Membrane Durability and Tissue Response of Different Bioresorbable Barrier Membranes: A Histologic Study in the Rabbit Calvarium

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Purpose: *The objective of the present study was to histologically evaluate barrier durability and host tissue response of new prototype collagen membranes in comparison to clinically available collagen and synthetic polymer membranes.* ***Materials and Methods:*** *The experimental study was conducted in 20 rabbits with 4 different healing periods of 2, 6, 12, and 28 weeks. Following surgical exposure of the calvarium, 6 circular bone defects (diameter 4 mm, depth 1.5 mm) were drilled into the outer cortex. After the bone had been removed, each defect was covered with 1 of 6 different membranes: 3 collagen prototype membranes, a Bio-Gide collagen membrane (BG), a glycolide-lactide-trimethylene carbonate Osseoquest membrane (OQ), and a polylactide Atrisorb membrane (AS). Histological analysis was performed following staining with toluidine blue and transversal sectioning of the calvarial bone.* ***Results:*** *All collagen membranes showed similar tissue integration characterized by fibrous encapsulation with differentiation of a periosteumlike tissue upon the external bony surface. One prototype collagen membrane displayed clearly longer membrane integrity. The evaluated synthetic membranes demonstrated extended barrier durability but also exhibited inflammatory foreign-body reactions.* ***Discussion:*** *Recent experimental investigations have shown that degradation of collagen membranes may begin within days to weeks of membrane placement. This was confirmed in the present study. However, 1 of the chemically modified collagen prototype membranes exhibited prolonged membrane integrity in the absence of an inflammatory tissue response.* ***Conclusion:*** *Further investigation of the prototype membrane that showed prolonged membrane integrity to evaluate its potential in GBR procedures is needed.* Int J Oral Maxillofac Implants 2005;20:843–853

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The application of barrier membranes in guided bone regeneration (GBR) has become a widely used surgical technique in implant dentistry.[1,2] In the initial phase of establishing this surgical technique, membranes made of expanded polytetrafluoroethylene (ePTFE) were widely used.[3] Although clinical and experimental studies have shown excellent treatment results using ePTFE membranes in GBR procedures,[4–6] the outcome was influenced by wound healing complications, in particular, soft tissue dehiscence.[7–9] A number of studies have reported wound infection sequelae following the exposure of ePTFE membranes and subsequently a poor outcome in bone regeneration.[10–12] The ePTFE material is not subject to biologic resorption; thus, the membrane must be surgically removed. This implies discomfort and increased cost to the patient, as well as the risk of losing some of the regenerated bone because of resorption following flap elevation and membrane removal.[13]

For these reasons, clinicians and researchers have advocated the use of resorbable barrier membranes in GBR procedures. Currently the 2 materials most commonly used to produce resorbable membranes are collagen derived from an animal source and synthetic aliphatic polyesters.[14] Although both substances are considered biomaterials, each has distinctive features and biologic effects.[15,16] Important aspects of clinical significance are duration of membrane integrity (ie, duration of barrier function) and tissue response (ie, barrier biocompatibility), as related to membrane breakdown. Several investigations have drawn attention to the fact that membranes made of aliphatic polyesters elicit an inflammatory tissue response following degradation.[17–19] In addition, superficial resorption of newly-formed bone may occur following breakdown of membranes containing aliphatic polyesters.[20,21] Conversely, while collagen membranes have been deemed as more "tissue-friendly," these membranes have also been reported as having unfavorable mechanical properties[22] and inadequate barrier durability.[23–25]

The objective of the present study was to evaluate new prototype membranes (1 physically cross-linked and 2 chemically cross-linked porcine type I and type III collagen membranes) relative to barrier durability and tissue response following coverage of circular bony defects in the rabbit calvarium in comparison to presently available collagen or synthetic polymer membranes.

MATERIALS AND METHODS

Study Design

The study protocol was approved by the national authorities (Department of Agriculture, Section Veterinary Service, Experimental Animal Studies, study number 55/01, Bern, Switzerland). The experimental study was conducted in 20 adult Burgundy rabbits, each at least 5 months old and weighing between 4 and 5 kg. Initially, 5 groups each containing 4 animals were created for analysis of the membranes after healing periods of 2, 6, 12, 24, and 36 weeks.

Medication of Animals

All surgeries were performed under intravenous general anesthesia. The animals were premedicated with ketamine (65 mg/kg; Narketan; Vétoquinol, Bern, Switzerland) and xylazine (4 mg/kg; Xylapan, Vétoquinol) mixed and injected intramuscularly into the hind leg. Subsequently, a canula was placed into the lateral ear vein. General anesthesia was maintained with an intravenous infusion of ketamine and xylazine (double quantity of premedication dosage) in 100 mL physiologic saline. Postoperatively, the animals were given analgesics for 3 days (50 mg/kg, once a day intramuscularly; Novalgin; Aventis, Zurich, Switzerland). No antibiotics were administered.

Surgical Protocol

The animals were shaved on the top of the head between the eyes and the ears. The skin was disinfected using a povidone-iodine solution (Betadine; Mundipharma, Basel, Switzerland). After a local anesthetic (1 mL Ultracain DS; Aventis Pharma, Frankfurt am Main, Germany) was administered, a midline incision was made, and the skin and periosteum were reflected to expose the vault of the skull. Using a bone trephine, circular bone defects (diameter 4 mm, depth 1.5 mm) representing a non-critical size were drilled into the outer cortex (*tabula externa*). Caution was taken not to perforate the inner cortex (*tabula interna*) and thereby to avoid contact with the *dura mater*, although this was not always possible. A total of 6 bony defects were created, 3 on each side of the sagittal suture (Fig 1a). After removing the cortical bone (Fig 1b), the 6 defects were covered with 6 different membranes (Fig 1c and Table 1).

Each membrane was cut and placed individually to extend beyond the defect margins by approximately 2 mm. No additional material was used to stabilize the membranes. All the membranes were somewhat hydrophilic and therefore adhered to the bony surface after becoming soaked with blood. Wound closure was accomplished in a 2-layer technique. The periosteum (*galea aponeurotica)* was closed using ePTFE-suturing material (Gore-Tex Suture CV-5, WL Gore & Associates, Flagstaff, AZ). This suture material was chosen to avoid interference with membrane tissue reactions, since ePTFE is an inert and biocompatible material. The skin was closed with interrupted sutures (Vicryl 5-0, Johnson & Johnson/Ethicon, Somerville, NJ).

Sacrifice

Following the induction of general anesthesia with the same medications used presurgery, a canula was placed into the lateral ear vein. Animals were euthanized with Nembutal (1.4 mL/kg; Abbott Laboratories, North Chicago, IL). A rectangular skin incision was made, and the calvarium was removed with an oscillating autopsy saw. The retrieved specimens were immediately immersed in a solution of 4% formaldehyde and 1% calcium.

Histologic Analysis

The nondecalcified specimens were embedded in methylmethacrylate resin and stained with toluidine blue. Transversal sections with a thickness of approxi-

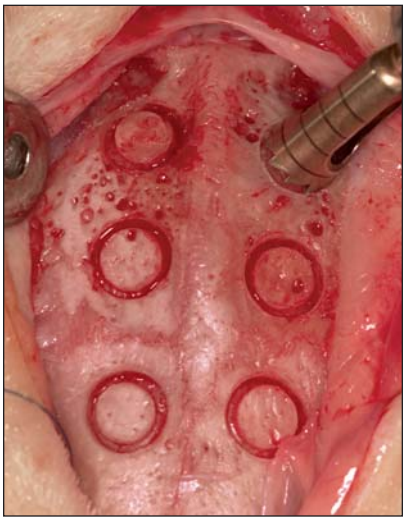


Fig 1a Six circular defects (diameter 4 mm) were drilled into the outer cortex of the skull using a trephine.

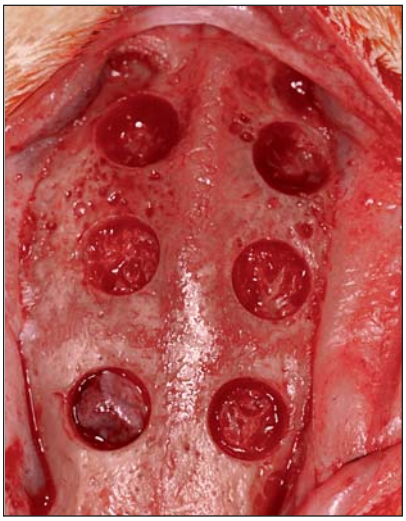


Fig 1b Following removal of the cortical bone, the cancellous bone was visible.

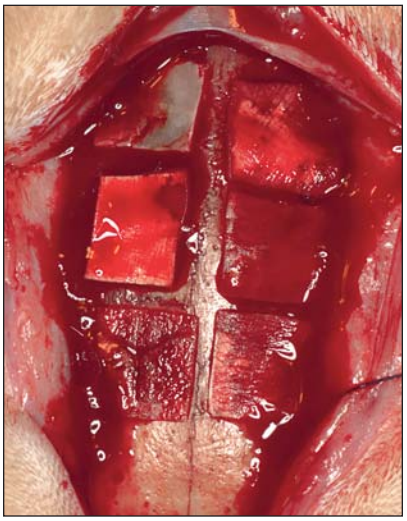


Fig 1c The 6 defects covered with the control and test membranes.

Table 1 Characteristics of Tested Membranes

Membrane	Manufacturer	Material	Origin
Bio-Gide (BG)	Geistlich Wolhusen, Switzerland	Collagen types I and III (without cross-linking or chemical treatment)	Porcine
VN p (prototype)	Geistlich	Physically cross-linked collagen types I and III	Porcine
VN c/EN (prototype)	Geistlich	Chemically cross-linked collagen types I and III	Porcine
VN c/E (prototype)	Geistlich	Chemically cross-linked collagen types I and III	Porcine
Osseoquest (OQ)	Gore & Associates Flagstaff, AZ	Glycolide, lactide, and trimethylene carbonate copolymer	Synthetic
Atrisorb (AS)	Atrix Laboratories Fort Collins, CO	Polylactide dissolved in methyl-pyrrolidone	Synthetic

mately 80 mm were obtained. The sections showing the greatest defect diameters were chosen for descriptive histology.

RESULTS

Clinical Healing

All animals in the 2-, 6-, and 12-week groups healed without complications and were sacrificed as scheduled. Two animals from the 24-week healing group and 1 animal from the 36-week healing group developed a systemic infection and were excluded from further analysis. To have a sufficient number of animals with an extended healing period, the remaining 5 animals were combined to become a single 28-week healing group and were sacrificed accordingly.

Descriptive Histology

The histologic analysis is reported separately for each type of membrane accounting for the different healing periods. However, 2 of the 3 prototype collagen membranes, types VN c/E and VN p, demonstrated similar findings as compared to the commercially available collagen membrane (BG). Therefore, detailed description of these 2 specific prototypes has been omitted. The histologic findings are summarized in Table 2.

BG

2 Weeks. The membrane was fully intact and maintained the defect space (Figs 2a and 2b). It appeared intensely stained due to a network of thick fiber bundles apparently consisting of collagen type III. The outer, denser part of the membrane was covered by

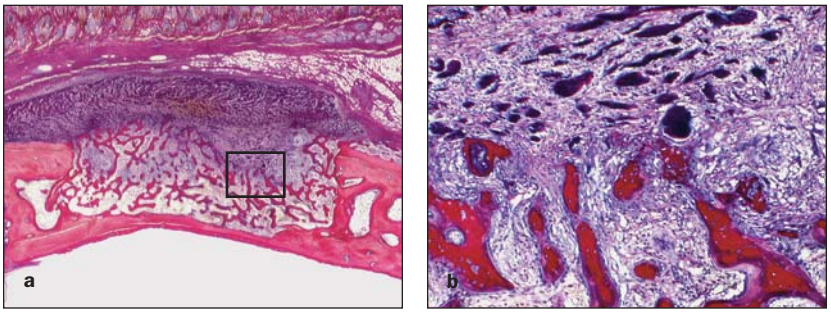
Table 2 Summary of Histologic Findings for Each Membrane and Healing Period

Membrane	2 weeks' healing	6 weeks' healing	12 weeks' healing	28 weeks' healing
BG	Membrane intact, outer part covered with fibrous capsule, inner part in direct contact with newly formed bone.	Membrane reduced in width, capillaries frequently seen in the interfibrillar meshes.	Differentiation between membrane and host collagen almost impossible, dense collagen fibers parallel to the reconstituted *tabula externa.*	Continuous connective tissue layer over former defect covered by adipose tissue, subdivided like a mattress by interposition of fibrous tissue sheets.
VN c/EN	Membrane fully intact, outer portion of membrane covered by fibrous capsule.	Thickness of membrane still maintained, interfibrillar space invaded by capillaries and cellular elements.	Collagen membrane was largely integrated in host tissue. Defect covered by periosteum, possibly including remnants of the collagen membrane.	Dense connective tissue functioned as a periosteum. Matrix was dense, almost hyaline, and included disseminated groups of adipocytes. Matrix layers possibly contained remnants of porcine collagen.
OQ	Membrane integrity intact, but ingrowth of macrophages and fibroblasts. Fibrous capsule covered membrane surface, inflammatory cells quite frequent in this location.	Membrane still intact, showed fibrous encapsulation, foreign-body giant cells could be seen in contact with membrane material.	Degradation of membrane material results in formation of separate globular elements. Cellular response included foreign-body giant cells and macrophages, fibrous capsule still present around membrane.	Membrane still existed. Degradation products smaller and enclosed in fibrous tissue, invaded by blood vessels, macrophages, and foreign-body giant cells. Osteoclasts and Howship's lacunae were seen, indicating bone resorption activated by breakdown products of the polymers.
AS	Membrane with variable thickness and partially subdivided by connective tissue septae, fibrous capsule present around the membrane.	Shape and integrity of membrane maintained, surface of membrane completely surrounded by dense fibrous capsule. Marginal inflammatory infiltration could be seen.	Shape and integrity of membrane preserved, encapsulation and cellular response had not changed.	Degradation throughout the membrane space, blood vessels had entered the membrane space, accompanied by inflammatory cells, macrophages, and giant cells.

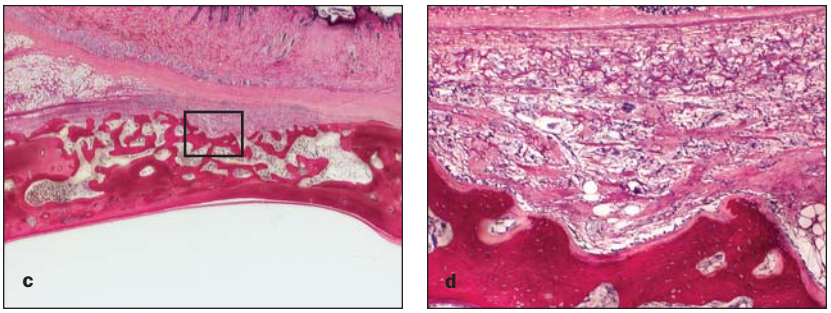
a fibrous capsule, whereas the inner, looser portion was in direct contact with newly formed bone and the marrow space. The cellular response of the bone marrow was dominated by the formation of granulation tissue. The space between the thick fiber bundles was invaded by capillaries, mesenchymal cells, fibroblasts, macrophages, and round cells. This cellular and vascular invasion engaged both the outer and inner surfaces of the membrane and extended throughout its full thickness. The outer and inner compartments met approximately one third of the way from the external membrane surface. The bony defect was already bridged by a scaffold of newly formed woven bone. Woven bone trabeculae emerged from the defect floor and lateral walls and spread as an array of interconnected bars and plates throughout the defect cavity. The intertrabecular space was filled with fibrous primary bone marrow. The interface between membrane and bone marrow was clearly demarcated.

6 Weeks. The membrane was reduced in thickness, with a slight collapse in the center (Figs 2c and 2d). The dark stained fiber bundles had disappeared, while strands of wavelike fibers, probably collagen type I, persisted. A few foreign-body giant cells were present. Blood vessels, mostly capillaries, were frequently seen in the interfibrillar meshes. The bone density in the defect area had increased. A periosteumlike tissue including vascular and fibrous components had differentiated upon the external bony surface. The bone marrow had matured, as indicated by the development of a larger number of adipocytes, intermingled with blood-forming islands.

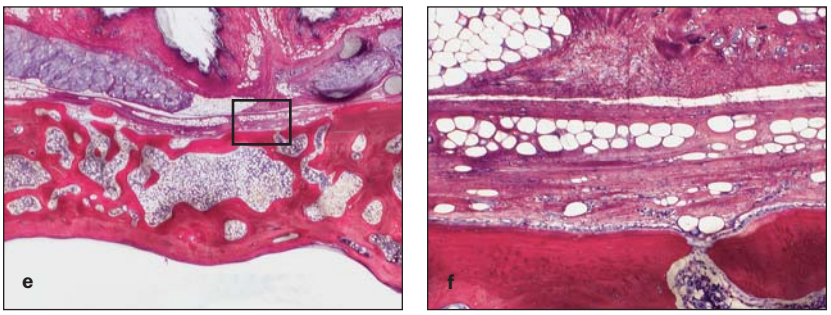
12 Weeks. Differentiation between membrane and host collagen was virtually impossible (Figs 2e and 2f). The collagen fibers were arranged in rather dense layers that ran parallel to the reconstituted *tabula externa*. A periosteum had developed that continued with osteoblastic bone apposition. Adipose tissue had appeared in the connective tissue at some distance to the bone, and some scattered adipocytes were also located in the periosteal layer. The structure of the bone marrow was, compared to the 6-week specimen, unchanged. Both cortical tabu-



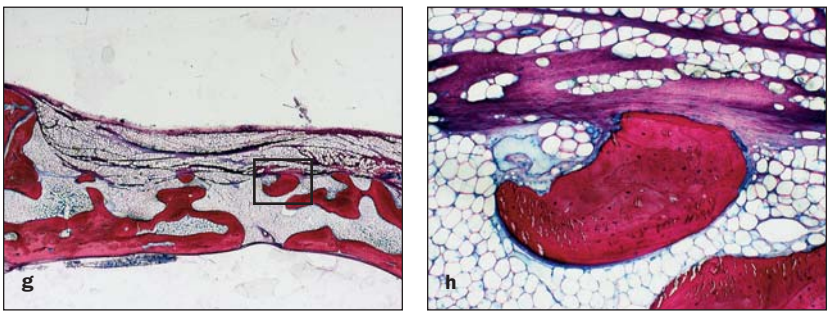
Figs 2a and 2b Transversal histologic sections of defects covered with BG membrane at 2 weeks. *(a)* An overview of the sample (toluidine blue; original magnification ×18). *(b)* Enlargement of marked area (original magnification ×120).



Figs 2c and 2d Transversal histologic sections of defects covered with BG membrane at 6 weeks. *(c)* An overview of the sample (toluidine blue; original magnification ×18). *(d)* Enlargement of marked area (original magnification ×120).



Figs 2e and 2f Transversal histologic sections of defects covered with BG membrane at 12 weeks. *(e)* An overview of the sample (toluidine blue; original magnification ×18). *(f)* Enlargement of marked area (original magnification ×120).



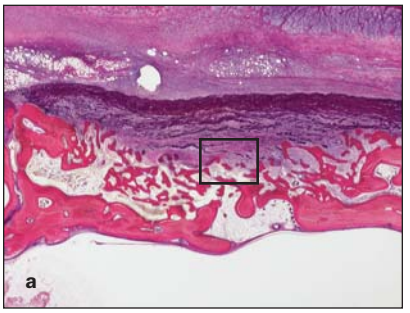
Figs 2g and 2h Transversal histologic sections of defects covered with BG membrane at 28 weeks. *(g)* An overview of the sample (toluidine blue; original magnification ×20). *(h)* Enlargement of marked area (original magnification ×120).

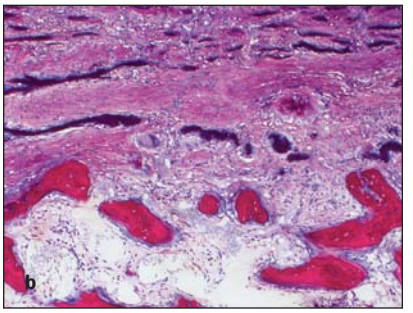
lae had further increased in width, and the diameter of the interconnecting trabeculae had also increased.

28 Weeks. The inner surface of the membrane could be located as a continuous fibrous tissue layer that was suspended like a hammock between the upper end of the bony pillars that emerged from the *tabula interna* and traversed the entire defect space (Figs 2g and 2h). This connective tissue layer was covered by adipose tissue that again was subdivided like a mattress by the interposition of fibrous tissue sheets. The adipose tissue had some peculiar aspects: in the marrow region, small adipocytes were tightly packed into the intertrabecular space, whereas in the compartment that was derived from the membrane area, the adipoctes were somewhat larger.

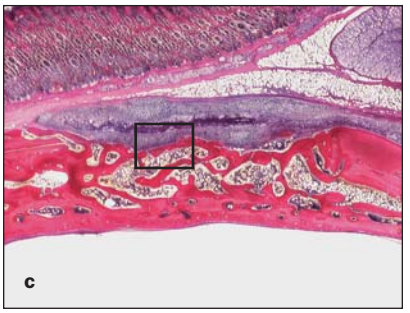
VN c/EN

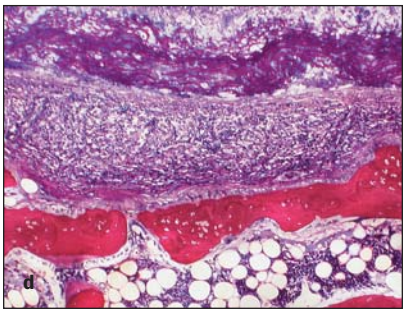
2 Weeks. Fully intact, the membrane exhibited a denser meshwork and darker staining in the outer compartment as compared to BG (Fig 3a). The outer portion of the membrane was covered with a fibrous capsule deposited by host tissue. The bone formed in the defect space presented as a rather loose scaffold of woven bone, with primary bone marrow filling the intertrabecular space. In those areas where the bone marrow and the collagen membrane were in direct



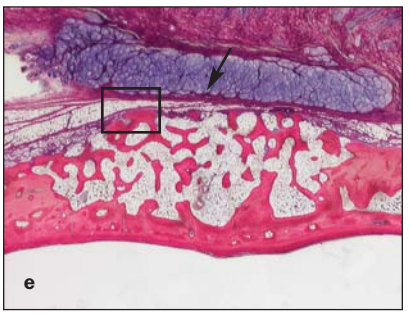


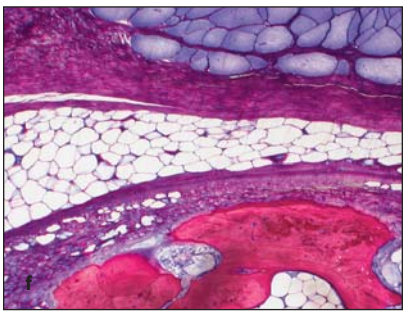
Figs 3a and 3b Transversal histologic sections of defects covered with prototype Vn c/EN membrane at 2 weeks. *(a)* An overview of the sample (toluidine blue; original magnification ×18). *(b)* Enlargement of marked area (original magnification ×120).



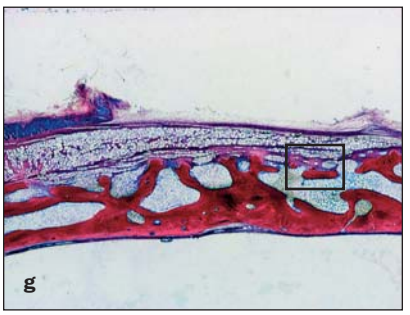


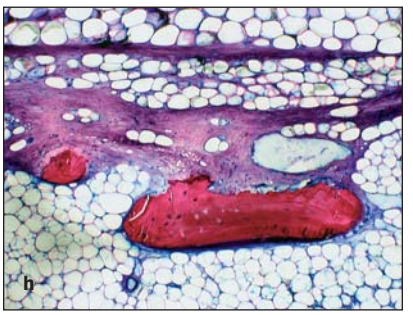
Figs 3c and 3d Transversal histologic sections of defects covered with prototype Vn c/EN membrane at 6 weeks. *(c)* An overview of the sample (toluidine blue; original magnification ×18). *(d)* Enlargement of marked area (original magnification ×120).





Figs 3e and 3f Transversal histologic sections of defects covered with prototype Vn c/EN membrane at 12 weeks. *(e)* An overview of the sample (toluidine blue; original magnification ×18). *(f)* Enlargement of marked area (original magnification ×120).



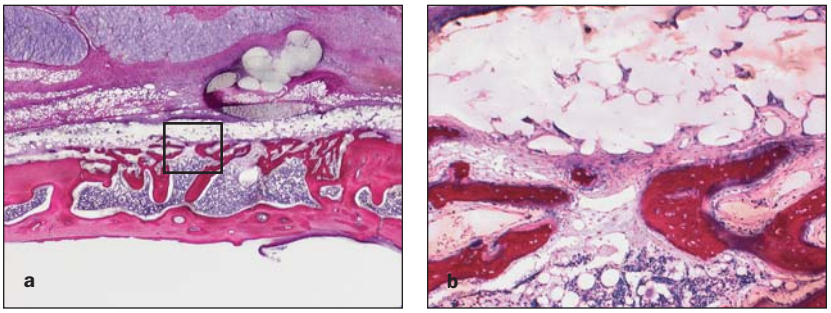


Figs 3g and 3h Transversal histologic sections of defects covered with prototype Vn c/EN membrane at 28 weeks. *(g)* An overview of the sample (toluidine blue; original magnification ×20). *(h)* Enlargement of marked area (original magnification ×120).

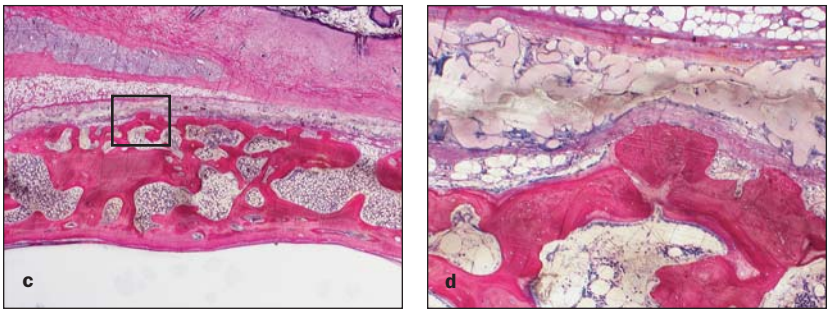
contact (Fig 3b), there was a gradual structural transition as a result of differentiation of a granulation tissue and the proliferation of capillaries that invaded the interfibrillar space of the collagen meshwork. In addition, osteoblasts produced a framework of woven bone that bridged the defect to a full extent.

6 Weeks. The thickness of the membrane was still fully maintained but had now acquired a trilayer structure (Fig 3c). The middle layer was rather narrow, and the heavy staining showed that most of the original thick fiber bundles were preserved (Fig 3d). Both in the outer and inner zones, most of this material was degraded or resorbed. The interfibrillar space had been invaded by capillaries and other cellular elements provided by the host, and the overall tissue density was greatly reduced. Bone formation in the defect had resulted in a coherent cancellous framework that connected the *tabula interna* to an almost completely reconstructed *lamina externa*.

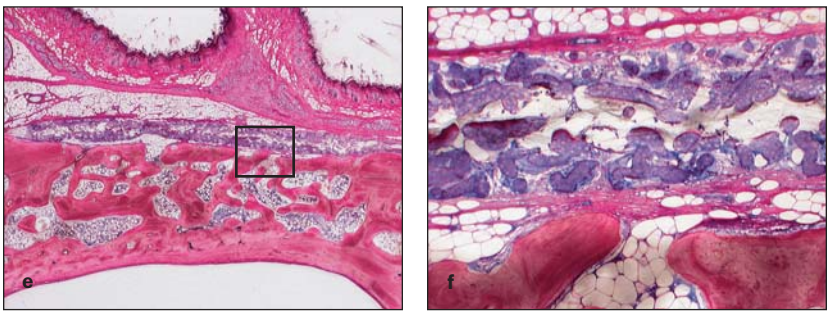
12 Weeks. The collagen membrane had integrated to a large extent within the host tissue (Figs 3e and 3f). One of the craniofacial muscles had become incorporated in the *galea aponeurotica* (Fig 3e). Beneath was the cranial vault with the fully repaired defect (Fig 3e). It was covered by periosteum that may have included remnants of the colla-



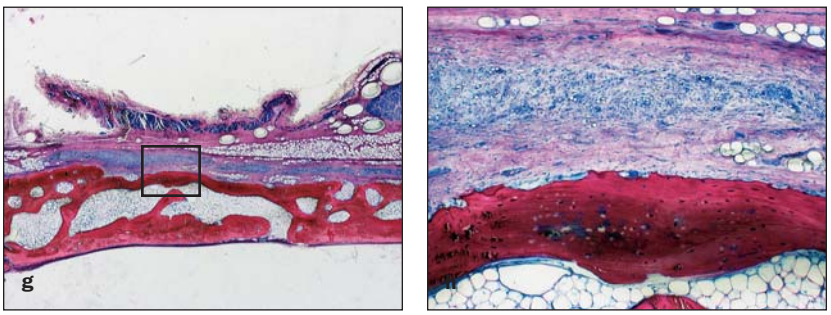
Figs 4a and 4b Transversal histologic sections of defects covered with prototype OQ membrane at 2 weeks. *(a)* An overview of the sample (toluidine blue; original magnification ×18). *(b)* Enlargement of marked area (original magnification ×120).



Figs 4c and 4d Transversal histologic sections of defects covered with prototype OQ membrane at 6 weeks. *(c)* An overview of the sample (toluidine blue; original magnification ×18). *(d)* Enlargement of marked area (original magnification ×120).



Figs 4e and 4f Transversal histologic sections of defects covered with prototype OQ membrane at 12 weeks. *(e)* An overview of the sample (toluidine blue; original magnification ×18). *(f)* Enlargement of marked area (original magnification ×120).



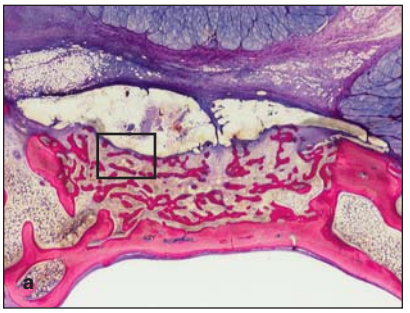
Figs 4g and 4h Transversal histologic sections of defects covered with prototype OQ membrane at 28 weeks. *(g)* An overview of the sample (toluidine blue; original magnification ×20). *(h)* Enlargement of marked area (original magnification ×120).

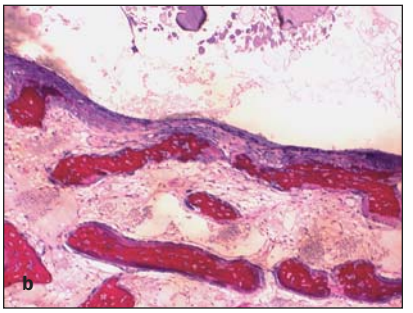
gen membrane (Fig 3f). Cancellous bone and the newly formed *lamina externa* were separated from the periosteum by adipose tissue, which may have facilitated gliding motions between the 2 stiffer components.

28 Weeks. The bone defect was filled with rather thick trabeculae that emerged from the thick, reinforced *lamina externa* (Fig 3g). The outer ends formed footlike horizontal plates that were covered with a rather dense connective tissue membrane that functioned as a periosteum. The matrix of the connective tissue was dense, almost hyaline, and included disseminated small groups of adipocytes (Fig 3h). Thinner fibrous tissue sheets ran parallel to the periosteal surface and subdivided the adipose tissue into flat cushions. It is possible that these matrix layers contained remnants of porcine collagen, but they were impossible to identify without immunohistochemistry.

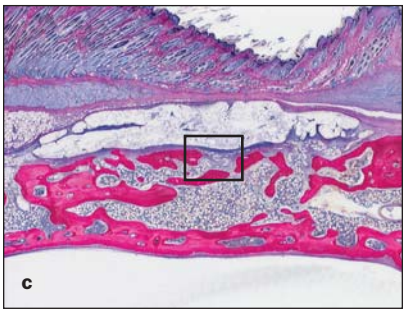
OQ

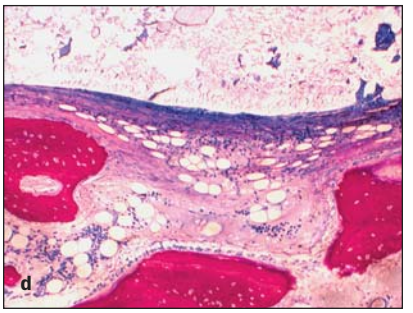
2 Weeks. The membrane was intact, and no collapse could be seen (Fig 4a). The polymer was unstained but not homogenous. Small notches at the surface were connected to fissures and allowed an ingrowth of cells, presumably macrophages and fibroblasts (Fig 4b). A fibrous capsule had formed and covered



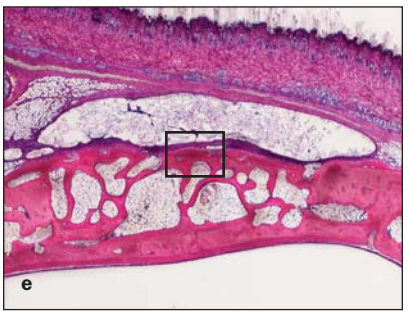


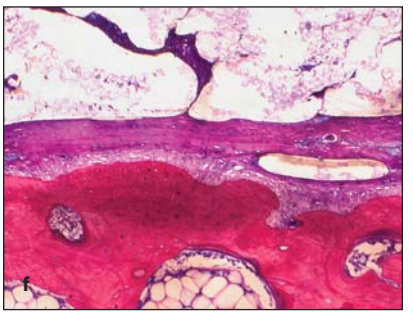
Figs 5a and 5b Transversal histologic sections of defects covered with the AS membrane at 2 weeks. *(a)* An overview of the sample (toluidine blue; original magnification ×18). *(b)* Enlargement of marked area (original magnification ×120).



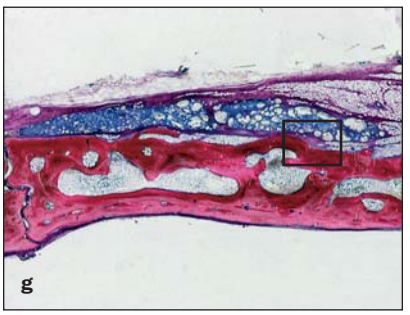


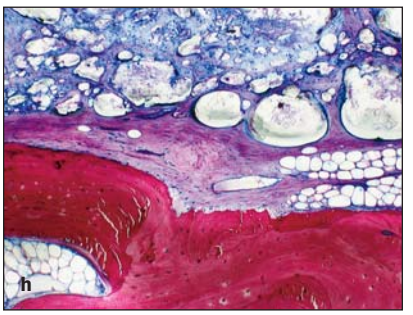
Figs 5c and 5d Transversal histologic sections of defects covered with the AS membrane at 6 weeks. *(c)* An overview of the sample (toluidine blue; original magnification ×18). *(d)* Enlargement of marked area (original magnification ×120).





Figs 5e and 5f Transversal histologic sections of defects covered with the AS membrane at 12 weeks. *(e)* An overview of the sample (toluidine blue; original magnification ×18). *(f)* Enlargement of marked area (original magnification ×120).





Figs 5g and 5h Transversal histologic sections of defects covered with the AS membrane at 28 weeks. *(g)* An overview of the sample (toluidine blue; original magnification ×20). *(h)* Enlargement of marked area (original magnification ×120).

the outer surface of the membrane, and inflammatory cells were quite frequent in this location. Along the interface between membrane and bone marrow, the fibrous capsule consisted of finer collagen fibrils and was well vascularized (Fig 4b). Woven bone formation resulted in a net of thin, bifurcating trabeculae that enclosed primary marrow.

6 Weeks. The membrane remained intact and showed fibrous encapsulation (Fig 4c). Foreign-body giant cells could be seen in contact with the membrane material (Fig 4d). Bone formation was characterized by dense scaffold of mature cancellous bone. A thin layer of connective tissue separated the newly formed bone from the membrane (Fig 4d). This layer was periosteal; it included bone matrix apposition and eroded parts of bone surface.

12 Weeks. There was evidence of degradation of the membrane material, resulting in the formation of separate globular elements (Figs 4e and 4f). These were heavily stained with toluidine blue, which binds to acidic breakdown products of the polylactides and polyglycolides (Fig 4f). The membrane appeared to disintegrate from both surfaces toward the center. This gave it a trilayer appearance with a less stained, still highly polymerized middle zone. The heavily stained partially degraded breakdown

products were toward the surface. The cellular response included foreign-body giant cells and macrophages.

28 Weeks. The membrane still existed. It formed a barrier between the soft tissues (scalp) of the skull and the periosteum, which covered the reconstructed *tabula externa* in the roof of the bony defect (Fig 4g). The degradation and resorption of the material had profoundly altered the internal structure and the staining properties. The degradation products were much smaller and enclosed in fibrous tissue that was invaded by blood vessels and numerous round cells, macrophages, and foreign-body giant cells (Fig 4h). The interface with the bone was formed by a periosteum. Osteoclasts and Howship's lacunae were frequently seen and indicated some activation of bone resorption by the molecular breakdown products of the polymers (Fig 4h).

AS

2 Weeks. The membrane showed a variable thickness and was partially subdivided by connective tissue septae (Fig 5a). A fibrous capsule was present around the membrane, with a denser structure above and a thin periosteumlike structure below the membrane (Fig 5b). New woven bone formation had led to a nearly complete filling of the defect.

6 Weeks. The shape and integrity of the membrane were maintained (Fig 5c). The surface of the membrane was still completely surrounded by a dense fibrous tissue capsule. Only a marginal inflammatory infiltration was seen. Foreign-body giant cells were present in the fissures of the membrane that were open to the surface (Fig 5d). New bone formation continued.

12 Weeks. The shape and integrity of the membrane were preserved (Fig 5e). Encapsulation and cellular response had not changed as compared to the 6-week stage. Staining of the material was nearly absent, indicating that no internal changes of the membrane structure had occurred. Bone formation had resulted in a complete reconstruction of the *tabula externa;* however, this bone was separated from the membrane by a stripe of darker stained connective tissue (Fig 5f), representing the capsule blended with the *stratum fibrosum* of the periosteum.

28 Weeks. Profound structural changes occurred between the 12th and 28th weeks (Figs 5g and 5h). Degradation began and spread throughout the membrane space, which was still clearly demarcated by the capsule. The polymer decayed into small particles that, again, had a high affinity to basic dyes like toluidine blue. Blood vessels entered the membrane space, accompanied by inflammatory cells, macrophages, and giant cells. In spite of these metabolic activities, bone formation and remodeling continued, with a preference for osteoclastic resorption by the periosteum that lined the outer surface of the *lamina externa*.

DISCUSSION

The present experimental study describes the histologic observations of 6 different barrier membranes placed over circular defects (4 mm wide × 1.5 mm deep) in the rabbit calvarium. The size of the defect created was considered noncritical. However, the objective of the study was to analyze the tissue reactions toward the tested membranes rather than the bony healing of the created defects. Three of 20 animals developed a systemic infection and had to be euthanized prematurely. No obvious reasons were found for these incidents. None of the animals had received antibiotic prophylaxis.

Historically, bioresorbable membranes have been developed for periodontal regeneration purposes and may not be adequate to promote extensive bone healing.[15] In guided tissue regeneration (GTR) procedures, a short membrane barrier function of only 4 to 6 weeks has been advocated for periodontal regeneration,[26] in contrast to the prolonged period of at least 6 months recommended for GBR procedures.[1,27,28] It is therefore likely that the duration of membrane integrity is a key issue for the formation and maturation of new bone in membrane-protected defects.[4,28]

Recent experimental investigations have shown that degradation of collagen membranes may begin within 4 to 28 days after membrane placement.[24,25] An experimental study in mongrel dogs failed to prove any beneficial effects of a prototype hybrid membrane consisting of 2 collagen layers with an intermediate layer of polylactide for GBR in lateral ridge augmentation.[21] The objective of the trilayer membrane configuration and the addition of polylactide was to enhance membrane stiffness and to retard the resorption rate of the prototype barrier. At the completion of the study after 4.5 months, no remnants of the collagen layers were discernible, while the internal polylactide layer showed fragmentation.

In the present study the bilayer collagen membrane (BG) and 2 prototype collagen membranes (types VN c/E and VN p) demonstrated histologic signs of biodegradation at 6 weeks and had disappeared after 12 weeks. In contrast, components of the chemically modified prototype membrane VN c/EN were still visible in the 12-week specimens. In addition, this membrane seemed to promote bone formation even above the level of the original *tabula*

externa. The 2 synthetic membranes (OQ, AS) also persisted beyond 12 weeks, but both membranes exhibited early fibrous encapsulation and a less favorable pattern of new bone formation.

The favorable effect of collagen membranes on bone formation seems to be supported by a recent in vitro study comparing the effects of resorbable membranes made of either collagen, hyaluronic acid, or poly DL-lactide on human osteoblasts.[29] Collagen and hyaluronic acid membranes cultured on osteoblasts greatly enhanced the secretion of type I collagen, alkaline phosphatase, and transforming growth factor-β_1 (TGF-β_1). Additionally, the proliferation of osteoblasts was accelerated, which suggests that these membrane types promote bone regeneration through a direct effect on osteoblasts.

Another recent study compared the biocompatibility of 4 different types of collagen membranes in cultures of human PDL fibroblasts and human osteoblast-like cells.[30] The authors concluded that 3 membranes, Bio-Gide, Tutodent (Tutogen Medical, Neunkirchen, Germany), and Ossix (3i/Implant Innovations, Palm Beach Gardens, FL), promoted, whereas BioMend (Integra, Plainsboro, NJ) inhibited the attachment and proliferation of human PDL fibroblasts and human osteoblasts. The cross-linking of glutaraldehyde and bovine type I collagen in the BioMend membrane was cited as a possible reason for the decreased biocompatibility of this collagen membrane. Although these findings seem to demonstrate that collagen membranes have a direct effect on bone formation, the ideal time period that the membrane should retain its barrier function to maximize the healing results has still not been precisely determined.

An important and often mentioned reason for the reduced osseous defect fill with bioresorbable membranes in comparison to nonresorbable (ePTFE) membranes is the generation of resorption products such as polylactic acid or citric acid, which can negatively affect bone formation because of cytotoxic effects.[31,32] Depending on the type of material used for the bioresorbable membrane, inflammatory reactions ranging from mild[33–35] to severe[17] have been documented in the adjacent tissues. In a recent experimental study in dogs evaluating a bioresorbable prototype trilayer membrane, histologic analysis demonstrated an inflammatory reaction triggered by the polylactide component of the prototype membrane.[21] A plasma cell-rich infiltrate was found adjacent to the separated polylactide fragments, and foreign-body giant cells lined these fragments. This inflammatory response seemed to provoke osteoclastic resorption of newly formed bone facing the prototype membrane.

An inflammatory foreign-body reaction due to the degradation and resorption process of a bioresorbable synthetic membrane containing poly(d,l-lactic)-co-trimethylene carbonate has been previously reported.[18] Similarly, multinucleated giant cells have been also described following another experimental GBR study that evaluated a porous polylactide membrane.[20] Acidic degradation and the release of resorption products appear to induce an inflammatory reaction to bioresorbable polymers such as polylactide.[14]

In the present study, similar findings were observed for the 2 synthetic membranes tested. At 2 weeks, AS demonstrated fibrous membrane encapsulation and at 6 weeks a marginal inflammatory infiltration with foreign-body giant cells in the fissures of the membrane. Similarly, OQ displayed early fibrous encapsulation and foreign-body giant cells in direct contact with the membrane. In contrast, BG and the 3 collagen prototype membranes showed neither signs of fibrous membrane encapsulation nor a foreign-body reaction characterized by an inflammatory response and giant cell formation.

The results of this study encourage further investigation of the prototype VN c/EN membrane to evaluate its potential in GBR procedures.

CONCLUSIONS

The present experimental study compared 3 prototype collagen membranes to a collagen and 2 synthetic polymer membranes already in clinical use with respect to barrier durability and tissue response following coverage of bone defects in the rabbit calvarium. One prototype membrane (VN c/EN) displayed significantly longer duration of membrane integrity in the absence of a foreign-body reaction as compared to the 2 other prototypes (VN c/E and VN p) and the BG collagen membrane. The 2 synthetic membranes (OQ and AS) demonstrated longer barrier durability compared to the VN c/EN membrane. However, both OQ and AS exhibited fibrous encapsulation and an inflammatory foreign-body reaction.

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REFERENCES

1. Buser D, Dula K, Hess D, Hirt HP, Belser UC. Localized ridge augmentation with autografts and barrier membranes. Periodontol 2000 1999;19:151–163.
2. Malmquist JP. Successful implant restoration with the use of barrier membranes. J Oral Maxillofac Surg 1999;57:1114–1116.
3. Hardwick R, Hayes BK, Flynn C. Devices for dentoalveolar regeneration: An up-to-date literature review. J Periodontol 1995;66:495–505.
4. Schenk RK, Buser D, Hardwick WR, Dahlin C. Healing pattern of bone regeneration in membrane-protected defects: A histologic study in the canine mandible. Int J Oral Maxillofac Implants 1994;9:13–29.
5. Buser D, Ruskin J, Higinbottom F, Hardwick R, Dahlin C, Schenk RK. Osseointegration of titanium implants in bone regenerated in membrane-protected defects: A histologic study in the canine mandible. Int J Oral Maxillofac Implants 1995;10:666–681.
6. Buser D, Dula K, Hirt HP, Schenk RK. Lateral ridge augmentation using autografts and barrier membranes: A clinical study with 40 partially edentulous patients. J Oral Maxillofac Surg 1996;54:420–432.
7. Simion M, Baldoni M, Rossi P, Zaffe D. A comparative study of the effectiveness of ePTFE membranes with and without early exposure during the healing period. Int J Periodontics Restorative Dent 1994;14:167–180.
8. Zitzmann NU, Näf R, Schärer P. Resorbable versus nonresorbable membranes in combination with Bio-Oss for guided bone regeneration. Int J Oral Maxillofac Implants 1997;12:844–852.
9. Machtei EE. The effect of membrane exposure on the outcome of regenerative procedures in humans: A meta-analysis. J Periodontol 2001;72:512–516.
10. Gher ME, Quintero G, Assad D, Monaco E, Richardson AC. Bone grafting and guided bone regeneration for immediate dental implants in humans. J Periodontol 1994;65:881–891.
11. Augthun M, Yildirim M, Spiekermann H, Biesterfeld S. Healing of bone defects in combination with immediate implants using the membrane technique. Int J Oral Maxillofac Implants 1995;10:421–428.
12. Nowzari H, Slots J. Microbiologic and clinical study of polytetrafluoroethylene membranes for guided bone regeneration around implants. Int J Oral Maxillofac Implants 1995;10:67–73.
13. Rasmusson L, Sennerby L, Lundgren D, Nyman S. Morphological and dimensional changes after barrier removal in bone formed beyond the skeletal borders at titanium implants. A kinetic study in the rabbit tibia. Clin Oral Implants Res 1997;8:103–116.
14. Hutmacher DW, Hürzeler MB, Schliephake H. A review of material properties of biodegradable and bioresorbable polymers and devices for GTR and GBR applications. Int J Oral Maxillofac Implants 1996;11:667–678.
15. Zellin G, Gritli-Linde A, Linde A. Healing of mandibular defects with different biodegradable and non-biodegradable membranes: An experimental study in rats. Biomaterials 1995;16:601–609.
16. Aaboe M, Schou S, Hjörting-Hansen E, Helbo M, Vikaer D. Osseointegration of subperiosteal implants using bovine bone substitute and various membranes. Clin Oral Implants Res 2000;11:51–58.
17. Gotfredsen K, Nimb L, Hjörting-Hansen E. Immediate implant placement using a biodegradable barrier, polyhydroxybutyrate-hydroxyvalerate reinforced with polyglactin 910. Clin Oral Implants Res 1994;5:83–91.
18. Hürzeler MB, Quinones CR, Schüpbach P. Guided bone regeneration around dental implants in the atrophic alveolar ridge using a bioresorbable barrier. An experimental study in the monkey. Clin Oral Implants Res 1997;8:323–331.
19. Dupoirieux L, Pourquier D, Picot MC, Neves M. Comparative study of three different membranes for guided bone regeneration of rat cranial defects. Int J Oral Maxillofac Surg 2001;30:58–62.
20. Schliephake H, Dard M, Planck H, Hierlemann H, Jakob A. Guided bone regeneration around endosseous implants using a resorbable membrane vs a PTFE membrane. Clin Oral Implants Res 2000;11:230–241.
21. von Arx T, Cochran DL, Schenk RK, Buser D. Evaluation of a prototype trilayer membrane (PTLM) for lateral ridge augmentation: An experimental study in the canine mandible. Int J Oral Maxillofac Surg 2002;31:190–199.
22. Hürzeler MB, Kohal RJ, Naghshbandi J, et al. Evaluation of a new bioresorbable barrier to facilitate guided bone regeneration around exposed implant threads. An experimental study in the monkey. Int J Oral Maxillofac Surg 1998;27:315–320.
23. Miller N, Penaud J, Foliguet B, Membre H, Ambrosini P, Plombus M. Resorption rates of 2 commercially available bioresorbable membranes. A histomorphometric study in a rabbit model. J Clin Periodontol 1996;23:1051–1059.
24. Zhao S, Pinholt EM, Madsen JE, Donath K. Histological evaluation of different biodegradable and nonbiodegradable membranes implanted subcutaneously in rats. J Craniomaxillofac Surg 2000;28:116–122.
25. Owens KW, Yukna RA. Collagen membrane resorption in dogs: A comparative study. Implant Dent 2001;10:49–56.
26. Becker W, Becker BE, Berg L, Prichard J, Caffesse R, Rosenberg E. New attachment after treatment with root isolation procedures; report for treated class III and class II furcations and vertical osseous defects. Int J Periodontics Restorative Dent 1988;8:9–23.
27. Hutmacher DW, Kirsch A, Ackermann KL, Hürzeler MB. A tissue engineered cell-occlusive device for hard tissue regeneration– A preliminary report. Int J Periodontics Restorative Dent 2001;21:49–59.
28. Schenk RK. Bone regeneration: Biologic basis. In: Buser D, Dahlin C, Schenk RK, eds.: Guided Bone Regeneration in Implant Dentistry. Chicago: Quintessence, 1994:49–100.
29. Marinucci L, Lilli C, Baroni T, et al. In vitro comparison of bioabsorbable and non-resorbable membranes in bone regeneration. J Periodontol 2001;72:753–759.
30. Rothamel D, Schwarz F, Sculean A, Herten M, Scherbaum W, Becker J. Biocompatibility of various collagen membranes in cultures of human PDL fibroblasts and human osteoblast-like cells. Clin Oral Implants Res 2004;15:443–449.
31. Alpar B, Leyhausen G, Günay H, Geurtsen D. Compatibility of resorbable and nonresorbable guided tissue regeneration membranes in cultures of primary periodontal ligament fibroblasts and human osteoblast-like cells. Clin Oral Investig 2000;4:219–225.
32. Hämmerle CHF, Jung R. Bone augmentation by means of barrier membranes. Periodontol 2000 2003;33:36–53.
33. Aaboe M, Pinholt EM, Hjörting-Hansen E, Solheim E, Praetorius F. Guided tissue regeneration using degradable and non-degradable membranes in rabbit tibia. Clin Oral Implants Res 1993;4:172–176.
34. Piatelli A, Scarano A, Russo P, Matarasso S. Evaluation of guided bone regeneration in rabbit tibia using bioresorbable and non-resorbable membranes. Biomaterials 1995;17:791–796.
35. Sandberg E, Dahlin C, Linde A. Bone regeneration by the osteopromotion technique using bioabsorbable membranes. An experimental study in rats. J Oral Maxillofac Surg 1993;51:1106–1114.