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ORIGINAL RESEARCH

Socket Preservation using Enzyme-treated Equine Bone Granules and an Equine Collagen Matrix: A Case Report with Histological and Histomorphometrical Assessment

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ABSTRACT

Aim: To histologically assess the effectiveness of a socket-preservation technique using enzyme-treated equine bone granules as a bone-graft material in combination with an equine collagen matrix as a scaffold for soft-tissue regeneration.

Background: Enzyme-treated equine bone granules and equine collagen matrix recently have been developed to help overcome alveolar bone deficiencies that develop in the wake of edentulism.

Case report: The patient had one mandibular molar extracted and the socket grafted with equine bone granules. The graft was covered with the equine collagen matrix, placed in a double layer. No flap was prepared, and the gingival margins were stabilized with a single stitch, leaving the matrix partially exposed and the site to heal by secondary intention. The adjacent molar was extracted 1 month later, and that socket was left to heal by secondary intention without any further treatment. Three months after each surgery, an implant was placed and a biopsy was collected. The two biopsies underwent histological processing and qualitative evaluation. Histomorphometric analysis was also performed to calculate the percentage of newly formed bone (NFB) in the two cores. Healing at both sites was uneventful, and no inflammation or other adverse reactions were observed in the samples. Soft-tissue healing by secondary intention appeared to occur faster at the grafted site. The corresponding core showed a marked separation between soft and hard tissue that was not observed in the core from the nongrafted site, where soft-tissue hypertrophy could be observed. Newly formed bone at the grafted and nongrafted sites was not significantly different (27.2 ± 7.1 and 29.4 ± 6.2% respectively, p = 0.45).

Conclusion: The surgical technique employed in this case appeared to facilitate postextraction soft-tissue healing by second intention and simplify soft-tissue management.

Clinical significance: Using a collagen-based matrix to cover a postextraction grafted site may facilitate second intention soft-tissue healing and proper soft-tissue growth.

Keywords: Case report, Equine bone graft, Equine collagen matrix, Secondary intention healing, Socket preservation.

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INTRODUCTION

Partially or totally edentulous patients' increasing demands for complete functional and esthetic restoration have made implant-supported prosthetic-driven rehabilitation the elective treatment for edentulism. Edentulous patients often present with deficient bone, either due to trauma or atrophy. Bone loss may occur to the extent that implant placement becomes unfeasible. If implants can be placed, but bone loss is present, achieving complete esthetic restoration may be difficult. Bone loss occurs even as a consequence of tooth extraction. After extraction, the first healing stages involve clot formation, clot maturation, clot infiltration by fibroblasts, extracellular matrix stabilization, and subsequent bone formation.[1] The overlying mucosa heals either by primary or secondary intention, depending on the conditions and juxtaposition

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of the gingival margins. During later healing stages, as tissue regeneration occurs in the socket volume, alveolar bone undergoes changes leading to both vertical and horizontal loss. This may complicate subsequent implant insertion.

The structural and dimensional alveolar bone changes may be significant[2] and have been extensively studied both in animals[3-5] and humans.[6-9] By 12 months after extraction, horizontal bone loss may reach 5 to 7 mm, a deficiency corresponding to about 50% of the original volume.[2,10-12] Resorption is greater on the buccal side and affects mainly the coronal third of the socket. The greater buccal resorption is thought to be due to the presence of bundle bone only there, a condition that might favor osteoclastic activity.[5] In contrast, bundle and woven bone are found on the palatal/lingual side. Whatever the cause, buccal resorption often causes buccal soft-tissue collapse, resulting in marked vestibular esthetic alterations.[10] These represent a real challenge, especially in the anterior, where achieving complete esthetic restoration is pivotal.

Different treatment techniques have been proposed to limit volumetric bone loss consequent to tooth extraction, including placement of biomaterials into the fresh postextraction socket.[13] Grafting aims to stabilize the blood clot and facilitate bone regeneration, prevent soft-tissue collapse into the defect, and preserve the alveolar bone from atrophy, at least partially.[14] Autogenous bone is still regarded as the gold standard grafting material for bone regeneration. Yet its use exposes the patient to additional discomfort and risks. Bone allografts eliminate the need for a second surgical site to collect autogenous bone, but the availability of allografts is subject to proper donor screening and functioning of tissue banking.

To overcome these limitations, alternative synthetic or natural-derived bone grafting materials are extensively used. Among the natural materials, mammal-derived processed bone represents an interesting option, given the well-known similarities of bone structure and composition among mammals. Recently, evidence has been reported about an enzyme-treated equine bone graft material featuring bone collagen preserved in its native state and having remodeling properties that lead to the formation of significant amounts of newly regenerated bone.[15,16] Yet no evidence has yet been provided about its effectiveness in alveolar bone preservation. The effectiveness of bone grafting in preserving buccal alveolar bone after tooth extraction is still debated,[17,18] as several variables condition its success, including achievement of soft-tissue healing by primary intention.[19] The latter requires careful and skillful soft-tissue management.

Recently, it has been observed that collagen matrices work as effective scaffolds that favor soft-tissue healing. In the clinical setting, they have been shown to be useful in treating both class I and II gingival recessions, where they might replace palatal connective tissue grafts,[20] and helping with closure of postextraction sockets.[21] Recently, an equine collagen-based matrix has become available on the market, but still no evidence has been provided in published literature about its safety and effectiveness. In the following case, the performance of this matrix as a scaffold promoting soft-tissue healing by secondary intention in a socket-preservation surgery was assessed histologically by comparison with the histological healing of a postextraction socket that received no grafting.

CASE REPORT

A nonsmoking 55-year-old woman with no significant medical history presented with two compromised teeth (3.7 and 3.6) (Figs 1 and 2). She was seeking pain relief and definitive rehabilitation. To enable comparison of a grafted *vs* nongrafted extraction socket, equine bone

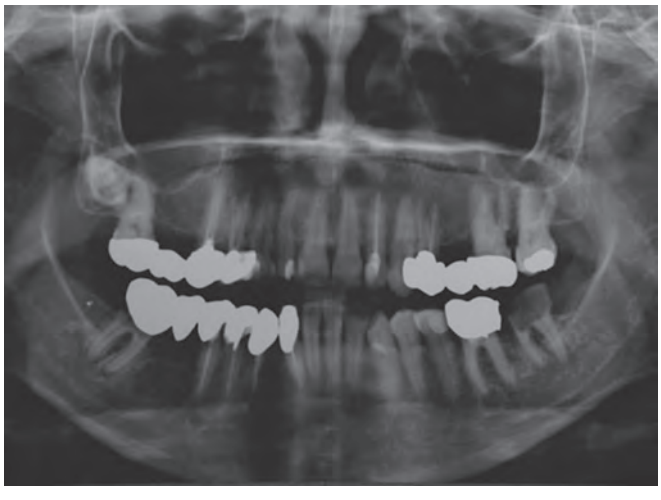


Fig. 1: Panoramic radiograph. Both element 3.6 and 3.7 are compromised and call for extraction

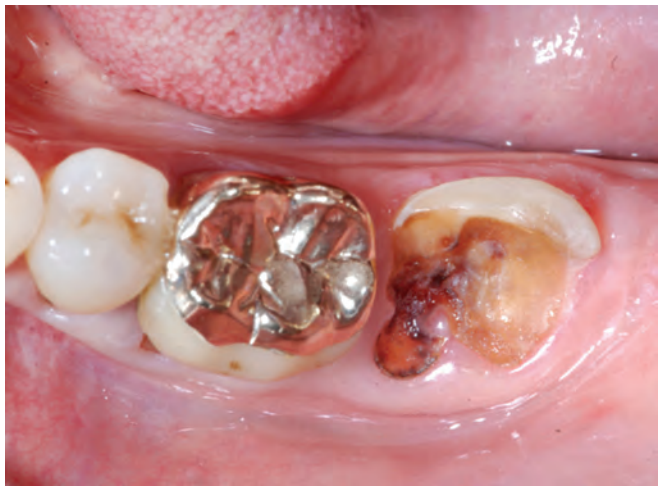


Fig. 2: Occlusal view of the 3.7 and 3.6 teeth before extraction

granules and an equine collagen matrix would be used at the 3.7 extraction socket, but at the 3.6 site, where the tooth would be extracted 1 month after the first extraction, the socket would not be grafted. An implant to support a single crown would be placed 3 months after each extraction. The patient provided informed consent to the treatment and collection of a biopsy, along with the use of histologic data for a possible publication.

Equine bone granules to be used (Osteoxenon, OX-31, Cortical-Cancellous bone granules, 0.5–1 mm, Bioteck, Vicenza, Italy) are manufactured by subjecting equine bone to the action of digestive enzymes that allow for selective elimination of equine antigens. As the process occurs at 37°C, and no collagenases are used during the process, the graft materials retain bone collagen in its native form, a characteristic that may favor bone regeneration given the well-known properties of the collagen molecule. Both the equine bone graft and the collagen matrix undergo sterilization by e-beam irradiation at 25 kGy.

The equine collagen matrix (Xenomatrix, BCG-XC10, Bioteck, Italy, Fig. 3) is a two-part 4-mm-thick homogeneous collagen sheet. One part is octagonal and approximately 20 × 10 mm, while the other is a 14-mm-wide circular patch. The sheet is made of collagen extracted from equine Achilles tendon using an enzymatic process that involves collagen partial digestion and subsequent precipitation in a mildly acidic (pH 5) environment to create a collagen gel. Subsequent gel lyophilization produces a dehydrated homogenous collagen sheet.

The patient had a thorough oral hygiene session prior to the first extraction. Before both surgeries, antibiotic prophylaxis (amoxicillin/clavulanic acid, Augmentin, Glaxo-SmithKline, Verona, Italy, 2 gm 1 hour before surgery and then every 12 hours for 8–10 days) was initiated, and the patient underwent mouth rinsing with chlorhexidine 0.2% (Corsodyl, Glaxo-SmithKline), to be continued for 2 weeks after surgery. Sodium naproxen 500 mg (Synflex, Recordati, Milano, Italy) was also administered 1 hour before surgery and then twice a day for 7 days. The surgical area was anesthetized using articaine hydrochloride 40 mg/mL with epinephrine 1:100,000.

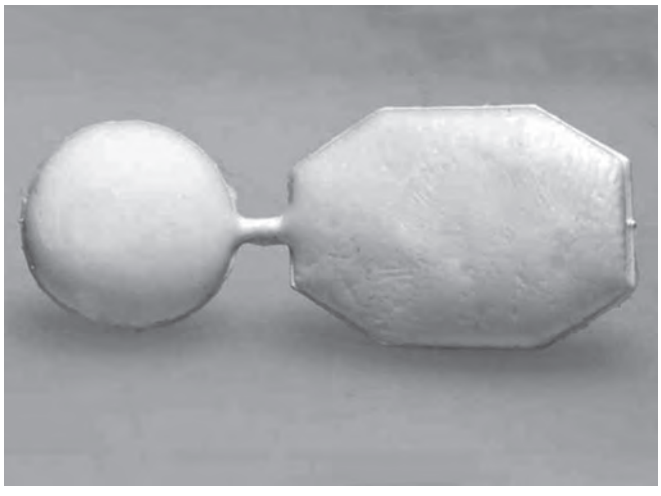


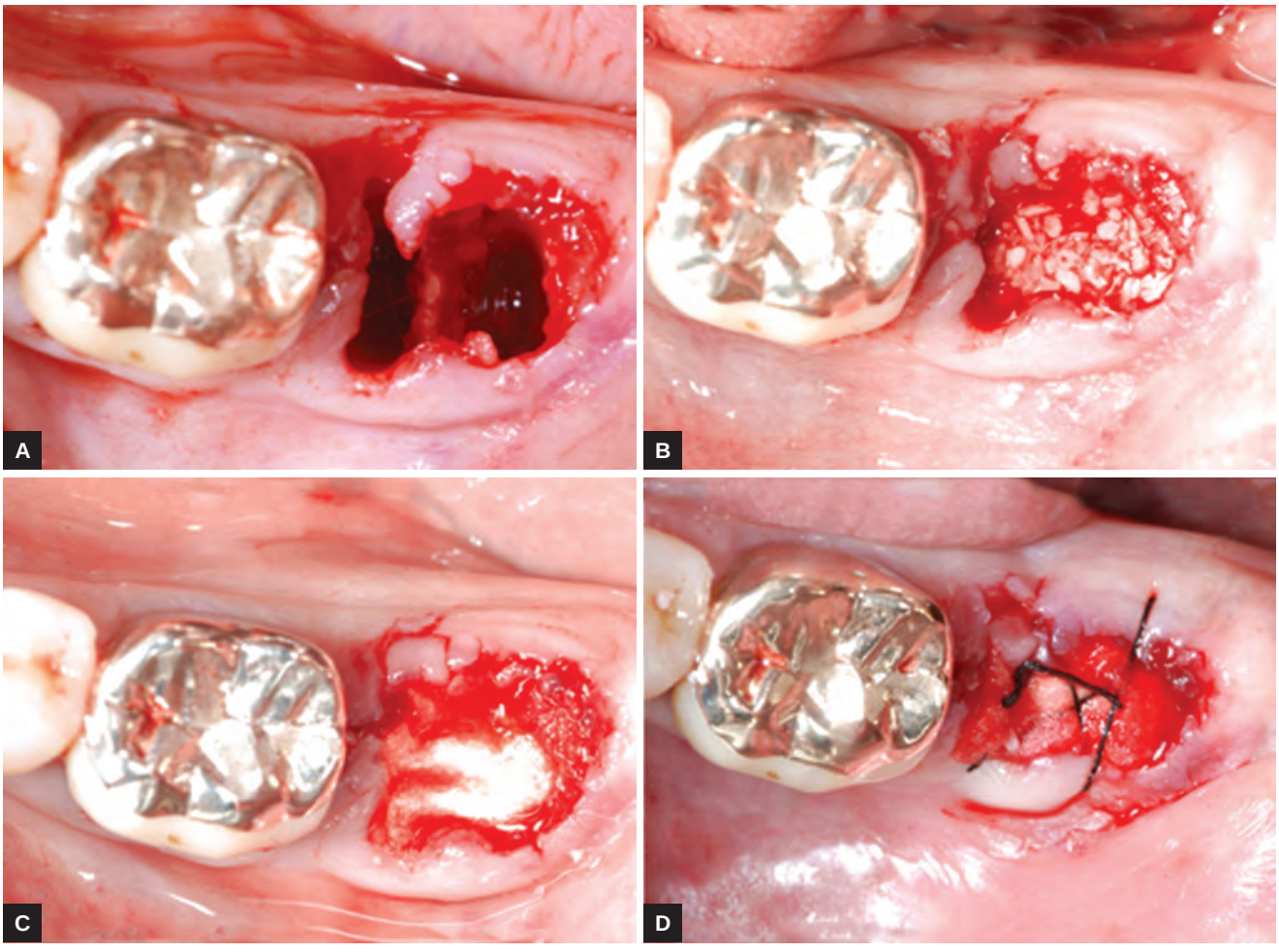
Fig. 3: The collagen matrix used in the present study. The matrix consists of two portions, an octagonal and a round one that are placed in two layers to cover the graft

Figures 4A to D and 5A to C illustrate both surgeries. A single experienced surgeon (AL) performed the atraumatic extractions. Each socket was gently debrided using a curette and irrigated using sterile saline. After gently detaching the periosteum from the alveolar bone edge all around the gingival margin, the 3.7 socket was grafted with a mixture of cortical and cancellous equine enzyme-treated bone granules. A first collagen matrix layer was placed under the gingival margins of the socket to cover the graft, and the second part of the collagen matrix was then placed upon the first. A month later at the 3.6 site, no bone graft or matrix layer was used. No releasing incisions were used at either site.

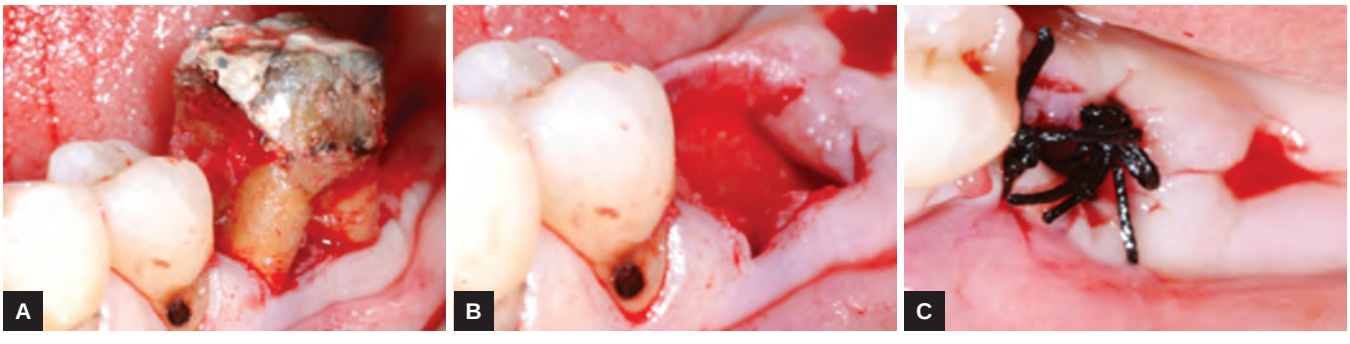
A single cross stitch using nonresorbable suture material (3-0 Silk Suture, Ethicon, USA) was placed at each socket to stabilize the gingival margins. The collagen matrix at the 3.7 site was therefore left exposed to the oral cavity. The patient was controlled 7 days after each surgery, when the sutures were removed. Control visits followed 14 days thereafter, and then once a month up to implant placement.

Three months after each surgery, after being subjected to the same antibiotic prophylaxis, anesthesia, and pain management treatment previously described, the patient had a 5.5 × 10 mm implant placed in each site (Way Milano, Synthegra surface, Nuova Geass, Pozzuolo del Friuli, Italy). Both times, before implant site preparation, a transmucosal trephine was used to collect a biopsy composed of both the upper soft tissue layer and the lower bone tissue. Site preparation and implant placement followed, according to the implant manufacturer's instructions. Three and a half months after implant placement, a single provisional prosthesis was delivered at each site; after one additional month, the definitive restoration was delivered. The patient was recalled every 3 months for clinical and radiographic follow-up.

For the histological analysis, each core was placed in a test tube containing buffered 10% formalin. The tube was marked with a unique alphanumeric code and sent to the histologists (LC, LP) who did not know, therefore, which of the two sockets the sample came from. Bone cores were decalcified for 21 days in a 0.76 M sodium formate and 1.6 M formic acid solution (Panreac Quimica, Barcelona, Spain). Samples were subsequently dehydrated in ascending concentrations of ethanol and embedded in paraffin. The cores were cut into 5 µm-thick sections, mounted on



Figs 4A to D: (A) The 3.7 socket after the extraction; (B) the socket has been filled with the equine-derived granules. The socket after the first; (C) and second; (D) collagen layer have been placed. A single stitch holds the gingival margin firm. The collagen matrix is left exposed



Figs 5A to C: (A) The 3.6 tooth during the extraction: (B) the postextraction socket; and (C) a single stitch is placed to hold the gingival margin firm

slides, and stained with hematoxylin-eosin. One histologist (LC) provided, for each sample, a qualitative report aimed at providing a general sample description and identifying any sign of inflammatory or immune reactions.

Morphometric measurements were performed on digital photomicrographs collected at 10× magnification, analyzing the bone part of the core only, as follows. Each whole sample image was analyzed by two investigators (LC, LP) independently and in triplicate using the Image J 1.33 analysis software (National Institute of Health, Bethesda, USA). For each image, after digitally delimiting the hard tissue area only, the total hard tissue area (THA), the total bone area (TBA), newly formed bone area (LBA), and residual bone substitute area (RBA) were measured. Average newly formed bone (NFB) was then calculated and expressed as the percentage over the THA (%NFB = LBA × 100/THA). Newly formed bone is given as the mean percentage of all sections.

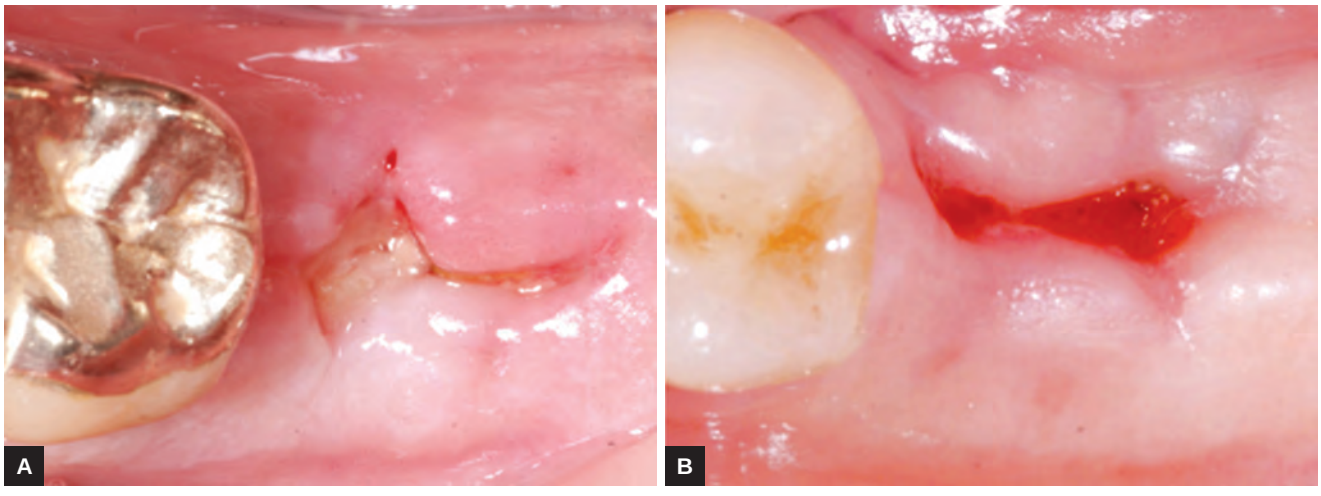
A two-way t-test was performed to test for significant differences in NFB in the two cores. The significance level of the test was 0.05. A dedicated software program (Origin 9.0, MicroCal, USA) was used for all statistical analyses. All values are presented as mean ± standard deviation (SD).

The patient healed uneventfully at both implant sites. At present, the two definitive crowns have been loaded for 17 months and are completely functional. No bone resorption was observed around the implants at the 12-month follow-up. Consequently, the two implants are currently 100% successful, according to the Albrektsson and Zarb criteria.

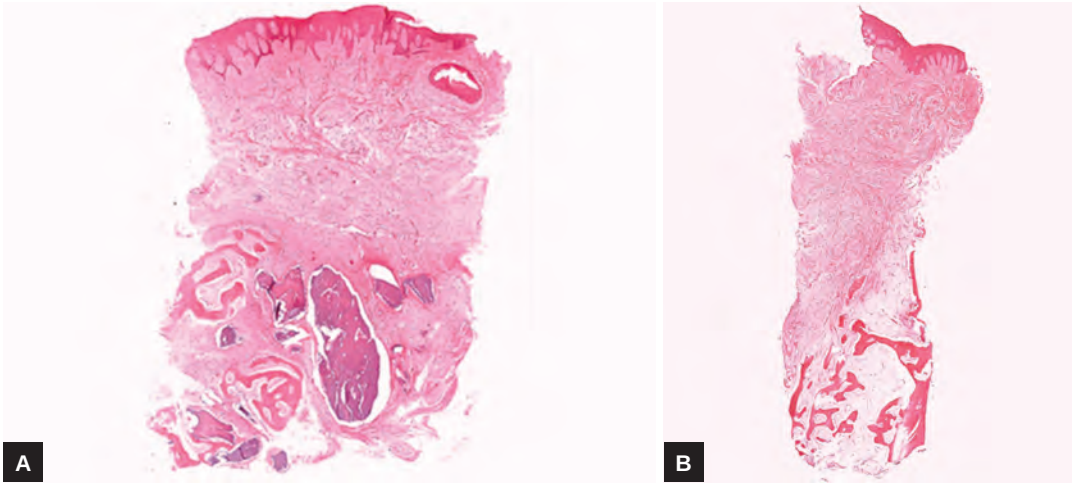
Soft-tissue healing appeared to occur faster at the grafted site than at the nongrafted one (Figs 6A and B).

Histological sections of the biopsy sample collected at the grafted site showed two distinctly separate portions corresponding to soft (upper) and hard (lower) tissue respectively (Fig. 7A). A thick layer corresponding to a stratified squamous epithelium could be observed in the upper side of the core. The underlying soft-tissue layer consisted of a thick portion of fibrous connective tissue. No matrix residuals could be observed. In the lower portion of the core, residual particles of the xenograft material could be identified as hematoxylin-stained areas in which the bone lacunae were devoid of osteocytes. They were in close contact with the living, patient-derived NFB tissue (eosin-stained and osteocytes-rich), which showed a trabecular-like organization. Medullary spaces were filled with fibrous connective tissue and displayed no areas with cartilage-like tissue. Osteoblasts could be observed lining both the bone substitute and the NFB. No inflammatory cells could be observed in any portion of the core.

In the core collected at the nongrafted site, no marked separation was observed between the soft and hard tissues. Instead, the soft tissue appeared to have widely invaded the left portion of the core and was quite hypertrophic (Fig. 7B). The soft-tissue portion was again organized in two layers, i.e., a stratified squamous epithelium



Figs 6A and B: Healing at 21 days: (A) 3.7 socket; and (B) 3.6 socket



Figs 7A and B: Hematoxylin-eosin staining: (A) Core from the 3.7 (grafted) socket; and (B) core from the 3.6 (nongrafted) socket. Soft tissue hypertrophy and invasion of the bone area can be observed in the 3.6 socket

overlying fibrous connective tissue. In the hard-tissue portion, eosin-stained and osteocyte-rich NFB, showing a trabecular-like organization, was observed, and as in the other core, osteoblasts were observed lining the NFB areas. Again, no inflammatory cells were observed in any portion of the core. Newly formed bone values in the cores corresponding to the grafted and nongrafted sites were 27.2 ± 7.1 and 29.4 ± 6.2% respectively, without a significant difference (p = 0.45).

DISCUSSION

The present case provides histological evidence that grafting a postextraction socket with an equine bone graft and an equine collagen matrix allows proper regeneration of both soft and hard tissue. In the histologic slides of both cores, the presence of a thick squamous pluri-stratified epithelium is consistent with the clinical observation of an appreciable growth of keratinized tissue over both sockets. The fact that the two soft-tissue layers in the cores appear to have the same spatial organization is consistent with the fact that cores were collected 3 months after the tooth extractions, i.e., at a time when epithelial and connective tissues have had enough time to complete healing. Yet the core corresponding to the nongrafted site showed a marked soft-tissue hypertrophy. Further studies, to be performed on animal models, should aim at assessing whether the collagen matrix actually facilitates soft-tissue healing at earlier postsurgical stages, as it appeared to do in this case. Both the equine bone graft and the collagen matrix appeared to be biocompatible, as no inflammatory cells or other histological findings indicating adverse tissue reactions were observed.

Observations of graft granules possibly undergoing remodeling and the NFB percentage observed in the present case are consistent with the results of previous investigations of such equine bone graft,[16,17] showing that it undergoes remodeling and is replaced by NFB soon after grafting. As the NFB in the two cores was not significantly different, it follows that at least in the clinical situation described here, the bone graft used did not modify the physiological bone regeneration that occurred at the grafted site. The clinical behavior that was observed in the present study is consistent with that of published studies on similar matrixes. The facilitation of healing by secondary intention that could be observed in the grafted site is consistent with the collagen matrix working as a scaffold and guide for epithelial and connective cells. The marked separation of the soft from the hard tissue portion that was observed in the histologic slides of the regenerated core is consistent with a barrier effect that the matrix might have exerted, possibly enhanced by the fact that the two matrix layers were actually grafted, enhancing occlusivity and, therefore, the matrix barrier effect. As no residual matrix could be observed in the histologic slides 3 months after surgery, the matrix appears to have a complete resorption time of less than 3 months and be totally resorbable.

The clinical technique applied in this case appears to be easily employed, not requiring flap elevation, mucoperiosteal release, or other soft-tissue management that demands extraordinary skill. It is noteworthy that even though the matrix was left exposed to the oral cavity, this did not hinder proper soft-tissue regeneration. The present case did not permit assessment of the ridge volume preservation over time. Additional specifically designed prospective studies should investigate the effectiveness of this double-layer matrix.

CONCLUSION

Observations of the present case suggest that using an equine collagen matrix in a double layer to protect an equine bone graft may facilitate postextraction soft-tissue healing by second intention and simplify soft-tissue management when performing socket preservation through bone grafting. Prospective studies should be undertaken to investigate the clinical effectiveness of this equine collagen matrix and socket preservation technique.

CLINICAL SIGNIFICANCE

Using a collagen-based matrix to cover a postextraction grafted site may facilitate second intention soft-tissue healing and proper soft-tissue growth.

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