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# Interface evaluation of experimental dental adhesives with nanostructured hydroxyapatite incorporation

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## Abstract

The aim of this study was to evaluate the adhesive interface with dentin of an experimental adhesive resin with nanostructured hydroxyapatite addition. The organic phase of the adhesive resin was produced by mixing 50 wt.% Bis-GMA, 25 wt.% TEGDMA and 25 wt.% HEMA. CQ and EDAB were added at 1 mol% to all groups, according to the monomer moles. $HA_{nano}$ was added at the following two concentrations: 0 and 2 wt%. One commercial adhesive system was used as control. Nine lower incisor bovine teeth were used to produce interfaces of adhesive resin and dentin. Tooth slices were analysed using the following micro-Raman parameters: a 100 mW diode laser with 785 nm wavelength and spectral resolution of ~ 3–5 $cm^{-1}$. One-dimensional mapping was performed over a 150 μm line across the adhesive-dentine interface at 1 μm intervals using a computerized XYZ stage. These areas covered the composite resin, adhesive layer, hybrid layer, partially demineralised and un-affected dentine and were visualised and focused at x500 magnification. Raman analysis showed the penetration of experimental and commercial adhesive systems into dentin. $HA_{nano}$ was observed into the hybrid layer. Based on results of the present study, is possible to observe resin and nanostructured hydroxyapatite penetration at the hybrid layer.

**Keywords:** Adhesive resin; Dentistry; Micro Raman

## Background

Degradation of hybrid layer at dentin adhesive interface is a concern for long term success of restorative procedures [1]. Achievement of a more hydrolytic stable hybrid layer is a recurrent goal of adhesive systems development nowadays. Comonomer blends with higher hydrophilicity leads to a hybrid layer more prone to degradation [2,3]. Furthermore, addition of fillers to adhesive resin could increase mechanical properties and decrease long term degradation of hybrid layer. The addition of fillers reduces the amount of organic matrix in the same volume of material. Considering that fillers are less prone to degradation by hydrolysis [4], the materials with filler addition could present a decreased long term degradation.

Different types of filler have been incorporated to adhesive systems, such as $SiO_2$ [5], $Ta_2O_5$ [6] and $Nb_2O_5$ [7] to increase its mechanical properties. However, few studies evaluated addition of apatite fillers at adhesive resin properties. Since hydroxyapatite

(HA) is a biological material, its presence in hybrid layer is desirable to reconstruct the HA depleted zone after acid etching. Hydroxyapatite has been evaluated in adhesive resins in different morphologies like spherical particles [8], nanorods [9] and nanostructured hydroxyapatite ($HA_{nano}$) [10]. $HA_{nano}$ addition to adhesive resin showed reliable properties in a recent published study [11]. However, the dentin/adhesive interface was not characterized regarding its infiltration. The aim of this study was to evaluate the adhesive interface with dentin of an experimental adhesive resin with nanostructured hydroxyapatite addition.

## Methods

### Formulation

Experimental dental adhesives were produced using bisphenol A glycol dimethacrylate (BisGMA), triethylene glycol dimethacrylate (TEGDMA), 2-hydroxyethyl methacrylate (HEMA), provided by Esstech Inc (Essington, PA, USA) and camphorquinone (CQ) and ethyl 4-dimethylaminobenzoate (EDAB) (Sigma Aldrich, USA), used without further processing. The organic phase of the adhesive was produced by mixing 50 wt.% Bis-GMA, 25 wt.% TEGDMA and 25 wt.% HEMA. CQ and EDAB were added at 1 mol% to all groups, according to the monomer moles. Nanostructure hydroxyapatite ($HA_{nano}$) was produced according to previous studies [10-12]. $HA_{nano}$ was added at the following two concentrations: 0 and 2 wt%. No radical scavenger was added. To improve the adhesion interface between filler particles and the matrix, $HA_{nano}$ was subjected to a silanisation process with 5% of silane (γ- methacryloxypropyltrimethoxysilane, Aldrich Chemical Co., Milwaukee, WI, USA) and 95% of solvent (acetone), in weight. After the silanisation process, the particles were stored for 24 hours at 37°C to allow the solvent to evaporate. All components were weighed using an analytical balance (AUW220D, Shimadzu, Kyoto, Japan), mixed and ultrasonicated for 1 hour. One commercial adhesive system was used as control (Scotchbond Multipurpose Plus, 3 M ESPE, St. Paul, USA). To perform monomer photo-activation, a light-emitting diode unit (Radii Cal, SDI LTD., Bayswater, VIC, Australia) was used. An irradiation value of 1200 $mW/cm^2$ was confirmed with a digital power meter (Ophir Optronics, North Logan, UT, USA).

### Interface characterisation by micro-Raman

Nine lower incisor bovine teeth were cleaned and stored in distilled water at 4°C. The labial enamel was removed using a water-cooled, low-speed diamond saw (Low Speed Saw; Buehler, Lake Bluff, IL, USA) to expose the superficial dentin. A smear layer was produced by grinding the flat surface with a 600-grit silicon carbide (SiC) disc under water for 30 s. The dentin was etched with phosphoric acid 37% (Condac 37, FGM, Joinvile, SC, Brazil) for 15 s and washed for an additional 15 s. A commercial primer (Primer Scotch bond multi-purpose, 3 M ESPE, St Paul, MN, USA) was applied in all groups and the solvent was dried for 5 s with an air spray. Adhesive resin was applied according the experimental group and photocured for 20 seconds. A commercial composite resin (Z350, 3 M ESPE, St Paul, MN, USA) was inserted in two increments of 2 mm and photocured for 40 seconds each to simulate tooth restoration.

The bonded specimens were stored in distilled water in a light-proof container at 37°C for 24 h. Sections (1 mm in thickness) were prepared by sectioning perpendicular to the

flat adhesive-dentine surface, using a precision cutting machine under constant water-cooling (Low Speed Saw, Buehler, Lake Bluff, IL, USA).

Micro-Raman spectroscopy was performed using a SENTERRA Raman Microscope (Bruker Optics, Ettlingen, KA, Germany). The samples were analysed using the following micro-Raman parameters: a 100 mW diode laser with 785 nm wavelength and spectral resolution of ~ 3–5 $cm^{-1}$. One-dimensional mapping was performed over a 150 μm line across the adhesive-dentine interface at 1 μm intervals using a computerized XYZ stage. These areas covered the composite resin, adhesive layer, hybrid layer, partially demineralized and un-affected dentine and were visualized and focused at x500 magnification. Accumulation time per spectrum was 5 seconds with 2 co-additions. Post-processing was performed in Opus6.5 (Buker Optics Ettlingen, KA, Germany) and consisted of analysis with modeling, which distinguished spectral components of the adhesive and dentine. One correspondent peak of each substance was used for integration. For the hydroxyapatite, 960 $cm^{-1}$ was used and for adhesive monomer, 1610 $cm^{-1}$ was used.

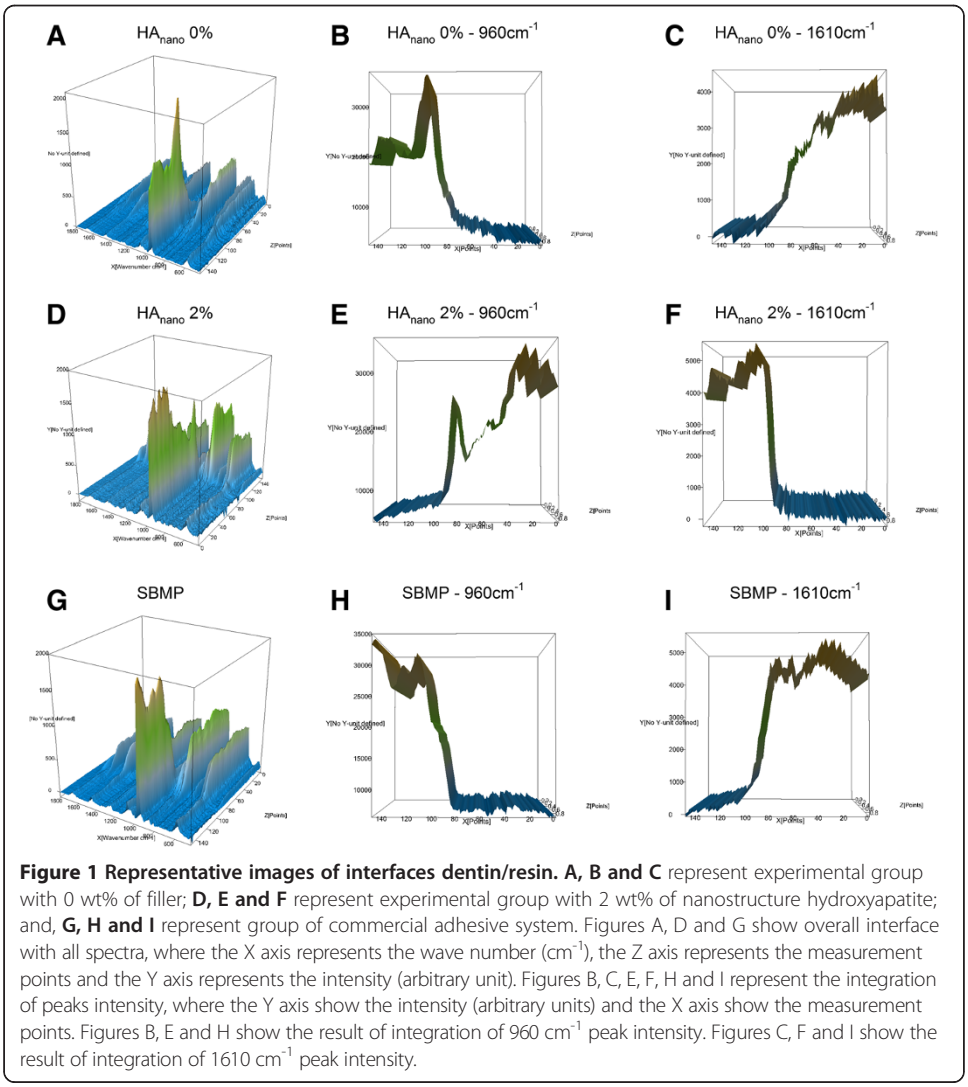


**Figure 1 Representative images of interfaces dentin/resin. A, B and C** represent experimental group with 0 wt% of filler; **D, E and F** represent experimental group with 2 wt% of nanostructure hydroxyapatite; and, **G, H and I** represent group of commercial adhesive system. Figures A, D and G show overall interface with all spectra, where the X axis represents the wave number ($cm^{-1}$), the Z axis represents the measurement points and the Y axis represents the intensity (arbitrary unit). Figures B, C, E, F, H and I represent the integration of peaks intensity, where the Y axis show the intensity (arbitrary units) and the X axis show the measurement points. Figures B, E and H show the result of integration of 960 $cm^{-1}$ peak intensity. Figures C, F and I show the result of integration of 1610 $cm^{-1}$ peak intensity.

## Results and discussion

The evaluation of interface dentin/restorative materials could illustrate the behavior of materials within the hybrid layer. In the present study, Raman analysis showed the penetration of experimental and commercial adhesive systems into dentin. Representative images of interfaces are shown in Figure 1. The presence of adhesive can be observed across the hybrid layer, and it is possible to observe the penetration of nanostructured hydroxyapatite ($HA_{nano}$) at almost the same extension of dentin demineralization (Figure 1D, E and F). The presence of inorganic fillers into adhesive layer could promote an increase the resin/dentin bond strength [11]. Inorganic fillers increase mechanical properties of adhesive resin [7] and the use of stronger polymers could result in stronger hybrid layer [13,14]. Complete penetration of polymer across mineral-depleted dentin is important to avoid collagen degradation, and consequently, the reduction of durability of resin/dentin bond strength [15].

The evaluation of the adhesive interface promote a more detailed understanding of relationship of different components of adhesive resin. The addition of nanofillers increase the viscosity of the resin, as shown elsewhere [16], and could difficult material penetration between demineralized collagen network. On the other hand, nanofillers are more prone to penetrate than micrometer particles. The collagen interfibrillar spaces are around 20 nm [17]. In the present study, the used nanostructured hydroxyapatite has a mean diameter size of 26.9 nm, as shown elsewhere [11]. Considering the distribution of particle size, at the suggest experimental adhesive resins, could be found particles with less than 20 nm of diameter, allowing the penetration into collagen network spaces. In the present study, the group with 2 wt% hydroxyapatite addition exhibited penetration across the hybrid layer. Experimental group with 0 wt% of $HA_{nano}$ and commercial adhesive system presented penetration of monomers across the interface (Figure 1A, B, C, G, H and I). The evaluation of a commercial adhesive could indicate the correct production of experimental adhesive (without filler - Group 0%) and evaluate the real contribution of added filler.

In the present study, the group with incorporation of 2 wt% of nanostructured hydroxyapatite was evaluated since this group presented the best behaviour in other study [11]. The highest bond strength achieved by group with 2 wt% in other study [11] could be explained by penetration of $HA_{nano}$ through the hybrid layer. Further studies should be conducted to evaluate the longitudinal bond strength and the interface behaviour after long term hydrolysis and enzymatic degradation. The present results represent an important increment at adhesive resin behavior, comparing to non filler adhesive resin.

## Conclusion

Based on results of the present study it is possible to observe resin and nanostructured hydroxyapatite penetration at the hybrid layer.

#### Competing interests

The authors declare that they have no competing interests.

#### Authors' contributions

FMC participated in the study design development, manuscript construction and manuscript final review, CP participated in the HA production and laboratory analysis, VCBL participated in the study design development and manuscript construction, RT participated in the HÁ production, study development and manuscript construction, CPB participated in the study design development and manuscript final review, and SMWS participated in the study design development and manuscript final review. All authors read and approved the final manuscript.

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