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Research Paper

Mechanical and chemical characterisation of demineralised human dentine after amalgam restorations

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ABSTRACT

Objectives: The purpose of this study was to evaluate the ability of Zn-free *vs* Zn-containing amalgams to induce remineralisation at the dentine interface.

Methodology: Sound and caries-affected dentine surfaces (CAD) were subjected to both Zn-free and Zn-containing dental amalgam restorations. Dentine surfaces were studied by nano-indentation, Raman spectroscopy/cluster analysis, X-ray diffraction (XRD), field emission electron microscope (FESEM) and energy-dispersive analysis (EDX), for mechanical, morphological and chemical characterisation. Analyses were performed before and after placement amalgam restorations.

Results: Zn-containing amalgams restorations promoted an increase in the nano-mechanical properties of sound and CAD surfaces. In samples from sound or CAD restored with Zn-containing amalgams, it was evidenced: (a) new mineral calcium–phosphate deposits (intratubular and intertubular) with augmented crystallographic maturity; these crystals were identified as hydroxyl-apatite, and (b) a generalised crosslinking reduction plus an increase in those values testing nature and secondary structure of collagen. It indicates an optimal preservation, molecular organisation and orientation of collagen fibrils.

Significance: Zn-containing amalgams promote remineralisation of subjacent dentine, which is more evident in caries affected dentine surfaces.

1. Introduction

Dental caries is the localised destruction of susceptible dental hard tissue by acidic products from bacterial fermentation of dietary carbohydrates (Longbottom et al., 2009). Two layers are described in carious dentine. The superficial layer is called caries-infected dentine (CID), and it refers to the heavily infected and physiologically irreversible demineralised tissue. The deeper layer is the caries-affected dentine (CAD), which is bacteria-free, potentially and physiologically remineralisable dentine with expanded odontoblastic processes, sound or intact collagen fibers, and apatite crystals bound to the fibers. The

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modern concept of minimally-invasive dentistry calls for more conservative elimination of the CID in order to preserve as much as possible sound or potentially remineralisable tooth tissue (Mount, 2003).

Dental amalgam, a dental restorative filling material alloy that consists of approximately 50% mercury with the balance including silver, tin, copper, and other trace metals, has been used by dentists globally to restore teeth diseased by dental caries. Amalgam is being discontinued in response to global concerns about mercury in the environment. Resin composite is the most common alternative to dental amalgam, but moderate to large composite restorations have higher failure rates, more recurrent decay and increased frequency of replacement as compared with amalgam (Simecek et al., 2009; Rathore et al., 2012; Spencer et al., 2014). This emphasis on replacement therapy will increase as dentists discontinue their use of dental amalgam (Spencer et al., 2014). The amalgam restorations have shown both the best marginal integrity and the smallest artificial wall lesion, if compared to resin based restorations, reducing secondary caries formation (Lai et al., 2013). In comparison to amalgam, caries is the most frequent reason for failure of composite. In vivo biodegradation of the bond between the composite and tooth is considered a particularly critical contributor to secondary loss of adhesion, microleakage and decay (Opdam et al., 2010; Spencer et al., 2014).

Improved clinical performance of amalgam restorations, as measured by a reduction in marginal fracture, may be related to the presence of zinc in the amalgam alloy (Anusavice, 2003). Alloys containing more than 0.01 wt% zinc are identified as Zn-containing amalgams; those containing less than 0.01 wt% of zinc are known as Zn-free amalgams (Anusavice, 2003). Zn-containing amalgams enhanced the physical, mechanical and electro-chemical properties of the restoration. Zn increases the heterogeneity and so, the fatigue resistance of the amalgam microstructure (Watkins et al., 1995). Additionally, retrieved Zn-containing amalgam restorations contain Zn-rich products at the tooth-amalgam interface. Some of these zinc-rich products are $ZnSn(OH)^6$, ZnO_2,and Zn^{++} free (Scholtanus et al., 2013). These Zn-rich products improved the sealing of the dentine–amalgam interface because of the formation of a zinc-corrosion phase, enhancing the clinical lifetime of this restoration (Marshall et al., 1992).

Zinc is thermodynamically the most active component of dental amalgams; a fast preferential initial dissolution of zinc from zinc-containing amalgams has been reported (Marek, 1997). Zinc incorporation into the dentine adhesive resin and its further release and presence at the resin–dentine interface has been shown to promote the formation of apatite crystallites on the partially demineralised collagen fibrils (Toledano et al., 2012). Specific Zn-containing amalgams might also have therapeutic/protective effects for inducing mineral precipitations within the partially demineralised caries affected dentine–amalgam interface. This particular approach might, in addition, be suitable for Conservative Dentistry where minimally invasive operative treatments are followed by therapeutic restorations which stabilise and remineralise the carious lesion and create an optimal environment to repair the demineralised dental hard tissues, as it occurs in the CAD substratum.

The term remineralisation of dentine refers to the process of restoring the inorganic matrix, which is clinically applicable for prevention strategies, therapy of dentine caries, hypersensitivity, erosion of cervical area, affected dentine and exposed collagen incompletely infiltrated (Xu and Wang, 2011; Toledano et al., 2012). Collagen fibrils contain intrafibrillar and extrafifrillar mineral, completely covering their structure. During demineralization, the mineral is removed much more slowly from within the collagen fibrils than from the extrafibrillar compartment. Intrafibrillar mineral is rather resistant to demineralization, and thus influencing remineralization, as partially demineralized dentin may contain intact collagen fibrils with remnant mineral plus insoluble noncollagenous proteins in association with the remaining crystallites that could perform as sites for re-crystallization (Bertassoni et al., 2012) Turnover changes in the matrix composition, especially non-enzymatic intra- and extracellular post-translational modifications of the collagen network, may contribute to changes in the mechanical properties of the hard tissues (Garnero, 2012; Toledano et al., 2015). Enzymatic modifications included the formation, among others, of pyridinium within the N- and C-terminal telopeptides (Garnero, 2012), affecting the crosslinking capacity and further precipitation of minerals Toledano et al., 2014b). Presence of nonreductible and reductible crosslinking formation in dentin collagen collagen has been previously reported (Toledano et al., 2014a).

The purpose of this study was to evaluate the ability of Zn-free *vs* Zn-containing amalgams to induce mineral formation at the dentine–amalgam interface. The null hypothesis is that remineralisation is not produced at the sound dentine or CAD surfaces after placement of Zn-free or Zn-containing amalgam restorations.

2. Material and methods

2.1. *Specimens preparation*

Eight extracted carious third molars without opposing occlusion and eight sound third molars, that were stored in 0.01% (w/v) thymol at 4 °C for less than 1 month were employed for the study. Teeth were collected after written patients' informed consent (20 to 40 yr of age) (Nazari et al., 2009), under a protocol approved by the Institution Review Board. Specimens were randomly assigned to two groups ($n=4$) according to the type of amalgam to be used: with or without zinc. The inclusion criteria for carious teeth were that the caries lesion, surrounded by sound dentine, was limited to the occlusal surface and it extended at least half the distance from the enamel–dentine junction to the pulp chamber. To obtain caries affected dentine, 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 dentine. It was left the relatively hard, caries-affected non staining dentine, on the experimental side. Flat mid-coronal sound dentine, or caries affected dentine surfaces surrounded by normal dentine, were 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).