Experiment Report A28-1-1295

Title: Extracellular matrix control of crystal nucleation and growth in dental tissue health and disease

Duration: 28/10/21 to 02/11/21

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The aim of our experiment was to understand how the extracellular organic matrix plays a crucial role in the mineralization process of teeth. We've undertaken a comprehensive approach that combines crystallography and structural analysis of dental hard tissues with insights from human genetics and clinical observations. The overarching aim was to pinpoint the developmental pathways crucial for the normal development of hard tissues, particularly focusing on crystal nucleation and growth mediated by the organic matrix. This research seeks to enhance our understanding of these processes, providing valuable insights for the diagnosis and treatment of oral-systemic dental and skeletal conditions.

Despite being held remotely, the beamline scientists have helped immensely in the subsequent experimental configuration which was an ideal setup for our experiment:

- Detector employed: Pilatus 1M with dimensions of 981 x 1043 pixels.
- Wavelength: 0.652548 A, energy was set at 19 keV in multi-bunch mode.
- Beam spot size of 50 X 50 µm
- a sample-to-detector distance of 250 mm.
- Counting time: 5 seconds proved sufficient to attain a robust signal while minimizing noise.

The beamtime was dedicated more specifically toward measuring the hydroxyapatite 2D texture distribution of dental enamel with a resolution of 50 µm (X-direction) X 100 µm (Y-direction) in dental enamel thin slices approximately 200-300 µm in thickness. These teeth have been previously diagnosed with an underlying gene mutation that is known to cause Amelogenesis imperfecta diseases AI). This experiment is important is it allows us to understand the impact of the gene disruption on the enamel structure.

Day and night shifts were employed during the beamtime to scan teeth in designated regions of interest (ROI). The enamel was divided into buccal, lingual, and occlusal ROIs with the assistance of a telescope's crosshairs. A script was developed during the beamtime to outline the dimensions and locations of each ROI, and it was tasked with scanning 5-7 ROIs in each shift.

A total of 38,210 diffractions patterns were collected, including some LaB6 patterns used as a caliberant. For texture analysis, the 002 reflection will be extracted as it doesn't overlap with other reflections by Fit2D software through azimuthal integration in order to create 1D graphs of 002 intensities versus azimuthal angle. The 1D graphs belonging to the 002 reflection will then be used for a Gaussian fit in order to determine the FWHM (texture), crystallite orientation, existent crystallite populations and their spatial distribution using an inhouse built software developed by Matlab (Al-Mosawi et al., 2018).

We consider this experiment successful, as the experimental setup was optimised to perform XRD mapping of the enamel of whole crowns of 8 dental samples during this beamtime, two belonging to the SLC24A4 genotype and 6 samples belonging to the WDR72 genotype. The SLC24A4 protein is known as a membrane transport protein responsible for calcium ion transport by the ameloblasts into enamel matrix during enamel maturation. Whereas, WDR72 is believed to be part of vesicle coat protein, expressed specifically during the maturation stage of amelogenesis and is believed to be critical for protein transport. Both genotypes are known to cause the hypomature subtype of amelogenesis imperfecta (Smith et al., 2017). The intention was to incorporate samples from the LAMB3 genotype; however, we encountered challenges in locating healthy type-matching control teeth within a suitable timeframe prior to the experiment.

One issue we faced during this beamtime is that we have noticed a fluctuation in diffraction intensity as a function of time, this was evident after constructing composite maps of the ROIs scanned. This may restrict our plans for Rietveld analysis, but we shall discuss this with the beamline scientist in order to plan on how best to normalize this data.

We would like to highlight the support we received by the beam line scientists especially to Dr Laurence Bouchenoire who has been extremely helpful, even above and beyond her normal working hours.

References

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