Histologic and Micro–Computed Tomographic Analyses of Replanted Teeth Stored in Different Kind of Media

Manuella Verdinelli de Paula Reis, DDS, MSc, Camilla Christian Gomes Moura, DDS, MSc, PhD,* Priscilla Barbosa Ferreira Soares, DDS, MSc,* Graziela Bianchi Leoni, DDS, MSc,† Manoel Damião Souza-Neto, DDS, MSc, PhD,† Darceny Zanetta Barbosa, DDS, MSc, PhD,‡ and Carlos José Soares, DDS, MSc, PhD**

Abstract

Introduction: Coconut water (CW) and soy milk (SM) have been proposed as storage media for avulsed teeth because of their nutrients that preserve cell viability. The present study investigated the periodontal healing process of dog teeth replanted after storage in CW, SM, and whole milk (WM) using micro–computed tomographic (μCT) and histologic analyses compared with immediate tooth replantation. **Methods:** Forty roots of 10 adult beagle dogs were extracted and subjected to the following protocols: immediate replantation after extraction (control), stored in CW with an adjusted pH, and SM and WM for 50 minutes before replantation. The animals were euthanized 28 days postoperatively, and the obtained specimens were scanned using a μCT scanner and subjected to routine processing for histometric analyses under an optical microscope. **Results:** CW and SM performed similarly to WM; however, SM showed significantly higher ankylosis than the control group. **Conclusions:** Additionally, this study showed that the combined use of histologic analysis and μCT is a promising method to better identify tooth resorption and the repair process and to evaluate the total extension of the periodontium. CW as a storage medium is a promising transport media for avulsed teeth. *(J Endod 2014;40:665–669)*

Key Words

Avulsed tooth, histologic analysis, micro–computed tomographic analysis, replantation, storage media

From the *Biomechanics Group, Department of Operative Dentistry and Dental Materials and †Oral and Maxillofacial Surgery and Implantology Department, School of Dentistry, Federal University of Uberlândia, Uberlândia, Brazil; and ‡Department of Restorative Dentistry, Faculty of Dentistry, University of São Paulo, Ribeirão Preto, Brazil.

Address requests for reprints to Dr Carlos José Soares, Biomechanics Group, Department of Operative Dentistry and Dental Materials, School of Dentistry, Federal University of Uberlândia, Av República do Piratini S/N, Bloco 4LA, Sala 4LA-32, Campus Umuarama, Uberlândia, MG 38402-028, Brazil. E-mail address: carlosjsoares@umuarama.ufu.br

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The ideal treatment for tooth avulsion is immediate replantation. However, in most cases of avulsion, the teeth are replanted after significant extra-alveolar time and dry or inadequate wet storage (1, 2). To minimize future complications such as ankylosis and root resorption, which are the major causes of replantation failure and tooth loss (3), the teeth should be stored in a medium capable of maintaining periodontal ligament (PDL) cell viability (3, 4). The ideal storage medium must be of appropriate pH and physiological osmolality and should include the presence of nutritional substances that allow cell survival (5). Furthermore, this storage medium should be readily available and simple to use (6). Taking requisites into consideration, bovine whole milk (WM) is widely accepted as a suitable medium for avulsed teeth because it maintains PDL cell viability for longer periods of time than many other types of media (3, 7).

Regarding these requirements for defining the most adequate storage medium, studies have proposed soymilk (SM) as an alternative storage medium (8–10) because of the increased consumption of soy products in many countries and the fact that SM is a good source of minerals and vitamins. Recently, studies have indicated that coconut water (CW) can be used as a transport medium for avulsed teeth (3, 4, 11). CW is rich in essential amino acids, proteins, vitamins, minerals, electrolytes, and sugars, all of which are important factors in nourishing cells and preserving PDL cell viability (4). However, these properties of CW may be compromised by its acidic pH, which requires adjustment before use as storage solution for avulsed teeth (11, 12). However, no *in vivo* study of the use of CW and SM for the storage of avulsed teeth has been conducted.

Although several studies have analyzed the histologic effects of storage media using histomorphometric analysis (13, 14), no systematic *in vivo* study has been performed analyzing the effects of CW and SM using histomorphometric analysis in conjunction with micro–computed tomographic (μCT) analysis. Although SM is widely consumed in eastern countries and CW is a typical product of tropical countries, the evaluation of these products as alternatives to CM is necessary for the advancement of research in dentoalveolar trauma. Considering that the PDL of dogs is one of the closest to humans and this animal is a devoted model to testing storage solutions on avulsed studies (15), the purpose of this study was to evaluate the healing process of dog teeth replanted after storage in WM, SM, and CW with adjusted pH using histomorphometric and μCT analysis. It was hypothesized that CW and SM used as storage media for avulsed teeth would exhibit better performance than WM and that these storage media are similar as the immediate replantation.

Materials and Methods

This research protocol was approved by the Animal Research Ethics Committee of Federal University Uberlândia (protocol no. 101/11). The animal experimental procedures were performed at the Veterinary Hospital of Federal University of Uberlândia, Uberlândia, Brazil. The animals were acquired from the Federal University of Santa Catarina, Santa Catarina, Brazil; maintained in a kennel during the experimental period; and observed daily by the veterinarian.

Ten healthy 18-month-old beagle male dogs weighing 5.55–7.10 kg were used. Forty alternate mature incisors with closed apices were selected for extraction and

TABLE 1. Definition of Terms Used to Assess the Roots

Terms	Definition
Normal periodontium	No evidence of inflammatory cells, presence of an intact cementum layer surface surrounded by connective tissue
Surface resorption	Shallow areas of resorption in root, possibly extending to dentin, without inflammatory cells
Inflammatory resorption	Extensive resorption of root accompanied by inflammatory cells, with osteoclasts generally present
Ankylosis	Obliteration of the periodontal ligament space resulting in bone and dentin contact
Replacement resorption	Areas of direct bony union with dentin with resorption of cementum and dentin
Repaired resorption areas	Resorption cavities filled with cementum or cementum-like tissue

were randomly assigned to the experimental groups (n = 10 in each group). The animals were preanesthetized using an atropine sulfate base by intramuscular injection (Atropion; Ariston Indústrias Químicas e Farmacêuticas, São Paulo, SP, Brazil; 0.04 mg/kg body weight). After 5 minutes, the animals were anesthetized intramuscularly with xylazine hydrochloride (Anasedan; AgriBrands Ltda, São Paulo, SP, Brazil; 1 mg/kg body weight) and a 1:1 combination of tiletamine hydrochloride and zolazepam hydrochloride (Zoletil 50; Virbac SA, Carros, France; 50 mg/kg body weight). A local anesthetic complementation was administered with 2% mepivacaine (DFL; Indústria de Comércio SA, Rio de Janeiro, RJ, Brazil) (13). The whole procedure of sedation and anesthesia followed the guidelines of the committee on ethics in animal research to prevent pain and suffering to animals. The teeth were removed using elevators and forceps as atraumatically as possible and immediately stored in the following experimental solutions for 50 minutes: CW (Ducoco, Linhares, ES, Brazil) with pH adjusted to 7.0 by triethanolamine (Pharma Nostra, São Paulo, SP, Brazil), SM (Sollys Original, Nestlé, Araras, SP, Brazil) with a pH of 6.91, and WM (Italac Integral, Italac, Corumbaíba, GO, Brazil) with a pH of 6.63. For the positive control group, the teeth were irrigated with a 0.9% saline solution (0.9% sodium chloride; Drogasil SA, Uberândia, Brazil) and immediately replanted.

All teeth were replanted in the socket with finger pressure and splinted with semirigid contention for 14 days. Intraoral radiographs were taken, and mobility was assessed postoperatively every 2 weeks. After 28 days, the dogs were euthanized through a massive dose of intravenous sodium pentobarbital at 100 mg/kg body weight, and the frontal portion of the maxillary and mandible containing the replanted teeth was removed in block. The blocks were fixed in a 10% formalin solution for 48 hours. During the experimental period, the animals were housed in individual pens, received water ad libitum, and were balanced feed (Royal Canin, São José do Rio Preto, SP, Brazil) 3 times a day.

μCT Analysis

The blocks were attached in a custom attachment and scanned in a μCT scanner (SkyScan 1174v2; SkyScan NV, Kontich, Belgium) at an isotropic resolution of 22.6 μm. Images of each specimen were reconstructed with dedicated software (NRecon v1.6.4, SkyScan NV) that provided transaxial, coronal, and sagittal cross-sections of the inner structure of the samples. Data Viewer v.1.4.4 software (SkyScan) was used to reconstruct the images in a 3-dimensional (3D) profile; 3D images of each sample as well as transaxial images were qualitatively

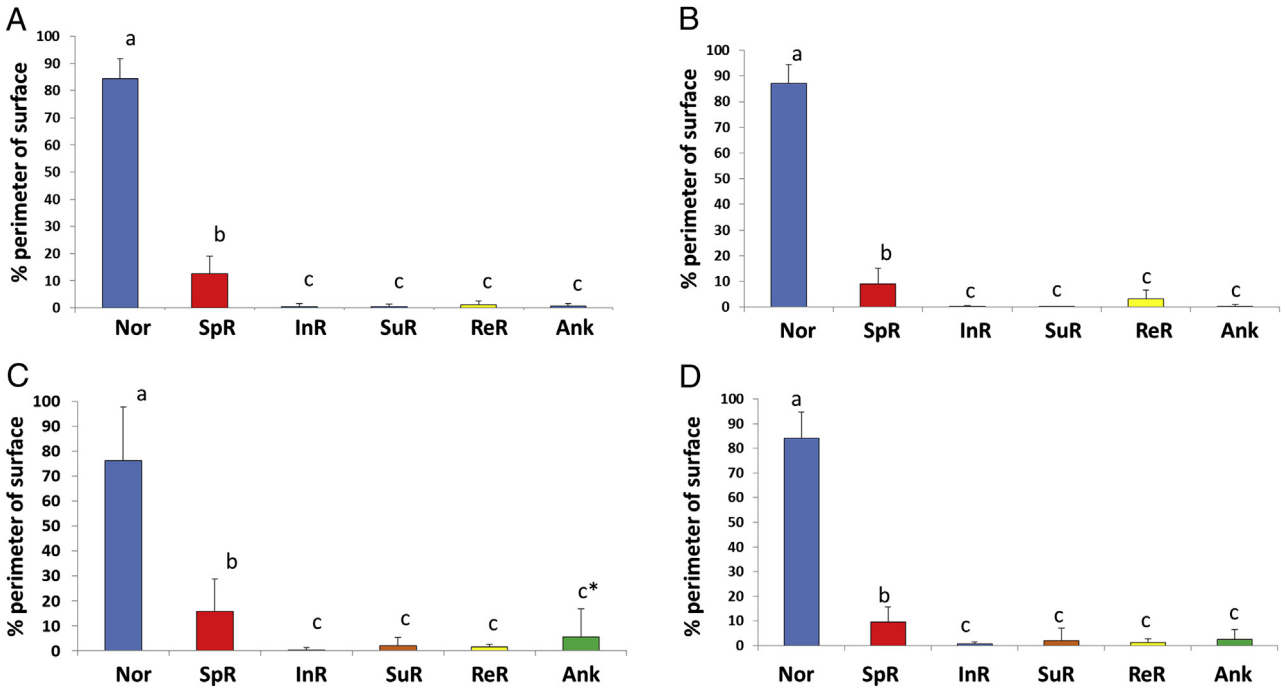

Figure 1. Results of histologic analyses of the (*A*) control group, (*B*) CW group, (*C*) SM group, and (*D*) WM group showing the prevalence of normal periodontal conditions followed by surface resorption for all groups ($P < .001$, Kruskal-Wallis). *The soy milk group had significantly higher ankylosis than the immediately replanted teeth group (control) ($P = .034$, Dunn test). Error bars represent standard deviation. Periodontal conditions: Ank, ankylosis; InR, inflammatory resorption; Nor, normal condition; ReR, repaired resorption; SpR, surface resorption; SuR, replacement resorption.