Porcine Dermis-Derived Collagen Membranes Induce Implantation Bed Vascularization Via Multinucleated Giant Cells: A Physiological Reaction?

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In this study, the tissue reactions to 2 new porcine dermis-derived collagen membranes of different thickness were analyzed. The thicker material (Mucoderm) contained sporadically preexisting vessel skeletons and fatty islands. The thinner membrane (Collprotect) had a bilayered structure (porous and occlusive side) without any preexisting structures. These materials were implanted subcutaneously in mice to analyze the tissue reactions and potential transmembranous vascularization. Histological and histomorphometrical methodologies were performed at 4 time points (3, 10, 15, and 30 days). Both materials permitted stepwise connective tissue ingrowth into their central regions. In the Mucoderm matrix, newly built microvessels were found within the preexisting vessel and fatty island skeletons after 30 days. This vascularization was independent of the inflammation-related vascularization on both material surfaces. The Collprotect membrane underwent material disintegration by connective tissue strands in combination with vessels and multinucleated giant cells. The histomorphometric analyses revealed that the thickness of Mucoderm did not decrease significantly, while an initial significant decrease of membrane thickness in the case of Collprotect was found at day 15. The present results demonstrate that the 2 analyzed collagen membranes underwent a multinucleated giant cell-associated vascularization. Neither of the materials underwent transmembraneous vascularization. The microvessels were found within the preexisting vessel and fatty island skeletons. Additional long-term studies and clinical studies are necessary to determine how the observed foreign body giant cells affect tissue regeneration.

Key Words: collagen membrane, porcine, vascularization, foreign body giant cells, guided tissue regeneration (GTR), guided bone regeneration (GBR)

INTRODUCTION

Collagen-based biomaterials are a material class with the highest potential for replacing autologous connective tissue in guided tissue regeneration (GTR) and guided bone regeneration (GBR). The potential of collagen is based on its role as a practically ubiquitous biological molecule with a major contribution to wound healing in humans via different cellular interactions. Furthermore, collagen-based materials are able to support tissue repair in dentistry, oral implants, and maxillofacial surgery, because they increase the thickness and stability of gingiva or subcutaneous connective tissue.2–4 Collagen-based materials of different types function within the host tissue as a scaffold and enhance tissue regeneration.5–7

Several different collagen materials have been successfully introduced for application in GTR and GBR with many varying material characteristics, such as different donor tissues, structures, thicknesses, and purification methods.8,9 The understanding of these parameters is of great relevance, as they form the basic knowledge for a successful clinical application of the materials.

Recently, a systematic in vivo analysis using a standardized subcutaneous implantation model in CD-1 mice was performed. In addition, the influence of the physicochemical properties of porcine-derived collagen membranes/matrices that might lead to a specific cellular inflammatory pattern and
contribute to a potential transmembranous vascularization was investigated.5,6,10

Previously the in vivo functionality of Bio-Gide (BG, Geistlich, Switzerland), which is a bilayered membrane originating from porcine peritoneum, was analyzed using a subcutaneous implantation model in CD-1 mice.6 The data revealed that this material induced a tissue reaction that mainly involved mononuclear cells and did not undergo transmembranous vascularization. During the observation period, vessels were unable to penetrate the membrane completely and form a vascular network between the collagen fibers.6 However, in the literature, such a vascularization pattern was previously described for this material.11,12 Several authors have used the term “transmembranous vascularization” for this and other collagen-based materials.13

The analysis of Mucograft (MG, Geistlich, Switzerland), which is a bilayered matrix derived from porcine peritoneum and dermis using the same in vivo methods, revealed that this material also remained stable for 60 days.5 MG retained its volume stability and underwent good tissue integration within the peri-implant tissue. There was no premature dissolution or breakdown observed for either BG or MG membranes. Interestingly few multinucleated giant cells were observed within the implantation bed of both BG and MG.5,6

The application of 2 xenogenic collagen materials made from porcine dermis (BEGO Collagen Fleece, BEGO Implant Systems, Germany) and from porcine pericardium (BEGO Collagen Membrane, BEGO Implant Systems) induced different cellular reactions.10 The dermis-derived fleece underwent early tissue integration by stepwise disintegration and incorporation within the surrounding peri-implant tissue. Thereby the collagen fleece induced a relatively mild vascularized granulation tissue that contained tartrate-resistance acid phosphatase (TRAP)-negative mononuclear and multinucleated cells at both material surfaces.10 In contrast, the application of the bilayered pericardium-derived membrane induced 2 different tissue reactions that were associated with its 2 different components. A combination of mononuclear and multinucleated cells was observed on the compact surface of the membrane and resulted in its nearly complete degradation within the first 30 days. The latter was associated with the expression of a mild granulation tissue. The spongy surface of the membrane induced a mononuclear cell-driven tissue response that was similar to the response observed for BG and MG. This membrane served as a stable barrier for up to 60 days.10 Data indicate that the presence of multinucleated giant cells was associated with a stepwise vascularization of these materials and it is based on granulation tissue without transmembrane vascularization. Nevertheless, the achievement of transmembranous vascularization is still a desired clinical goal because it is believed that materials with this characteristic will permit better communication between material-covering gingival mucosa/skin and the underlying connective tissue. The result would be a more mature regeneration of the keratinized gingiva and consequently a better clinical outcome. However, from the previously-mentioned study, it can be concluded that biologically-derived collagen membranes do not necessarily require transmembranous vascularization for successful tissue integration.5,10 The central portions of these materials can be supplied with nutritive elements by processes such as diffusion.5,6,10 The conditions for such nutrition are present in well-vascularized tissues, such as the oral cavity.

The present study investigated the in vivo tissue reaction to Mucoderm (MD, Botiss Biomaterials, Berlin, Germany), a porcine dermis-based collagen membrane that contains vessel skeletons enclosed within the decellularized animal collagen structure. The main aim of the present study was to apply the described systematic study protocol to investigate the cellular response to this material. As a control, we used Collprotect (CP, Botiss Biomaterials, Germany), which is a much thinner bilayered porcine-derived dermis-based collagen membrane from the same animal tissue region that lacks vessel skeletons.

MATERIALS AND METHODS

Biomaterials

Mucoderm

According to the manufacturer, Mucoderm (MD, Botiss Biomaterials) is a collagen-based tissue matrix derived from porcine dermis. This membrane passes through a multistep cleaning process to remove all potential tissue components from the dermis. This procedure is then followed by lyophilization and final gamma radiation sterilization. This preparation process results in a 3-dimensional stable matrix consisting of collagen and elastin without additional cross-linking or chemical treatment. MD has a mean thickness of approximately 1.2 to 1.7 mm. The medical device is Conformité Européenne (CE)-marked.

Collprotect

According to the manufacturer, Collprotect (CP, Botiss Biomaterials) is a bilayered membrane based on naturally cross-linked Type I and III collagen that is derived from porcine dermis. The native tissue passes through a multistep cleaning process to remove all potential tissue remnants and is then lyophilized and gamma radiation sterilized. This process results in a 3-dimensional and stable membrane that is similar to MD and consists of collagen and elastin. CP has a mean thickness of approximately 0.2 to 0.5 mm and is also CE-marked.

Histological analysis of blank material samples

A specimen of each material (sized 10 × 10 mm) was fixed in 4% buffered formalin for 24 hours and then dehydrated in a series of alcohol and xylene and embedded in paraffin. The samples were sectioned in slices measuring 3 to 4 µm in thickness, deparaffinized and histochemically stained with Mayer’s hema-toxylin and eosin (H&E). These sections were also stained with previously described special (connective) tissue stains such as azan, Movat pentachrome, Masson Goldner, and sirius red.5,6,10 The ultrastructures and the 3-dimensional porous system of the materials were then assessed prior to implantation.
**In vivo study**

**Experimental design of the in vivo study**

The present study was approved by the Committee on the Use of Live Animals in Teaching and Research of the State of Rhineland-Palatinate, Germany. CD-1 mice were obtained from Charles River Laboratories (Sulzfeld, Germany). The 40 study animals were randomly divided into 2 experimental groups (MD and CP, n = 16) and 1 control group (n = 8). Animals in the first experimental group were given a subcutaneous implantation of MD. The animals in the second experimental group received implantation of CP. The control group consisted of 8 sham-operated animals. The tissue reactions to each biomaterial were subsequently assessed at 4 explantation time points (3, 10, 15, and 30 days after implantation) in 4 randomly selected animals (n = 4 animals per time point for experimental and n = 2 animals per time point for the sham-group).

**Subcutaneous implantation procedure**

The surgical procedure for the biomaterial implantation was performed as previously described by the authors. After shaving, animals were sedated with 10 mL of ketamine (50 mg/mL) in combination with 1.6 mL of 2% xylazine. A sterile incision was made in the rostral portion of the interscapular region and a sample of each biomaterial with an approximate size of 10 x 10 mm was inserted under the skin and muscle in preformed subcutaneous pockets. The wound margins were closed with 6.0 prolene sutures (Ethicon, Somerville, NJ). The study animals were kept postoperatively under artificial conditions with an adapted day/night cycle and regular nutrition with mouse pellets (Laboratory Rodent Chow, Altromin, Germany) and water ad libitum.

**Tissue preparation for histochemistry**

Animals were sacrificed at the end of each observation period by an overdose of ketamine and xylazine. The implanted biomaterial samples were explanted with the surrounding peri-implant tissue according to histological methodologies that have been well established in previous studies on collagen-based biomaterials. The explanted tissue samples within the materials were cut into 3 portions representing the left and right margin, and the center of the implantation site. The sections were subsequently fixed in 4% buffered formalin for 24 hours, dehydrated in a series of alcohol and xylene, and embedded in paraffin. The processed specimens were cut in sections with a thickness of 3 to 4 μm, deparaffinized and histologically stained as previously described. The first slide was stained with standard Mayer’s hematoxylin and eosin (H&E). The second and third slides were stained with azan and Movat pentachrome. Finally, Slides 4 and 5 were stained with Masson Goldner and Sirius red, respectively.

**Morphological evaluation of the biomaterial-specific inflammatory response**

The tissue reaction and the biomaterial-specific inflammatory response to the investigated biomaterials were evaluated histologically and histomorphometrically at the REPAIR Lab in vivo laboratory (Institute of Pathology, University Medical Center of the Johannes Gutenberg University Mainz) using a Nikon ECLIPSE 80i microscope (Nikon, Tokyo, Japan). Microphotographs of the analyzed biopsies were recorded with a connected digital camera DS-Fi1 together with a Nikon digital sight control unit (Nikon, Tokyo, Japan). A qualitative analysis was performed by histological evaluation of the tissue response and the degradation and integration behavior of both implanted biomaterials. Thereby the cells participating in the process of biomaterial vascularization and possible adverse reactions such as fibrotic encapsulation or necrosis were observed microscopically.

**Material thickness measurement**

The thickness of both collagen membranes was measured at each experimental time point to determine the biodegradation and volume stability of the biomaterials in the animal connective tissue. The analysis was performed according to previously published methods using a special scanning microscope (Eclipse 80i histological microscope, Nikon, Tokyo, Japan) connected to a DS-Fi1 digital camera (Nikon, Tokyo, Japan). The measurements were performed with the “annotations and measurements” tool of the NIS-Elements software on the previously generated total scans. Total scans are large images of the whole implantation bed and the peri-implant tissue that are generated from 100 to 120 single images at a magnification of x100 and a resolution of 2500 x 1200 pixels with the previously-described microscope assembly. To evaluate the mean membrane thickness at every time point, the thickness of every membrane in each biopsy was measured at 15 different points and the thickness was calculated in μm by the NIS Elements software. This allowed the statistical analysis of the respective material thickness. Furthermore, the percent thickness was calculated for each material and time point by relating the values of the later study time points to that of day 3 (100%).

**Measurement of the multinucleated giant cell activity**

To analyze the occurrence of multinucleated giant cells in the implantation beds of the collagen-based biomaterials, the number of these cells was histomorphometrically measured based on a previously published method. Briefly, after digitizing the slides according to the previously-mentioned method, the number of biomaterial-adherent multinucleated giant cells was measured using the counting tool of the NIS Elements software. The number of multinucleated giant cells within an implantation bed was calculated in relation to its total area (multinucleated giant cells/mm²) for statistical comparisons.

**Measurements of membrane vascularization**

The measurements of implant bed vascularization for both biomaterials were conducted using the previously-mentioned scanning microscope in combination with the NIS Elements software following previously published methods. Briefly, after digitization of the histological slides, the area tool of the “Annotations and Measurements” section of the software was used to manually mark the vessels within the implantation beds of the biomaterials. For further comparison of the values
the vessel density (vessels/mm²) and the vascularization percent were calculated using the number and the total area of vessels in the implantation bed.

Statistics

The values from the histomorphometric measurements were assessed for statistical intra- and inter-individual differences using an analysis of variance (ANOVA) followed by LSD post hoc assessment in SPSS 16.0.1 software (SPSS Inc, Chicago, Ill). The intra- (●) and the interindividual (●●●) differences were considered significant if the P-values were less than 0.05 (●●● P < .05), and highly significant if the P-values were less than 0.01 (●●● P < .01) or less than 0.001 (●●● P < .001). All of the graphs were generated using GraphPad Prism 5.0 software (GraphPad Software Inc, La Jolla, Calif).

Results

Histological results of the blank biomaterials

Mucoderm

The histological assessment of the Mucoderm blanks revealed a porous structure, in which the collagen fibers were aligned in vertical, horizontal, and diagonal directions (Figures 1a and b). Furthermore, the histological assessment of the material revealed the presence of pores within the membrane body that were evocative of collagenous correlates of former vessel muscle rings and sporadic fatty tissue-like islands in some parts of the membranes (Figure 1b). Thus, these structures have been supposed to be formed by preexisting vessels and fat tissue in the donor tissue (Figures 1c and d). There were no cellular tissue components found in any of the histologically analyzed blank materials.

Collprotect

The histological analysis of Collprotect blanks revealed a porous structure with collagen fibers arranged in a more organized pattern than the Mucoderm blanks (Figures 1e and f). Neither vessel skeletons nor fatty tissue islands were observed within this membrane (Figure 1f).

In vivo results

All animals of each group survived the material implantation. There were no changes in animal feeding habits and housing behaviors observed for any of the analyzed groups. There was no wound dehiscence, and no macroscopically detectable wound healing disorders were found in any of the animals.

Histological results after subcutaneous implantation

Mucoderm. Mononuclear cells were found to be aligned on the material surface at day 3 after implantation (Figure 2a). Some of these cells showed evidence of slight penetration into the body of the membrane (Figure 2a). At this time point, the Mucoderm membrane was present as a volume-stable membrane within the subcutaneous connective tissue containing the above-described collagenous vessel skeletons and fatty tissue islands. The latter did not show any evidence of cell or tissue element ingrowths (Figure 2b).

At day 10 after implantation, the observed mononuclear cell wall was increased and there was a progression to mild cell penetration into the membrane (Figure 2c). The analyzed membrane still appeared as a volume-stable material. Interestingly, the inner surface of the decellularized vessel skeletons and the previous fatty tissue islands within the membrane body were found to have been lined with single mononuclear cells (Figure 2d). However, no cell penetration was found within the central regions of the membrane (Figure 2d).

By day 15 after implantation there were only mononuclear cells observed on both surfaces of the material (Figure 2e). These cells continued to slowly penetrate the peripheral material region in the direction of the central material regions (Figure 2e). However, cellular penetration into the membrane was only observed in the regions, in which vessel skeletons or fat tissue remnants were located (Figure 2f). At this time point, the Mucoderm membrane still appeared as a volume-stable barrier membrane, but no cellular colonization of the deeper matrix regions was observed (Figure 2f).

At day 30 after implantation, a combination of mono- and multinucleated giant cells was found on both membrane surfaces and mostly mononuclear cells penetrated into deeper peripheral regions of the material (Figure 3a and a1). At this time point the fibers on both material surfaces were embedded within a vascularized connective tissue (Figure 3a). Thereby the matrix was still detectable within the subcutaneous tissue as a volume-stable material without signs of breakdown (Figure 3b), while the lumina of the decellularized vessel skeletons were filled with connective tissue and a new microvessel system could be observed (Figures 3c and d). At this time point only a few cells had penetrated in the central regions of the material body (Figures 3c and d).

Collprotect. At day 3 after implantation, Collprotect induced only a thin mononuclear cell layer on both membrane surfaces, while the membrane body was free of cells and vessels (Figures 4a and b). At this time point the membrane was detectable as a volume-stable material within the subcutaneous connective tissue (Figure 4b).

On day 10, the observations showed the beginning of cell penetration into the superficial regions of the membrane (Figure 4c). There was a thin layer of mononuclear cells located on the surfaces (Figure 4c). However, the volume stability of the material remained intact (Figure 4d). There were no observable signs of vessel ingrowth (Figures 4c and d).

By day 15 after implantation, the occurrence of sporadic multinucleated giant cells in addition to an accumulation of mononuclear cells could be observed on both material surfaces (Figure 4e). At this time point, cell penetration into the superficial regions of the membrane was increased (Figure 4e). However, there was no cell penetration into the central regions and the membrane was still detectable as a stable material (Figures 4e and f). There was no vessel invasion into the membrane at this time point (Figures 4e and f).

At day 30 after implantation, the material showed sporadic penetration by connective tissue strands thorough the whole membrane body (Figures 4g and h). A combination of vessels and mono- and multinucleated cells was found within these.
**Figure 1.** This figure shows histological images of the blank biomaterials prior to implantation. (a) shows a cross-section through the body of the Mucoderm matrix (MD = double head arrow) with its fiber distribution and microstructure (hematoxylin and eosin [H&E], magnification ×40, scale bar = 100 μm). (b) shows the enclosed fatty tissue-like islands (black arrows) and the enclosed vessel skeletons (blue arrows) within the matrix (MD; H&E, magnification ×200, scale bar = 10 μm). (c) and (d) show the enclosed vessel skeletons (blue arrow heads) that exhibit histological signs of muscle ring-like assembly around their lumina (MD = Mucoderm matrix) (c: sirius stain, magnification ×200, scale bar = 10 μm; d: Masson Goldner staining of collagen, magnification ×400, scale bar = 10 μm). (e) and (f) show the morphology and microstructure of the Collprotect membrane (CP = double head arrows), which exhibits more consistently arranged collagen fibers without any signs of other tissue structures (H&E, e: magnification ×100, scale bar = 100 μm; f: magnification ×200, scale bar = 10 μm).
FIGURE 2. This figure shows the tissue reactions and the integration behavior of the Mucoderm matrix (MD) at days 3 (a and b), 10 (c and d) and 15 (e and f) after implantation in subcutaneous connective tissue (CT) of the CD-1-mice. On days 3, 10, and 15 after implantation (a, c, and e) a thin wall of mononuclear cells (black arrow heads) was observed at the surfaces of the membranes (MD), while a comparatively low number of cells was found within the body of the matrix (green arrow heads; CT = connective tissue; a: hematoxylin and eosin [H&E], c: Movat pentachrome stain, e: Movat pentachrome stain; magnifications ×400; scale bars = 10 μm). Within this time frame the matrix remained stable within the subcutaneous connective tissue (CT) and no signs of tissue ingrowth were observable (b, d, and f). The described fatty tissue-like islands (black arrows) and the vessel skeletons (blue arrow heads) were invaded by single mononuclear cells at these time points (b: H&E; d: Movat pentachrome stain; f: sirius stain, magnifications ×100; scale bars = 100 μm).
strands and a pervading, but not “transmembranous,” vascularization was clearly established (Figures 4f and g). However, the membrane could still be detected within its implantation bed and showed volume stability (Figure 4g).

**Histomorphometrical results of membrane thickness analyses**

*Mucoderm.* The histomorphometrical analysis revealed that the Mucoderm matrix at day 3 after implantation had a mean thickness of $1889.92 \pm 112.27 \mu m$ (Figure 5a). At day 10 after implantation the matrix had a thickness of $1836.01 \pm 200.81 \mu m$. No statistically significant decrease was measurable between these 2 study time points (Figure 5a). At day 15 after implantation, Mucoderm had a mean thickness of $1757.23 \pm 129.78 \mu m$ (Figure 5a). There were no statistically significant differences compared with day 3 and 10 after implantation (Figure 5a). However, on day 30 after implantation the matrix showed a statistically significant decrease of the membrane thickness ($1715.99 \pm 90.46 \mu m$) compared only with day 3 ($^{*}P < .05$) (Figure 5a).

The analysis of the percent thickness of the Mucoderm matrix showed no significant decrease of its thickness at day 10 ($97.15 \pm 10.62\%$) compared to day 3 (100%; Figure 5b). A further decrease was found at day 15 after implantation ($92.98 \pm 6.87\%$), which also showed no significant differences to the
values of day 3 and 10 after implantation (Figure 5b). The analysis showed that also a decrease of the percent thickness was found at day 30 after implantation (90.79 ± 4.79%) and no significant differences of the percent thickness were found compared to the values of the former time points (Figure 5b).

Collprotect. At day 3 after implantation the Collprotect membrane showed a mean thickness of 398 ± 38.1 µm (Figure 5c). There was no significant decrease of membrane thickness at day 10 after implantation, the mean thickness being 382.8 ± 53.8 µm (Figure 5c). By day 15 after implantation the thickness of the Collprotect membrane showed a highly significant decrease (322.8 ± 46.9 µm) compared to days 3 and 10 (**P < .001 / ***P < .01) (Figure 5c). Furthermore, the histomorphometrical analysis revealed a further significant decrease in membrane thickness at day 30 after implantation (298.5 ± 57.5 µm) compared to days 3 and 10 (**P < .001; Figure 5c).

The analysis of the percent thickness of the Collprotect membrane revealed that a decrease was found at day 10 after implantation (96.19 ± 13.52%) without significant differences compared to day 3 (100%; Figure 5d). At day 15 after implantation a further decrease of the percent thickness of the Collprotect membrane was measured (81.10 ± 11.81%), which significantly differed from the values of day 3 (**P < .05) and day 10 (**P < .01; Figure 5d). At day 30 after implantation also a decrease of the percent thickness (74.99 ± 14.45%) was found that still was significantly lower compared to the values of day 3 (**P < .05) and day 10 (**P < .01) but not compared to that of day 15 after implantation (Figure 5d).

**Histomorphometrical results of the multinucleated giant cell activity**

The comparative measurements of the occurrence of multinucleated giant cells revealed that no multinucleated giant cells were found at day 3 and day 10 after implantation within the implantation beds of the collagen-based biomaterials (Figure 6a). By day 15 after implantation, there was a moderate number of multinucleated giant cells within the implantation beds of Collprotect (1.23 ± 0.54 multinucleated giant cells/mm²). However, there were no multinucleated giant cells found within the implantation beds of Mucoderm (0.17 ± 0.04%). The statistical analysis showed there were no significant differences between the values of the study groups and there was no significant intraindividual increase of the cell numbers in the Collprotect group (Figure 6a).

The analysis at day 30 after implantation revealed comparable amounts of multinucleated giant cells within the implantation beds of both materials (Mucoderm: 2.81 ± 1.05 multinucleated giant cells/mm²; Collprotect: 3.69 ± 1.28 multinucleated giant cells/mm²). There were no significant differences between the values of the study groups (Figure 6a). The intraindividual analyses showed that the values of both study groups at the last time point were significantly higher compared to all other values of the previous study time points in each material group (**P < .001; Figure 6a).

**Histomorphometrical results for vascularization**

The histomorphometrical measurements of the vessel density showed that no vessels were found in the implantation beds of either collagen-based biomaterial at day 3 after implantation. The control group showed a mild vascularization (0.69 ± 0.22 vessels/mm²). There were no statistically significant differences found between the 3 study groups (Figure 6b).

No vessels were found within the implantation beds of either biomaterial group on day 10 after implantation. The tissue of the control group showed an increase of vessels (1.19 ± 0.54 vessels/mm²) without any intra- or interindividual significance (Figure 6b).

The analysis on day 15 after implantation showed no vessel ingrowth for the 2 collagen-based materials (Figure 6a). Furthermore, the control group showed similar amounts of vessels (1.29 ± 0.31 vessels/mm²) compared to day 10 (Figure 6b). At this study time point there were no significant differences between the 3 groups (Figure 6b).

At day 30 the implantation beds of Mucoderm (3.16 ± 1.89 vessels/mm²) and Collprotect (2.09 ± 0.77 vessels/mm²) showed slightly increased vascularization. However, no significant differences were found between these 2 groups (Figure 6b). The number of vessels in both groups were significantly increased compared to their corresponding early study time points (**P < .001; Figure 6b). Furthermore, the measurements showed that the control group had comparable numbers of vessels (1.66 ± 0.45 vessels/mm²). Thus, there were no significant differences compared to the other 2 study groups (Figure 6b).

The percent vascularization at day 3 after implantation showed no significant differences for the collagen-based materials as no vessels were found within their implantation beds (Figure 6a). At this time point the control group showed a mildly increased percent vascularization (0.05 ± 0.01%). However, there were no significant differences compared to the other groups (Figure 6c).

There were no vessels found within the implantation beds of both biomaterials at day 10 after implantation (Figure 6c). At this time point the control group showed a relatively increased percent vascularization (0.09 ± 0.01%) that was statistically significantly different (**P < .01) (Figure 6c). However, no intraindividual significance was found compared to the values of the control group at day 3 after implantation (Figure 6c).

There was no vessel ingrowth into the biomaterial implantation beds at day 15 after implantation. There were similar values for the percent vascularization found in the control group (0.09 ± 0.03%) compared to the day 10 values. Thus, these values were also significantly higher than the values for the collagen-based materials (**P < .01; Figure 6c).

Comparable vessel fractions were found within the implantation beds of Mucoderm (0.17 ± 0.04%) and Collprotect (0.19 ± 0.07%) at day 30 after implantation, but the differences were not significant. The values of both groups were significantly higher compared to day 15 (**P < .001; Figure 6c). Furthermore, a similar value was also measured for the control group (0.12 ± 0.03%). This value was not significantly different than the material study groups or former study time points (Figure 6c).

**Discussion**

Vascularization is one of the key elements for successful biomaterial integration and tissue regeneration.14 Several
FIGURE 4. This figure shows the tissue reactions to the Collprotect membrane (CP). (a) and (b) show that a thin layer of mononuclear cells (black arrow heads in a) and small-sized vessels (read arrows in a) were located at the surface of the membrane (CP) at day 3 after implantation. The membrane (CP = double arrow in b) was detectable within the subcutaneous connective tissue (CT) and shows no signs of tissue ingrowth (CT = connective tissue; hematoxylin and eosin [H&E]; a: magnification ×400, scale bar = 10 μm; b: magnification ×100, scale bar = 100 μm). (c) and (d) show the tissue reaction to the Collprotect membrane (CP = double arrow) at day 10 after implantation.
material classes, such as silk fibroin (SF), starch polycaprolactone fibers (SPCL), and polylactic acid (PLA)-based materials, have been introduced to enhance vascularization and induce a transmembranous vascularization by creating proinflammatory conditions. Multinucleated giant cells between the material fibers acted as a source of vascular-inducing signal molecules (eg, VEGF) and contributed to the generation of a granulation tissue within the implantation bed of these materials.15,19–22 Despite induction of multinucleated giant cells, the previously-mentioned material classes are good candidates for complex tissue engineering. By cultivating scaffolds with endothelial and mesenchymal cells, cell-cell communication with the surrounding host tissue could be achieved and this results in bone matrix production and enhanced vascularization of the implantation bed.21,23–29

Animal-based collagen materials have been used in humans for soft and hard tissue engineering in oral and maxillofacial surgery. This application is on account of their “natural” origin. Additionally, animal collagen I, III, V, and VI can be used to mimic the extracellular matrix molecules required for both soft and hard tissue regeneration.30,31 The focus of the industry in oral and maxillofacial surgery is on collagen-based materials and matrices or membranes derived from different animal species such as equine,32 bovine,33 and porcine collagenous materials.5–7

A wide range of different membranes and matrices have been analyzed systematically with the aim of clarifying the tissue reactions to the different materials.5,6,10 This forms the scientific basis to decide if these materials are suitable for specific clinical applications. Previous studies have shown that some collagen membranes derived from porcine peritoneum and those from porcine dermis and peritoneum do not require transmembranous vascularization to contribute to a successful material integration.5,6 Within the implantation bed of these materials there are mostly mononuclear cells and a few multinucleated giant cells. Additionally, no material breakdown was observed until the end of the observation period at day 60 after implantation.5,6 The materials served as a porous guide for the surrounding connective tissue and persisted within the implantation bed without any disintegration. These results led to the conclusion that these types of collagen membranes become integrated within their implantation bed and contribute to the overall tissue regeneration by serving as pathways through which nutrients diffuse.5,6 Another study has shown that a collagen membrane derived from porcine dermis induced a tissue reaction that involved a comparatively high number of multinucleated giant cells. A pericardium-derived collagen membrane induced a combined cell reaction with the involvement of mononuclear and multinucleated giant cells.10 In these studies, the presence of the multinucleated giant cells was associated with the formation of a granulation tissue. These results led to the conclusion that for the latter 2 collagen membranes the tissue integration occurs by induction of granulation tissue, which then contributes to a stepwise material breakdown with time.10 According to the observations “transmembranous vascularization,” in which vessels form between the collagen fibers does not take place.5,6,10

The present study has investigated the tissue reaction to a new porcine-based collagen membrane, Mucoderm, which is extracted and processed from deeper regions of the xenogeneic demis. Another comparable collagen membrane with a thinner thickness is Collprotect, which is derived from the upper dermis region and was used as a control. The histological results of blank probes of these materials showed that Mucoderm contained decellularized vessel skeletons and fatty tissue islands from its xenogeneic origin. Collprotect had neither vessels nor fatty tissue islands present within the membrane. Histomorphometry showed that Mucoderm has a thickness of approximately 1800 μm and was more than fourfold thicker than the Collprotect, which had a mean thickness of 400 μm.

Our study of the tissue reaction to the Mucoderm matrix revealed that no relevant tissue ingrowth within its central regions could be observed during the observation period of 30 days. Accordingly, no breakdown of this material was observed for this time period. The material induced a cellular reaction with a combination of mononuclear and multinucleated cells and the latter cell types were observed at day 30 after implantation. The occurrence of this cell type at the late stage is in accordance with the inability of the mononuclear cells to degrade the material.34,35

The assessment of tissue ingrowth into the matrix has shown that the blood vessel skeletons and the fatty tissue islands allow a stepwise “internal” connective tissue ingrowth into the scaffold that enables microvessels to penetrate into the membrane. This specific vascularization pattern is different from vascularization associated with a multinucleated cell-triggered inflammatory process, in which vessels spread out as a response to biomaterial cellular degradation.

The overall results of the present study on Mucoderm demonstrated a stepwise tissue penetration through the relatively thick material. A transmembranous vascularization was only possible through the preexisting vessel-skeletons and...
fatty tissue islands. The 2 material surfaces underwent vascularization associated with multinucleated giant cells and the generation of a perimembranous granulation tissue. The histological tissue analysis of the Collprotect membrane, which is more than 4 times thinner than Mucoderm, showed that 30 days are required for sequential material breakdown. The breakdown was associated with granulation tissue formation, which was formed around and within the material after 30 days. Both mononuclear and multinucleated giant cells were involved in material degradation.

**Figure 5.** This figure shows the results of the histomorphometrical thickness measurements of the Mucoderm matrix (a and b) and the Collprotect membrane (c and d). (a) Membrane thickness of the Mucoderm matrix was significantly reduced on day 30 compared to day 3 (*P < .05). (b) The percent thickness of the Mucoderm matrix did not significantly reduce over the study time. (c) Membrane thickness of the Collprotect membrane was significantly reduced between day 15 and day 3, between day 30 and day 3, between day 30 and day 10 (**P < .001) and between day 15 and day 10 (**P < .01). (d) The comparison of the percent thickness showed also a significant decrease starting with day 15 (*P < .05) and remaining at day 30 (**P < .01) as well as between day 10 and 15 (**P < .01) and day 10 and 30 (**P < .001). Decrease of membrane thickness is stated as significant if P-values were less than .05 (*P < .05), and highly significant if P-values were less than .01 (**P < .01) or less than .001 (**P < .001).
The results of the present study together with our previously published data on porcine-based materials indicate that the tissue source, the tissue structure and, more importantly, the tissue processing technique all affect the tissue response. Whether animal-derived collagen membranes can be designated as natural-based collagen is unclear, especially when considering that the multiple processing techniques used for licensing it in human applications might change their surface characteristics and induce foreign-body type tissue reactions. Nevertheless, none of the previously-mentioned membranes was able to induce a transmembranous vascularization.5,6 This result suggests that transmembranous vascularization occurs either by generating granulation tissue that penetrates the membrane and leads to its breakdown over time or by the presence of a pore-like system that connects the 2 membrane surfaces. The latter forms the basis for the process of connective tissue ingrowth-associated angiogenesis and is primarily independent of the inflammation-associated generation of a granulation tissue on collagen material surfaces.

It is possible that for a matrix or membrane implanted within the oral mucosa, the “desired” transmembranous vascularization is not necessarily mandatory for successful tissue regeneration.5,6 It is known that mechanisms such as diffusion from both material surface regions can contribute to the nutrition supply of a biomaterial.20,36,37

This study as well as the previous studies with porcine-based materials demonstrated the presence of multinucleated giant cells and the induction of granulation tissue. Thus, it has to be questioned to what extent these animal-derived materials are superior to other biologically derived materials, such as silk fibroin (SF) or starch polycaprolactone fibers (SPCL). The latter also showed generation of granulation tissue combined with the formation of multinucleated giant cells.19,23,25 Synthetic materials can be generated by techniques such as 3D plotting or solution-based micronet structuring and might be easier to design with microchannels of different dimensions. Additionally, it is still unclear if the induction of multinucleated foreign body giant cells by animal-derived collagen is a result of differences in the manufacturing procedures, which might change material surface characteristics and allow the materials to be recognized as foreign bodies. Moreover, it has to be questioned to what extent the multinucleated giant cells are needed to enhance the early implantation bed vascularization as shown for SF and SPCL. The present study data do not provide answers concerning the meaning, benefit, or potential harm of the described multinucleated giant cells within the implantation bed. Based on previously published data on different synthetic biomaterials and collagen-based membranes it has to be questioned whether the trend is towards accepting the presence of cells within the implantation bed of all materials and especially of biological-derived collagen membranes as a “physiological” reaction.7,10 By definition, each substance that does not belong to the body is recognized by the immune system as a foreign body and is targeted either to be degraded or to be sequestered by encapsulation.38 Additional in vitro and in vivo studies are necessary to elucidate the molecular mechanisms of how multinucleated giant cells contribute to the integration of synthetic or biologically derived materials. Without a deeper understanding of these biological processes, the targeted application of biomaterials that induce a multinucleated giant cell response in patients with specific needs will not be possible. This issue continues to be a focus of research.

**Conclusions**

In the present study, the tissue reaction to 2 different porcine-derived, collagen-based membranes was evaluated by means of subcutaneous tissue implantation and subsequent histological analysis. The 2 materials differed in membrane thickness and structure. The thicker biomaterial contains preexisting decellularized channel-like structures and fatty tissue islands. Two distinct patterns of vascularization took place in the course of material integration in the examined membranes. Transmembranous vascularization was only observed in the thicker membrane within the preexisting vessel skeleton and fatty islands, while the vascularization on both material surfaces was attributed to a multinucleated giant cell-triggered inflammatory condition. Collagen-based biological materials undergo multiple processing techniques before application in humans. Accordingly, this might explain their ability to induce multinucleated giant cell formation similar to that observed for
synthetic or other natural materials such as silk fibroin and starch polycaprolactone (SPCL). It is unclear if channel-like structures similar to the channels in the investigated collagen membrane can be transferred to other biomaterials and if they improve the clinical outcome.

**ABBREVIATIONS**

BG: Bio-Gide  
CE: Conformite´ Europe´enne  
CP: Collprotect  
CT: connective tissue  
GBR: guided bone regeneration  
GTR: guided tissue regeneration  
H&E: hematoxylin and eosin  
MD: Mucoderm  
MG: Mucograft  
PLA: polylactic acid  
SF: silk fibroin  
SPCL: starch polycaprolactone fibers  
TRAP: tartrate-resistant acid phosphatase

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