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Application of a FIB/SEM to Study the Occlusion of Dentine Tubules from a Calcium Sodium Phosphosilicate Bioactive Glass (Novamin)

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Dentine hypersensitivity (DH) which is a common but painful condition affects up to 42% of the adult population. It can occur if the nerve endings associated with the dentine tubules of a tooth are exposed to the environment due to damage to the enamel or gum recession. Dentine tubules are microscopic tubular structures that radiate outwards from the pulp and are typically 0.5-2 microns in diameter. It is the changes in the flow of the plasma-like biological fluid present in the tubules due to, for example a hot or cold stimuli, that can trigger mechanoreceptors present eliciting the pain response.

Here we report on the study of a bioactive glass (45S5) a calcium sodium phosphosilicate (supplied by GSK) to treat dentine hypersensitivity. It was found that the bioactive glass when in an aqueous environment not only resulted in tubule occlusion with a hydroxyapatite-like material but remineralised demineralised dentine

The FIB/SEM has been a key tool in this project and we will outline how we have been using it to study the tubule occlusion and the effects of the bioactive glass on the mechanical properties of the dentine. Figure 1 shows a SE image of a treated dentine disc and the insert is the surface of a control untreated disc. The FIB/SEM has been used to prepare TEM lamellae through the center of the tubules to study the occluding material visible in Figure 1. Figure 2 shows a BF TEM image of an occluded tubule while the inset shows a SE image of a FIB lamella. The dentine matrix consisting of collagen and bound hydroxyapatite (HA) crystals was found to be very susceptible to FIB damage. It was found that using a 1 kV beam energy and top down milling enabled lamellae to be prepared with less than 2 nm of damage to the dentine matrix and no visible localized ppts/areas of Ga. Chemical and structural analysis (HREM, EDS and EELS) showed the occluding material was hydroxyapatite-like, the same as in the dentine matrix. It was also observed that the demineralized layer due to acid etching was remineralised with a similar crystalline structure to that of the dentine.

FIB/SEM slice and view has been used to study the percentage filling and depth of filling of the tubules. Figure 2(a) and (c) shows a slice from an S&V set and a 3D reconstruction of a single tubule. We have also been combining the SE and BSE signals with those from EDS to generate 3D physical and chemical data cubes. We will discuss the difficulties in this owing to the different interaction volumes, the porous and irregular nature of the tubule distribution and because the formed occluding material has a chemical composition similar to the background dentine material. To aid the reconstruction of the EDS data TEM lamellae of the dentine were FIB prepared with windows of different thickness (something only possible with the FIB) and cylinders were FIB milled in hydroxyapatite discs with controlled separations.

We have also been studying the occlusion of the tubules using X-ray tomography which enables 3D information of the same area before and after treatment. FIB milling and *insitu* lift-out has been used to prepare 40 μm sized cubes. We will show have we have been combining the X-ray tomography data and the 3D FIB S&V work to aid the chemical and structural analysis to help interpret the mode of action of the bioactive glass and to perform correlative microscopy for TEM analysis.

In the final part of the talk we will show how we have been using the FIB to aid characterizing the effect of the bioactive glass on the mechanical properties of the treated dentine. FIB machining has been used to prepare pillars and cantilevers to study the adhesion and fracturing of the treated dentine and S&V has been used to study the crack distribution underneath nanoindents.

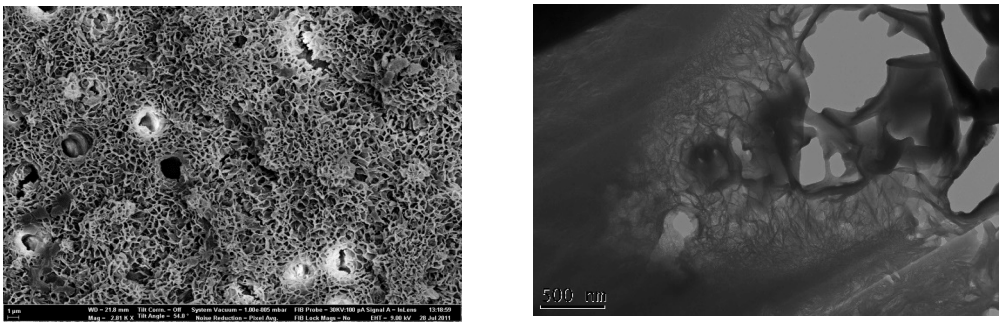

Figure 1. SE image of a treated dentine disc (insert shows control).
Figure 2. BF image of a FIB prepared lamella through a dentinal tubule showing the occluding material. (Insert shows a SE image of a lamella showing the material formed on the surface.)

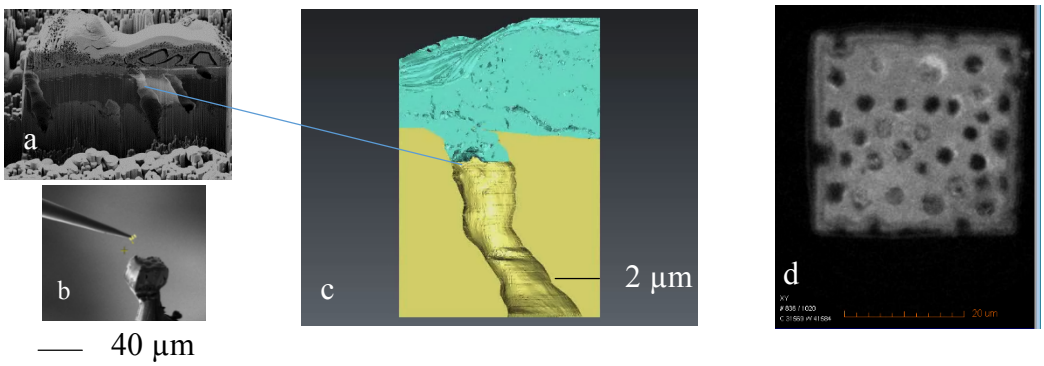

Figure 3. (a) FIB slice and view through a treated sample, (b) a FIB cut cube mounted on a pin for tomography (c) a reconstruction through a single tubule and (d) a plan-view from 5 µm beneath the surface from the X-ray tomography data showing the occlusion of the tubules

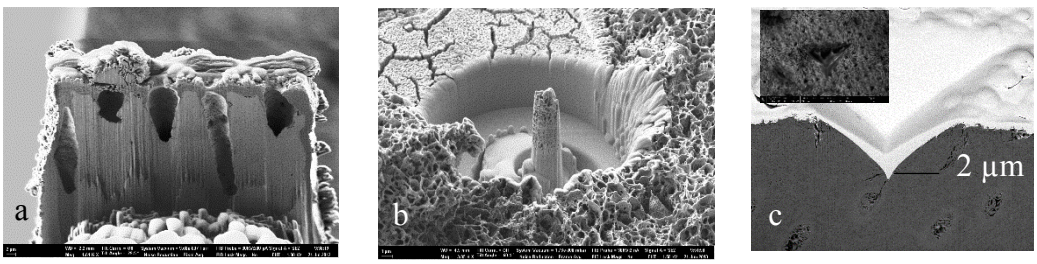

Figure 4. (a). FIB slice through cube after X-ray tomography, (b) FIB prepared micropillar, and (c) slice from a slice and view set looking at crack propagation (insert shows SE image of indent).

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