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Masticatory function induced changes, at subnanostructural level, in proteins and mineral at the resin–dentine interface

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ABSTRACT

Objective: This study evaluated the ability of different *in vitro* mechanical loading tests to promote new mineral formation at the bonded dentine interfaces created with a two-step self-etching resin adhesive.
Methodology: Restored teeth were divided in the following groups: (1) unloaded, load cycling with (2) sine waveform, (3) square waveform, and hold waveform for (4) 24 h, and (5) 72 h. Raman spectroscopy and cluster analysis were used to assess the resin–dentine interface.
Results: Mechanical loading in CSEB-treated samples promoted a generalized increase of relative presence of minerals and ratio of phosphate peaks, except in square waveform, where the nature of collagen resulted damaged. Crystallinity of carbonate was higher than phosphate. The organic component showed, in general terms, an increase in crosslinking. Molecular orientation (α-helices) peaks augmented in all tests. Pentosidine vibration increases in all tests, except in hold 72 h. Ratios amide I and II/CH_2 incremented, in general. Non uniform parameters of Bis-GMA and adhesive penetration were encountered, as both increased at the bottom of the hybrid layer when loading square and hold 72 h were applied.
Significance: Functional remineralisation at the resin–dentine interface was attained after in vitro mechanical stimuli application. When loading in square waveform, the lowest vibrations to favor remineralisation were attained.

1. Introduction

Dentine is a composite of an organic matrix and an inorganic mineralised component. It is composed of approximately 70% mineral, 20% organic matrix and 10% water by weight. The organic matrix is 85–90% fibrous protein (primarily type I collagen fibrils), which provides a supporting matrix upon which the mineral crystals grow. Minor proteins provide additional structural strength as well as regulatory and signaling functions. The mineral fraction mainly consists of carbonated-substituted hydroxyapatite (Xu and Wang, 2012a).

To promote adhesion to dentine, the mineral phase from the substrate has to be removed, by conditioning or etching, and the voids left by mineral should be filled with the adhesive resin that undergoes complete *in situ* polymerization to form the

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List of symbols and acronyms

A	amide.
ADH	adhesive.
AGEs	advanced glycation end products.
BHL	bottom of hybrid layer.
Bis- GMA	bisphenol A diglycidyl methacrylate.
CQ	camphorquinone.
CSEB	Clearfil SE Bond (Kuraray Medical Inc., Tokyo, Japan).
DC	degree of conversion of adhesive.
DEN	dentine.
DMP	dentine Matrix Protein 1.
FWHM	Full-width half-maximun
GMC	gradient in mineral content.
HAP	hydroxyapatite.
HEMA	2-hydroxyethyl methacrylate.
HL	hybrid layer.
KMC	K-means cluster analysis.
MDP	methacryloyldodeylphosphate.
MMP-2	metalloproteinase 2.
MMPs	metalloproteinases.
ODMAB	2-(ethylhexyl)-4-(dimethylamino) benzoate (co-initiator).
PAM	polyacrylic acid methacrylated.
PCA	principal components analysis.
PCs	principal components.
PPR	phosphate peaks ratio.
Pyrid	pyridinium.
RMC	relative mineral concentration between mineral/phenyl.
UDMA	urethane dimethacrylate.

hybrid layer (HL) (Van Meerbeek et al., 2003). The self-etching adhesive systems facilitate the etching and priming of the dentine at the same time without rinsing. Self-etch adhesives are a promising development in the adhesive dentistry, especially in terms of reduction of the necessary application steps and the possibility of a chemical interaction with hydroxyapatite-coated collagen fibers. The self-etching systems can be presented in two-steps with a separate etching-priming liquid, followed by the application of an adhesive resin. In these adhesives, hydrophobic and hydrophilic monomers are mixed together with a high content of solvent to keep them in solution (Toledano et al., 2012a). Functional monomers (10-methacryloyloxi-decyl-dihydrogen-phosphate/MDP) play the most essential role on the bonding performance and on the physicochemical properties of self-etch adhesives, as they are capable of chemically bonding to calcium in dentine (Van Landuyt et al., 2008). Nevertheless, a reduced volume of demineralised/unprotected collagen layer remains at the base of the hybrid layer (BHL) which may become the sites for collagen hydrolysis by host-derived matrix metalloproteinases (Toledano et al., 2012b), reducing the clinical success of restoratives because of the loss of this integrity at the resin–dentine interface (Spencer et al., 2010).

Teeth are continuously subjected to stresses during mastication, swallowing, and parafunctional habits. Weakly bonded tooth–material interfaces are more prone to suffer from the effects of oral environment in the short and long term (Erdilek et al., 2009). Occlusal trauma is caused by conditions such as premature contacts, bruxism and clenching (Noma et al., 2007) and can affect both restorations and restorative strategies involving dentine. Bruxism, a diurnal or nocturnal parafunctional activity, includes clenching (continuous or sustained loading), bracing, gnashing and grinding of teeth, in other than chewing (cyclid loading) movement of mandible. Thereby, minimally invasive dentistry comprises the philosophy of preservation of the maximum quantity of reparable dental tissues and utilizing remineralisation approaches (Milly et al., 2014), to obtain the integrity of the resin–dentine interface. In vitro load and thermal cycling have been demonstrated to promote mineralisation at the resin-infiltrated dentine and mineral depleted areas of the resin–dentine interface, at 24 h and 3 weeks of storage (Toledano et al., 2014a–2014c).

Confocal Raman spectroscopy analysis with the micro-Raman mapping technique have the considerable advantage of being sensitive to both the mineral and organic components of dentine, thus allowing for the study of mineral–matrix interactions as well as each individual component's properties. Sample preparation is relatively simple; this non nondestructive approach allows for spatial distribution mapping of resin–dentine interface's components as well as compositional analysis. Raman spectroscopies enable studies at micron-scale spatial resolution, and holds great promise for generating high definition chemical-state images that may prove invaluable in the study of resin–dentine interfaces. In this in vitro study we attempted to describe the morpho-chemical influence of chewing and clenching on the resin–dentine interface created by a self-etching adhesive. The purpose of this study was to evaluate the ability of different in vitro mechanical loading tests to promote dentine remineralisation at subnanoestructural levels. The tested null hypothesis is that structural changes of proteins and minerals are not produced at the resin–dentine interface created by a self-etching adhesive after some in vitro mechanical loading tests.

2. Material and methods

2.1. *Specimen preparation, bonding procedures and mechanical loading*

Ten non-carious human third molars were obtained with informed consent from donors (20 to 40 yr of age), under a protocol approved by the Institution Review Board. Molars were stored at 4 °C in 0.5% chloramine T for up to 1 month before use. A flat mid-coronal dentine surface was exposed using a hard tissue microtome (Accutom-50; Struers, Copenhagem, Denmark) equipped with a slow-speed, water-cooled