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Demineralized deciduous tooth as a source of bone graft material: its biological and physicochemical characteristics

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Objective. To examine structural and physicochemical characteristics of demineralized deciduous tooth powder (DDTP) in relation to demineralization time and to present potential of using DDTP as a bone graft material.
Study design. For structural and physicochemical analysis, scanning electron microscopy, inductively coupled plasma spectrometry, energy-dispersive X-ray analysis, X-ray diffraction analysis, differential scanning calorimetry, and Brunauer−Emmett−Teller surface area analysis were performed. In in vivo experiments, DDTP was grafted in 20 Sprague-Dawley rats' calvarial defects, and radiographic and histologic examination and histomorphometric analysis were performed.
Results. In vitro studies confirmed physicochemical demands for collagen-based bone graft material, such as lowered calcium content, lowered crystallinity of hydroxyapatite, and exposed organic structures to demineralization. In vivo experiment indicated new bone formation in DDTP-grafted sites and gradual resorption of the grafted particles. Defect closure rate was significantly higher in the 8-week DDTP-grafted group compared with control ($P < .05$).
Conclusions. Deciduous teeth had structural and physicochemical characteristics suitable for grafting with appropriate demineralization. Bone healing was observed to have successfully occurred in DDTP-grafted sites. (Oral Surg Oral Med Oral Pathol Oral Radiol 2015;120:307-314)

Bone graft is commonly applied in dentistry to induce bone formation, resulting in increased bone thickness and quality that can support oral functions. In the field of pediatric dentistry, bone regeneration could be aimed at filling defects caused by trauma, tumor excision, or congenital defects such as cleft alveolus.

Different bone graft materials have been introduced over time. Among them, autogenous bone is considered to be the most ideal material because it promotes osteogenesis, osteoinduction, and osteoconduction. However, the drawback of using autogenous bone are secondary defects caused at the donor site, limited availability, increased operation time, and inevitable resorption of the graft. To overcome these limits, allogeneic bone, xenogeneic bone, and synthetic bone materials have been developed, but concerns about infection and the high cost associated with allogeneic or xenogeneic bone and low functional activities in synthetic bone limit their usage, especially in adolescents.

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Recently, bone graft materials using permanent teeth have come to light, and clinical use and positive safety profile of this material have been confirmed by various studies.[1-8] Tooth components have been found to be very similar to those of alveolar bone,[3,9] allowing for novel bone graft materials utilizing the inorganic and organic components of extracted teeth. Studies have indicated that bone graft substitution involving dentin of animals and humans can be used to restore hard tissue defects in the oral and maxillofacial areas.[1,4-6,10]

Although sufficient basis has been developed for using extracted teeth as bone graft materials, permanent teeth can only be used on an individual basis for patients who can provide an extracted tooth, and the number of intact teeth available for extraction is limited. If deciduous teeth collected at the time of natural exfoliation could be used for bone graft, the method would be applicable in many more cases with less cost and sacrifice. Chemical and mechanical properties of permanent and deciduous teeth are different, yet not much study has been performed to establish the possibility of using deciduous teeth as bone graft materials.

Statement of Clinical Relevance

In this study, we provide a basis for assessing the potential use of deciduous teeth as an alternative bone graft material by examining the structural and physicochemical characteristics of demineralized deciduous tooth powder (DDTP) and presenting histologic evidence of bone formation.

In this study, we present the structural and physicochemical characteristics of demineralized deciduous tooth powder (DDTP) in relation to demineralization time, and provide histologic evidence for the potential of using DDTP as a bone graft material.

MATERIALS AND METHODS

This study was approved by the Institutional Review Board of Ewha Womans University Mokdong Hospital (ECT 14-04 B-21).

Sample preparation

Exfoliated deciduous teeth were collected and washed with 4% hydrogen peroxide and 70% ethanol for 10 minutes each and stored at −20°C. All steps, including demineralization, washing, and sterilization, were processed in a vacuum-ultrasonic device (VacuaSonic System, CosmoBioMedicare Co., Seoul, Korea) following manufacturer's instructions. Teeth were crushed into powders of 800-1000 μm and defatted with ether solution. Contaminants and remaining soft tissues were removed by 4% hydrogen peroxide and 70% ethanol. Demineralization using 0.6N hydrochloride was done for 0, 10, 15, 20, 25, 30, 60, and 90 minutes for in vitro analysis and 15 minutes for in vivo experiment. The sample was then washed with phosphate buffered saline (PBS), sterilized with peracetic acid−ethanol solution, and consecutively washed again with PBS and distilled water. The prepared sample was kept at 4°C before use.

Structural and physicochemical analysis

Scanning electron microscopy. A scanning electron microscope (S-4800, Hitachi, Ibaraki, Japan) was used to examine the surface structure of DDTP samples demineralized for 0, 10, 30, 60, and 90 minutes.

Inductively coupled plasma spectrometry. For quantitative analysis of calcium in DDTP, inductively coupled plasma spectrometry (ICP; Optima8300, PerkinElmer, MA, USA) was performed for samples demineralized for 0, 10, 30, 60, and 90 minutes.

Energy-dispersive X-ray analysis. Energy-dispersive X-ray analysis (EDS; X'PertPro, Panalytica, Almelo, Netherlands) was performed to examine the relative ratio of chemical elements such as calcium and phosphate (inorganic components) and carbon, nitrate, and oxygen (organic components) from the surface of DDTP samples demineralized for 0, 10, 30, 60, and 90 minutes.

X-ray diffraction analysis. To examine crystallinity change in inorganic components of DDTP according to demineralization time, individual samples were inserted into an analytical glass holder and diffraction patterns were obtained using an X-ray diffractometer (Ultima IV, Rigaku, Tokyo, Japan). After X-ray diffraction analysis (XRD) was performed with 0-, 10-, 30-, 60-, and 90-minute samples, additional XRD was performed with 15-, 20-, and 25-minute samples for more detailed observation between 10 and 30 minutes.

Differential scanning calorimetry. Differential scanning calorimetry (DSC; DSC131 evo, Setaram, Caluire, France) was performed at a constant rate of 2°C/min to examine the phase transition pattern. Phase transition pattern is related to structural stability, which again estimates crystallinity of the material. The analysis was performed with 15-, 20-, and 25-minute samples.

Brunauer−Emmett−Teller surface area analysis. Brunauer−Emmett−Teller (BET) analysis (Quadrasorb, Quantachrome Instruments Co., FL, USA) was performed to obtain surface area and examine pore size distribution in deciduous teeth that had undergone demineralization for 15, 20, and 25 minutes.

In vivo experiment

The experiment was performed in 20 Sprague-Dawley rats that weighed 300 grams (g). Animal selection and management, surgical protocols, and preparation procedures were approved by the Institutional Animal Care and Use Committee of Ewha Medical Center.

Anesthesia. Before surgery, 2% xylazine hydrochloride (Rumpun, Bayer Vetchem-Korea Ltd., Seoul, Korea) was mixed with tiletamine/zolazepam (Zoletil, Virbac S.A., Carros, France) at a ratio of 3:2 and administered by intraperitoneal injection at a dose of 0.01 mL/kg.

Cranial bone defect formation and DDTP graft. A full thickness skin flap was elevated from the midline of the calvarium. Two bicortical cranial bony defects of 5 mm diameter were created on both sides. Care was given to avoid trauma to the dura mater. The experimental side was grafted with DDTP and covered with a thin demineralized dentin sheet protecting the particle in situ, and the control side was left with no graft (Figure 1). Rats were allocated into 2 groups and allowed to heal for 2 ($n = 10$) or 8 ($n = 10$) weeks.

Necropsy and sample harvest. Rats were euthanized in a carbon dioxide chamber at 2 weeks or 8 weeks postoperatively. Bone specimens were harvested and fixed in 10% neutral formalin.

Histologic examination. The specimens were decalcified, embedded in paraffin, and sagittally sectioned passing the center of the defect site. Hematoxylin-eosin staining was performed, and histologic examination was done under the optical microscope (BX51, Olympus, Tokyo, Japan).

Histomorphometric analysis. For histomorphometric analysis, the images were captured with a charge-coupled device (CCD) camera (Eclipse 50i, Nikon, Tokyo, Japan) attached to the microscope and analyzed using Image-Pro Plus (Media Cybernetics, Silver