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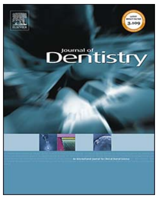

Real-time observations of tooth demineralization in 3 dimensions using X-ray microtomography

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ABSTRACT

Objectives: The immediate aim of this study was to develop and test a method to record, visualize and quantify real-time demineralization (or remineralization) of teeth in vitro using X-ray microtomography (XMT or micro-CT). The longer term objective is to improve understanding of demineralization processes, allowing the creation of better artificial models of dental caries and better quantification of the efficacy of caries treatment and prevention regimes.

Methods: For demineralization studies, the tooth is mounted in a plastic container through which the demineralizing solution is circulated during simultaneous repeated scanning. Key features of the method are safe circulation of the demineralizing solution, periodic interruption to adjust X-ray filament current and re-focus, accurate beam-hardening correction and calibration, alignment of reconstructed scans, and normalization of grey-levels to compensate for changes in the X-ray spectrum. The method was tested by scanning an extracted third molar during 8 days of demineralization.

Results: From the reconstructed 3D images, the progression of an artificial carious lesion could be visualized and quantified. The lesion progressed at approximately 0.1 mm per day and appeared to be more erosive in nature.

Conclusions: A successful method has been developed to monitor real-time demineralization in 3 dimensions. Further work is now needed to create better models of true carious lesions.

Clinical Significance: Observation of the dynamics of demineralization and remineralization will aid in the development of therapies to treat and prevent dental caries.

1. Introduction

The use of X-ray microtomography (XMT or micro-CT) is well established in dental research [1]. XMT has been shown to be an excellent tool to evaluate morphological features, such as changes in shape [2,3] and the presence of voids [4,5] in *ex vivo* studies of root canal instrumentation and obturation. With appropriate beam-hardening correction and calibration, the technique can also be used to study dental hard tissue loss following acidic challenge, by quantification of mineral concentration [6–8]. Repeated scanning allows changes to be observed over time. Previously, this has been associated with very rapid scanning, such as for observing a beating heart [9]; it may be referred to as dynamic or 4D micro-CT. However, for slower processes, e.g. *in vitro* tooth mineral loss or gain experiments during acid attack, the scan time can be longer, allowing good quality images to be obtained with no special reconstruction algorithm. In order to do this, it is necessary to immerse the tooth in a circulating acid or remineralization solution throughout the scanning period, which may be several days, or even weeks. In terms of hardware, the main consideration is ensuring that there is no risk of leakage within the scanner, especially since scans may be left unattended overnight. In addition to the usual tomographic reconstruction, processing involves aligning each reconstructed volume (to compensate for positional drift) and calibrating to allow for slight changes in the X-ray spectrum that may occur over time. Although synchrotron based micro-CT systems may be able to deliver higher quality images, their use for such slow dynamic processes is logistically impractical and thus only impact source based systems, with their inherent problems with beam-hardening and low flux, are suitable.

In the past, X-ray absorption studies to measure changes in tooth mineral have been carried out on 2D sections of enamel exposed to acid challenges [10]. This involves considerable destruction and therefore potential damage to the specimen, which could modify the progress of acid. Further, as the architecture of enamel is complex, reducing the progress to effectively two dimensions will not be a true reflection of the overall process.

The aim of this paper is to describe the hardware and software

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necessary for 3D real-time observation of tooth demineralization and to demonstrate this method with an 8-day continuous scan of tooth mineral loss in an experimental acid challenge.

2. Method

2.1. Scanner

The scanner used was the MuCAT 2 [11] system developed at Queen Mary University of London, employing time-delay integration (TDI) to avoid ring artefacts [12] and facilitate high signal-to-noise ratio imaging. In this case, the relatively short scan times meant that photon statistics were the primary limitation on image quality and thus a conventional X-ray imaging system could have been used, and indeed may have been more efficient. The TDI system does, however, have the advantage that it does not suffer from burn-in artefacts caused by regional variations in scintillator light output that change over time according to exposure.

The X-ray generator and enclosure was an HMX 225 system (Nikon Metrology, UK) which, for the experiment described, was operated at 90 kV and 180 mA, with an internal air temperature stabilization system set to 22 °C. The X-ray enclosure was of sufficient size to allow the circulation system to be housed internally, thus maintaining the temperature of the circulating solution. Note, an alternative would be to run tubing though the cable labyrinth to allow the pump to be mounted outside the X-ray enclosure.

An 800S series camera (Spectral Instruments, Tucson, Arizona) with a 16 megapixel Fairchild CCD485 detector was used, coupled to a CsI scintillator. For TDI readout, this is mounted on a horizontal linear stage that moves the camera through the X-ray shadow at a velocity synchronized to the CCD readout.

2.2. Circulation

One litre of standard acetic acid demineralizing solution was prepared at pH = 4.0 using analytical grade reagents [13]. A large volume was used to prevent the accumulation of dissolution products reducing the reaction kinetics. To minimize the risk of spills within the X-ray enclosure, a “double-safe” pumping method was used (Fig. 1). The first principle of this is that the circulating solution is drawn through the specimen container by the pump from the reservoir (consisting of a 1 litre bottle with two holes drilled in the cap for the tubes and a further hole to prevent changes in pressure). This creates a negative relative pressure within the circulation tubes and specimen container, whereby a small leak would result in air being drawn into the system and a major leak would simply stop the circulation altogether. The second principle in the double-safe methodology is that the solution is drawn up through a tube that protrudes just below the surface in the reservoir. If for any unforeseen reason, the solution should leak into the cabinet, the reservoir level would drop and circulation would cease. The pump and reservoir were themselves placed in a large plastic tray, providing a further level of protection. PVC tubing of 2.79 mm inner diameter [Altek, UK] was used, except through the peristaltic pump, which used silicone tubing [Altek, UK] with an inner diameter of 4 mm. The tubes to and from the specimen container were passed through an overhead guide, with sufficient slack to allow them to twist through 360° throughout the course of a single scan. The scanner software was configured to “rewind” the specimen rotation stage after each scan.

2.3. Specimen preparation

A caries free third molar was selected from a human tissue bank; ethical approval was obtained from the Queen Mary Research Ethics Committee (QMREC2008/57). The tooth was then coated with acid resistant nail varnish, except for a 5 mm window on the buccal surface, as is done in conventional tooth mineral loss microradiographic studies [14]. The tooth was then secured in the XMT circulation cell using soft wax (6969 from Poth Hille & Co Ltd, Rainham, Essex, UK) around the roots.

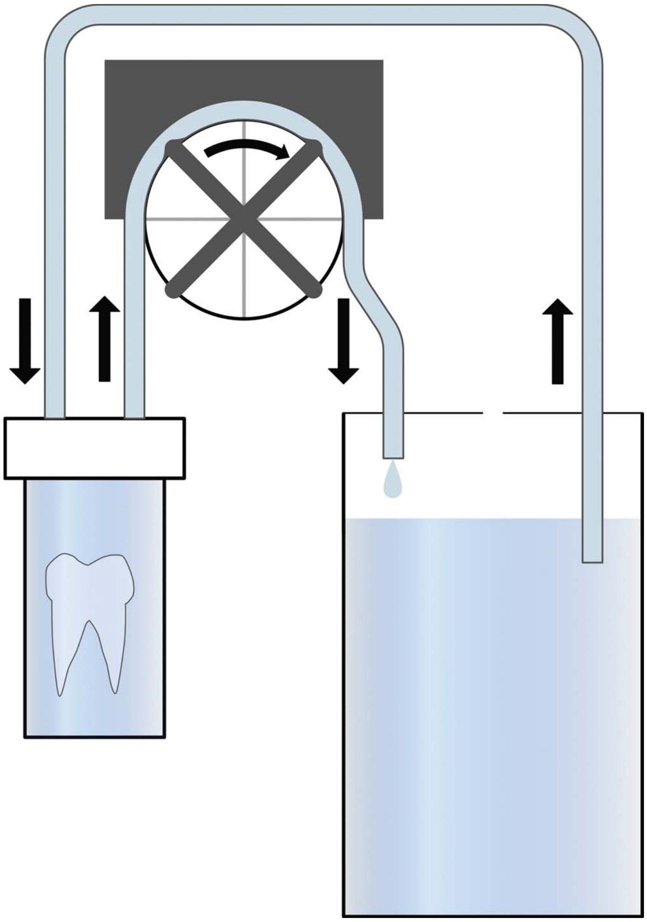

Fig. 1. Double safe pumping principle. Demineralizing solution is drawn through the specimen from a tube descending just below the surface in the reservoir.

2.4. Procedure

The X-ray source was of the demountable type, with a filament lifetime of around two weeks of continuous use. However, throughout its lifetime, the filament becomes thinner and requires less current to maintain its temperature. The lifetime is considerably reduced if the current is not reduced throughout its lifetime. There is also a need to change the reservoir solution periodically to remove reaction products. For these reasons, scanning was briefly interrupted approximately every 24 hours to reduce the filament current to the minimum level that provided an acceptable focus and to refresh the reservoir; this took approximately 10 minutes. A calibration carousel was also scanned at the end of each 24-hour period [15]. The voxel size was set to 30 µm, with 927 projections around 360°, taking 2.6 hours, allowing 9 scans per day. The first scan began as soon as the circulating solution had filled the specimen container. After four 24 hour periods, the scans were set to run continuously for four days to cover an un-attended period (there was less concern over filament failure at the end of the experiment when sufficient data would have been collected). In total, 73 scans were completed.

2.5. Processing

The calibration carousel scans (9 projections) were used to optimize a model of the X-ray spectrum which was then used to create a 2D