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Influence of phosphoproteins' biomimetic analogs on remineralization of mineral-depleted resin–dentin interfaces created with ion-releasing resin-based systems

Salvatore Sauro[a,*], *Raquel Osorio*[b], *Timothy F. Watson*[c], *Manuel Toledano*[b]

[a] *Dental Biomaterials and Minimally Invasive Dentistry, Departamento de Odontología, Facultad de Ciencias de la Salud, Universidad CEU-Cardenal Herrera, Valencia, Spain*
[b] *Dental Materials, School of Dentistry, University of Granada, Colegio Máximo, Campus de Cartuja, Granada, Spain*
[c] *Biomaterials, Biomimetics and Biophotonics, King's College London Dental Institute at Guy's Hospital, London, UK*

ARTICLE INFO

Article history:
Received 14 October 2014
Received in revised form
2 December 2014
Accepted 27 March 2015

Keywords:
Biomimetic
Bioactive
Remineralization
Dentin
Bonding
Resin-based systems

ABSTRACT

Objective. The study aimed at evaluating the remineralization of acid-etched dentin pre-treated with primers containing biomimetic analogs and bonded using an ion-releasing light-curable resin-based material.
Methods. An experimental etch-and-rinse adhesive system filled with Ca^{2+}, PO_4^{3-}-releasing Ca-Silicate micro-fillers was created along with two experimental primers containing biomimetic analogs such as sodium trimetaphosphate (TMP) and/or polyaspartic acid (PLA). Dentin specimens etched with 37% H_3PO_4 were pre-treated with two different aqueous primers containing the polyanionic biomimetic analogs or deionized water and subsequently bonded using the experimental resin-based materials. The specimens were sectioned and analyzed by AFM/nanoindentation to evaluate changes in the modulus of elasticity (Ei) across the resin–dentin interface at different AS storage periods (up to 90 days). Raman cluster analysis was also performed to evaluate the chemical changes along the interface. The phosphate uptake by the acid-etched dentin was evaluated using the ATR-FTIR. Additional resin–dentin specimens were tested for microtensile bond strength. SEM examination was performed after de-bonding, while confocal laser microscopy was used to evaluate the interfaces ultramorphology and micropermeability.
Results. Both biomimetic primers induced phosphate uptake by acid-etched dentin. Specimens created with the ion-releasing resin in combination with the pre-treatment primers containing either PLA and TMA showed the greatest recovery of the Ei of the hybrid layer, with no decrease in μTBS ($p > 0.05$) after 3-month AS storage. The ion-releasing resin applied after use of the biomimetic primers showed the greatest reduction in micropermeability due to mineral precipitation; these results were confirmed using SEM.

* *Corresponding author*. Tel.: +34 96 136 90 00.
E-mail address: salvatore.sauro@uch.ceu.es (S. Sauro).

http://dx.doi.org/10.1016/j.dental.2015.03.013

Significance. The use of the ion-releasing resin-based system applied to acid-etched dentin pre-treated with biomimetic primers containing analogs of phosphoproteins such as poly-L-aspartic acid and/or sodium trimetaphosphate provides a suitable bonding approach for biomimetic remineralization of resin–dentin interfaces.

1. Introduction

Dental caries represents the major reason for placement and replacement of restorations [1,2]. The concept of minimally invasive (MI) was applied for the treatment of deeper carious lesions by adopting the atraumatic restorative treatment (ART) approach (1990s) with the aim of selective caries removal to excavate infected carious tissue leaving as much caries-affected dentin as possible for therapeutic remineralization [3].

Contemporary ART concepts coupled with the advent of the latest generation of adhesive systems have radically influenced modern operative management of dental caries [4,5]. The application of resin-based materials on demineralized dentin (e.g. acid-etched dentin) followed by placement of esthetic light-curing resin composites represents a revolution in adhesive dentistry [6,7]. Acid-etching is the first step in resin–dentin bonding. Using 32–37% phosphoric acid in etch-and-rinse adhesive systems, this step removes smear layers and completely demineralizes dentin to a depth of 5–8 μm. That step also uncovers and activates all preforms of the endogenous proteases of dentin matrices metalloproteinases (MMPs) and cathepsin [8]. As the mineral phase of dentin is 50 vol.% mineral, complete demineralization replaces all that minerals with 50 vol.% of water. Because the proteases are hydrolases, that is, they hydrolyze peptide bonds by enzymatically adding water across specific peptide bonds, it is important that resins replace all of that water, so that the proteases cannot degrade collagen.

Unfortunately, resin infiltration of water-saturated dentin matrices is not uniform, in particular, in thick layers of caries-affected dentin. Indeed, some areas of hybrid layers are well infiltrated and exhibit little residual water, while adjacent regions are not properly infiltrated and may contain very little resin, but 30–40% residual water [9,10]. It is thought that the resin-sparse, water-rich zones in resin-bonded interfaces degrade over 1–2 years and their stiffness is so low that they may undergo excessive cyclic strain under normal function and fatigue failure [11].

Clinicians only have one chance for resin infiltration. If normal resin–dentin bonds include 30–50% of their volume filled with water instead of resin, how can these water-filled defects be corrected? One answer to that problem is to displace the residual water by filling the voids with nano-sized crystals of apatite. Remineralization is a form of dehydration [12,13]. By removing residual water by remineralization, the activated MMPs in the matrix can be inactivated [13].

It has been recently proposed that all resin bonds be covered with a flowable resin composite containing amorphous calcium phosphate (ACP)/calcium silicates/and biomimetic polyanions that can slowly diffuse through any water-filled channels (e.g. water trees in adhesives and hybrid layers, residual water in un-infiltrated dentin) and "back-fill" any such defects with apatite-like mineral crystals, displacing all residual water and inactivating all proteases by mineralizing the enzymes; this approach was shown to increase the durability of such resin–dentin bonds [14].

One more critical problem associated with such esthetic restorations is the absence of therapeutic remineralization of caries-affected dentin and the poor durability/integrity of the resin–dentin interface during aging [15]. ART is considered to have a combined technique–material effect. It requires removal of the caries-infected dental tissues in order to arrest the caries progression (Massler's theory) and induces dental remineralization while utilizing the healing potential of glass ionomer cements (GICs) [2].

Unfortunately, Yiu et al. [16] demonstrated that, even though specific GICs developed for the ART treatments of carious dentin may favor the penetration of particular ions deep into caries-affected dentin [17,18], they fail to remineralize apatite-depleted dentin due to a lack of nucleation of new apatite. This lack of remineralization has also been confirmed by Kim et al. [19] who reported the failure of GICs to remineralize apatite-depleted dentin, even in the presence of biomimetic remineralizing analogs. Also in this case, the application of biomimetic analogs as therapeutic remineralizing strategies has been advocated as a possible method to achieve a more reliable approach to restore the elastic modulus values due to the creation of apatite crystallites similar to those of sound dentin [20–22]. The advent of these new therapeutic concepts in minimally invasive restorative/adhesive dentistry has captured the attention of many researchers so far, showing encouraging results both in terms of healing properties (i.e. remineralization) [23] and longevity of the bonding interface [24,25]. Nevertheless, it is still necessary to generate bioactive/biomimetic strategies with enhanced therapeutic remineralization properties at the hybrid layer.

Thus, the present study aimed at evaluating the remineralizing of water-saturated collagen that remains uninfiltrated with resin when using an experimental light-curable resin-based etch-and-rinse dental adhesive containing ion-releasing micro-fillers applied on acid-etched dentin pre-treated with one of the different biomimetic primers containing either sodium trimetaphosphate [26] or polyaspartic acid as a nano-precursor cluster stabilizer [27]. The null hypothesis to be tested was that the pre-treatment of acid-etched dentin with biomimetic primers does not improve the remineralization ability of an experimental light-curable resin-based system containing ion-releasing micro-fillers.