International Journal of Adhesion & Adhesives 69 (2016) 91–109

Contents lists available at ScienceDirect

International Journal of Adhesion & Adhesives

journal homepage: www.elsevier.com/locate/ijadhadh

Efficacy and micro-characterization of pathophysiological events on caries-affected dentin treated with glass-ionomer cements

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ARTICLE INFO

Available online 28 March 2016

Keywords:
Glass ionomer cements
remineralization
interface
load cycling
resin-modified GICs
sound dentin
caries-affected dentin

ABSTRACT

The aim of this study was to evaluate if mechanical cycling influences bioactivity and bond strength at the glass-ionomer cement-dentin interface, after load cycling. Microtensile bond strength (MTBS) was assessed with Ketac-Bond (conventional glass ionomer/GIC) or Vitrebond Plus (resin-modified/RMGIC), in sound dentin or in caries-affected dentin (CAD). Debonded dentin surfaces were studied by field emission scanning electron microscopy (FESEM), and remineralization was evaluated through nanohardness (Hi) and Young's modulus (Ei), Raman spectroscopy, and Masson's trichrome staining technique. Load cycling did not affect MTBS, except when Ketac-Bond was applied on sound dentin, which attained 100% pretesting failures. Minerals precipitated in porous platforms. GIC promoted total occlusion of tubules, and RMGIC originated empty or partial occluded tubules. In sound dentin, load cycling produced an increase of the relative presence of crystalline minerals after using Ketac-Bond (Phosphate peak, from 18.04 up to 81.29 cm^{-1} at hybrid layer, and from 19.28 up to 108.48 cm^{-1} at the bottom of the hybrid layer; Carbonate peak, from 8.06 up to 15.43 cm^{-1} at the hybrid layer, and from 7.22 up to 19.07 cm^{-1} at the bottom of the hybrid layer). Vitrebond Plus, in sound dentin, attained opposite outcomes. In CAD treated with Ketac-Bond, the highest Hi (1.11 GPa) and Ei (32.91 GPa) values were obtained at the hybrid layer after load cycling. This GIC showed increased and immature mineral components (an average of 25.82 up to 30.55 cm$^{-1)}$, higher frequencies of crosslinking (considering the pyridinium ring at hybrid layer, from 4.1 up to 6.86 cm^{-1}; at bottom of the hybrid layer, from 7.55 up to 8.58 cm^{-1}) and worst collagen quality (considering the ratio amide I/AGEs-pentosidine at the hybrid layer, from 0.89 up to 0.69 cm^{-1}; at the bottom of the hybrid layer, from 1.39 up to 1.29 cm^{-1}) after load cycling, at the interface of the CAD samples. Both Hi and Ei of CAD treated with RMGIC were not affected after load cycling, though phosphates, carbonates and crystallinity increased. The organic components showed a dissimilar crosslinking and an improvement of the nature of collagen. Trichrome staining showed lower signs of demineralization or exposed proteins after mechanical loading, though Vitrebond Plus exhibited a slight increment in red intensity at the interface. The null hypothesis to be tested is that bond strength, chemical bonding and mechanical performance of the tested ionomer-based cements would not be influenced by the application of load cycling on restorations of sound and caries-affected dentin substrates.

1. Introduction

Sound dentin is mainly composed of type I collagen fibrils with associated non-collagenous proteins, forming a three-dimensional matrix that is reinforced by mineral [1], but non-carious dentin is not the substrate most frequently involved in clinical dentistry. Instead, dentists usually must bond adhesive materials to irregular dentin substrates such as carious dentin [2]. Caries-affected dentin (CAD) is partially demineralized and more porous than non-carious dentin with a predominantly intact collagen matrix, whose collagen fibrils retain their banded structure and intermolecular crosslinks [3]. It should be preserved during clinical treatment because it is remineralizable and serves as a suitable substrate for dentin adhesion [4] and physiologic remineralization [5]. Adhesive materials such as glass ionomer cements (GICs) have been clinically proposed for this aim; their potential for chemical interactions with both resin and water-based cement-like materials have been stated [5]. One of the more interesting uses of GICs has been the atraumatic restorative technique for stabilization of caries lesions in countries where routine dental care is not available [6].

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http://dx.doi.org/10.1016/j.ijadhadh.2016.03.023
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Conventional GIC is formed of fluoro-aluminosilicate glass (powder) and an aqueous solution of polyalkenoic acid, such as polyacrylic acid (liquid). Upon mixing, an acid–base reaction takes place between the polyalkenoic acid and the ion-leachable glass particles. During the dissolution phase, ions such as aluminum, fluoride, calcium and strontium are released. Cross linking between polyacid's carboxylic groups and ions are facilitated in the latest stage of gelation [5,7]. GICs are characterized by their self-adhesiveness, claimed to depend upon chemical interaction of polyalkenoic acids with inorganic tooth constituents [8]. Previous application of a respective conditioner is mandatory, in order to remove the smear layer and partially demineralize the dentin substrate, with exposure of a micro-porous collagen fibrils network in which hydroxyapatite crystals remains attached [9]. Consequently, formation of a shallow hybrid layers (~1–2 μm depth) [10,11] definitely contribute to micro and nano-mechanical bonding, as with resin-based adhesives [12,13]. The most important disadvantages of conventional GICs are poor performance as a restorative material particularly regarding esthetics, the lack of sufficient strength, toughness and wear resistance, decrease setting time, and attenuate moisture sensitivity. To offset this drawback, resin-modified glass-ionomers (RMGIs) have been introduced [13].

Resin-modified glass-ionomer cements (RMGICs) are typically powder/liquid formulations which set, when mixed, through an acid–base reaction between ion-leachable glasses and polyalkenoic acids, as well as upon light-polymerization of water-soluble (metha)crylate, such as HEMA and photoinitiators [14]. In both GICs and RMGICs, aluminium, fluoride and calcium or strontium leach out of the cement as the glass is being dissolved by the polyacid, while calcium and phosphate ions also move out of the underlying dentin as a result of the self-etching effect of the setting cement on mineralized dentin [15]. Chemical interaction is the primary bonding mechanism for RMGICs, in addition to micro-mechanical interlocking into surface irregularities [16], hybridizing dentin. When RMGICs are applied without separate conditioners, micro-mechanical bonding is limited to retention provided by the intrinsic surface roughness of dentin, and porosity created by the RMGIC self-etching characteristics [9]. Hypomineralized and porous CAD substrate may allow deeper penetration of the acidic compounds, leading to a deeper demineralization with diffused monomer [2], creating a much thicker hybrid layer (HL) over a zone of non-infiltrated but demineralized collagen substratum, the bottom of the hybrid layer (BHL). This unprotected collagen may become the sites for collagen hydrolysis by host-derived matrix metalloproteinases (MMPs) enzymes [17], and it deserves attention to know if the adhesive material occupies all the space left by the mineral after conditioning [18].

The longevity of adhesive restorations and its clinical success are influenced by the mechanical and chemical properties of the materials and by the restorative technique, which directly affect hybrid layer quality and bond strength [19,20]. In the oral environment, teeth are subjected to challenges provided by mechanical stresses from mastication and para-functional habits. Mechanical cycling has been widely used as potential aging methods that, additionally, stimulate challenges *in vitro*, *i.e.*, dentin remineralization [21]. Remineralization is the process of delivery of mineral (calcium and phosphate), from outside the tooth into previously demineralized hard tissue. Fluoride, calcium and phosphate are the three major ions for tooth remineralization [22]. Additionally, the application of GIC to a caries lesion induces a reduction in lesion depth. This may be due not only to the fluoride-releasing but to the recharging ability of GICs. Fluoride can be taken up into the cement from the oral cavity and released again. Similarly, GIC could act as a fluoride reservoir [23].

There exists scarce information on the nature of the dentin-restorative (GIC/RMGIC) interactions, since the subnanostructural characteristics of those interfaces have not been investigated to any great extent. This study used microtensile bond strength (MTBS), field-emission SEM (FESEM), nanohardness and Young's modulus (Hi & Ei), Raman analysis, and Masson's trichrome staining to examine the regional differences in the ultra-structure of both a GIC and a RMGIC onto sound and caries-effected dentin substrata. Thereby, this investigation assessed the bond strength, chemical interaction and mechanical performance of sound and caries-affected dentin surfaces treated with both a conventional glass-ionomer and a resin-modified glass-ionomer cement, submitted or not to mechanical loading. The null hypothesis to be tested is that bond strength, chemical bonding and mechanical performance of the tested ionomer-based cements would not be influenced by the application of load cycling on restorations of sound and caries-affected dentin substrates.

2. Material and methods

2.1. Specimen preparation, bonding procedures and mechanical loading

Eighty human third molars (forty sound specimens and forty with occlusal caries) were obtained with informed consent from donors (20–40 year of age), under a protocol approved by the Institution Review Board. Molars were stored at 4 ºC in 0.5% chloramine T for up to 1 month before use. A flat mid-coronal sound or carious 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). The inclusion criteria for carious teeth were that the caries lesion, surrounded by sound dentin, should be limited to the occlusal surface, that it extended at least half the distance from the enamel–dentin junction to the pulp chamber. To obtain caries-affected dentin, grinding was performed by using the combined criteria of visual examination, surface hardness using a dental explorer, and staining by a caries detector solution (CDS, Kuraray Co., Ltd., Osaka, Japan). Using this procedure it was removed all soft, stainable, carious dentin. It was left the relatively hard, caries-affected non staining dentin, on the experimental side. 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 [24]. A conventional glass ionomer cement, Ketac-Bond (3M Deustchland GmbH, Neuss, Germany) and a resin-modified glass ionomer cement, Vitrebond Plus (3M Deustchland GmbH, Neuss, Germany) were tested. The chemical components and descriptions of the materials are provided in Table 1. Glass ionomer cements were applied in sound or caries-affected dentin following the manufacturer´s instructions, and a flowable resin composite (X-FlowTM, Dentsply, Caulk, UK) 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. Half of the carious teeth were stored for 24 h in simulated body fluid solution (SBF) (ISO 23317 method), and the other half were submitted to mechanical loading, in SBF (100,000 cycles, 3 Hz, 49 N) (S-MMT-250NB; Shimadzu, Tokyo, Japan) [25]. The load cycling lasted for 9 h and 15 min; the rest of the time until complete 24 h, the loaded specimens were kept in SBF, at 37 ºC. Diagram 1 shows how samples were prepared for testing.