ORIGINAL ARTICLE

Cracks in dentin and enamel after cryopreservation

Sebastian Kühl, DMD,[a] Hans Deyhle, PhD,[b] Melanie Zimmerli, DMD,[a] Giulio Spagnoli, PhD, MD,[c] Felix Beckmann, PhD,[d] Bert Müller, PhD,[b] and Andreas Filippi, MD, PhD, [a]Basel, Switzerland, and Hamburg, Germany
UNIVERSITY OF BASEL AND INSTITUTE OF MATERIALS RESEARCH

Objective. The objective of this study was to investigate if cryopreservation of teeth for long-term storage leads to cracks in enamel and dentin.
Study Design. Three teeth, which were extracted for orthodontic reasons, were cryopreserved in liquid nitrogen (temperature –196°C) and thawed according to standard protocols after 4 months. Micro computed tomography using synchrotron radiation was performed to detect cracks in the tooth hard tissues.
Results. Cracks were found in the enamel of all teeth, which are associated with forceps application during extraction. Cracks with a width larger than 0.8 μm were not identified in dentin and cementum.
Conclusion. Although cryopreservation of teeth according to the standard protocol does not generate cracks more than 0.8 μm wide, the use of forceps can result in prominent cracks. (Oral Surg Oral Med Oral Pathol Oral Radiol 2012;113:e5-e10)

One major requisite for the success of tooth replantation or transplantation is the survival of the periodontal cells (PDL) during extraoral tooth storage. Special cell nutrient media are available in which the periodontal cells can survive for about 24 hours and can be successfully used to store teeth after avulsion.[1]

In some indications, such as the extraction of healthy teeth for orthodontic reasons or tooth avulsion with extended damage of hard and soft tissues enabling an immediate replantation and transplantation, long-term storage of the extracted teeth (weeks up to years) is desireable.[2] Long-term storage of tissues and cells, without the loss of biological activity, can be successfully achieved by cryopreservation.[2,3] When cells are cooled under controlled conditions at temperatures lower than –130°C, the biological time can effectively be stopped.[4] Cryopreservation of tissues for transplantation is generally performed at temperatures lower than –196°C, because chemical reaction rates are massively elongated. At these temperatures, intracellular crystallization can occur, which is associated with the mechanical expansion of the intracellular liquid. To avoid intracellular ice crystallite formation with consecutive irreversible cell damage, cryoprotective agents, such as dimethyl sulfoxide or glycerol, which have shown to successfully avoid this incidence, are applied. The sufficient penetration of the pulp soft tissue by cryoprotective agents represents a major problem during cryopreservation, as the apical foramen is often too small to guarantee a sufficient inflow. Schwarz[5] proved in the 1980s that, in contrast to PDL, the cells in the pulp do not survive cryopreservation because of insufficient penetration of cryoprotective agent and subsequent intracellular crystallization. The expansion of the pulp tissue might directly cause irreversible cracks in the hard tissues, having a negative impact on the prognosis of cryopreserved teeth used for transplantation. It is unknown whether cryopreservation causes cracks in dentin and enamel, as the proof is technically challenging. Histologic methods provide only 2-dimensional (2D) information; the sample preparation may result in a significant alteration or destruction of the 3-dimensional (3D) integrity of the specimen and as a result of sawing and grinding procedures a considerable part of the sample is lost and therefore inaccessible for evaluation.[6] The identification of cryopreservation-induced cracks is difficult owing to the preparation artifacts. Modern sawing and grinding techniques may avoid additional fractures but are extremely time-consuming and allow only 2D evaluation. Therefore, nondestructive and 3D imaging methods are needed. Micro computed tomography (μCT) allows for the nondestructive visualization of hard tissues and therefore also for the cracks formed after treatments, such as cryopreservation. Synchrotron radiation–based μCT (SRμCT) takes advantage of highly intense, monochro-

[a]School of Dental Medicine, Department of Oral Surgery, Oral Radiology and Oral Medicine, University of Basel, Basel, Switzerland.
[b]Biomaterials Science Center, University of Basel, Basel, Switzerland.
[c]Institute for Surgical Research and Hospital Management and Department of Biomedicine, University of Basel, Basel, Switzerland.
[d]Institute of Materials Research, Helmholtz-Zentrum Geesthacht, Hamburg, Germany.
Received for publication May 25, 2011; returned for revision Jun 13, 2011; accepted for publication Jun 23, 2011.

2212-4403/$ - see front matter
doi:10.1016/j.tripleo.2011.06.020

matic x-rays and, therefore, yields much better contrast than conventional laboratory-based x-ray sources. Consequently, cracks smaller than the voxel size cannot only be made visible, but using the mean absorption values within the crack area, one may deduce the average crack width with subvoxel precision. This estimate gives a lower limit for the detectable cracks of submicrometer width.

MATERIAL AND METHODS

One incisor (12) and 2 unfilled premolars (14 and 24), termed tooth A, tooth B, and tooth C, were extracted for orthodontic reasons from a 21-year-old female patient and included in this investigation.

The teeth were removed under local anesthesia using a forceps at balanced forces. For the extraction of tooth A, the necessary forces were higher than for tooth B and tooth C. A detailed value, however, cannot be given. Immediately after extraction, the teeth were immersed in cell nutrition medium (Dentosafe; Dentosafe, GmbH, Iserlohn, Germany).

The teeth were cryopreserved within 2 hours after extraction. The teeth were removed from the cell nutrition medium (Dentosafe box) and transferred into Petri dishes filled with phosphate-buffered saline (PBS). To wash out blood and proteins adjacent to the tooth surfaces, the Petri dishes were gently agitated for several seconds manually. This procedure was repeated 3 times per tooth. Then, the teeth were transferred into special cryo-containers, filled with an optimized cell nutrition medium, including 50% RPMI 1640 medium (Invitrogen, Basel, Switzerland), 40% fetal-calf serum (FCS; Invitrogen), and 10% cryoprotective agent in form of dimethyl sulfoxide (DMSO, SIGMA, St. Louis, MO). The cryo-containers were sealed by a screw cap after immersing the teeth and stored in an isopropanol container at –70°C for 12 hours for a slow and controlled freezing procedure. Subsequently, the cryo-containers were stored in the gas phase above liquid nitrogen (–196°C).

After a storage period of 4 months, the cryo-containers were removed from nitrogen tanks and transferred into a water bath with a temperature of 37°C. The specimens were kept under continuous agitation until the thawing process started. The teeth were subsequently removed from the cryo-containers and transferred to a Petri dish containing PBS to dilute and remove the cryoprotective agent. After 3 sessions of gentle agitation for 1 minute in PBS, the teeth were stored in a Dentosafe box for transport to the location of the synchrotron radiation source.

The SRμCT-measurements were performed at the beamline W2 at HASYLAB (DESY, Hamburg, Germany), operated by the HZG Research Center in the standard absorption contrast tomography setup.[7] The teeth were removed from the Dentosafe box and each tooth was transferred into a 1.5-mL Eppendorf tube filled with Dentosafe solution. The teeth were clamped into Eppendorf tubes based on their fitting size and shape. The specimen containers were glued onto the high-precision manipulator. Tooth A was measured at a photon energy of 36 keV with an asymmetric rotation axis.[8] There were 1440 projections acquired over 360° with a pixel size of 2.8 μm, resulting in a 3D dataset of 2988 × 2988 × 1020 isotropic voxels. Tooth B and tooth C were measured at a photon energy of 41 keV with an asymmetric rotation axis in 4 height steps each. The 360° scan with a pixel size of 4.1 μm generated a total of 1440 projections resulting in a dataset of 2884 × 2884 × 4966 and 2940 × 2940 × 4940 isotropic voxels, respectively. The data reconstruction was carried out by means of the filtered back-projection algorithm and with a binning factor of 2.[9]

The dataset of each tooth was converted into DICOM format to be visually analyzed. Two oral surgeons, experienced in the evaluation of 3D data, scrolled through the 3D data in x-, y-, and z-directions to detect the cracks. After this evaluation, each examiner described the cracks.

Selected cracks were further examined to determine their average width. First, a volume of interest was determined. The histogram of this region of interest was quantitatively evaluated by means of dedicated Matlab code (MATLAB, MathWorks, Natick, MA).

RESULTS

The composition of hard tissues of teeth (dentin and enamel) are known and the related local x-ray absorption values allow segmenting dentin, enamel, and surrounding PBS, as qualitatively illustrated in Figures 1 to 3. Consequently, cracks with a width above specific thresholds become visible in the virtual cuts and 3D representations of the computed tomography (CT) data (see Figure 1).

Tooth A contained numerous characteristic features identified as cracks in the enamel. The CT slices in Figure 1 reveal discontinuities of the gray-scale–related x-ray absorption values within the enamel, identified as micrometer-wide cracks. The cracks are generally aligned perpendicular to the dentin-enamel junction and seem to stop at this internal interface. In the diagonally opposite periapical regions, a high density of cracks is present, as distinctly demonstrated by the 3D image to the right in Figure 1.

Figure 2 shows slices and 3D renderings of tooth B. Again, one can perfectly discriminate between enamel and dentin as well as surrounding PBS. The virtual cut along the tooth axis shows areas of reduced intensity, which originated from the stacking and can be regarded