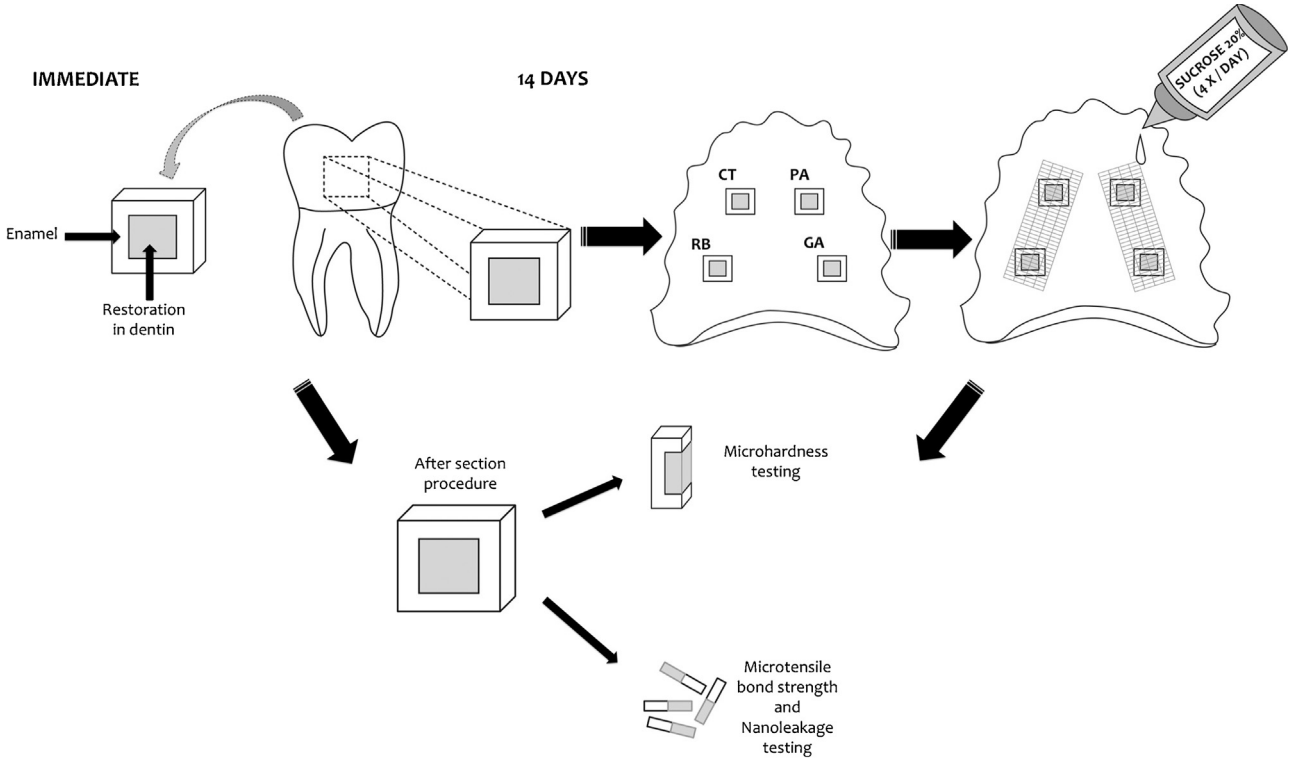

Fig. 1. Representation of experimental design used in this study. CT: control group; PA: proanthocyanidin group; RB: riboflavina group and GA: glutaraldehyde group.