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Development of an in situ root caries model. A. In vitro investigations

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Summary *Objectives*. The paper describes preliminary in vitro investigations, the objectives of which were to examine the influence of certain experimental parameters on artificial carious lesion formation in root hard tissues, and their remineralisation. These experiments formed part of a wider study that aimed to develop an in situ model of root caries, based on the existing coronal caries model used in Liverpool. The present studies examined the effects (a) of the anatomical origin of the dentine, the presence or absence of cementum, the exposure time and the type of demineralising system, on lesion development, and (b) of baseline lesion size on the extent and location of mineral re-precipitation.

Methods. Mineral content parameters in plano-parallel sections taken from dentine lesions were determined by computer-controlled transverse microradiography.

Results. The importance of the anatomical origin of the dentine on lesion formation was investigated by comparing in vitro lesion formation in premolar and molar dentine, and in dentine from apical, middle and coronal thirds of the root: no difference was observed between these sites. Lesions formed more rapidly in acid buffer solutions than in acid gel systems, and were more reliably produced when cementum was removed. The effect of baseline lesion size on subsequent in vitro remineralisation demonstrated that a small baseline mineral content was associated with a larger percentage mineral gain. The location of mineral deposition throughout the lesion was also influenced by baseline mineral content parameters.

Conclusions. The results form a basis for the further development of an in situ dentinal caries model, providing data to suggest that manipulation of parameters involved in the preparation of artificial carious lesions has a significant effect on the behaviour of the lesion, particularly the phenomenon of remineralisation. Further work is needed to investigate the behaviour of the model in situ.

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Introduction

The retention of the dentition into later life, associated with prevention and increasingly sophisticated treatment of coronal caries, has thrust into prominence the prevalence of root caries. This prominence is also highlighted by the aging of the population and the difficulties of effective restorative treatment of root hard tissues.[1,2] In order to understand the mechanisms of root caries, and to be able to test preventive agents, a model system for root caries is required.

Extensive work has been carried out on coronal caries models, and much information is therefore available regarding both the mechanistic aspects of the caries process in enamel, the optimal preparation of the substrate and the reliable quantification of the processes of demineralisation and remineralisation. For root caries, however, there is much less information available. Previous work[3–8] has investigated aspects of caries in dentine, and the effects of fluoride and non-fluoride preventive agents, but much work remains to be done before the models can be regarded as established. In particular, the optimum treatment of the dentinal substrate needs to be evaluated to allow the greatest sensitivity, and thus statistical power, in applications of the model.

The in situ enamel caries model used in Liverpool has been described in another paper in this symposium.[9] This consists of an attachment fixed to the tooth surface, which carries a slab of enamel which is covered by surgical gauze to entrap plaque. The model has been shown to respond to demineralisation and remineralisation conditions. The purpose of this paper is to describe progress towards the use of the same method to model root caries by replacing the enamel slab with a dentinal sample. As a first step, the in vitro behaviour of root dentine during de- and remineralisation has been studied to determine the importance of the anatomical origin of the dentine, the presence of cementum, the demineralising system, exposure time and temperature on demineralisation, and the effect of the baseline lesion mineral content parameters on subsequent remineralisation.

Materials and methods

Effect of demineralising system, exposure time and presence of cementum

Two demineralising systems were tested: an acetic acid buffer system as described by ten Cate and Duijsters[10] consisting of a 50 mM acetate buffer solution at pH 4.5 containing 2.2 mM each of KH_2PO_4 and $CaCl_2$ and 0.5 ppm fluoride in the form of NaF, and a 50 mM lactate gel at pH 4.5 containing 6% (w/v) methylcellulose.[11]

Human dentine samples were obtained from 40 extracted molar teeth stored in 0.1% thymol in distilled/deionised water (DDW) immediately after extraction following debridement and brushing with a fluoride-free prophylactic paste (Associated Dental Products, UK). The crowns were removed using a water-cooled diamond-wafering blade (Isomet, Buehler, UK) and the roots divided mesiodistally to provide 80 root halves. These were assigned randomly to four groups each of 20 root halves: groups A and C were with cementum removed using a dental curette followed by wet abrasion with a 1.20 grit paper; groups B and D had cementum intact. Preliminary studies showed that cementum removal was achieved by the method described.

Groups A and B were exposed to the buffer system, while groups C and D were exposed to the gel system. The root halves were further divided saggitally using a water-cooled diamond wire saw (Well, Walter Ebner, Switzerland) into three parts, and assigned to exposure periods of 5, 6 or 7 days in the buffer system, or 7, 14 or 21 days in the gel system (Fig. 1).

All samples were covered with two layers of acid-resistant nail varnish (Diamond Hard, Max Factor) leaving an exposed window of approximately 2 mm$\times$5 mm on the middle third of the root surface. They were then attached with dental modelling wax to glass rods fitted in the caps of 25 mL universal tubes containing 20 mL of buffer or gel. The demineralisation took place at room temperature; the buffer tubes were stirred continuously while the gels were undisturbed. After the demineralisation period, the samples were washed thoroughly in DDW, and varnish removed using acetone.

With the water-cooled diamond saw, the dentine under the exposed window was sectioned to form transverse slabs approximately 250 μm thick. The slabs were ground with a custom-made diamond disc grinder to form plano-parallel sections approximately 130 μm thick prior to transverse microradiography (TMR). The sections were microradiographed while wet on Kodak 1A HR plates alongside a calibration aluminium step-wedge using a monochromatic Cu Kα X-ray source (Philips, Eindhoven) with an exposure time of 5 min, at 25 kV and 10 mA and a focus distance of 30 cm.

The microradiographs were examined with a Leitz DMRB microscope under standardised lighting and magnification. The images (microradiographs