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Effects of fresh mineralized dentin and cementum on socket healing: a preliminary study in dogs

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Abstract (J Korean Assoc Oral Maxillofac Surg 2015;41:119-123)

Objectives: Dentin is composed of many minerals and growth factors. Based on this composition, we studied its effect as a possible regenerative material for alveolar healing.

Materials and Methods: This study was conducted using four 2.5-year-old mongrel dogs (male; weight, 25 to 30 kg). The third mandibular premolars were carefully mobilized with a dental elevator and then removed using forceps. The crown portions of the extracted teeth were removed with cutters, and the root portions of the remaining teeth were collectively trimmed as closely as possible to 350 to 500 μm. Dentin and cementum (DC) chips harvested from the extracted teeth were soaked in blood and packed into the fresh sockets (autograft). Biopsies were performed at the ends of day 14 and day 56 following implantation. Data were expressed as mean±standard deviation and compared with t-test results.

Results: The ratio of S_A(bone) to total area of each probe was determined and was 170±16 μm^2 for the control group and 71±14 μm^2 for the DC group, a significant difference ($P<0.05$).

Conclusion: DC particulate grafts offered no improvement in bone regeneration in alveolar extraction sockets.

Key words: Dentin, Cementum, Tooth extraction, Healing

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I. Introduction

The preservation of the alveolus for jawbone health has been widely discussed in the scientific literature. In the majority of cases, when a tooth is extracted, the surrounding alveolar bone undergoes a series of resorptive processes[1]. Studies have shown that natural healing post-extraction is associated with a significant loss in vertical and horizontal bone[2,3]. Moreover, a variety of factors, including trauma and endodontic pathologies, contribute to the loss of alveolar bone and subsequently complicate restorative treatments such as dental implants and dentures[4]. Many studies have demonstrated the potential merits of socket preservation following tooth extraction. To date, various materials and techniques have been used to preserve bone after tooth extraction[5]. For instance, the use of dense hydroxyapatite (HA) and bovine bone mineral integrated in collagen matrix have been reported after tooth extraction[6,7].

Extracted teeth can be used as grafting materials[8], and dentin is of particular interest for this purpose due to its unique chemical composition[9]. Dentin is an acellular, avascular, collagen-rich tissue matrix, compared to bone that is a cellular tissue with vessels. However, dentin and bone have similar components; they both consist of 10% body fluid, 20% organic materials, and 70% minerals (mainly HA) and contain bone morphogenetic proteins (BMPs), insulin-like growth factor (IGF)-II, and transforming growth factor (TGF)-β. Cementum, a bone-like material surrounding tooth roots, also

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contains TGF-β, IGF-I, and type I and type III collagen[10-14]. The osteoinductive potential of dentin was discovered in 1967[15]. Since then, several lines of studies have shown that animal demineralized dentine matrix induces ectopic bone formation in subcutaneous and intramuscular pockets in rodents[9,11]. Regarding the biochemical properties of dentin, we sought to assess the regenerative properties of fresh dentin as a novel graft material for alveolar bone regeneration.

II. Materials and Methods

This study was conducted on four 2.5-year-old mongrel dogs (male; weight, 25 to 30 kg). These animals were housed in temperature-controlled rooms and lived under a standard 12-hour light/dark cycle. The protocol of this prospective, randomized controlled trial was approved by the ethics committee of the Dental Research Center at Shahid Beheshti University of Medical Sciences (Tehran, Iran). The animals were sedated with an intramuscular injection of 2% acepromazin (0.1 mg/kg; Ingelheim Vetmedica Inc., St. Joseph, MO, USA) and subsequently anesthetized with 10% ketamine hydrochloride (25 mg/kg; Parke-Davis, Morris Plains, NJ, USA) administered intravenously and maintained during anesthesia; 2% lidocaine with 1:80,000 epinephrine (Darouphakhsh, Tehran, Iran) was injected to control any hemorrhaging. The third mandibular premolars on both sides were carefully mobilized with a dental elevator and then removed using forceps without the elevation of a muco-periosteal flap or compromising the marginal gingiva. The sockets were checked after extraction and thoroughly debrided with a curette to remove the periodontal ligament. After irrigation with saline solution, the depth of each socket was measured with a probe. The pulp tissues of the roots were extirpated with a hand instrument, and the root surfaces were cleaned with sterile gauze and a soft periodontal curette. The crown portions of the extracted teeth were removed with cutters, and the root portions of the remaining teeth were collectively trimmed as closely as possible to 350 to 500 μm using a calibrated mesh filter. In one quadrant, dentin and cementum (DC) chips harvested from the extracted teeth were soaked in blood and packed into the fresh sockets (autograft) so that they were completely filled with graft material. In the other quadrant, no bone substitute was placed. The sockets were sutured (Seralene 4-0s; Serag-Wiessner, Naila, Germany), and the animals were observed once a day for any clinical abnormality. Antimicrobial prophylaxis (cephalosporin 15 mg/kg, twice a day) was continued for 48 hours after surgery. Postoperative pain control was achieved via carprofen (50 mg/os/day, Rimadyls; Pfizer Santé Animale, Orsay, France) for 13 days. The dogs were placed on a soft diet throughout the entire observation period. The sutures were removed under a brief period of general anesthesia two weeks after the surgery.

Experiments performed and conducted in accordance with Regional Committee of Ethic complied with the regulations of the European Convention on Vertebrate Animals protection (2005).

1. Collection and storage of the specimens

The biopsy procedure was performed at the ends of 14 and 56 days after implantation[16]. At that time, short-lasting general anesthesia was induced, and local infiltration anesthesia was administered. A crestal incision was made, and the mucoperiosteal flap was elevated on both the buccal and lingual/palatal sides using a microsurgical periosteal elevator (P-TROM; Hu-Friedy, Chicago, IL, USA) whenever the concomitant surgical procedure required a full-thickness flap. When this was not the case (e.g., implant positioning with a flapless approach), the soft tissues overlying the extraction socket were included. The tissue specimens were collected with a trephine bur (2 mm internal diameter and 8 mm length; Hu-Friedy) from the centers of the sockets. The depth of trephine bur insertion was related to the measurements previously made by the stent. Tissue was obtained from the center of each socket, with the insertion axis of the trephine kept parallel to the long axis of the adjacent tooth. The apical portion of the specimen was marked with a fine indelible pen. The specimens were immediately fixed in 10% formalin.

2. Histological analysis

The specimens were rinsed thoroughly with water. After decalcification[6], the tissue blocks were embedded in paraffin. Several transverse sections with a diameter of 5 μm were cut through the center of each defect using a microtome (Jung, Frankfort, Germany). We used systematic random sampling to select sections with 20-μm intervals[17]. The samples were then stained with H&E and examined with an optical microscope at 40× magnification (Nikon Eclipse E400; Nikon, Tokyo, Japan) that was linked via a digital camera (Nikon Fuji HC-300 ZI; Nikon) to a personal computer. For quantification of the studied fields, a simple morphometric analysis was used; tissues of the specimens were divided into two compartments: newly formed bone and connective tissue.

A stereological frame was superimposed on each randomized field.(Fig. 1) Based on the tissue contained within each randomized field, the boundary of each phase was defined on the image. And then the frame was superimposed on it; the hit points or intersections for each phase were counted and recorded. Using this method, the proportions of the histological phases (new bone and connective tissue) were obtained for each studied field. The surface area (SA) of each counting frame (150×120 μm) was 18,000 μm^2. Finally, the number of hit of points was inserted into the following formula:

SA=I phase A/total number

where *SA* indicates the proportion of tissue, and *I* indicates the number of intersections for each phase. As mentioned earlier, the surface ratios for each section were calculated[17].

3. Statistical analysis

All data are expressed as mean±standard deviation. The comparison was made by paired t-test, and *P*-values<0.05 were considered statistically significant. Calculations were performed using the SPSS Statistics version 17.0 software (SPSS Inc., Chicago, IL, USA).

III. Results

Histological examination of group DC at the end of the second week showed particles of unreacted ground dentin and some dilated vessels.(Fig. 2) On the control side, no specific reaction was noted. At the end of eight weeks in group DC, granular tissue with fibroblast cells and collagen fibers was the dominant histological feature, and sparse bone formation was noted in the vicinity of the particles.(Fig. 3) On the control side after eight weeks, the histological examination showed collagen fiber deposition, a few scattered osteoblasts, bone formation in a centripetal pattern, and venules packed with red blood cells.(Fig. 4, 5)

1. Morphometry

The ratio of S_A(bone) to total area of each probe was as-

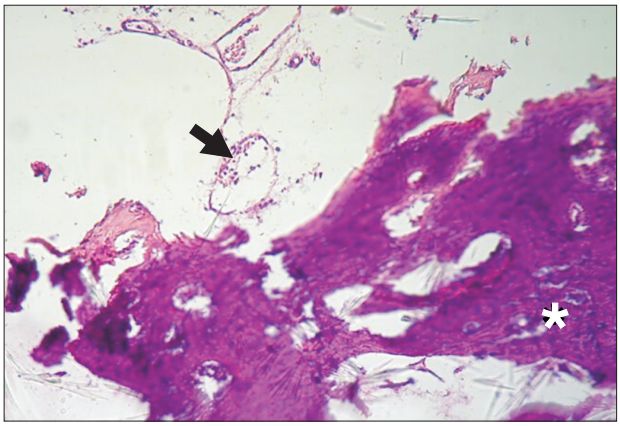

Fig. 2. Dentin/cementum particle (asterisk) and a blood vessel (arrow) in an alveolar socket at the end of the second week. H&E staining, ×40.
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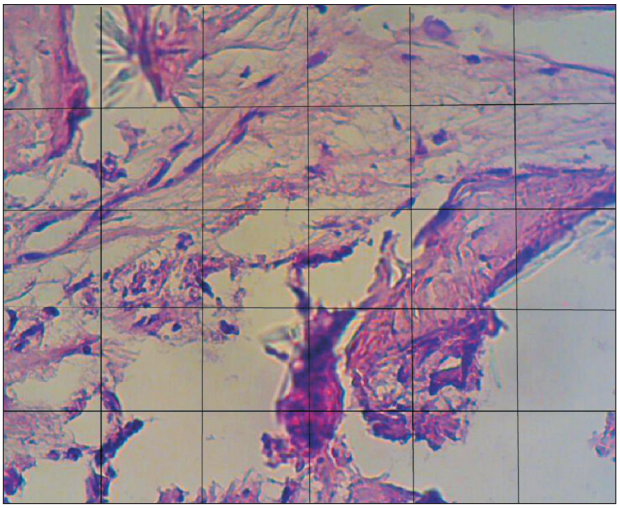

Fig. 1. A stereological counting frame was superimposed on each randomized field. This frame is composed of 30 squares (each square, 25 μm). ×40.
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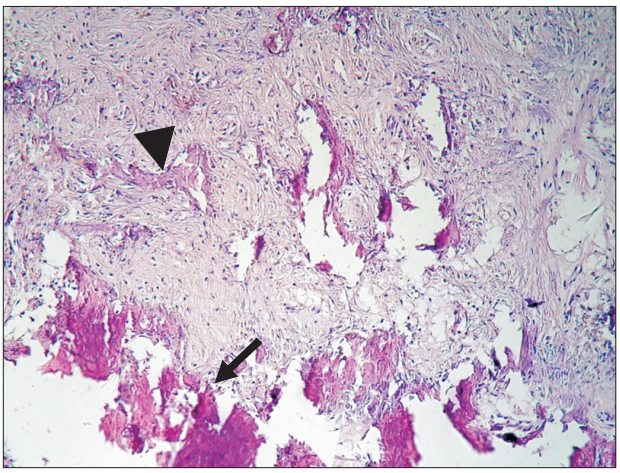

Fig. 3. New bone formation (arrowhead), particles of dentin/cementum (arrow) in the periphery, and newly formed bone adjacent to the particles at the end of the eighth week. H&E staining, ×20.
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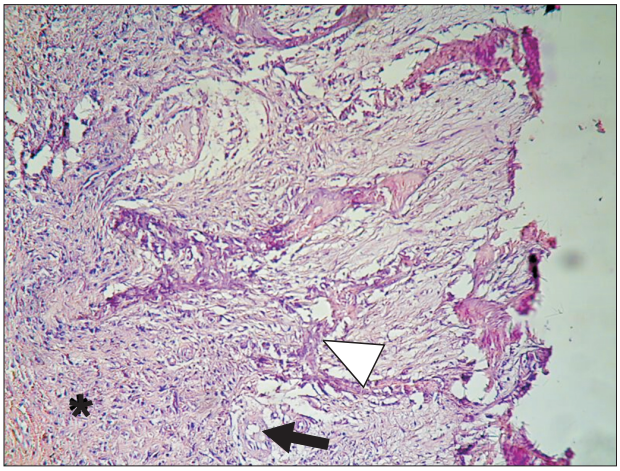

Fig. 4. Bone formed in a centripetal pattern. Bone spicules (arrowhead), connective tissue (asterisk) and a blood vessel (arrow) in the control group at the eighth week. H&E staining, ×20.
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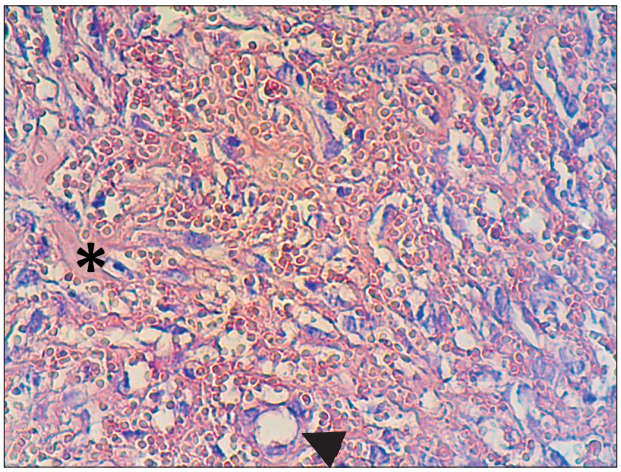

Fig. 5. Red blood cells, venules (arrowhead), and connective tissue (asterisk) in the control group at the eighth week. H&E staining, ×20.
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sessed and was 170±16 μm^2 for the control group at the end of eight weeks and 71±14 μm^2 for the DC group, a significant difference (P<0.05).

IV. Discussion

This animal study was conducted to assess the healing of tooth extraction sockets using fresh DC. The results showed that the application of DC induced no effects on the healing process before two weeks. However, after eight weeks, granular connective tissue was prominent in DC sockets. We used descriptive examination in conjunction with morphometric analysis to estimate the SA of newly formed bone. This method provides tangible and quantitative results from histological sections and therefore enables the precise comparison of data[18]. The results of morphometric analysis revealed that bone formation proceeded at a lower rate in sockets treated with fresh DC. The present results are likely the first report on the use of fresh DC in reconstructive surgery. The application of biomaterials such as dentin has been studied extensively in the field of reconstructive surgery. Our data suggest that the route of preparation is a crucial step in the osteoinductivity of biomaterials[19]. Studies have shown that demineralizing treatments of bone and dentin increase their osteoinductivity and decrease their antigenicity[20,21]. Nampo et al.[22] studied the effect of iliac bone and teeth on new bone formation in the jawbones of rats. The results of their study showed that both types of materials could be used as suitable grafting materials. Kim et al.[21] reported that a mixture of heat-pulverized dentin and plaster repaired large jaw defects 57 months after surgery. They concluded that a dentin/plaster mixture was a useful biomaterial in the reconstructive surgery of jaw defects[13]. Register found that dentin allografts activated bone formation[14], and Jeong et al.[23] reported that autogenous teeth and bone could be used as good alternatives to autogenous bone. Collectively, the aforementioned studies have reported the potential of dentin as a grafting material. However, our results are not compatible with these studies. The discrepancies between our results and those of other studies may be explained by the wounds or defects, species, age, and method of material preparation. For instance, Nampo et al.[22] used the extracted tooth with its pulp as a source of stem cells and neural crest cells. Devecioğlu et al.[24] studied the effects of demineralized, freeze-dried DC on periodontal ligaments and fibroblasts. Their results implied that the applied materials had greater potential to form mineral-like nodules than did other applied materials. Gomes et al.[25] studied the effects of demineralized dentin matrix on bone repair in diabetic rabbits and showed that dematerialized dentin was biocompatible in diabetic rabbits.

Given the results of these studies and our findings, fresh DC has little capability to induce significant bone formation in the alveolar socket. This result may be explained by the fact that such mineral acts as a barrier to chemotactic and morphogenic molecules of dentin, while acid initiates a demineralizing process that exposes osteoinductive molecules like BMPs. It seems that demineralization of biomaterials such as DC would be a key determinant factor in subsequent

inductive/conductive potential of *in vivo* grafted materials[19-21]. Studies have documented that demineralization of dentin exposes the sequestrated BMPs in the insoluble collagen of the dentin matrix. Decalcified dentin has revealed better bone induction activity due to the activation of BMPs, which bind to collagen matrices through the demineralization process[17-21,26]. Recent data provide evidence that addition of BMP2 to demineralized dentin accelerates its osteoinductivity[16,26].

V. Conclusion

In conclusion, the strength of our pilot study is the evidence that fresh mineralized DC autografts have little to no effect on the induction of new bone in the alveolar socket milieu. We suggest using ultrastructural and molecular approaches in similar studies in order to determine the underlying mechanisms.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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