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Effect of *in vitro* chewing and bruxism events on remineralization, at the resin–dentin interface

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ABSTRACT

The purpose of this study was to evaluate if different *in vitro* functional and parafunctional habits promote mineralization at the resin–dentin interface after bonding with three different adhesive approaches. Dentin surfaces were subjected to distinct treatments: demineralization by (1) 37% phosphoric acid (PA) followed by application of an etch-and-rinse dentin adhesive, Single Bond (SB) (PA+SB); (2) 0.5 M ethylenediaminetetraacetic acid (EDTA) followed by SB (EDTA+SB); (3) application of a self-etch dentin adhesive, Clearfil SE Bond (SEB). Different loading waveforms were applied: No cycling (I), cycled in sine (II) or square (III) waves, sustained loading hold for 24 h (IV) or sustained loading hold for 72 h (V). Remineralization at the bonded interfaces was assessed by AFM imaging/nano-indentation, Raman spectroscopy and Masson's trichrome staining. In general, *in vitro* chewing and parafunctional habits, promoted an increase of nanomechanical properties at the resin–dentin interface. Raman spectroscopy through cluster analysis demonstrated an augmentation of the mineral–matrix ratio in loaded specimens. Trichrome staining reflected a narrow demineralized dentin matrix after loading in all groups except in PA+SB and EDTA+SB samples after sustained loading hold for 72 h, which exhibited a strong degree of mineralization. *In vitro* mechanical loading, produced during chewing and bruxism (square or hold 24 and 72 h waveforms), induced remineralization at the resin–dentin bonded interface.

1. Introduction

Tooth contact is not a dominant activity over a 24-h cycle. It has been estimated that tooth contact occurs for approximately 17.5 min over a 24-h period; sleep bruxism (Toledano et al., 2014a) related with muscle activity lasts approximately 8 min over a complete sleep period that usually remains between 7 and 9 h (Lavigne et al., 2008; Okeson et al., 1990). The prevalence of bruxism is reported to be 6–20% among the adult population, 20% for the clenching type and it occurs predominantly among females (Lavigne et al., 1996, 2008; De Laat and Macaluso, 2002). Most theories adhere to multifactorial etiology and discriminate between peripheral factors (anatomy, dental occlusion, receptor input), central (central nervous system), and psychological factors (De Laat and Macaluso, 2002). Bruxism is commonly considered to be the main contributor to dental attrition, periodontal disease and temporomandibular joint disorders (Nishigawa et al., 2001). Duration of the chewing cycle ranges from 0.7 to 2 s, with a contact time between 0.2 and 0.3 s. During clenching, the occlusal force was observed to be as high as 520–800 N. The duration of bruxim episodes varies from 2 to 375 s (Jantarat et al., 2001; Abbink et al., 1999). Chewing and occlusal trauma can affect restorative strategies involving dentin.

Two main strategies are used to create dentin bonding: (a) etch-and-rinse adhesives which require previous acid etching of the dentin surface, or (b) self-etching adhesives, based on the use of polymerizable acidic monomers that simultaneously condition/prime the dentin. With both strategies, a resin–dentin inter-diffusion zone or hybrid layer (HL) (Nakabayashi et al., 1982) is created, but a volume of demineralized/unprotected collagen remains at the bottom of the hybrid layer (BHL). This unprotected collagen may become the sites for collagen hydrolysis by host-derived matrix metalloproteinases (MMPs) enzymes (Toledano et al., 2012).

It has been stated that dentin is a relatively inert tissue and that mastication or parafunctional habits have limited influence in tissue response. Occlusal loads have been pointed out to be accountable to some degree of irreversible mechanical disruption of the poorly infiltrated collagen fibrils (Tjäderhane et al., 2013). When chewing or bruxing, dental materials used in restorative dentistry not only should be resistant to wear and breakage, but promoters of the protection of resin–dentin interfaces, triggering the bioactive nature of dentin matrix, by releasing bound bioactive molecules and growth factors (Smith et al., 2012). The precise

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interaction of dentin matrix with these signaling molecules deserves more research.

Information on biomechanical properties of the demineralized dentin and their influence on remineralization are lacking (Xu et al., 2011; Brauer et al., 2011). AFM nano-indentation is the most commonly applied means of testing the mechanical properties of materials or substrates (Poon et al., 2008), and it was deemed to be a suitable method for the determination of the visco-elasticity of the demineralized dentin and its effective remineralization (Balooch et al., 2008; Bar-On and Wagner, 2012). Various modes of micro-Raman multivariate spectra of principal components (PCs) (Almahdy et al., 2012) have been established for analyzing three dimensional *data*. Finally, Masson's trichrome stained sections of dentin interfaces may show or not an exposed and unenveloped-stained collagen network at the BHL, confirming any demineralization at this site of the resin–dentin interdiffusion zone, indicating or not signs of interface degradation (Sauro et al., 2012).

In this study we attempted to clarify the experimental morpho-functional influence of chewing and bruxism events on the resin–dentin interface. The purpose of this study was to evaluate the ability of different *in vitro* mechanical loading stimuli, as chewing and bruxism, to induce remineralization at the bonded dentin interface created by using different demineralization procedures and two dentin adhesive systems (etch-and-rinse *vs* self-etch). The tested null hypothesis is that remineralization is not produced at the resin–dentin interface after the application of some *in vitro* mechanical loading stimuli, reproducing parafunctional habits.

2. Material and methods

2.1. Specimens preparation, bonding procedure and mechanical loading

Forty-five 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 dentin surface was exposed using a hard tissue microtome (Accutom-50; Struers, Copenhagem, Denmark) equipped with a slow-speed, water-cooled diamond wafering saw (330-CA RS-70300, Struers, Copenhagen, Denmark). A 180-grit silicon carbide (SiC) abrasive paper mounted on a water-cooled polishing machine (LaboPol-4, Struers, Copenhagem, Denmark) was used to produce a clinically relevant smear layer (Koibuchi et al., 2001). The specimens were divided into three main groups ($n=15$) based on the tested adhesive and dentin-etching procedure: (i) two-step etch-and-rinse adhesive system, Adper™ Single Bond Plus (SB, 3 M ESPE, St. Paul, MN, USA) applied on 37% phosphoric acid (PA)-etched dentin, 15 s (PA+SB); (ii) SB applied on EDTA-treated dentin, 0.5 M, 60 s (EDTA+SB); (iii) Clearfil SE Primer and SE Bond (SE, Bond, Kuraray Medical Inc., Tokyo, JAPAN) (SEB). A flowable resin composite (Tetric EvoCeram®-Ivoclar Vivadent, Schaan, Liechtenstein-) was placed incrementally in five 1 mm layers and light-cured with a Translux EC halogen unit (Kulzer GmbH, Bereich Dental, Wehrheim, Germany), for 40 s. The detailed composition and application mode is shown in Table 1.

The specimens were divided into five sub-groups, based on the type of mechanical loading that were applied: (1) Restored teeth stored in simulated body fluid (SBF), for 24 h (no cycling). (2) Load cycling with sine waveform (259,200 cycles, 3 Hz,) (S-MMT-250NB; Shimadzu, Tokyo, Japan). (3) Load cycling with square waveform (6171 cycles, 0.072 Hz,) (S-MMT-250NB; Shimadzu, Tokyo, Japan). (4) Load with hold waveform, for 24 h (Instron 3345, Instron Corporation, Canton, MA, USA), and (5) Load with hold waveform, for 72 h (Instron 3345, Instron Corporation, Canton, MA, USA) (Graph 1). To proceed with the mechanical loaded samples, specimens were mounted in plastic rings using dental stone. A compressive load of 225 N was applied to the flat resin composite build-ups using a 5-mm diameter spherical stainless steel plunger, while immersed in SBF and proceeded as in Toledano et al. (2014b).

2.2. AFM imaging and nano-indentation

An atomic force microscope (AFM—Nanoscope V, Digital Instruments, Veeco Metrology group, Santa Barbara, CA, USA) equipped with a Triboscope indentor system (Hysitron Inc., Minneapolis, MN, USA) and a Berkovich indenter (tip radius ~20 nm) was employed for the imaging and indentation processes in a fully-hydrated status. Five indentations with a load of 4000 nN and a time function of 10 s were performed in a straight line starting from the adhesive layer down to the intertubular dentin in order to evaluate hardness (Hi) and the modulus of elasticity (Ei); five indentation lines were executed along the interface. The distance between each indentation was kept constant by adjusting the distance intervals in 5 (± 1) μm steps and the load function (Sauro et al., 2012). ANOVA was performed including Hi and Ei as dependent variables. Adhesive procedure and mechanical loading were considered independent variables. Analysis of interactions was included in the analysis. Multiple comparisons were performed with Student–Newman–Keuls and Student *t* tests. Statistical significance was set at $\alpha=0.05$.

Table 1
Composition and application mode of the adhesive resins.

Single bond (3 M ESPE, USA)	***Composition***	
	HEMA	***Dentin conditioning***
	Bis-GMA	37% H_3PO_4 (15 s) or
	Ethanol	0.5 M EDTA (60 s)
	PAM	***Adhesive application***
	UDMA	Rinse with water
	CQ	Air-dry (5 s)
	ODMAB:	Adhesive application (30 s)
	Tripheylantimonydicaroxylates	Light activation (15 s)
	Phosphine	
	Water	
	Quartz	
Clearfil SE primer and bond (Kuraray, Japan)	***Composition primer***	
	MDP	
	HEMA	
	CQ	***Adhesive application***
	N,*N*-diethanol *p*-toluidine	Rinse with water
	Water	Air-dry (5 s)
	Composition bond	Primer application (20 s)
	Bis-GMA	Air-dry (3 s)
	MDP	Adhesive application (10 s)
	HEMA	Light activation (15 s)
	CQ	
	N,*N*-diethanol *p*-toluidine	
	Silanated colloidal silica.	

Abbreviations: Bis-GMA: bisphenol A diglycidyl methacrylate; HEMA: 2-hydroxyethyl methacrylate; CQ: camphorquinone; ODMAB: 2-(ethylhexyl)-4-(dimethylamino)benzoate(co-initiator); PAM: polyacrylic acid methacrylated; UDMA: urethane dimethacrylate; MDP: Methacryloyldodeylphosphate.