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Angiogenesis pattern of native and cross-linked collagen membranes: an immunohistochemical study in the rat

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Abstract: The aim of the present study was to immunohistochemically evaluate angiogenesis pattern of native and cross-linked collagen membranes after subcutaneous implantation in rats. Five commercially available and three experimental membranes (VN) were included: (1) BioGide® (BG), (2) BioMend® (BM), (3) BioMend Extend® (BME), (4) Ossix® (OS), (5) TutoDent® (TD), and (6–8) VN(1–3). Specimens were randomly allocated in unconnected subcutaneous pouches ($n = 4$) separated surgically on the back of 40 wistar rats, which were divided into five groups (2, 4, 8, 16, and 24 weeks), including eight animals each. Pattern of angiogenesis was labelled using primary mouse monoclonal antibody to transglutaminase II. For each membrane, the period of time, needed for a complete and homogeneous transmembraneous vascularization, was assessed immunohistomorhometrically. Differences between the membranes were found regarding the initial pattern of transmembraneous angiogenesis, as evaluated 2 weeks following implantation. Mean cross- and longitudinal-sectional area of blood vessels (%) was highest for VN(3) (5.27 ± 2.73), followed by BG (2.45 ± 0.88), VN(1) (2.07 ± 0.29), VN(2) (1.91 ± 0.55), TD (1.44 ± 0.53), BME (0.35 ± 0.29) and BM (0.25 ± 0.4). In contrast to BG and VN(1–3), BM, BME and TD exhibited a homogeneous transmembraneous formation of blood vessels merely 4–8 weeks following implantation. OS, however, exhibited no signs of angiogenesis throughout the whole study period. Within the limits of the present study, it may be concluded that pattern of transmembraneous angiogenesis markedly differs among the membranes investigated.

The concept originally proposed to account for guided bone regeneration (GBR) and guided tissue regeneration (GTR) emphasized the establishment of a secluded space by means of membrane barriers in order to prevent the apical downgrowth of undesired cells inside the wound area and allow for ingrowth of cells with the capacity of regenerating the desired tissue (Gottlow et al. 1986; Dahlin et al. 1988; Karring et al. 1993; Hämmerle & Karring 1998; Hämmerle & Lang 2001). Several design requirements have been proposed for a material that is used as a barrier for GBR/GTR procedures. In particular, these are biocompatibility, tissue integration, cell-occlusivity, nutrient transfer, space making ability and also ease of use in the clinic (Hardwick et al. 1994). A variety of different membrane materials have been successfully used for GBR/GTR procedures, ranging from non-resorbable materials such as expanded polytetrafluorethylene (e-PTFE) to bioabsorbable membranes composed of dura-mater, polylactic acid, polyglycolic acid and polyurethane (Magnusson et al. 1988; Greenstein & Caton 1993; Hardwick et al. 1994; Hutmacher et al.

1996; Kohal et al. 1998). Most recently, many investigations focused on the use of products derived from type I and type III porcine or bovine collagen (for a review see Bunyaratavej & Wang 2001). Some advantageous properties of collagen over other materials include hemostatic function, allowing an early wound stabilization, chemotactic properties to attract fibroblasts, and semipermeability, facilitating nutritient transfer (Postlethwaite et al. 1978). In this context, it must be pointed out that angiogenesis has been emphasized to be an important factor strongly influencing outcome of healing following GBR/GTR procedures (Hardwick et al. 1994). Indeed, in a case report of a rabbit experiment, Schmid et al. (1997) have indicated that formation of blood capillaries precedes the formation of new bone. This observation may be explained by the fact that osteogenic cells have been observed to arise from pericytes adjacent to small blood vessels in connective tissue (Long et al. 1995; Rickard et al. 1996; Reilly et al. 1998). Based on these findings, it might be hypothesized that a GBR/GTR membrane allowing earlier anastomosis of the vasculature of the flap and the regenerated tissue may enhance *de novo* bone/tissue formation. However, because a major drawback of native collagen is the fast biodegradation by the enzymatic activity of macrophages and polymorphonuclear leucocytes, several cross-linking techniques such as ultraviolet light, glutaraldehyde, diphenylphosphorylazide or hexmethylenediisocyanate have been nowadays used in order to prolong biodegradation (Kodama et al. 1989; Minabe et al. 1989; Quteish & Dolby 1992; Brunel et al. 1996; Zahedi et al. 1998; Bunyaratavej & Wang 2001). Indeed, animal studies have demonstrated that biodegradation of chemical and enzymatic cross-linked collagen membranes was significantly slower compared with non-cross-linked membranes (Pitaru et al. 1988; Paul et al. 1992). However, as cross-linking with glutaraldehyde resulted in a decreased membrane biocompatibility because of cytotoxic effects (Speer et al. 1980; Wiebe et al. 1988; Rothamel et al. 2004), an alternative chemical cross-linking technique has recently been developed to prolong biodegradation of porcine-derived collagen types I and III (experimental VN membranes, Geistlich Biomaterials, Wolhusen, Switzerland). The influence of different cross-linking techniques on biodegradation over time, foreign body reactions, tissue integration, and vascularization has recently been examined in a histomorphometric study in rats (Rothamel et al. 2005). It was observed that cross-linking of bovine and porcine-derived collagen types I and III seemed to be associated on the one hand with prolonged biodegradation, but on the other hand with foreign body reactions and decreased tissue integration and vascularization (Rothamel et al. 2005). Accordingly, in view of nutritient transfer, cross-linking of collagen seems to delay transmembraneous vascularization, which has been hypothesized to be an important factor in the process of tissue regeneration (Schmid et al. 1997). However, experimental studies using conventional histology may not be appropriate to evaluate the complex process of vascularization. Therefore, the aim of the present study was to immunohistochemically evaluate angiogenesis pattern of commercially available and also experimental native and cross-linked collagen membranes after subcutaneous implantation in rats.

Material and methods

Animals

The animals, used in this experiment, have been described previously (Rothamel et al. 2005). Briefly, forty albino rats of the wistar strain (age 3.0 ± 0.5 months, weight 350 ± 20 g) were used in the study. Animal selection, management, and surgery protocol were approved by the Animal Care and Use Committee of the Heinrich Heine University, Düsseldorf, Germany. The animals were divided into five groups (2, 4, 8, 16, and 24 weeks), including eight rats each.

Membranes examined

Five commercially available and three experimental (VN) GBR/GTR collagen membranes were included: (1) BioGide® (BG) (Geistlich Biomaterials) (non-cross-linked porcine types I and III collagen, bilayered), (2) BioMend® (BM) (Zimmer Dental GmbH, Freiburg, Germany) (glutaraldehyde cross-linked bovine type I collagen), (3) BioMend Extend® (BME) (Zimmer Dental GmbH) (glutaraldehyde cross-linked bovine type I collagen), (4) Ossix® (OS) (3i, Colbar R&D Ltd., Ramat Husharon, Israel) (enzymatic cross-linked bovine type I collagen), (5) TutoDent® (TD) (Tutogen, Alachua, FL, USA) (non-cross-linked bovine type I collagen, bilayered), (6) VN(1), (7) VN(2), and (8) VN(3) (Geistlich Biomaterials) (1, 3, 4 × chemical cross-linked porcine types I and III collagen, bilayered, respectively). In order to account for the different membrane designs (single vs. bilayered), each specimen was folded (i.e. exposure of the cell occlusive surface of the bilayered membranes), trimmed to a uniform size (octagon-shaped specimens of 1.7 cm^2), and separated by a non-resorbable polycarbonate spacer (Isopore®, Millipore Corporate, Billerica, MA, USA) in the middle. Furthermore, all membranes were sutured circumferentially using non-resorbable sutures to avoid an ingrowth of the surrounding tissue (Polyester®, Resorba, Nürnberg, Germany). Ten minutes prior to implantation, each membrane was rehydrated in sterile 0.9% physiological saline (Braun, Melsungen, Germany).

Surgical procedure

All surgical procedures have been described previously (Rothamel et al. 2005). Briefly, the animals were anesthetized by intraperiotoneal injection of 9 mg/kg ketamine 10% (Ketanest®, Pfizer GmbH, Karlsruhe, Germany) and 5 mg/kg xylazine 2% (Sedaxylan®, Pfizer GmbH). An area of approximately 8 cm in length and 4 cm in width was depilated on the back of each rat using an electric shaver and a razor blade. Following disinfection with polyvidone iodine (Betaisodona®, Mundipharma, Limburg/Lahn, Germany), a skin incision was made right paramedian along the spinal column followed by the separation of four unconnected subcutaneous pouches. The membranes were randomly allocated in the resulting 160 pouches, including eight rats ($n = 32$ pouches) per observation point. Accordingly, each membrane was tested fourfold per observation point. Randomization was performed according to a computer-generated protocol (RandList®, DatInf GmbH, Tübingen, Germany). Primary wound closure was achieved using horizontal mattress 3.0 Polyester® sutures (Resorba, Nürnberg, Germany). During the experiment, the animals were fed *ad libitum* with standard laboratory food pellets.

Animal sacrifice and retrieval of specimens

Animals were sacrificed in a carbon dioxide euthanasia chamber after 2, 4, 8, 16, and 24 weeks. Residues of the membranes were removed with the surrounding connective tissue and fixed in 4% formalin. All specimens were embedded in paraffin.

Immunohistochemical labelling – angiogenesis

After deparaffinization and rehydration of 5-μm-thin tissue sections, antigen unmasking was performed by heating for 15 min in target retrieval solution (DakoCytomation, Hamburg, Germany). After quenching the activity of endogenous peroxidase with 0.9% hydrogen peroxide in phosphate-buffered saline (PBS) for 15 min at room temperature, non-specific binding sites were blocked with blocking solution for 30 min (Dako). Following a 5 min wash with PBS, the primary mouse monoclonal antibody to transglutaminase II in a dilution of 1 : 110 (Labvision, Fremont CA, USA) or unspecific antibodies, respectively, as negative control were applied to tissue sections in a humidified chamber at room temperature (Haroon et al. 1999; Buemi et al. 2004). The slides were washed in PBS and incubated with secondary biotinylated anti-mouse antibody (Dako) in a dilution of 1 : 60 for 1 h. The presence of antibody–antigen complexes was visualized using a streptavidin–peroxidase solution (dilution 1 : 300) with AEC as the chromogen (Dako). All sections were counter-stained with hematoxylin.

Immunohistomorphometric analysis

From each specimen, three sections representing basal, central and peripheral areas were systematically, uniformly randomly sampled for analysis. For image acquisition a color CCD camera (Color View III, Olympus, Hamburg, Germany) was mounted on a binocular light microscope (Olympus BX50, Olympus). Digital images (original magnification × 100) were evaluated using a software program (analySIS FIVE docu®, Soft Imaging System, Münster, Germany). For each specific time point, the membrane body of each specimen was divided into three equal layers (external, medial, internal) at four fields selected at random. In cases of the bilayered collagen membranes, the external layer represented the cell occlusive surface. For each layer, the cross- and longitudinal-sectional area (CLSA) of immunohistochemically labelled blood vessels was calculated as a percentage of the respective surface (Fig. 1). For each membrane, the period of time needed for a complete and homogeneous transmembraneous vascularization was assessed. All measurements were performed by one blinded and calibrated examiner.

Statistical analysis

A software package (SPSS 12.0, SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. Mean values and standard deviations were calculated for CLSA of blood vessels for each group. Normal distribution was looked for by the Kolmogorov–Smirnow test. As the data were normally distributed, analysis of variance (ANOVA) and *post hoc* testing using Bonferroni's correction for multiple comparisons was used for comparisons between groups. Results were considered statistically significant at $P<0.05$.

Results

Evaluation of biodegradation over time, vascularization, tissue integration, and foreign body reactions utilizing conventional histology has been described previously (Rothamel et al. 2005). Briefly, three wistar rats had to be euthanized prematurely because of severe wound infection (groups: 2, 8, and 24 weeks). The specimens implanted in these rats were excluded from the statistical analysis. In all other cases, postoperative wound healing was uneventful (i.e. no abscesses, no allergic reactions). Histomorphometric analysis revealed that membrane thickness of BG was significantly reduced between 2 and 4 weeks following implantation. In contrast, BM, BME, TD, VN(1), and VN(2) exhibited significant changes of membrane thickness 8 weeks following implantation. For the rest of the observation period, no statistically significant changes were noted for BM, BME, and VN(1). In contrast, 8 weeks following implantation, TD and VN(2) exhibited approximately 60% of membrane thickness as measured after 2 weeks. A significant reduction of membrane thickness was observed after 16 and 24 weeks. Histometric analysis of VN(3) showed that membrane thickness was reduced 16 and 24 weeks following implantation. Histometric analysis failed to demonstrate a reduction of membrane thickness for OS during the entire study period of 24 weeks (Rothamel et al. 2005).

Immunohistomorphometric analysis

CLSA's of blood vessels for each group and each layer at different time points are presented in Figs 1a–h. With exception for OS, immunohistochemical analysis revealed for all membranes investigated a complete and homogeneous transmembraneous vascularization not later than 8 weeks following implantation. However, differences between the membranes were found regarding the initial pattern of angiogenesis. In particular, 2 weeks following implantation, BG and VN(1–3) exhibited comparable mean CLSA's of blood vessels in the external, middle, and internal layer, indicating an almost complete and homogeneous vascularization of the membrane body (Figs 2a and b). While mean CLSA's of blood vessels per layer were comparable for BG and VN(1–2) ($P>0.05$, respectively), VN(3) seemed to allow for a pronounced angiogenesis of the membrane body 2 weeks following implantation ($P<0.01$, respectively) (Figs 3a and b). In contrast, initial angiogenesis pattern was completely different for BM, BME and TD. At 2 weeks following implantation, TD exhibited a similar formation of blood vessels within the external layer of the membrane body when compared with BG and VN(1–3) ($P>0.05$, respectively). The middle and internal layers, however, exhibited only a minute amount of invading blood vessels ($P<0.01$, respectively) (Figs 4a and b). Within the same period of time, BM and BME exhibited the least amount of mean CLSA's of blood vessels in the respective layers of the membrane body ($P<0.05$, respectively) (Fig. 5). For BM, BME and TD, immunohistochemical analysis revealed increasing mean CLSA's of blood vessels within the middle and internal layers between 4 and 8 weeks following implantation. Throughout the study period of 24 weeks, there were no signs of angiogenesis noted for OS (Fig. 6). Immunohistochemical analysis revealed, to a certain extent, a direct correlation between pattern of angiogenesis and biodegradation of each membrane over time. This was particularly

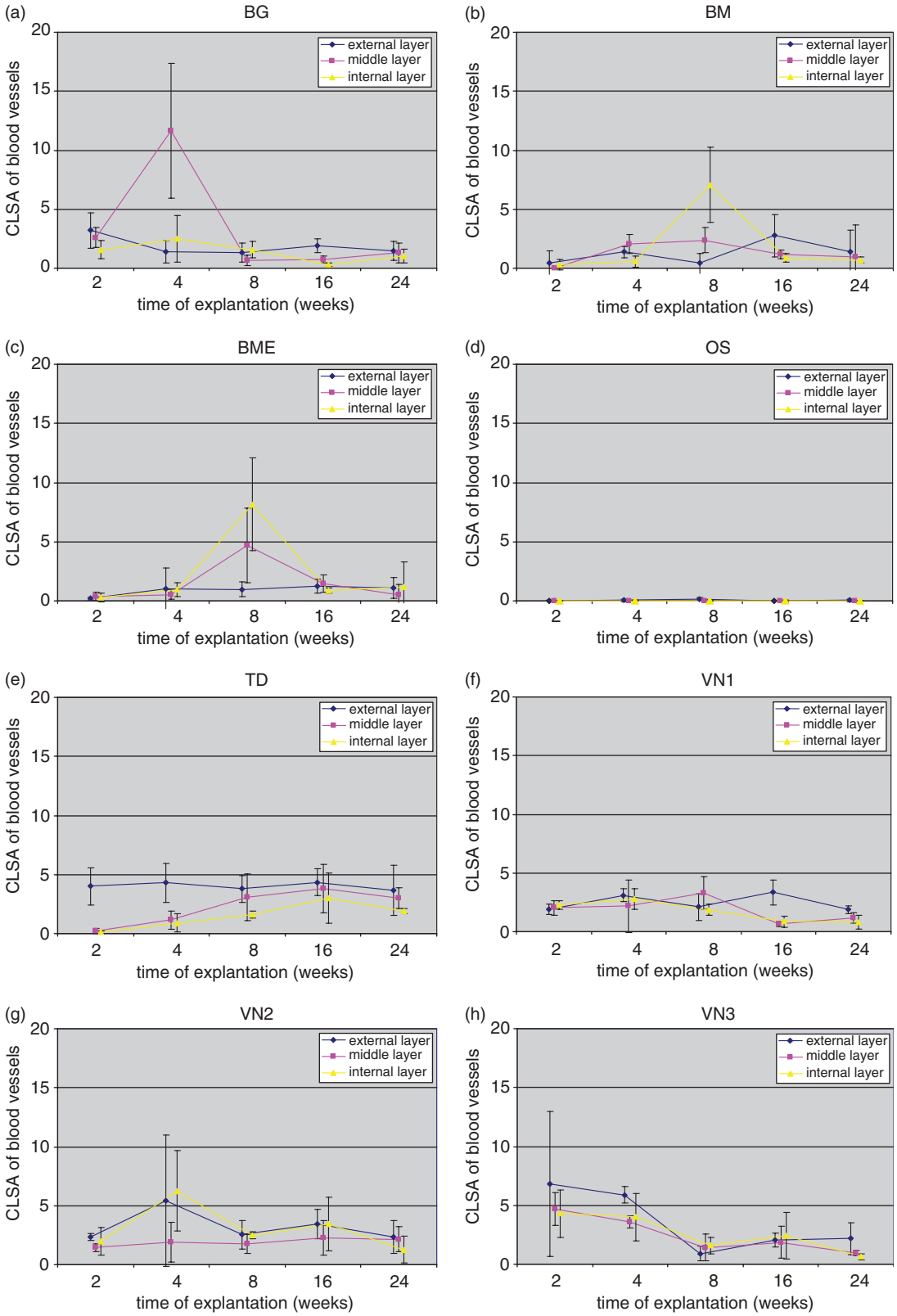

Fig. 1. (a–h). Mean cross- and longitudinal-sectional area of blood vessels (%) ± SD as evaluated 2, 4, 8, 16 and 24 weeks following implantation. Immunohistochemical analysis failed to demonstrate transmembraneous angiogenesis for Ossix® during the entire study period of 24 weeks (d).

true for BG, BM, BME, and TD, as the reduction of the membrane body seemed to be proportional to the extent of transmembraneous formation of blood vessels. In cases of VN(1–3), however, biodegradation of the membrane body seemed to follow transmembraneous angiogenesis with a certain latency. In particular, VN(1–3) exhibited a complete and homogeneous trans-

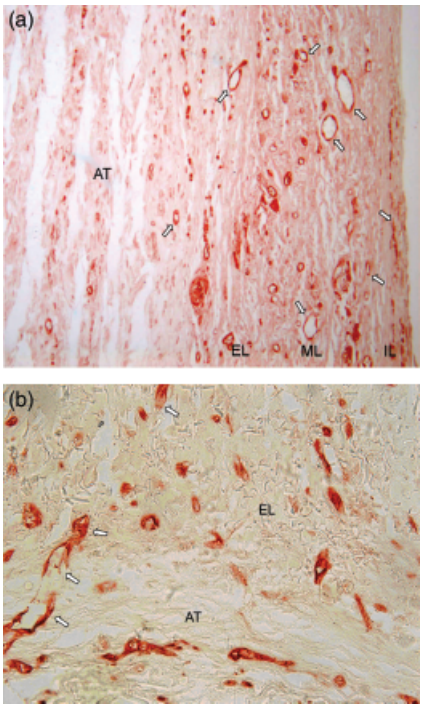

Fig. 2. (a) For Bio Gide® (BG), immunohistochemical staining revealed a homogeneous and complete transmembraneous vascularization 2 weeks following implantation (original magnification × 20). (b) Higher magnification of invading blood vessels from the adjacent tissue into the interconnective porous system of the membrane body of BG (2 weeks, original magnification × 40). AT, adjacent tissue; EL, external layer; ML, middle layer; IL, internal layer; PS, polycarbonate spacer. Arrows indicate the formation of blood vessels in the respective layers.

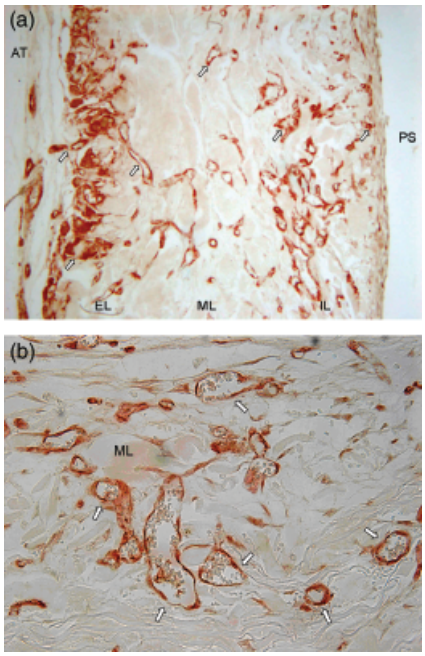

Fig. 3. (a) In comparison with BioGide®, mean cross- and longitudinal-sectional area of blood vessels seemed to slightly increase with the degree of cross-linking in the VN membrane groups (VN(3–2) weeks, original magnification × 20). (b) Higher magnification of the middle layer of VN(3) revealed a homogenous formation of blood vessels (2 weeks, original magnification × 40). For abbreviations see Fig. 2.

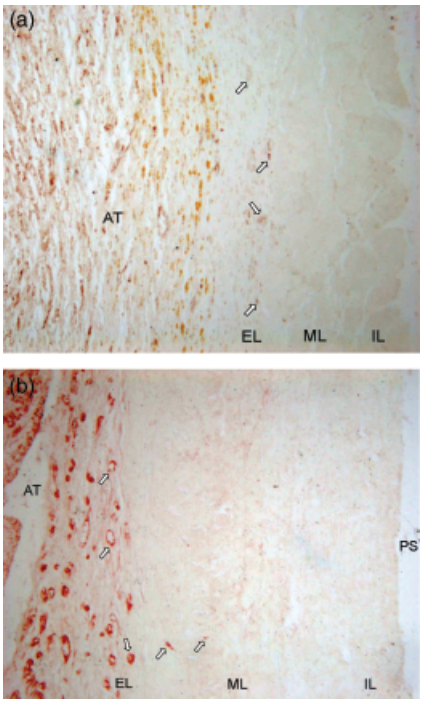

Fig. 4. Two weeks following implantation, angiogenesis merely reached the external layer of the respective membrane bodies of BioMend® (a), BioMend Extend® and TutoDent® (b) (original magnification × 20). For abbreviations see Fig. 2.

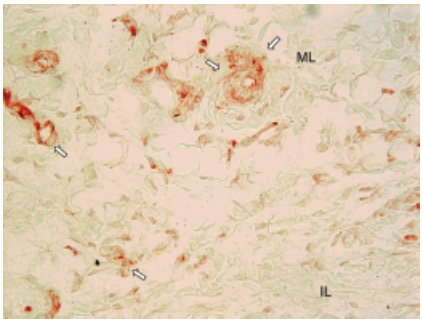

Fig. 5. Higher magnification of BioMend Extend® revealed that blood vessels reached the middle layer of the membrane body merely 4 weeks following implantation (original magnification × 40). For abbreviations see Fig. 2.

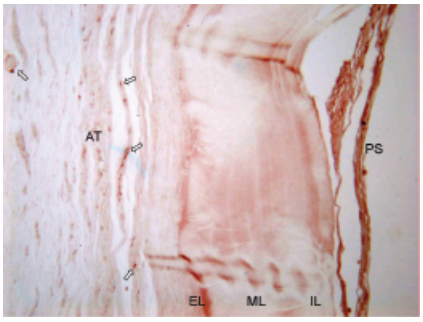

Fig. 6. Throughout the whole study period, there were no signs of angiogenesis noted for Ossix® (24 weeks, original magnification × 20). For abbreviations see Fig. 2.

membraneous vascularization 2 weeks following implantation, while histomorphometric analysis revealed significant changes of membrane thickness after 8 (VN(1) and VN(2)) and 16 weeks (VN(3)), respectively (Rothamel et al. 2005). As a consequence of decreasing mean values of membrane thickness over time (i.e. decreased surface areas of respective layers), each specimen exhibited increasing mean CLSA's of blood vessels. However, these values tended to zero when an almost complete biodegradation of the membrane body was reached (Figs 1a–h).

Discussion

The present immunohistochemical study was designed to evaluate transmembraneous angiogenesis pattern of commercially available and also experimental native and cross-linked collagen membranes after subcutaneous implantation in rats. Within its limits, it was observed that transmembraneous pattern of angiogenesis seemed to markedly differ among the membranes investigated. In particular, it was observed that BG and VN(1–3) exhibited a homogeneous and nearly complete transmembraneous formation of blood vessels 2 weeks following implantation. In contrast, angiogenesis of BM, BME and TD seemed to be delayed, because within the same period of time, blood vessels merely reached the external and middle layer of the membrane body. For these membranes, increasing mean CLSA's of blood vessels within the middle and internal layers have been observed not before 4 and 8 weeks following implantation. In this context, however, it must be pointed out that each membrane also exhibited increasing mean CLSA's of blood vessels as a consequence of decreasing mean values of membrane thickness over time. Furthermore, immunohistochemical labelling of OS failed to demonstrate any formation of blood vessels in the respective layers of this membrane during the whole observation period of 24 weeks. There might be several explanations for the present findings. First

of all, it has to be pointed out that a previous histological analysis revealed obvious differences in the structure of each membrane examined (Rothamel et al. 2005). The membrane body of BG, TD, and VN(1–3) seemed to be structured like an interconnective porous system, however, TD appeared to be more compact. In contrast, BM and BME had a more stratified appearance with large interstices, whereas OS exhibited a dense membrane body without the presence of any cognizable interstices (Rothamel et al. 2005). Based on these findings, angiogenesis pattern might be related to the specific structure of each membrane. Among all the membranes examined, the porous membrane properties noted for BG and VN(1–3) seemed to be most suitable for a premature transmembraneous formation of blood vessels as observed 2 weeks following implantation. In this context, however, it must be emphasized that one limitation of the present study was the lack of earlier examination points in order to evaluate initial pattern of angiogenesis. When interpreting the present results, it has also to be noted that angiogenesis of these membranes seemed to be positively correlated with the degree of cross-linking. Particularly VN(3) seemed to allow for a pronounced angiogenesis of the membrane body 2 weeks following implantation. In this context, it must be pointed out, that biodegradation of VN(3) has been reported to be initially associated with the presence of inflammatory cells in the adjacent connective tissue (Rothamel et al. 2005). Although pronounced foreign-body reactions may prevent or compromise connective tissue integration (Somerman et al. 1991), it is well known that an inflammatory process is mainly characterized by a pronounced hyperemia, which in turn might have ameliorated angiogenesis of VN(3). One possible explanation for the delayed invasion of blood vessels in cases of BM and BME may be because of a decreased membrane biocompatibility caused by cross-linking with glutaraldehyde (Speer et al. 1980; Wiebe et al. 1988). Indeed, results from a recent *in vitro* study have shown that BM appeared to be incompatible with attachment and proliferation of both, human PDL fibroblasts and human osteosarcoma derived (SaOs-2) osteoblasts throughout an observation period of 7 days (Rothamel et al. 2004). To the best of our knowledge, these are the first experimental data evaluating immunohistomorphometrically transmembraneous angiogenesis of native and cross-linked collagen membranes. However, the present results corroborate, to a certain extent, angiogenesis pattern of these specific membranes as evaluated by means of conventional histology (Rothamel et al. 2005). Histomorphometric analysis also revealed a complete vascularization of the membrane body of BG after 2 weeks, and TD, BM and BME between 4 and 8 weeks following implantation. Discrepancies, however, were reported for VN(1–3), as the transmembraneous vascularization of the respective membrane body was merely observed between 8 and 16 weeks (Rothamel et al. 2005). In contrast, immunohistochemical analysis of VN(1–3) revealed a complete transmembraneous formation of blood vessels after 2 weeks. In this context, it must be emphasized that the use of primary mouse monoclonal antibodies to transglutaminase II is a commonly accepted procedure for immunohistochemical labelling of angiogenesis (Haroon et al. 1999; Buemi et al. 2004). All these data, taken together with the results of the present study, seem to indicate that immunohistochemistry using monoclonal antibodies to transglutaminase II seems to provide results more precisely than conventional histology. As mentioned above, it was hypothesized that a GBR/GTR membrane allowing earlier anastomosis of the vasculature of the flap and the wound area may positively influence tissue regeneration (Hardwick et al. 1994). This hypothesis may be supported by the principle of bone formation. Osteogenic cells have been observed to arise from undifferentiated mesenchymal progenitor cells contained in the stroma of bone marrow and from pericytes adjacent to small blood vessels in connective tissue (Long et al. 1995; Rickard et al. 1996; Reilly et al. 1998). Indeed, the results from an animal study have emphasized the significance of angiogenesis in GBR, as the histologic examination revealed a close spatial and temporal correlation between newly formed blood vessels and *de novo* extraskeletal bone formation (Schmid et al. 1997). From a clinical point of view, this hypothesis might also be supported by the fact, that some authors have recommended a perforation of adjacent cortical bone during GBR/GTR procedures in order to open bone marrow spaces which in turn might ameliorate bone formation (Buser et al. 1990; Schmid et al. 1994). On the other hand, however, it must also be emphasized that vascularization may also contribute to membrane degradation, because the monocytes penetrating through the blood vessel wall may differentiate into macrophages. Indeed, the present results have shown that the fast transmembraneous vascularization of BG also seemed to be responsible for the premature biodegradation of this membrane. Although immunohistochemical analysis of VN(1–3) also revealed a complete transmembraneous formation of blood vessels after 2 weeks, the biodegradation of these specific membranes seemed to be prolonged (Rothamel et al. 2005). This observation might be explained by the specific chemical cross-linking procedure used for VN(1–3), facilitating a premature transmembraneous vascularization of the membrane body on the one hand and increasing the resistance against resorption on the other hand. With respect to vascularization, tissue structure and biomechanical conditions, however, it must be queried whether data obtained from an *in vivo* study performed subcutaneously in rats can be applied to the human oral cavity. Moreover, the clinical effect of early angiogenesis perfusion in the membranes and its impact upon the final clinical outcomes (e.g. bone gain) remains to be answered. In this context, another point of interest may be a comparison with e-PTFE membranes which are still considered to be the gold standard barrier membranes, even though this type of material totally excludes blood supply from the flap to the defect.

Within the limits of the present study, it may be concluded that pattern of transmembraneous angiogenesis markedly differs among the membranes investigated.

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要旨
本研究は、ラットの皮下に生来のコラーゲンと交差結合したコラーゲンのメンブレンを埋入した後に、血管新生のパターンを免疫組織化学的に評価した。市販のメンブレン５つと実験的メンブレン（VN）３つを評価した；(1) BioGIde ®(BG)、(2) BioMend® (BM)、(3) BioMend Extend® (BME)、 (4) Ossix® (OS)、 (5) TutoDent® (TD)及び (6-8) VN (1-3)であった。各メンブレンは、４０匹のウィスター・ラットの背中に外科的に剥離して作成した、連結していない皮下パウチに無作為に振り分けて入れ（ｎ＝４）、各群８匹ずつ、５群（２、４、８、１６、２４週）に分けた。血管新生のパターンは、トランスグルタミナーゼ II に対するマウス一次モノクローナル抗体を用いて標識した。各メンブレンについて、メンブレンを貫通した完全で均質な血管新生までに必要な期間を免疫組織形態学的に評価した。メンブレン埋入後２週間後の評価で、メンブレンを貫通した血管新生の初期パターンに、メンブレン間の差異が認められた。血管の平均断面積および縦断面積（%）はＶＮ（３）[5.27±2.73]が最大であり、続いてＢＧ[2.45 ±0.88]、ＶＮ（１）[2.07 ± 0.29]、ＶＮ（２）[1.91± 0.55]、ＴＤ[1.44 ± 0.53]、ＢＭＥ[0.35± 0.29]、ＢＭ[0.25 ± 0.4]であった。ＢＧ及びＶＮ（１－３）とは対照的に、ＢＭ、ＢＭＥ及びＴＤは埋入後わずか４－８週間でメンブレンを貫通した均質な血管形成を示した。しかしＯＳは全試験期間を通して血管新生を示さなかった。
本研究の制約内において、メンブレンを貫通した血管新生のパターンは、試験したメンブレン間で顕著に異なっていると結論される。

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