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Biodegradation of resin–dentin interfaces is dependent on the restorative material, mode of adhesion, esterase or MMP inhibition

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ABSTRACT

Objective. To measure the effect of simulated human salivary esterases (SHSE) and metalloproteinases (MMP) inhibition on the integrity of restoration–tooth interfaces made from traditional or polyacid-modified resin composites bonded to human dentin by either total-etch or self-etch adhesives.

Methods. Resin–dentin specimens, made from traditional (Z250) or polyacid-modified (Dyract-eXtra) composites were bonded to human dentin using total-etch (TE-Scotchbond) or self-etch (SE-EasyBond) adhesives. TE was applied with or without the MMP inhibitor galardin. Specimens were incubated in phosphate-buffer or SHSE (37 °C/pH = 7.0) for up to 180 days, then suspended in a continuous flow biofilm fermenter cultivating biofilms of *Streptococcus mutans* UA159. Interfacial bacterial penetration, biofilm biomass and viability were measured by confocal laser scanning microscopy and biomarker dyes and used as interfacial biodegradation markers.

Results. All specimens showed increased biofilm penetration and biomass with time regardless of incubation condition. SHSE increased bacterial penetration in all experimental samples after 180 days ($p < 0.05$). Galardin reduced interfacial bacterial ingress and bacterial biomass *vs.* non-MMP-inhibited TE-bonded specimens ($p < 0.05$). TE interfaces showed lower interfacial bacterial biomass *vs.* SE after 90-day and 180-day ($p < 0.05$). Dyract-eXtra specimens showed lower bacterial cell viability within the interface *vs.* Z250 ($p < 0.05$).

Significance. The biodegradation of resin–tooth interfaces is accelerated by esterases, modulated by MMP inhibition and is dependent on the material's chemistry and mode of adhesion. The *in vitro* bacterial growth model used in this study facilitates the elucidation of differences in interfacial integrity and biostability between different materials and techniques and is suitable for assessment of their performance prior to clinical evaluation.

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1. Introduction

Resin-based restorations are the most popular restorative materials in dentistry in large part due to their aesthetic properties, handling characteristics and modern adhesive technologies. While providing several beneficial properties, higher failure rates and more frequent replacements have been reported for resin-based restorations over amalgam [1,2]. One of the primary reasons (~70%) for composite restoration replacements is recurrent or secondary caries that develop at the compromised restoration–tooth interfacial margins [2–8].

Resin composites require to be bonded by adhesives to the tooth structure. This results with the formation of the restoration–tooth (resin–dentin) interface, which is characterized as a 3-D interlocking network consisting of resin polymer penetration and entanglement within the exposed collagen fibrils in the tooth dentin, also referred to as the hybrid layer [9]. The integrity of this interface becomes compromised with time due to several processes, including incomplete adhesive seal, combined with the effect of biological degradative factors [9–12]. The latter is thought to involve two main mechanisms: the hydrolysis of resinous components in both the adhesives and resin composites that is catalyzed by salivary and bacterial esterases [13–17], and the digestion of collagen fibrils within an incompletely resin-infiltrated collagen by dentinal matrix metalloproteinase (MMPs) [18,19].

The degradation of resin-based materials is largely a result of the hydrolysis of methacrylate-based resin monomers, such as the universal monomer 2.2-bis [4(2-hydroxy-3-methacryloxypropoxy)-phenyl] propane (BisGMA) and triethylene glycol dimethacrylate (TEGDMA) due to the presence of unprotected ester linkages in the monomers, leading to the release of biodegradation by-products (BBPs). This process is often referred to as biodegradation [15,16,20], and can be further catalyzed by salivary and bacterial esterases [14–17]. Human saliva contains cholesterol esterase (CE)-like and pseudocholine esterase (PCE) activities that show strong degradative ability toward resin composite and adhesives [16,21]. Accumulated BBPs in the resin–dentin interface promote bacterial growth and up-regulate expression of virulence genes and proteins that are associated with biofilm formation, acid production and acid tolerance, contributing to caries formation and progression [13,22–24].

Collagen degradation involves the breakdown of water-rich, resin-sparse collagen fibrils within the hybrid layer due to the activation of host-derived matrix metalloproteinase (MMPs) during bonding procedures [25]. MMPs are known as zinc- or calcium- depended proteolytic enzymes capable of degrading exposed collagen fibrils within the interface [18,26,27]. Dentin matrix has been shown to contain at least five MMPs: stromelysin-1 (MMP-3) [28], true collagenases (MMP-1 and MMP-8) [29,30] and gelatinases A and B (MMP-2 and MMP-9 respectively) [31]. Once activated, these peptidases are responsible for the intrinsic auto-degenerative process of dentinal degradation [18,32–36] and act in concert with other host-derived enzymes in breaking down components of the interfacial margin [16,37,38]. The MMP inhibitor, galardin, has been suggested for use as an inhibitor against MMP-1, -2, -3, -8 and -9 at low concentration (0.2 mM) while not having toxic effects toward bacteria [39–41].

As a result of the above enzymatic processes, the interface becomes compromised, allowing the passage of cariogenic bacteria such as *Streptococcus mutans* (*S. mutans*) [12], a major species associated with the initiation and progression of dental caries [42]. The size of the interfacial gap was reported as the major influencing factor on the development of caries lesion [43], since larger-sized marginal gaps provide the necessary space and access to the nutrients necessarily for cariogenic bacterial colonization [44]. Studies to date have demonstrated the effect of restorative materials [45–47] and adhesives [48–50] on the biostability of the bulk material and interfacial degradation [15,19,51,52], which could affect bacterial behavior and the development of secondary caries in the interface [22–24]. Strategies to improve the interfacial integrity and prevent cariogenic bacterial biofilm proliferation, including the application of MMP inhibitors [19,53] and antimicrobial restorative materials, were proposed [45,54]. However, the above studies have not concurrently investigated the effect of both endogenous and exogenous enzymes on the interfacial integrity of different restorative materials as they may exist in the oral environment.

Recently, Serkies et al. [39] investigated the combined effect of simulated human salivary esterase (SHSE) and MMP inhibition on the integrity of the restoration–tooth interface. The authors showed a mild modulating effect of MMP inhibition on the esterase-catalyzed degradation of bonded interfaces with the end-points being changes in the mode of fracture and/or fracture toughness values over time. The objective of the current study was to further explore the effects of SHSE and MMP inhibition on the biodegradation of the restoration–tooth interface made from various adhesive and restorative materials, with the end-points being the direct observation of bacterial invasion, biofilm formation and viability of interfacial cariogenic bacteria.

2. Materials and methods

2.1. *Preparation of resin–dentin specimens and interfacial degradation*

Standardized specimens (3 × 3 × 6 mm) were prepared from either traditional resin composite (Z, Filtek™ Z250 Shade A1, Z250, 3M™ESPE™, St. Paul, MN, USA) or polyacid-modified composite Dyract-eXtra (D, Dyract® eXtra Universal Compomer Restorative, Dentsply Caulk) bonded to human dentin (University of Toronto Human Ethics Protocol #25793) using total-etch (TE, Adper™ Scotchbond™ Multi-Purpose Plus, 3M™ESPE™, St. Paul, MN, USA) or self-etch (SE, Adper™Easy Bond, EB, 3M™ESPE™, St. Paul, MN, USA) adhesives under sterile conditions, as described previously [12]. Total-etch bonded specimens were prepared with (TE + G) or without (TE) the application of 0.2 mM of the MMP inhibitor galardin (USBiological, Swampscott, MA, USA) following the etching step associated with the total-etch adhesive application, for 30 s as previously described [39]. The latter study showed little to no effect of galardin on SE, hence the application of galardin in the current study was limited to TE. In total, there

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