DENTAL MATERIALS XXX (2018) XXX–XXX

Available online at www.sciencedirect.com

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In-vitro subsurface remineralisation of artificial enamel white spot lesions pre-treated with chitosan

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ARTICLE INFO

Article history:
Received 14 September 2017
Received in revised form
6 March 2018
Accepted 30 April 2018
Available online xxx

Keywords:
Bioglass
Polyacrylic acid
Chitosan
Raman
Remineralization
Dental caries

ABSTRACT

Objective. To test the null hypothesis that chitosan application has no impact on the remineralisation of artificial incipient enamel white spot lesions (WSLs).

Methods. 66 artificial enamel WSLs were assigned to 6 experimental groups (n = 11): (1) bioactive glass slurry, (2) bioactive glass containing polyacrylic acid (BG + PAA) slurry, (3) chitosan pre-treated WSLs with BG slurry (CS-BG), (4) chitosan pre-treated WSLs with BG + PAA slurry (CS-BG + PAA), (5) remineralisation solution (RS) and (6) de-ionised water (negative control, NC). Surface and cross-sectional Raman intensity mapping (960 cm^{-1}) were performed on 5 samples/group to assess mineral content. Raman spectroscopy and attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) were used to identify the type of newly formed minerals. Surface and cross-sectional Knoop microhardness were implemented to evaluate the mechanical properties after remineralisation. Surface morphologies and Ca/P ratio were observed using scanning electron microscopy (SEM) coupled with energy dispersive X-ray spectroscopy (EDX). Data were statistically analysed using one-way ANOVA with Tukey's test.

Results. BG + PAA, CS-BG, RS presented significantly higher mineral regain compared to NC on lesion surfaces, while CS-BG + PAA had higher subsurface mineral content. Newly mineralised crystals consist of type-B hydroxycarbonate apatite. CS-BG + PAA showed the greatest hardness recovery, followed by CS-BG, both significantly higher than other groups. SEM observations showed altered surface morphologies in all experimental groups except NC post-treatment. EDX suggested a higher content of carbon, oxygen and silicon in the precipitations in CS-BG + PAA group. There was no significant difference between each group in terms of Ca/P ratio.

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https://doi.org/10.1016/j.dental.2018.04.010

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Please cite this article in press as: Zhang J, et al. In-vitro subsurface remineralisation of artificial enamel white spot lesions pre-treated with chitosan. Dent Mater (2018), https://doi.org/10.1016/j.dental.2018.04.010

Significance. The null hypothesis was rejected. Chitosan pre-treatment enhanced WSL remineralisation with either BG only or with BG-PAA complexes. A further investigation using dynamic remineralisation/demineralisation system is required with regards to clinical application.

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1. Introduction

Dental caries is the most prevalent non-communicable oral disease [1,2]. The white spot lesion (WSL) is the earliest clinical sign of the dental caries process, manifesting as a white chalky lesion on a susceptible dental hard tissue enamel surface that could be observed with the naked eye which intensifies when carefully dried [3]. A WSL is caused by acid attack produced by bacteria from the overlying dental plaque biofilm which covers tooth surface [4,5]. Dissolved mineral ions, mainly Ca^{2+} and PO_4^{3-}, that leave a porous subsurface lesion, are partly retained on the newly demineralised enamel surface by the biofilm and reprecipitate to form a dense surface layer with reduced porosity (1–2 vol%), namely the surface zone [4].

Remineralising agents, including fluoride, nano-hydroxyapatites, functionalised tricalcium phosphates, casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), have all been applied to arrest and repair WSLs either *in vitro* or *in vivo* [6] [7–10]. NovaMin™ is a bioactive glass-based biomaterial that can function as a source of Ca/P ions during remineralisation to form hydroxycarbonate apatite [11]. In-vitro study has shown NovaMin™ to have potential in promoting remineralisation of incipient enamel lesions alone or in conjunction with other materials [12]. Milly et al. introduced polyacrylic acid (PAA) to enhance the bioactive glass' remineralisation potential [9]. After a 7 days *in-vitro* remineralisation without mechanical agitation, greater surface Knoop microhardness and higher phosphate content were recorded as well as obvious mineral depositions on lesion surface treated by BG-PAA compared to the untreated control, showing BG-PAA slurry effectively remineralised the surface of WSLs. A further study using BG-PAA as air-abrasion powder to pre-treat WSL surfaces before in-vitro remineralisation using BG-PAA slurry also found that BG-PAA successfully induced surface remineralisation [10]. Nevertheless, there is a paucity of evidence with regards to any subsurface remineralisation effect of such topical agents. It appears any remineralisation is limited to the superficial surface where the agent is applied, which is also an issue when using other materials such as fluoride [4]. When topical agents react and remineralise readily, the already limited superficial surface porosities will be blocked, thus hindering further penetration of ions into the deeper aspect of the lesion. Therefore, this delivery of mineral ions is pivotal to obtaining complete repair of the WSL [13].

Chitosan is an *N*-deacetylated derivative product of chitin found naturally in the shell of arthropods [14]. It has attracted much attention as a functional biomaterial due to its ready availability, biocompatibility, biodegradability and non-toxicity [15–19]. Its high nitrogen content makes chitosan a potential vehicle to carry ions such as calcium and phosphate for biomineralisation [16,20]. Chitosan causes a significant reduction in formation of the dental plaque biofilm through inhibition of the growth of mutans streptococci due to the positively-charged chitosan binding to negatively-charged s. mutans cell surfaces, so bridging adjacent cells thus preventing further colonisation [21,22]. The positive charge of chitosan allows it to adhere to negatively-charged surfaces, including demineralised enamel [23,24]. Despite being extensively used in tissue engineering, food and nutrition and drug delivery [25–28], reports of including chitosan in early enamel caries treatment are sparse. Recently, chitosan has been used to remineralise enamel lesions. Chitosan-amelogenin (CS-AMEL) hydrogel was shown to induce in-vitro biomimetic remineralisation on either etched enamel or artificial enamel lesions [29–31]. Enamel-like crystals formed, and lesion depth decreased. Remineralisation did not cease even when the pH decreased below 6.5 due to adhesion provided by the amino groups of chitosan through electrostatic interactions. This suggests chitosan may be a suitable candidate for repairing enamel white spot lesions.

The aim of this study was to evaluate the remineralisation effect of artificial incipient white spot lesions pre-treated with chitosan, using NovaMin™ or NovaMin™/PAA as remineralising agents. Raman spectroscopy including intensity mapping, ATR-FTIR, Knoop microhardness and scanning electron microscopy were used to assess mineral content, chemical composition, mechanical properties and morphologies, respectively, as reported in other studies [9,10]. The null hypothesis was that chitosan pre-treatment cannot enhance remineralisation of either NovaMin™ or NovaMin™/PAA.

2. Materials & methods

2.1. Lesion formation

Ethical approval was obtained from NHS Health Research Authority (Reference 16/SW/0220). Seventy-two caries-free enamel slabs were cut from healthy human molar teeth (buccal and lingual sides) stored in deionised water using a low-speed cutting machine with water-cooled diamond saw (Labcut 1010, Agar scientific Ltd, UK), and rinsed with deionised water. Each was included in acrylic resin (Oracyl™, Bracon, UK) in a customised mould for 1 h to set, with the natural outer enamel surface facing downwards. The surfaces were ground and polished (LabForce-100, Struers, Denmark) with SiC waterproof abrasive paper in the following sequence: P500 for 10 s, P1200 for 15 s, P2000 for 30 s and P4000 for 2 min. Ultrasonication was performed for 1 min between each step and 4 min after P4000. Finished surfaces were covered by red

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