Advances in Medicine and Engineering Interdisciplinary Research, 2023, Volume 1, Issue 2
DOI: 10.32629/ameir.v1i2.1240
ISSN Online: 2972-3825
ISSN Print: 2972-3833

Histological and Clinical Evidence of Soft Tissue Regeneration from a Porcine Collagen Matrix - Case Report

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Abstract: Tooth loss results in loss of hard and soft tissue volume, making it difficult to achieve aesthetically pleasing results. In order to decrease the morbidity caused by an autologous graft in the alveolar seal, it can be replaced by a resorbable matrix of collagen. The present case report evaluated clinically and histologically a porcine collagen matrix, in soft tissue regeneration, during the installation of an implant immediately after dental extraction. After 6 months, clinically, a tissue with an optimal final aesthetic appearance was obtained and histologically, the formation of an epithelial and connective tissue compatible with that of a normal mucosa was evidenced.

Key words: porcine collagen matrix; socket preservation; soft tissue graft

1. Introduction

Tooth extraction in the aesthetic field is a complex process, because it will lead to bone resorption and remodeling, resulting in changes in the size of the alveolar ridge, especially affecting vestibular contours [1, 2]. Therefore, it is difficult to obtain aesthetically satisfactory results for the volume loss of hard and soft tissues [2].

Currently, it has been described as an effective procedure to reduce the degree of horizontal and vertical resorption of the alveolar ridge after tooth extraction, to evaluate the patient's phenotype and to perform alveolar preservation using allografts, xenografts or alloplasts covered by a resorbable membrane [2].

In some clinical cases, immediate removal of implants can be installed together with bone filling materials, but if the implant does not have a healing abutment or temporary one over the implant,, the grafted hard tissue and implant will be exposed to possible chemical or bacterial contamination, which is the product of wounds exposed to the oral environment, leading to prolonged healing time and even damaging the aesthetic effect of treatment [3].

In order to improve the concept of alveolar preservation, soft tissue transplantation was used before or at the same time to seal alveolar, accommodate bone grafting materials and improve the function of soft tissue. This type of autotransplantation requires a second surgical site, which increases the incidence rate of surgery and has a risk of necrosis [4].

A few years ago, the clinical use of a three-dimensional resorbable collagen matrix of porcine origin (Geistlich

Mucograft® Seal, Geistlich Pharma AG, Switzerland) designed specifically for soft tissue regeneration in the oral cavity and for autologous graft replacement was introduced. It is manufactured as a matrix of type I and type III collagen matrix. It consists of two functional layers: a compact layer that allows suturing and protects the graft in open healing situations and another porous layer that favors the stabilization of a blood clot, promoting cell growth and early vascularization and accelerating soft tissue healing [5].

The purpose of this report is to evaluate clinically and histologically a porcine collagen matrix in soft tissue regeneration during the installation of an implant immediately after tooth extraction.

2. Case Report

2.1 Case selection.

A 61 years old female patient, ASA I, was consulted by the School of Dentistry of the University of Chile due to the alveolar bone trauma of teeth [1, 2], which were repaired by a single cermet fixed prosthesis. The imaging examination showed a radioactive transparent area around the root tip (Figure 1).

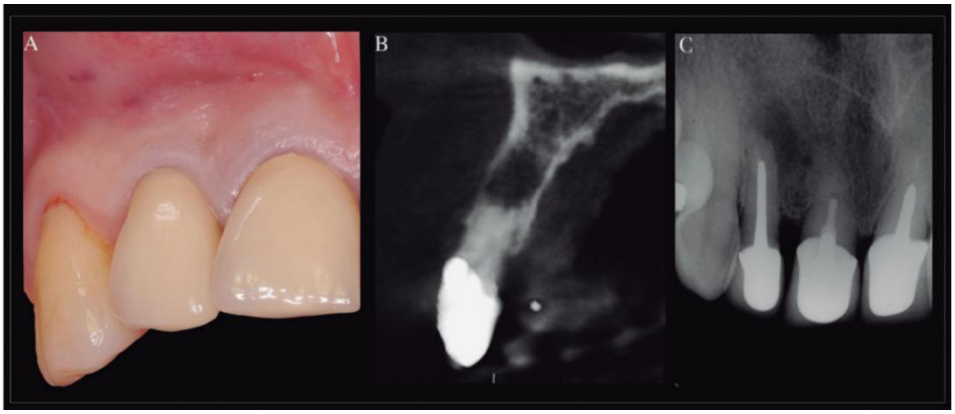

Figure 1. Initial situation.

Initial situation: (A) Tooth [1, 2] rehabilitated with metal-ceramic unitary fixed prosthesis with a history of dentoalveolar trauma. (B) Cone beam showing periapical radiolucent area and preserved vestibular table. (C) Retroalveolar radiograph of tooth [1, 2].

2.2 First surgical phase: atraumatic exodontia, immediate implant installation in the infected site and use of porcine collagen matrix.

The patient was prescribed antibiotic premedication with Amoxicillin 875 mg (Optamox, Pharma Investi Laboratory, Chile). The patient started the scheme 24 hours prior to surgery, taking 1 coated tablet every 12 hours for 7 days.

Once the surgical procedure is performed, an analgesic protocol begins with 125 mg of Lysine Clonixinate (Nefersil, Pharma Investi Laboratory, Chile) every eight hours for 3 days.

Syndesmotomy was performed with a peristome and then atraumatic vertical extraction of tooth [1, 2] with a Neodent® dental extractor (Neodent, Curitiba, Brazil), then the tooth socket was mechanically conditioned with a spoon and abundant irrigation with physiological saline solution to eliminate inflammatory and infectious tissue that might have remained after the extraction (Figure 2).

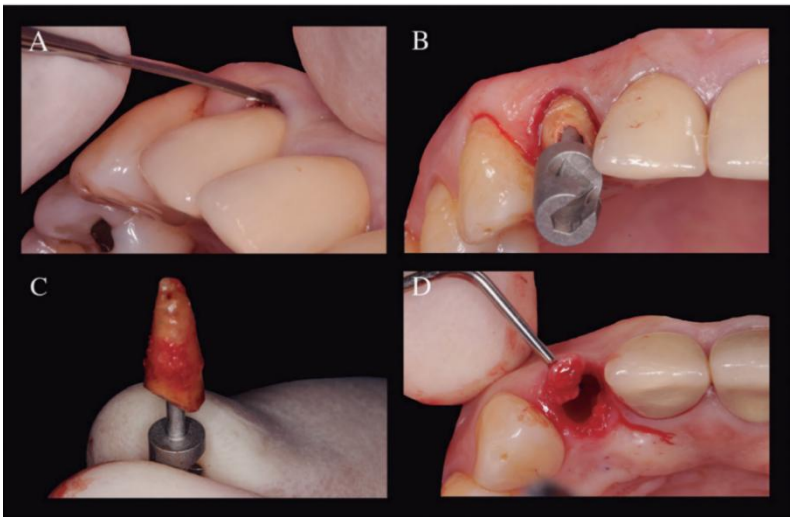

Figure 2. Tooth extraction and management of the alveolus.

Tooth extraction and management of the alveolus. (A) Syndesmotomy with periaptome. (B) Atraumatic vertical extraction of tooth [1, 2]. (C) Remaining root extracted. (D) Mechanical conditioning of the tooth alveolus with spoon and removal of periapical inflammatory tissue. (E) Removal of periapical inflammatory tissue.

In clinical practice, after tooth extraction, the alveoli remain intact and the gingival structure remains unchanged, but there is a small perforation in the vestibular plate related to apical injury at the root tip. Immediate juxtaosseous installation of a 3.3 × 12 mm Straumann Bone Level Tapered® implant (Basel, Switzerland) was performed, and subsequently the space between the vestibular bone table and the implant (gap) was filled with a xenograft of bovine origin (Geistlich Bio Oss®, Geistlich Pharma AG, Switzerland), which was carefully condensed (Figure 3). Finally, the entrance contour to the implant site was closed with an 8 mm porcine collagen matrix (Geistlich Mucograft® Seal, Geistlich Pharma AG, Switzerland), positioning its compact, thin and smooth layer in relation to the epithelium and its non-compact porous layer in relation to the implant site. The matrix was trimmed according to the dimensions of the alveolus, taking into account that it should be slightly larger, so that it would not be loose inside the alveolus, but with a slight retention and self-contained. To guarantee its position, a horizontal cross mattress stitch was sutured using Nylon 5-0 (Ethilon®, Johnson & Johnson de Chile S.A, Chile), without perforating the collagen matrix (Figure 4). Finally, to maintain the aesthetics of the anterior sector, a temporary Maryland type adhesive was installed.

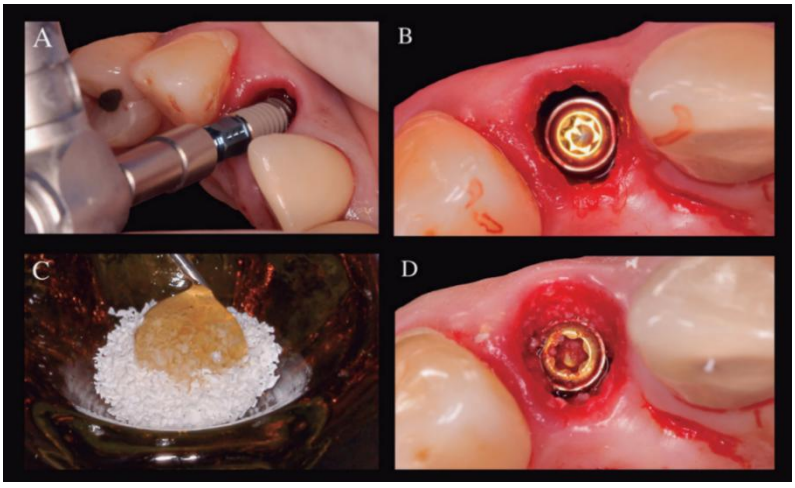

Figure 3. Implant installation and bone filling.

Implant installation and bone filling: (A) Immediate juxtaosseous installation of Straumann Bone Level Tapered® implant (Basel, Switzerland) 3.3 × 12 mm. (B) Undamaged socket with preserved gingival architecture and vestibular gap in relation to the implant. (C) Xenograft of bovine origin (Geistlich Bio Oss®, Geistlich Pharma AG, Switzerland) hydrated with autologous fibrin. (D) Vestibular gap with condensed xenograft.

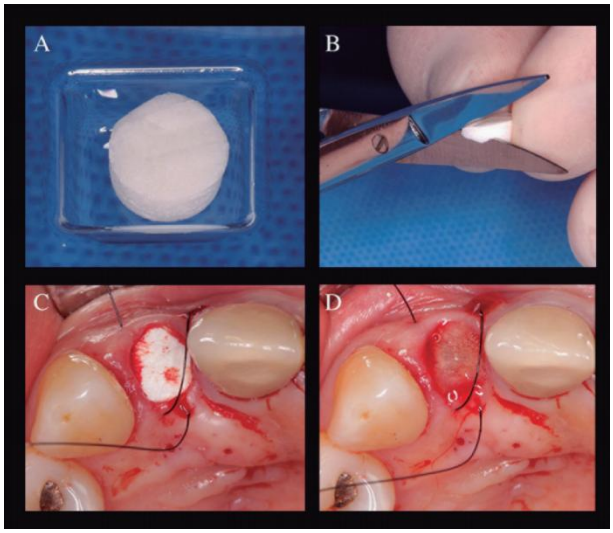

Figure 4. Sealing of the socket

Sealing of the socket: (A) 8 mm Geistlich Mucograft® Seal collagen matrix (Geistlich Pharma AG, Switzerland). (B) Matrix cut-out is shown, adapting to the contour of the implant site entrance. (C) Alveolus sealed with Geistlich Mucograft® Seal collagen matrix (Geistlich Pharma AG, Switzerland) with the compact, thin and smooth layer in relation to the epithelium and its non-compact porous layer in relation to the implant site. (D) Suture, horizontal mattress stitch crossed with Nylon 5-0 (Ethilon®, Johnson & Johnson de Chile S.A, Chile), without perforating the collagen matrix.

2.3 Clinical evaluation of regenerated tissue.

Soft tissue regeneration from the porcine collagen matrix placed in the alveolus was clinically evaluated using the Stony Brook Scar Evaluation Scale [7]. This scale describes 5 categories to be evaluated: 1) Width, 2) Height, 3) Color, 4) Suture marks and 5) Overall appearance. This scale was used for clinical evaluation during the controls at 1 week, 3 weeks, 5 weeks and 6 months after the placement of the porcine collagen matrix (Figure 5).

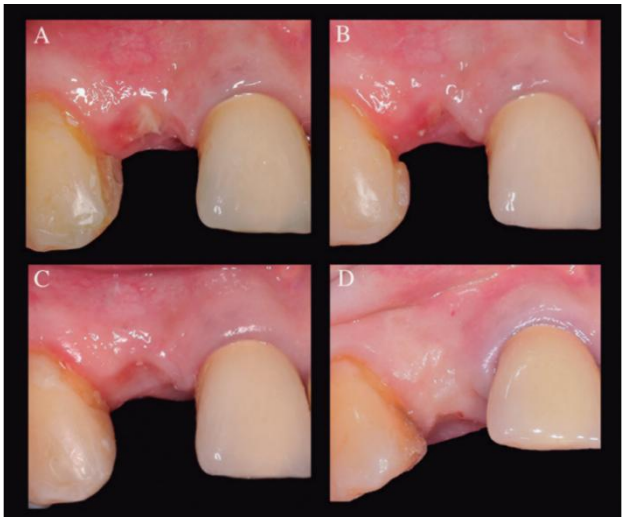

Figure 5. Clinical controls according to the Stony Brook Scar Evaluation Scale [7].

Clinical controls according to the Stony Brook Scar Evaluation Scale [7]. (A) 1st week control, central zone depression, reddening of the surgical site, suture marks and poor initial esthetic appearance. (B) 3rd week control, central zone depression, reddening of the surgical site, no suture marks and poor initial esthetic appearance. (C) 5th week control, a depression in the central zone, a slight reddening of the surgical site, no suture marks and a good initial esthetic appearance. (D) Control at 6 months, no depression in the central zone, no reddening of the surgical site, no suture marks and a good initial esthetic appearance.

2.4 Second surgical phase: connection surgery and biopsy.

Six months later, a connecting surgery was performed, and a linear incision was made on the spine using a miniature Buser elevator without touching adjacent nipples. A skin flap with a very small total thickness was lifted, revealing the sealing cover of the implant, and it was removed. A healing column with a height of 3.5 mm and a taper of 3.6 mm was placed using expansion techniques. After 2 minutes of expansion, the healing abutment was removed and replaced with a temporary Tam Straumann® Crown abutment (Basel, Switzerland) and the emergence profile was customized with Flow Resin according to the principles of Su H. et al. [6]. Simultaneously to the connection surgery, epithelial and connective tissue biopsies were performed on the mucosal tissue through a supracrestal incision parallel to the connecting incision, followed by histopathological studies of the sample. Before starting rehabilitation, a post alveolar control X-ray examination was performed to confirm the preservation of the alveolar bone, as the implant was in the position of the periosteal root crown. In addition, the images also confirmed the elimination of the apical process (Figure 6).

The final rehabilitation was carried out 2 months after the connection, consisting of fixed ceramic metal prostheses.

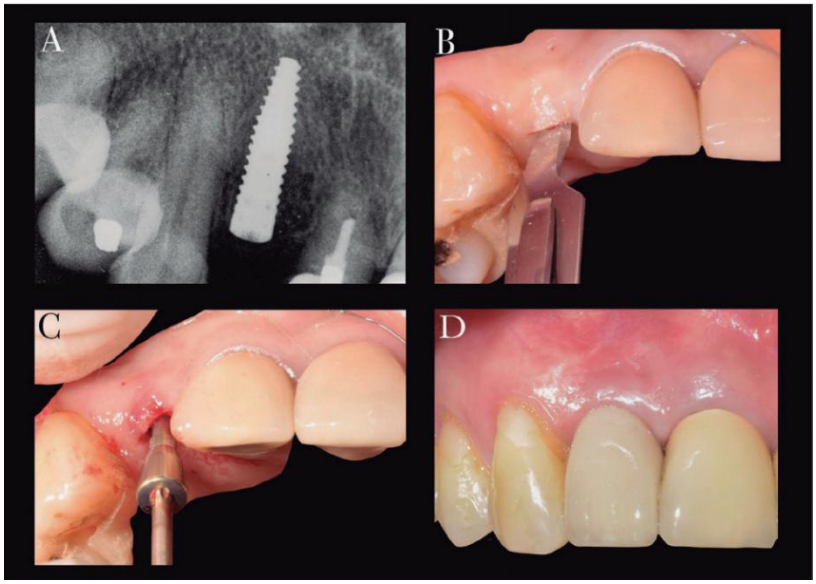

Figure 6. Second surgical phase.

Second surgical phase: (A) Control retroalveolar radiograph - implant presents a juxta-osseous apicocoronal position and absence of apical infectious process is observed. (B) Supracrestal linear incision without touching the adjacent papillae. (C) Expansion technique with a healing abutment 3.5 mm high and 3.6 mm taper. (D) A single metal ceramic prosthesis fixed on the implant in the area of the tooth was observed for final restoration [1, 2].

2.5 Histopathological study.

In the second surgical phase, a 2 × 3 mm deep biopsy was obtained. The sample was washed with physiological saline and fixed by immersion in 10% formalin pH7 for 48h at 4°C. The sample was then embedded in kerosene to obtain 5 µm thick serial histological sections. Subsequently, the sections were stained with routine Harris Hematoxylin/Eosin protocol and with Picrosirius red histochemical staining (Sirius red F3B or Rojodirecto 80, Aldrich) for the observation of Type I (yellowish orange to orange and red birefringence) and Type III (green or greenish-yellow birefringence) collagen fibers. Finally the images were acquired on a Zeiss Axio LAb A1 optical microscope, with the Canon EOS Rebel-T3 camera, EOS Utility software and in polarized light for Picrosirius red staining.

2.6 Clinical results.

The evolution of soft tissue regeneration from a porcine collagen matrix was gradual and the loss of the grafted matrix was not observed, with the consequent progressive migration of the epithelium over it. During the first weeks, particles of the xenograft (Geistlich Bio Oss®, Geistlich Pharma AG, Switzerland) were observed on the surface of the epithelium, which were removed.

According to the Stony Brook Scar Evaluation Scale [7], clinical findings ranged from a score of 0 for the 1st week control, where it was marked by a wound width greater than 2 mm, a depression in the central area, redness of the surgical site, suture marks and a poor initial esthetic appearance.

The 3rd week score was 2, marked by a wound width of less than 2 mm, a depression in the central area, a reddening of the surgical site, no suture marks and a poor initial esthetic appearance.

The 5th week score was 3, marked by a wound width of less than 2 mm, a depression in the central area, a slight reddening of the surgical site, no suture marks and a good appearance in the initial aesthetics.

At 6 months the score was 5, marked by a wound width of less than 2 mm, no depression in the central area, no redness of the surgical site, no suture marks and a good appearance in the initial aesthetics.

2.7 Histopathological results.

Biopsy of masticatory mucosa was performed at 10X H/E, and a normal parakeratinized stratified flat epithelium is observed (Figure 7A) with nuclear keratinocytes (Figure 7B). The epithelium has fine papillary projections into the connective tissue, which shows normal irregular fibrous connective tissue with fibroblasts (ovoid to fused cells) (Figure 7C) and blood vessels (Figure 7B). No inflammatory infiltrate is present.

Biopsy of masticatory mucosa was performed at 10X Picrosirius red (Figure 7D). In the lamina propria, papillary chorion (subepithelial) is identified, and the connective tissue is more loose and less dense. The main collagen fiber is type I with the presence of some areas of type III collagen (yellowish green) (Figure 7E). Towards the cut depth, in the reticular chorion, the collagen fibers are more organized compared to the papillary (surface) chorion. Again with high density of type I collagen fibers (yellowish red) and some scattered type III collagen aces (green) (Figure 7F).

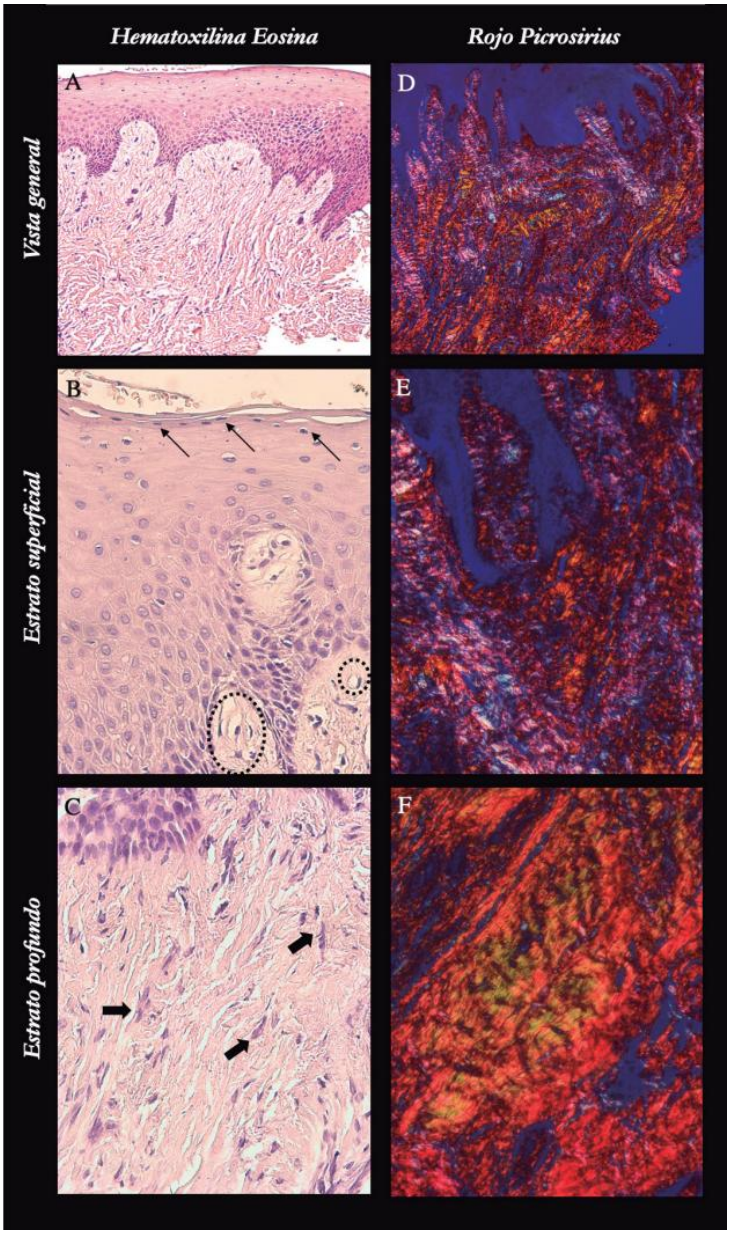

Figure 7. Histological analysis of masticatory mucosa grafted with Geistlich Mucograft® Seal (Geistlich Pharma AG, Switzerland).

Histological analysis of masticatory mucosa grafted with Geistlich Mucograft® Seal (Geistlich Pharma AG, Switzerland). (A) General view of mucosa, Hematoxylin/Eosin staining at 10X. (B) At 40X keratinocytes with nuclei (black arrow) characteristic of parakeratinized epithelium of the masticatory mucosa are observed, as well as the presence of blood vessels in the papillary chorion (dotted circle). (C) In the depth of the connective tissue it is observed how the fibroblasts invaded the collagen fibers of the graft. (D) General view of mucosa, Picrosirius red staining in phase contrast at 10X. The mucosal epithelium is at the top of the image and the subepithelial conjunctiva is composed mainly of type I collagen. (E) At 40X, in the papillary chorion, a mixture of mainly type I collagen fibers with orange-red hue and some type III fibers (green hue) is observed. The whitish portions correspond to the amorphous matrix composed mainly of non-fibrillar material. (F) In the reticular chorion, type I collagen fibers are more organized in aces, with the presence of type III collagen.

3. Discussion

Alveolar ridge preservation after tooth extraction has been reported to result in significantly less resorption both vertically and horizontally compared to natural healing of the alveolus [2, 8]. However, there is no clear scientific evidence for the superiority of either hard or soft tissue grafting procedures.

In 1994, Landsberg and Bichacho [9] used a gingival graft to close the socket post-extraction. Other authors [10] have described different modifications to the soft tissue grafting procedure to seal the socket, contain the bone filling material and protect the implant. In this regard, Cardaropoli et al. [11] described in a study of alveolar regeneration that the coronal portion of the post extraction socket had delayed healing, with a predominance of inflammatory infiltrate due to contamination from the oral environment and saliva, and that therefore regeneration in the apical and middle portion of the socket was faster than in the coronal portion.

The different soft tissue graft sealing techniques have different advantages according to the modification of the technique used, especially if connective tissue is tunneled in the vestibular area, since it changes the phenotype in this area. However, regardless of the modification used, all of them present a common disadvantage, which is the graft harvesting from a second surgical site, generally from the palate or the tuberosity.

Fickl et al. [12] in a retrospective case-control study reported that alveolar ridge preservation using a bovine xenograft and porcine collagen matrix to seal alveoli led to significantly lower scar tissue formation, costs, and treatment time for soft tissue regeneration compared with a bovine xenograft and a free gingival graft from the palate using the punch technique. However, there was no significant difference in the esthetic appearance of the 2 techniques.

In the present report, the clinical results of the use of a xenograft as a bone filler and a collagen matrix of porcine origin resulted in alveolar preservation, which was confirmed by radiographic control which showed the elimination of the pre-existing apical lesion and the maintenance of the bone level, since the implant maintained the initial juxta-osseous level of positioning. In addition to complete soft tissue regeneration, the clinical results ranged from a score of 0 for the first week of control, where it was marked by a wound width greater than 2 mm, a depression in the central area, redness of the surgical site, suture marks and a poor initial esthetic appearance, to a final score of 5 at 6 months, according to the Stony Brook Scar Evaluation Scale, with an optimal final esthetic appearance of the regenerated tissue.

Jung et al. [13] compared the preservation of alveolar bone in xenografts with the sealing of porcine derived collagen matrix and the sealing of soft tissue transplantation using puncture techniques. In both cases, they measured the bone parameters of alveolar bone after tooth extraction and at 6-month follow-up, and found no significant difference between the two techniques.

Another sealing option for alveolar preservation simultaneous to implant installation is described by Basualdo et al. [14] who described a modification to the regenerative alveolar preservation technique "ice cream cone", in which the collagen membrane was sutured by insertion vectors outside the vestibular table and an L-PRF membrane also tunneled with insertion vectors remained outside the collagen membrane, sealing and protecting the collagen membrane.

In the present work the histological sections show a mucosal tissue of normal characteristics, with the presence of a parakeratinized epithelium, blood vessels and absence of inflammatory infiltrate. In addition, the presence of type I and III collagenous fibers was confirmed. This is of special relevance, since it demonstrates histologically that the porcine collagen matrix was an adequate scaffold for epithelial migration and formation of an epithelium fully compatible with a normal masticatory mucosa. In the conjunctival thickness, invasion by fibroblasts from the patient is observed, suggesting conjunctival matrix synthesis by the latter. However, complementary studies are needed to evaluate the total or partial replacement of porcine collagen fibers by human collagen fibers.

The clinical results of the present case are in agreement with those of Sanz et al. [15], who showed in a clinical study of 20 patients that the use of a porcine collagen matrix increases the width of a band of keratinized tissue in a predictable manner.

4. Conclusion

The use of a collagen matrix of porcine origin to seal the alveolus and regenerate the soft tissue, allowed to appreciate at 6 months clinically a tissue with an optimal esthetic appearance and histologically showed the formation of an epithelial and connective tissue compatible with that of a normal mucosa.

Acknowledgments

Edgar Sandoval clinical photography. Histological photographs Prof. Dr. Maria Angélica Torres. Sample processing Prof. Cristian Peñafiel. Histological analysis Dr. Constanza Vera belonging to ANID Project PAI79160076, funded by Dr. Soledad Acuña-Mendoza.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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