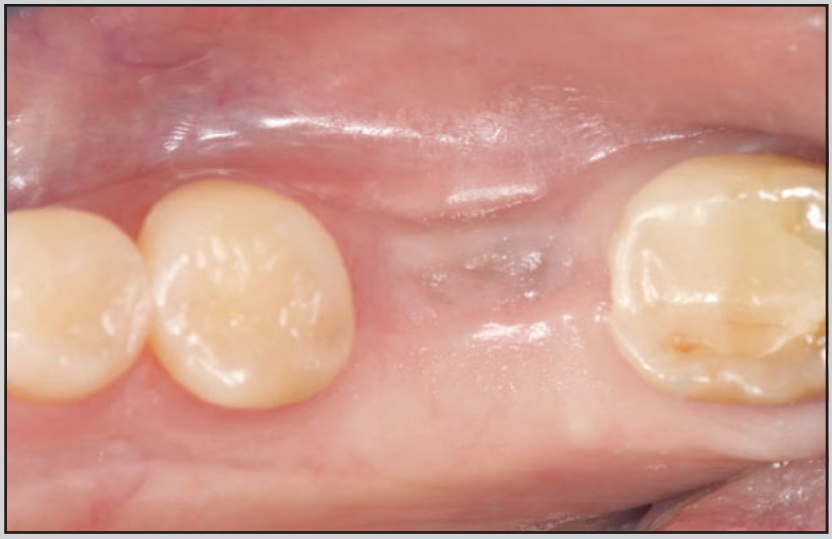
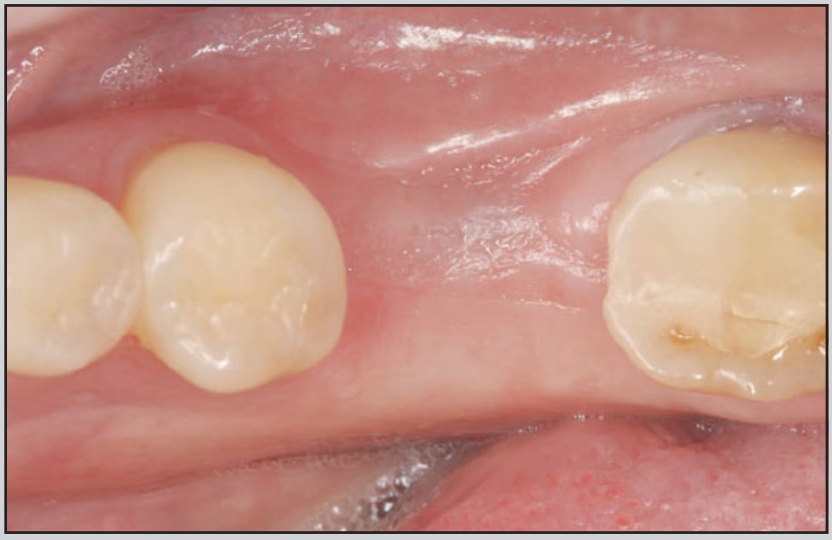
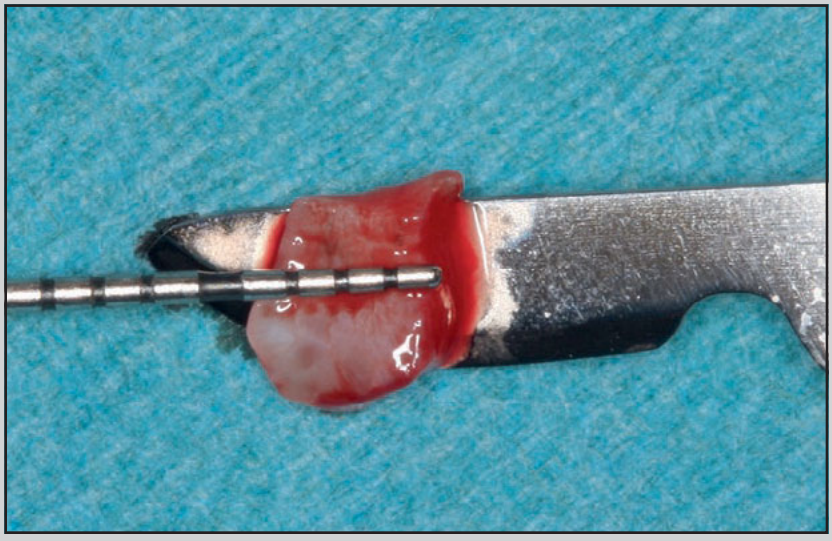
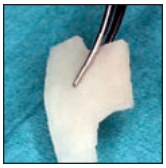






Clinical and Histologic Evaluations of Porcine-Derived Collagen Matrix Membrane Used for Vertical Soft Tissue Augmentation: A Case Series



Algirdas Puisys, DDS, Spec Perio, PhD[1]
Saulius Zukauskas, DDS[2]*/Ricardas Kubilius, DDS, PhD*[3]
Mike Barbeck, PhD[4]*/Dainius Razukevičius, DDS, PhD*[5]
Laura Linkevičiene, DDS, PhD[6]
Tomas Linkevičius, DDS, Dip Pros, PhD[7]

This case series aimed to clinically and histologically evaluate porcine-derived membrane used for vertical thickening of thin soft tissues. Twenty porcine-derived collagen membranes and bone-level implants were placed in 20 patients. After 2 months, thickened soft tissues were measured and biopsy samples were harvested. All xenografts healed successfully. The average thickness of thin soft tissue before vertical thickening was 1.65 ± 0.36 mm, while tissue thickness increased to 3.45 ± 0.52 mm after the procedure (P < .001); the mean thickness increase was 1.8 ± 0.13 mm. Histologic analysis showed complete integration of the graft and no differences (P = .4578) in vascularization between the host (39.74 ± 17.15 vessels/mm²) and graft (30.43 ± 11.26 vessels/mm²). It can be concluded that porcine-derived membrane can be used for vertical soft tissue thickening with substantial gain in tissue height.
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[1]Vilnius Research Group, Vilnius, Lithuania; Vilnius Implantology Center, Vilnius, Lithuania.
[2]Institute of Odontology, Faculty of Medicine, Vilnius University, Vilnius, Lithuania.
[3]Department of Oral and Maxillofacial Surgery, Lithuanian University of Health Sciences, Kaunas, Lithuania.
[4]Department of Oral and Maxillofacial Surgery, Laboratory for Regenerative Orofacial Medicine, University Hospital Hamburg-Eppendorf, Hamburg, Germany.
[5]Oral and Maxillofacial Surgeon, Hospital of Lithuanian University of Health Sciences, Kaunas, Lithuania.
[6]Institute of Odontology, Faculty of Medicine, Vilnius University, Vilnius, Lithuania.
[7]Institute of Odontology, Faculty of Medicine, Vilnius University; Vilnius Mokslo Grupe; Vilnius Implantology Center, Vilnius, Lithuania.

Correspondence to: Dr Tomas Linkevičius, Institute of Odontology, Faculty of Medicine, Vilnius University, Zalgirio str. 115/117, LT- 08217, Vilnius, Lithuania.
Email: linktomo@gmail.com

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It was proved that vertically thin soft tissues might be an important factor in the development of early crestal bone loss,[1,2] therefore several methods were suggested on how to increase peri-implant tissue thickness. Wiesner et al[3] were the first to suggest a clinical procedure for vertical soft tissue thickening, using autogenous tissues from the palate. However, autologous graft preparation leads to an increased patient morbidity[4] and ongoing pain.[5] Keeping feasible anatomical limitation in mind,[6] the situation calls for an alternative way to thicken soft tissue. A different procedure was developed by Puisys and Linkevičius, using allograft materials to change tissue dimensions.[7] However, allografts encounter inevitable drawbacks,[8] donation from another human being a major disadvantage. In this light, porcine-derived xenografts could be a viable alternative. However, there is no sufficient clinical or histologic data regarding how thin mucosal tissues would react to a porcine-derived collagen matrix membrane.

Therefore, the purpose of this study was threefold: *(1)* to evaluate clinical integration of porcine collagen matrix in vertical soft tissue thickening; *(2)* to measure the increase of vertical soft tissue thickness after the thickening procedure; and *(3)* to provide human histologic analyses of the xenograft.

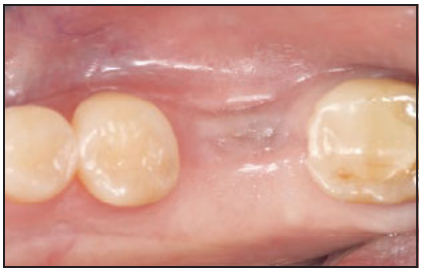


Fig 1 *Initial situation before grafting.*

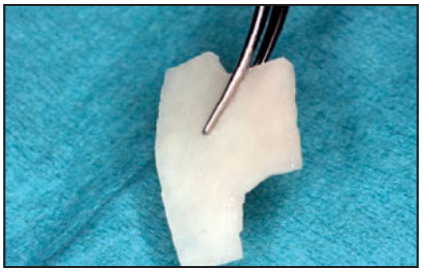


Fig 2 *Porcine-derived collagen matrix membrane, rehydrated and with V-shaped form.*

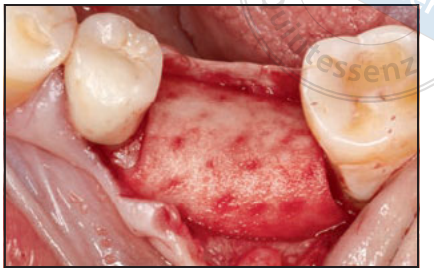


Fig 3 *Porcine-derived membrane buccolingually and crestally positioned on the alveolar ridge.*

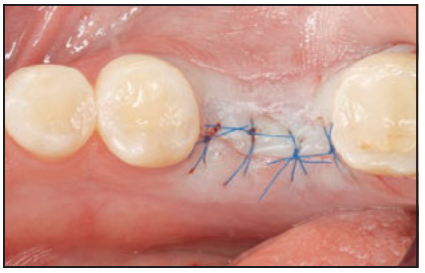


Fig 4 (left) *Released flaps with tension-free sutures.*

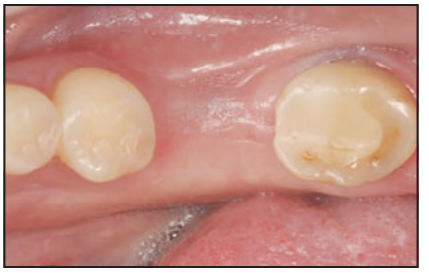


Fig 5 (right) *Membrane implantation site after 2 months of healing.*

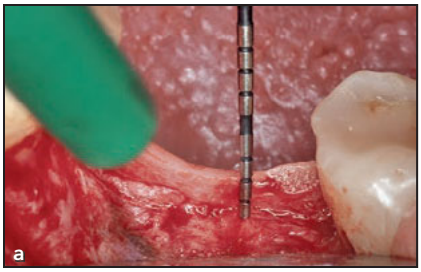
Materials and Methods

Patients

Partially edentulous patients requiring implant treatment were recruited for this study in Vilnius Implantology Center Clinic, Vilnius, Lithuania. The protocol was approved by Vilnius Regional Biomedical Research Ethics Committee (No. 158200-07-512-149). Patients were enrolled if they fulfilled the following inclusion criteria: thin mucosal tissues in vertical dimension (2 mm or less); missing teeth in posterior mandibular area; minimum bone width of 6 mm; healthy soft tissue; minimum of 4 mm of buccolingual keratinized gingiva; no bone augmentation procedures before/during implant placement; periodontally healthy; finally, provided consent for biopsy-sample harvesting procedure.

Soft Tissue Thickness: Measurement and Thickening

Surgery was performed under local anesthesia (40-mL solution of 4% articaine with adrenaline; Ubistesin, 3M ESPE). A crestal incision was performed in the edentulous ridge, a full-thickness buccal flap was raised, and the vertical soft tissue thickness was measured with a 1.0-mm marked periodontal probe (UNC, Hu-Friedy). If the vertical soft tissue thickness was 2 mm or less, the patient was included in the study (Fig 1). Bone-level platform-switched implants (Bone Level Tapered Implant, Straumann) were placed epicrestally. Flap releasing was performed to achieve passive mobility of the soft tissues. Porcine collagen matrix (mucoderm, botiss biomaterials) was used for vertical soft tissue thickening. A 2-mm–thick membrane with standard dimensions (15 × 20 mm) was immersed into sterile saline solution for 20 minutes to rehydrate (Fig 2). The tissue substitute was individually shaped to fit the implant site, avoiding neighboring teeth, and positioned on top of the newly placed implant. Membrane was extended buccally for about 10 mm and lingually for 5 mm beyond the implant margin to completely cover the implant site and achieve better stability (Fig 3). After positioning, flaps were approximated and sutured without tension with 6/0 sutures (Prolene, Ethicon), using a simple double suture technique (Fig 4). Patients were instructed to rinse the operated site for 1 minute with 0.12% chlorhexidine-digluconate solution (Perio-Aid, Dentaid) twice a day for 1 week. Sutures were removed 10 days after surgery. After 2 months of healing, the augmented area was inspected



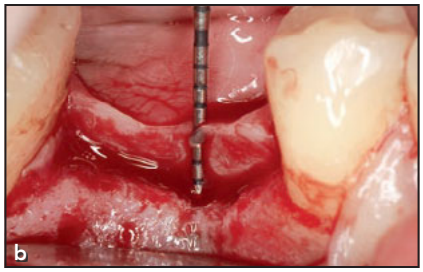


Fig 6 (a) *Initial vertical soft tissue thickness compared to* (b) *increased thickness measured at second-stage surgery.*

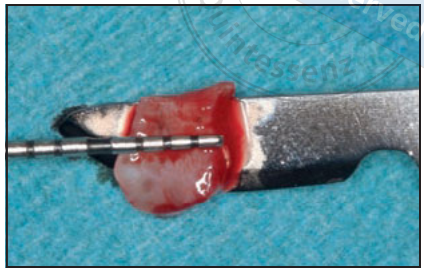


Fig 7 *Full-thickness soft tissue biopsy sample with dimensions of 3 × 1 × 4 mm.*

for mobility, graft integration, and tissue color and consistency. If there were no signs of inflammation and the operated site had the appearance of healthy immobile soft tissues, the patient was scheduled for second-stage surgery and biopsy-sample harvesting (Fig 5).

Biopsy Harvesting and Healing Abutment Connection

After infiltration of local anesthetic (40-mL solution of 4% articaine with adrenaline; Ubistesin, 3M ESPE), an incision was made in the center of the bone crest. A full-thickness buccal flap was raised, and the thickness of the augmented soft tissue over the implant was measured with a periodontal probe in a previously described manner (Fig 6). Then, full-thickness soft tissue biopsy samples (3 × 1 × 4 mm) were harvested from peri-implant tissues directly over the implant in the bone (Fig 7) using a specially designed splint for standardization of the procedure. Biopsy samples were immediately placed in a bottle of 4% buffered formalin for a minimum of 24 hours and sent to the laboratory in a sealed container for histologic analysis.

Healing abutment screw was covered with 0.12% chlorhexidine-digluconate gel (Perio-Aid Gel, Dentaid) and connected to the implant. The excess of gel was carefully washed off with sterile saline solution. Flaps were sutured with single interrupted 6/0 sutures without tension (Prolene, Ethicon). The sutures were removed 7 days after surgery.

Histologic Analysis

The histomorphometric and histologic analyses and workup were conducted at the Julius Wolff Institute of Charité – Universitätsmedizin, Berlin, using previously documented methods.[9,10] The histologic workup was performed via an increasing series of alcohol, xylol, and paraffin baths using a benchtop tissue processor (TP1020, Leica Biosystems). Subsequently, the tissue samples were embedded in paraffin via an embedding station (EG1150 Tissue Embedding Center, Leica Biosystems) and consecutively cut in 3- to 4-µm–thick paraffin slices using a rotation microtome (CUT 5062, SLEE Medical).

Slides were stained with hematoxylin-eosin (h&e), Sirius Red, and Masson Goldner. For the detection of blood vessels within the matrix and within the surrounding connective tissue, immunohistochemical staining by means of a monoclonal mouse anti-human CD31 antibody (Dako Clone JC70A, Agilent) was performed. For visualization of the antibody, the Dako REAL EnVision detection system (Agilent) was used.

Histopathologic and Histomorphometric Analysis

The analysis focused on the following parameters: fibrosis, hemorrhage, necrosis, vascularization, and presence of granulocytes, lymphocytes, plasma cells, monocytes/macrophages, and biomaterial-associated multinucleated giant cells (BMGCs). A light microscope (Eclipse 80i, Nikon) was used for the histopathologic analysis. Furthermore, microphotographs were taken by means of an Axiocam 105 color digital camera (Zeiss) connected to a computer running the ZEN 2 software, blue edition.

For histomorphometric analysis of vascularization, the slides were stained by the CD31 antibody. Every tissue sample was digitized

Table 1 Gingival Thickness Before and After Treatment with Statistical Difference

	Before treatment	After treatment	Difference*
N	20	20	20
Mean ± SD (SE)	1.65 ± 0.366 (0.082)	3.45 ± 0.510 (0.114)	1.80 ± 0.340 (0.076)
Median (min–max)	1.5 (1–2)	3 (3–4)	2 (1–2.5)
P	< .001		

SD = standard deviation; SE = standard error.
*Wilcoxon signed rank sum test, significant, when $P \leq .05$.

to generate "total scans" as a basis for the histomorphometric measurements. Therefore, a special scanning microscope consisting of an Axio Scope.A1 microscope (ZEISS) combined with an Axiocam 305 color digital camera (ZEISS) and an automatic scanning table (Märzhäuser Wetzlar) connected to a PC system running the ZEN core software (ZEISS) was used. Afterwards, the ZEN core was applied to measure both vascularization parameters (the vessel number and area) within the implantation area of the collagen-based matrix and within the surrounding connective tissue. Additionally, the total areas of the implant beds and the connective tissue were also determined. Based on this data, the number of vessels per square millimeter (vessels/mm^2) and the percent vascularization were calculated within both aforementioned zones.

Statistical Analysis

Data were analyzed using Prism 6.0c statistical software (GraphPad). Descriptive statistics for clinical measurements were calculated for the measurements as means, standard deviations, medians, and ranges of the measurements. Each patient was treated as a statistical unit. The normality of the distribution was tested with Shapiro-Wilk test and appeared to be nonparametric ($P < .001$). Wilcoxon rank-sum test was applied to find differences between the thickness of augmented and nonaugmented tissues. Distribution of the data from the histologic analysis was normal, and therefore the statistical analysis of the different tissue fractions was made by an unpaired *t* test. The mean differences were considered statistically significant at $P \leq .05$ with a confidence interval of 95%.

Results

Twenty patients, consisting of 15 males and 5 females with ages ranging from 21 to 53 years and averaging 42.5 ± 1.7 at the beginning of the experiment, were included in the study. Twenty porcine-derived collagen matrix membranes (mucoderm, botiss biomaterials) were placed. At 2 months after placement, all xenografts showed clinical signs of complete healing. Thin soft tissue before augmentation had an average thickness of 1.65 ± 0.36 mm (range: 1.0 mm to 2.0 mm), and after soft tissue augmentation with porcine xenograft, the average thickness increased to 3.45 ± 0.52 mm (range: 3.0 mm to 4.0 mm). This difference was found to be statistically significant ($P < .001$). The mean increase of soft tissue thickness was 1.8 ± 0.13 mm (range: 1.0 to 2.5 mm) (Table 1).

Histologic Results

The histologic analysis showed that the collagen matrix was completely integrated within the subgingival tissue in all analyzed cases without any severe inflammatory tissue responses. Analysis results are shown in Fig 8. In most cases, only slight microscopic differences were observed between the implantation area of the collagen matrix and the surrounding connective tissue. Thus, the distribution of the tissue components was comparable in both regions: the implant areas of the collagen matrix and the surrounding connective tissue. Besides high proportions of extracellular matrix components and mainly collagen fibers with only single associated cells, cell- and vessel-rich islands were observed in both regions. The remaining collagen fibers of the matrix showed a comparable appearance as the ingrown collagen fibers of the connective tissue (Figs 8b and 8c). In addition, the analysis of the cellular tissue reactions to the implanted collagen

matrix showed that low amounts of only mononuclear cells (such as macrophages and fibroblasts) were adherent to the matrix fibers; this further indicates the integration of the collagen matrix, as the same cell types and cellular distribution were also found within the surrounding connective tissue (Figs 8b and 8c). In the areas of the cell- and vessel-rich islands, mainly fibroblasts and macrophages were detected without histologic signs of severe inflammatory tissue reactions, indicating the further conversion of the collagen matrix into the patient's own connective tissue (Fig 8b). Moreover, no multinucleated giant cells were detected in the analyzed biopsy samples.

The analysis of the vascularization showed similar amounts of vessels within the implantation beds of the matrices and within the surrounding connective tissue (Fig 9). Additionally, the observations showed no visible differences in the vessel sizes between the implant area of the matrices and the surrounding connective tissue (Figs 9b and 9c).

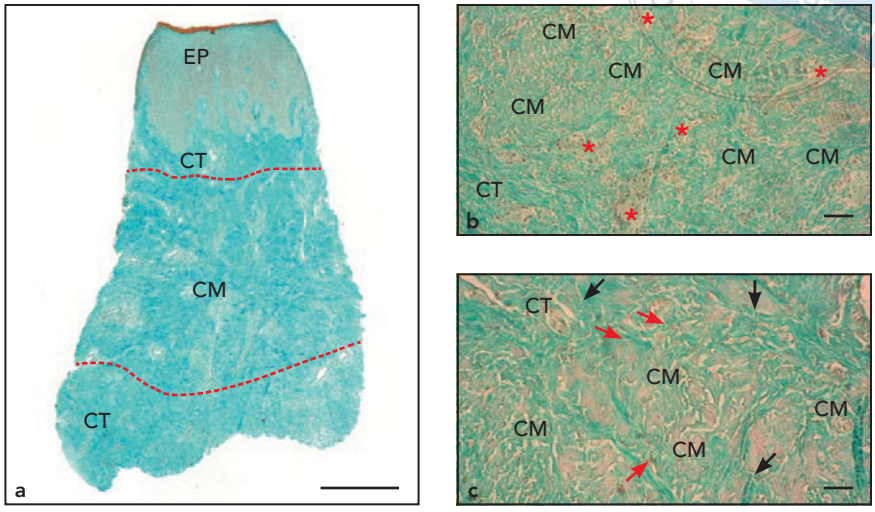


Fig 8 *Representative histologic images of the tissue reaction to the collagen-based matrix (CM) and its integration within the subgingival connective tissue (CT).* (a) *Overview of one side of the implanted CM* (red dashed lines) *that was completely integrated within the CT (EP = epithelium; Masson-Goldner stain, "total scan," ×5 magnification, scale bar = 500 µm).* (b) *The collagen fibers of the CM were directly neighboring CT. No severe inflammatory tissue responses were observed, and only some tissue islands* (red asterisks) *indicated further integration process (Masson-Goldner stain, ×10 magnification, scale bar = 100 µm).* (c) *Only mononuclear cells such as fibroblasts* (black arrows) *and single macrophages* (red arrows) *were involved in the tissue reactions to the fibers of the implanted CM, indicating good integration behavior (Masson-Goldner stain, ×20 magnification, scale bar = 100 µm).*

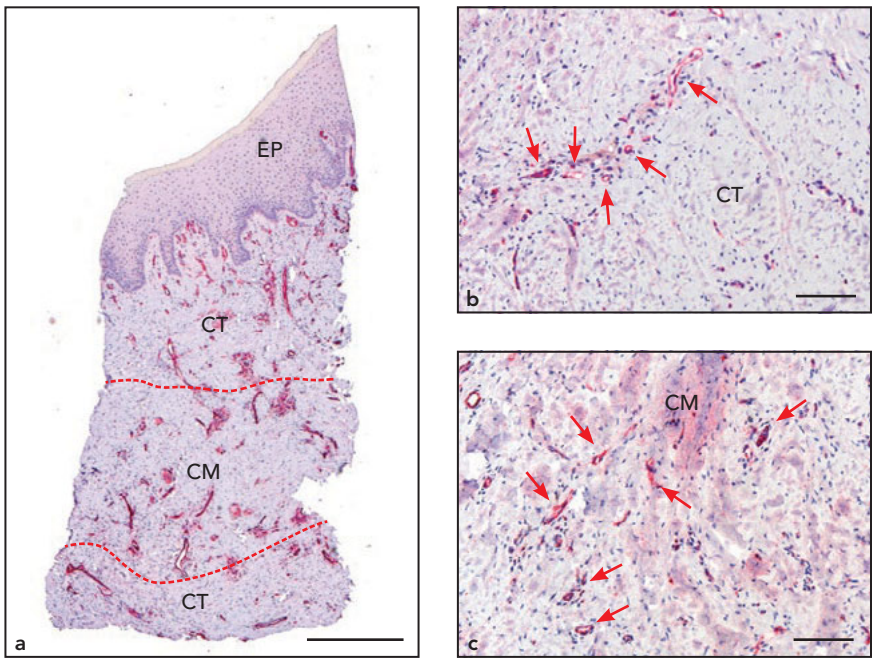
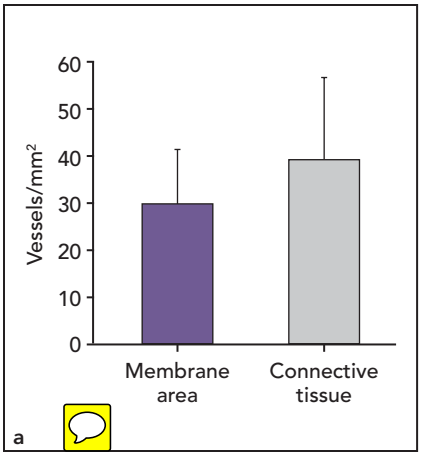


Fig 9 *Vascularization pattern of the analyzed biopsy samples.* (a) *Overview of one side of the collagen-based matrix* (CM; red dashed lines) *and the neighboring connective tissue (CT) showing a comparable distribution of vessels within both areas (EP = epithelium; CD31 immunostaining, "total scan," ×5 magnification, scale bar = 500 µm).* *Vascularization* (vessels = red arrows) *within the* (b) *neighboring CT and* (c) *implantation area of the CM (CD31 immunostaining, ×20 magnification, scale bars = 100 µm).*

Quantitative/ Histomorphometric Results

The histomorphometric analysis of the vascularization within the implantation beds of the collagen-based matrix and the surrounding connective tissue revealed no statistically significant differences (Figs 9a and 9b). Thus, similar values were found for the vessel numbers within the respective areas (the membrane area [30.43 ± 11.26 vessels/mm^2] and the



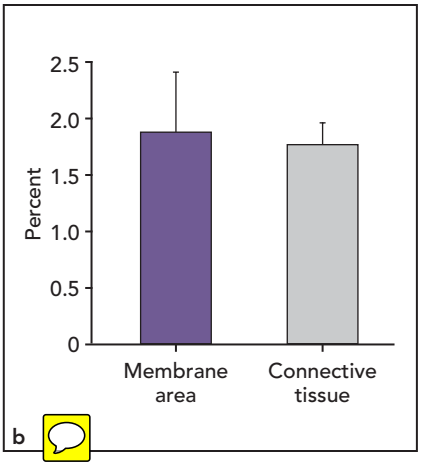


Fig 10 *Results of the histomorphometric measurements:* (a) *comparative vessels/mm² and* (b) *comparative percent vascularization.*

surrounding connective tissue [39.74 ± 17.15 vessels/mm²]) without statistically significant differences ($P = .4758$) (Fig 9a). Furthermore, similar percent vascularization values were also measured within the collagen membrane area (1.87% ± 0.54%) and the surrounding connective tissue (1.76% ± 0.19%) with no statistically significant differences ($P = .7640$) (Fig 10).

Discussion

The results of these cases demonstrate that thin soft tissues can be thickened vertically with porcine-derived collagen dermal matrix. This procedure allowed thin soft tissues with a mean thickness of 1.65 mm to form a 3.45-mm–thick soft tissue pattern, with a mean gain of 1.8 mm. A preceding study[8] that used allogenic instead of porcine-derived membranes showed a bigger increase in vertical soft tissue thickness; the mean soft tissue gain was equal to 2.21 mm. This difference between the outcomes of the studies can be explained: in the allograft study, every graft was folded once (double-layered) to reach a thickness of 2 to 3 mm, and therefore greater thickening of the tissues was achieved.

In a recently published case report, Puišys et al[11] showed the capability of this material to horizontally thicken soft tissues. Though weaknesses of the case report preclude drawing any conclusions, it might be interpreted as a promising result. Another case report showed that porcine-derived matrix can be successfully used to increase keratinized tissues around implants.[12]

Histologic analysis showed intense vascularization of the membrane and almost no cell inflammation after 2 months of healing. Substitute materials should optimally promote similar or equal conversion processes in the healing course. However, it has been shown that different collagen-based materials induce inflammatory reactions as part of a foreign-body response including multinucleated giant cells.[13] In the case of the analyzed collagen-based matrix in the present study, the histologic analysis showed that it promoted soft tissue healing without signs of inflammatory responses (ie, histologic correlates of a material-related foreign-body response including multinucleated giant cells). This conclusion is furthermore substantiated by the results of the vascularization measurements, which show comparable vascularization patterns within the implantation area of the collagen matrix compared to the unaffected surrounding subgingival connective tissue. The process of material fabrication might explain the described histologic integration. The process is a multi-step process and is applied for purification of the origin tissue, (ie, the removal of all potential immunogenic tissue components). Lyophilization and gamma-radiation sterilization finalize the procedure. Altogether, this preparation process results in a three-dimensional stable matrix consisting of collagen and elastin without additional cross-linking or chemical treatment and a mean thickness of approximately 2 mm.

Conclusions

Within the limitations of this study, it can be concluded that porcine-derived collagen matrix membranes can be successfully used for vertical soft tissue thickening. An average 1.8-mm gain in vertical soft tissue volume can be expected, depending on initial gingival thickness. Histologic integration of the material can be expected as early as 2 months postoperatively.

Acknowledgments

The authors declare no conflicts of interest.

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