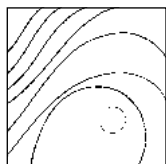


Soft Tissue Integration of a Porcine Collagen Membrane: An Experimental Study in Pigs



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Autogenous soft tissue augmentation procedures around natural teeth and dental implants are performed daily by clinicians. However, patient morbidity is often associated with the second surgical site; hence, research is moving toward an era where matrices may substitute autogenous grafts. The aim of this study was to assess the soft tissue response to a collagen matrix in an animal model. Nine pigs were included in this study. Each animal received four collagen matrices, two for each mandible. Three cohorts were included in the study: group A, where the matrix was applied as an onlay on a partial-thickness flap; group B, where the matrix was inserted under a partial-thickness flap; and group C, where the matrix was inserted in an inverted position under a full-thickness flap. Sacrifice occurred at 7, 15, and 30 days postoperatively for histologic assessment. The collagen matrix was seen in place for the first 2 weeks, and it was completely replaced by healthy connective tissue within 30 days in the inlay cohorts. No inflammatory adverse reactions were noticed in any specimen, resulting in optimal integration of the device. This study showed an optimal integration within 30 days postoperative of the placement of experimental collagen matrix in the soft tissues of an animal model. Its proven safety in this model provides an optimal starting point for further research projects considering its clinical applications. (Int J Periodontics Restorative Dent 2012;32:e34–e40.)

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Soft tissue augmentation with autogenous grafts is a widely used procedure in a variety of disciplines in dentistry.^{1–3} The most common surgical approach to increase the soft tissue volume involves the autogenous subepithelial connective tissue graft.^{4,5}

Autogenous grafting procedures present several disadvantages, mainly resulting from the harvesting procedure, which lead to increased patient morbidity.^{6,7} In addition, anatomical and individual limitations exist, which determine the quantity and quality of tissue that can be retrieved.⁸

Numerous alternative materials have been investigated to overcome the use of autogenous soft tissue. Materials primarily of allogenic origin have been developed and used in mucogingival surgery, starting in the late 1970s with freeze-dried skin allografts⁹ and moving toward allogenic dermal substitutes such as acellular dermal matrix graft (AlloDerm, LifeCell).¹⁰ The latter has been applied to increase keratinized tissue, for root coverage procedures, to deepen

the vestibular fornix, and to augment localized alveolar defects.¹¹⁻¹⁴

The advent of tissue engineering has recently introduced products based on isolated cells or cell substitutes, tissue-inducing substances (biologic mediators), and scaffolds of natural or synthetic origin.¹⁵ Materials such as human fibroblast-derived dermal substitute have been investigated in clinical trials in comparison with autogenous soft tissue to increase the width of keratinized tissue.^{16,17} A resorbable bilayer matrix made from type I/III porcine collagen without further cross-linking or chemical treatment (Mucograft prototype, Geistlich) has been investigated recently in patients to increase the height of keratinized tissue.¹⁸ The results reported the matrix prototype to be as effective as the traditional free connective tissue graft in gaining a band of keratinized tissue adjacent to prosthetic reconstructions. A degree of contraction of approximately 60% to 70% for both cohorts was also reported. A recent study by Wehrhan et al¹⁹ investigated the epithelialization, vascularization, scarring/fibrosis, and tissue integration of the same collagen matrix (Mucograft prototype) in a dermal pig model. The authors revealed this matrix as a plausible alternative to a full-thickness dermal replacement substitute because of an identical epithelialization, vascularization, and degradation as autogenous dermal grafts.

A recent review²⁰ systematically assessed the literature reporting on different graft materials used to increase tissue width and volume and

concluded that no ideal material is currently available. The aim of this study was to assess the soft tissue response to a porcine collagen matrix (Mucograft) in an animal model.

Method and materials

The study protocol was approved and conducted in accordance with both policies and principles of laboratory animal care and the European Union guidelines (86/609/EEC) approved by the Italian Ministry of Health (law 116/92).

Nine young pigs (8 to 10 months old) were included in this study. The anatomy and physiology of the cutaneous blood supply and the wound-healing characteristics have made the pig a standard model for plastic surgery and wound-healing studies.^{21,22} Each animal received four collagen matrices, two for each mandible. Three cohorts were included in the study: group A, where the collagen matrix was applied as an onlay on a partial-thickness flap; group B, where collagen matrix was inserted under a partial-thickness flap; and group C, where the collagen matrix was inserted in an inverted position under a full-thickness flap. In addition, a negative control was included in the study protocol. Areas adjacent to the allocated cohorts were left untreated and evaluated as controls.

A total of 36 defect sites were equally allocated to groups A, B, and C according to a predetermined rotating scheme. Three animals were sacrificed at 7 days postoperatively, three at 15 days, and the last three

animals at 30 days postoperatively, to have an early, intermediate, and late healing phase according to the animal model.

Surgical procedure

A soft tissue defect was artificially created by removing 3 mm of existing keratinized tissue in the buccal portion of the animals' mandibles. One month after the defect was created, the main surgery was performed.

Group A (12 sites)

After achieving general and local anesthesia, a buccal partial-thickness flap was performed extending 15 mm in the mesiodistal direction and 10 mm in the apicocoronal direction to create an adequate recipient site. The flap epithelium was repositioned apically and sutured to the apical underlying periosteum by means of resorbable sutures. The matrix (Mucograft) was secured to the recipient site by means of resorbable 5/0 sutures (Vicryl, Ethicon). Prior to this, the device was shaped according to the exact dimensions of the recipient site (15 × 10 mm) (Fig 1).

Group B (12 sites)

A partial-thickness trapezoidal flap was designed. The collagen matrix was adapted and sutured at the underlying periosteum by means of resorbable 5/0 sutures (Vicryl). The overlying flap was then repositioned to its original position covering the collagen matrix completely and sutured to the



Fig 1 (left) Clinical image of a group A site where the collagen matrix was sutured on a partial-thickness recipient bed and left exposed to the oral cavity. (right) Group B site where the collagen matrix was positioned and completely covered by the overlying flap. Group C specimens appeared clinically identical to those of group B.



Fig 2 Clinical image of two healing sites at 7 days postoperative. (left) Submerged healing result; (right) open healing area (group A).



Fig 3 Clinical appearance at 30 days postoperative. Sites treated with the collagen matrix exposed to the oral cavity (group A, right) seemed closer in appearance to the submerged groups.

alveolar crest with nonresorbable 5/0 sutures (PROLENE, Ethicon).

Group C (12 sites)

A full-thickness trapezoidal flap was performed. The buccal bone was exposed, and the adapted collagen matrix was applied in an inverted manner, ie, with its smooth, cell-occlusive layer in direct contact with the buccal bone. The overlying flap was then sutured back in its original position and secured by means of nonresorbable 5/0 sutures (Prolene).

Antimicrobial prophylaxis consisted of spiramycin (750,000 IU) and metronidazole 125 mg (1 tablet/10 kg per day) beginning at least 5 days before and continuing for at least 14 days after surgery. Sutures were removed after a healing period of 15 days. Following healing periods of 7, 15, and 30 days, clinical measurements of the buccal oral tissues were taken prior to animal sacrifice.

Histologic processing

The hemimandibles were removed en bloc and fixated by immersion in 4% buffered formaldehyde. Dehydration of the specimens was accomplished by increasing ethanol concentrations using a dehydration system with agitation and vacuum. The samples were embedded in Technovit 7200 VLC resin (Heraeus Kulzer) and sliced longitudinally on an Exakt cutting unit (Exakt). The slices were reduced by microgrinding and polishing using an Exakt grinding unit to an even thickness of 30 to 40 μm . These were stained with toluidine blue/pyronin G and examined using a Leica 6000DRB light microscope.

Results

Clinical observations

Healing proceeded uneventfully for all 36 surgical sites. The clinical appearance at 7 days postoperatively differed significantly between the submerged cohorts (groups B and C) and group A, where the device was left exposed to the oral cavity. The submerged groups showed a partially complete healing process compared to the cohort where the device was left uncovered, which indicated red hyperemic tissue (Fig 2).

The animals that were sacrificed at 15 days resulted in a similar clinical appearance, with the tissues appearing more mature in their healing phases. At 30 days postoperative, the three groups showed almost complete clinical healing (Fig 3).

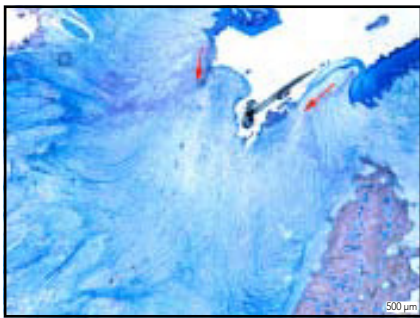


Fig 4a Group A specimen at 7 days. The arrows indicate the surgically treated area, also showing the interrupted keratinized epithelium.

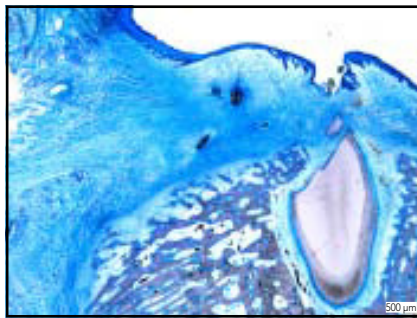


Fig 4b Group A specimen at 15 days showing partial closure of the surgical site by the in-growth of keratinized epithelium. No matrix could be detected.

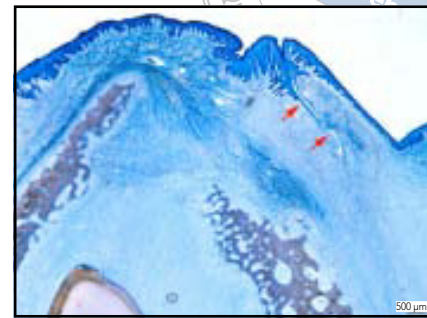


Fig 4c Group A specimen at 30 days showing complete healing of the treated area with the presence of keratinized epithelium covering the entire wound site (arrows).

Histologic observations

Group A

Samples retrieved at 7 days postoperative showed an interruption within the keratinized mucosa, demonstrating discontinuity of the oral epithelium. The interruption corresponded to the area of interest where the matrix was positioned; however, the matrix could not be clearly detected. A mild inflammatory reaction could be visualized in the connective tissue area.

The wound-healing process could be visualized already after 7 days in that the epithelium was seen to grow from the wound margin toward the grafted area, following an ongoing gradient of keratinization (Fig 4a).

After 2 weeks, the wound area appeared nearly completed by the growth of the keratinized epithelium. No collagen matrix could be detected at 15 days postoperative (Fig 4b). By 30 days postoperative, the entire wound area was healed

with keratinized epithelium, with a mild inflammatory reaction in the area of interest, which can be considered part of the normal wound-healing pattern. No collagen matrix was present (Fig 4c).

Group B

The overview section of the sample retrieved at 7 days postoperative clearly showed the collagen matrix in place (Fig 5a). The collagen matrix was perfectly visible in its entire length, surrounded by healthy connective tissue. The two layers of the matrix could be distinguished, with its compact portion and its porous structure. This could be easily detected in the high-magnification view (Fig 5b). The inflammatory infiltrate appeared more pronounced in close contact to the porous portion of the collagen matrix, whereas the rest of the connective tissue surrounding the membrane appeared healthy. Two weeks postoperatively, most of the collagen matrix was still present. Low magnification showed

an intact epithelium and the presence of remnants of suture material (Fig 5c). The 30-day specimens demonstrated complete collagen matrix resorption, and the histologic sections revealed healthy strands of collagen fibers (Fig 5d).

Group C

The 7-day postoperative specimens showed healed tissues with an intact keratinization of the oral epithelium and the collagen matrix in place. Suture remnants were clearly visible (Fig 6a). The samples retrieved after 2 weeks showed remnants of the collagen matrix in place with the most coronal portion resorbed. An inflammatory reaction was detected around the sutures (Fig 6b). Specimens examined at 30 days postoperative showed completely healed connective and epithelial tissue. A small portion of the collagen matrix could still be detected in its most apical portion. No inflammatory infiltrate could be seen (Fig 6c).

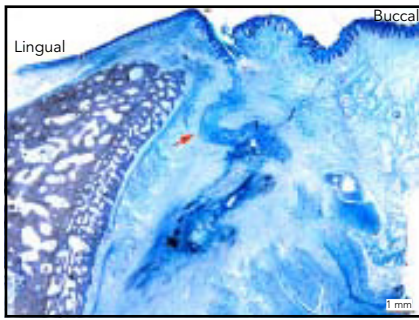


Fig 5a (left) Group B specimen at 7 days showing the complete integrity of the collagen matrix *in situ* (arrow). The surrounding connective tissue appears healthy.

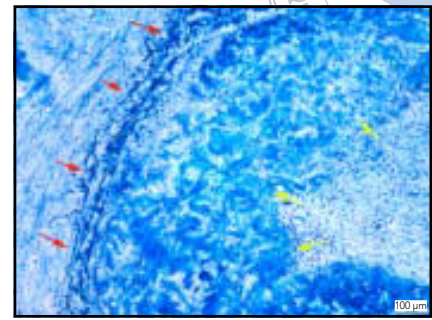


Fig 5b (right) High-magnification view of Fig 5a. Some pronounced inflammatory reaction was present contiguous to the porous layer of the matrix (yellow arrow). Red arrows = matrix.

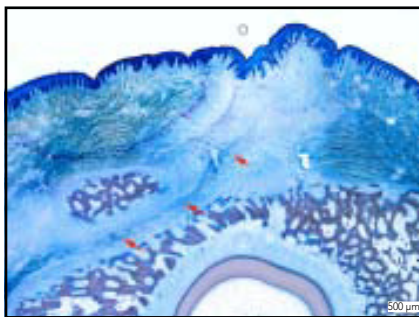


Fig 5c (left) Group B specimen at 15 days. The matrix was clearly visible at this time point. The matrix (arrows) was completely resorbed in its coronal portion under an intact epithelium, and sutures could be seen in the wound area.

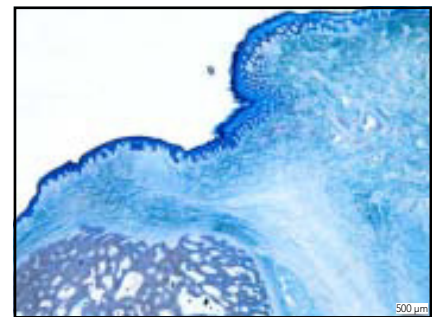


Fig 5d (right) Group B specimen at 30 days revealing healthy connective tissue and intact keratinized epithelium, with no remnants of the collagen matrix present.

Untreated group

Figure 7 shows the soft tissue specimens where no treatment was applied. The integrity of the oral epithelium was evident. The underlying connective tissue appeared healthy and free from any inflammatory reaction.

Discussion

Soft tissue augmentation procedures are currently performed by clinicians to augment the width of keratinized tissue or to increase the soft tissue volume. However, a limited number of studies provide data on soft tissue volume augmentation. The aim of this study was to observe the soft

tissue response using a resorbable porcine collagen type I/III matrix (Mucograft). The performance was evaluated by macroscopic and microscopic analysis after 7, 15, and 30 days of implantation.

Three cohorts were included in the study according to the surgical technique applied. An untreated specimen was included to examine the physiologic appearance of the soft tissues in the same animal model. This was not considered a control site. The goal of this study was primarily to establish the soft tissue integration and reaction of a collagen matrix to the soft tissues of the animal model. Hence, the authors considered it of interest to observe a healthy, untreated model.

Clinical results showed no macroscopic differences between the specimens treated with the collagen matrix under a partial- or full-thickness flap. The submerged techniques healed uneventfully. The open wound areas healed differently both clinically and histologically. The clinical appearance was similar to the traditional surgical treatment performed using a vestibuloplasty procedure.²³

The histologic sections of the open-wound specimens showed impaired healing for the first 2 weeks with absence of the collagen matrix. It is unknown if this result is because of a premature loss of the collagen matrix positioned and exposed to the oral cavity or rapid resorption of

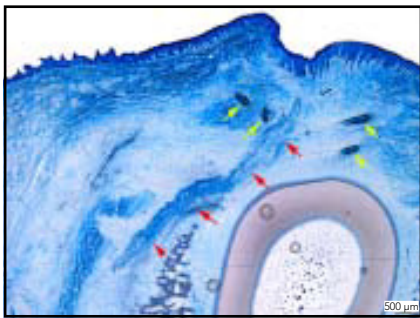


Fig 6a (left) Group C specimen at 7 days. The matrix was in place surrounded by the host connective tissue, and an intact keratinized oral epithelium was present (red arrows). Sutures could be detected (yellow arrows).

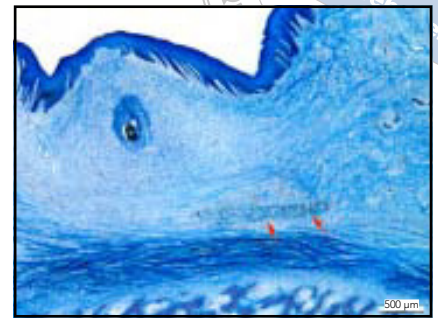


Fig 6b (right) Group C specimen at 15 days showing remnants of the collagen matrix in place (arrows). The coronal portion was resorbed. Note the inflammatory infiltrate surrounding the suture.

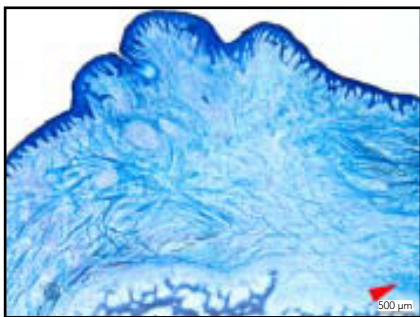


Fig 6c (left) Group C specimen retrieved at 30 days postoperative showing complete healing of the soft tissues. A small apical portion of the collagen matrix was detected (arrowhead).

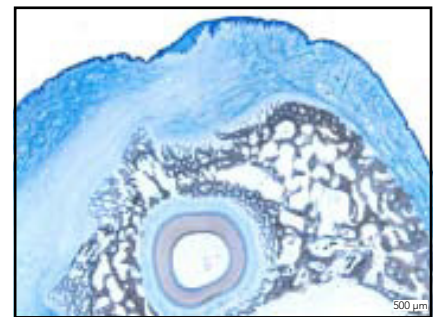


Fig 7 (right) Untreated area in a pig mandible. The integrity of the oral epithelium was evident as well as the healthy strands of connective tissue, with absence of inflammatory reaction.

the matrix itself to allow fast reepithelialization of the oral mucosa. This observation is in accordance with the study conducted by Wehrhan et al¹⁹ on pigs where the same collagen matrix was applied to a full-thickness dermal defect on the rear side of pigs' ears. The authors revealed collagen matrix degradation after 7 days. Their results showed that the matrix was clearly detectable at the early wound phases (from day 1 to day 7). On the 7th postoperative day, loss of the membrane stratification was observed, and by day 14, no differences in structure, orientation, fiber thickness, or architecture between the grafted collagen matrix and the graft bed could be detected. It must be noted, however, that the study

by Wehrhan et al¹⁹ was performed in a different model, ie, in a dermal full-thickness defect where the collagen device was in direct contact with nonvascularized cartilage, whereas this study was conducted with the same collagen device placed in direct contact with highly vascularized bone periosteum.

The two submerged techniques provided similar results both clinically and histologically. No difference was noticed with the matrix orientated in the correct position or inverted. The collagen matrix was seen in place for the first 2 weeks, and it was completely replaced by healthy connective tissue within 30 days. No inflammatory adverse reactions

were noticed in any specimen, resulting in optimal integration of the matrix. One month postoperatively, the histologic specimens of the two submerged techniques showed a strong similarity with the histologic specimens of the untreated sites, ie, the physiologic configuration of the animal model.

Conclusion

To the best of the authors' knowledge, there are no histologic published data using an analogous collagen matrix in an oral soft tissue environment. This study showed optimal integration within 30 days postoperative of the experimental

collagen matrix in the soft tissues of an animal model. The proven safety of this model provides an optimal starting point for further animal and clinical research projects considering its potential clinical application.

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