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THE INFLUENCE OF CRYOPRESERVATION ON THE SURFACE CHARACTERISTICS OF DENTAL HARD TISSUES: AN IN VITRO STUDY USING X-RAY MICRO-CT

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Summary: The objective of this in vitro study was to investigate the influence of cryopreservation on dental hard tissues by evaluating the formation and modification of enamel cracks by means of high-resolution X-ray computed tomography (micro-CT). Several cracks were found in the enamel. The modification of the pre-existing cracks varied depending on the crack volume.

1. INTRODUCTION

Cryopreservation is a process of prelevating and preserving cells and tissues by cooling them to sub-zero temperatures. The main goal of this therapy is to maintain the viability of these structures in order to be replanted in the future. However, a major disadvantage is intracellular crystallization which can lead to cell damage. This intracellular crystallization might cause irreversible cracks in dental hard tissues resulting in a negative impact on the survival of cryopreserved teeth used for autotransplantation [1].

Identification of cryopreservation-induced cracks is difficult. Histological methods provide only two-dimensional information. Tooth preparation for histological analysis leads to damage or destruction of tooth structures which impedes comparison before and after cryopreservation. X-ray computed tomography enables non-destructive three-dimensional visualization and analysis of a tooth and facilitates observation of a tooth over time or to detect changes due to processing. However, the detectability of cracks is limited with X-ray computed tomography and can be improved by the use of absorption histograms of a region of interest [1].

2. EXPERIMENTAL METHOD

The teeth were cryopreserved for 1 week and scanned in a custom-designed micro-CT setup [2] before and after cryopreservation. Different scans were taken before and after cryopreservation at an isotropic voxel size of 5.5^3 μm^3. After 3D rendering, image processing (Octopus imaging software) was performed by determining the volume of interest of enamel of a selected subvolume containing a part of a crack. Since enamel can be well approximated by a Gaussian [3], the obtained absorption histogram was fitted on the right part using a Gaussian because the left part (toward lower X-ray absorption) was affected by the crack [1]. The difference between the measured histogram and the Gaussian fit yielded the volume of the crack.

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3. RESULTS

The scans showed several cracks in the enamel but no new cryopreservation-induced cracks were formed. Most cracks were aligned perpendicular to the dentin-enamel junction (Fig. 1) while other cracks exhibited a more complex course.

The absolute results obtained by Gauss fitting were dependent on multiple parameters, particularly those concerning the Gaussian fit. However, the results showed the same trend for the different parameters. The modification of the pre-existing cracks varied depending on the crack volume. The mean volume of broad cracks before cryopreservation remained nearly unchanged after cryopreservation. For small cracks, the results were not conclusive. However, it should be noted that the standard deviations of these measurements were relatively large, and further research involving a larger sample is required to make general conclusions.

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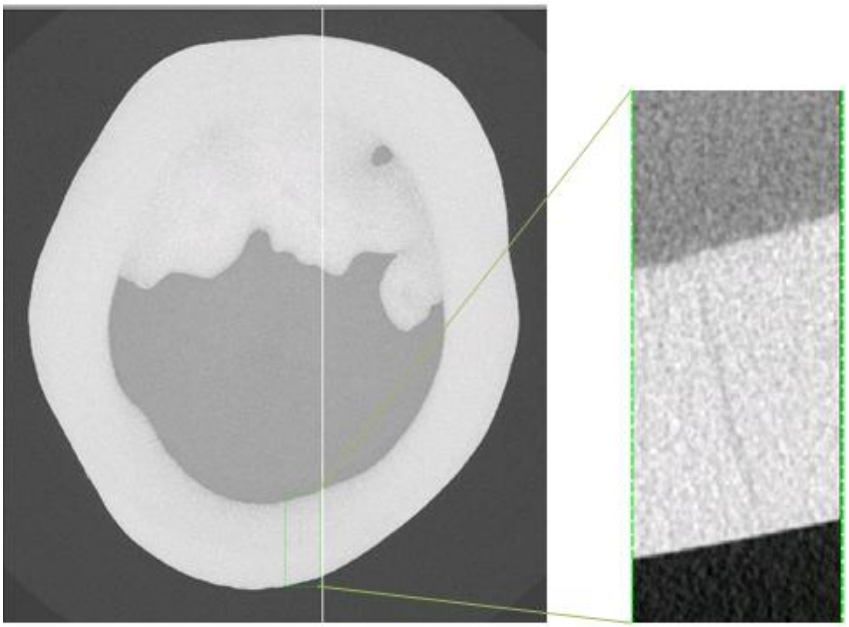

(a) *(b)*

Figure 1: (a) Horizontal slice through the 3D-data of a tooth. **(b)** A subvolume of enamel containing a perpendicular aligned crack to the dentine-enamel junction.