Membranes for Periodontal Regeneration

Andrej Aurer
Ksenija Jorgić-Srdjak

Department of Periodontics
School of Dental Medicine
University of Zagreb

Summary

This article reviews different types of membranes for guided tissue regeneration. They are used to cover defects and stimulate regeneration of osseous defects in periodontal pockets. A membrane should be biocompatible, enable cell exclusion separating the gingival flap from the fibrine clot and guard space for the new alveolar bone and the periodontal ligament.

Membrane can be non-resorbable and resorbable. When non-resorbable membranes are used, another surgical procedure for their extraction is needed. They are therefore used less frequently today. The majority of these membranes are made of polytetrafluoretylene, e.g. Gore-Tex membrane.

Resorbable membranes shorten the treatment since there is no need for their removal. They can be made from natural materials like collagen, laminar bone, dura mater or connective tissue transplants and from synthetic resorbable materials, most frequently derivatives of organic aliphatic thermoplastic polymers. Polyglycolic and polylactic acids are mostly used. This group includes the Atrisorb membrane that has to be prepared intraoperatively. The use of polyurethane membranes is presently being tested.

So far the perfect membrane has not been discovered. Collagen membranes are most popular due to their optimal biocompatibility, although their rate of resorption is difficult to predict.

Key words: *periodontal defect, guided tissue regeneration, resorbable membranes, non-resorbable membranes.*

Acta Stomat Croat
2005; 107-112

REVIEW
Received: May 10, 2004

Adress for correspondence:

Andrej Aurer
Zavod za parodontologiju
Stomatološki fakultet
Gundulićeva 5, 10 000 Zagreb

Introduction

Experimental and clinical application of the guided tissue regeneration (GTR) concept, led to the use of different materials in periodontal regenerative therapy. According to a hypothesis formulated by Melcher (1), certain cell populations residing in the periodontium have the potential to create new cementum, alveolar bone and periodontal ligament, provided they have the opportunuity to populate the periodontal wound. Collagen fibers need to be inserted into newly formed cementum on the one hand, and into alveolar bone on the other, in order to reinstate the normal function. This process requires fine coordination between these three tissues. The hypothesis was experimentally established and histologically verified by Karring et al. (2-4). It was shown that such conditions arise when epithelial cells or

fibroblasts are excluded from the wound space and periodontal ligament cells are allowed to migrate coronally. The necessity for exclusion of epithelial and connective tissue cells of the gingiva from the wound led to development and application of GTR membranes.

The first material clinically used in periodontal surgery, allowing regeneration of cementum, periodontal ligament and alveolar bone, was celulose-acetate laboratory filter (3, 4). Thus, for the first time periodontal regeneration as a response to GTR was histologically verified (4). Since that time, membranes from different materials for achieving periodontal regeneration have been developed and modified. Here, we will review the properties of different GTR membranes, as well as the results of some *in vitro* and *in vivo* studies addressing this topic.

Biomaterial is a nonviable material used in medicine and dentistry intended for interaction with biological systems (5). Any material introduced into the human organism, such as GTR membranes, has to fulfill two important requirements: safety and efficacy. Safety is assessed through a wide selection of *in vitro* and *in vivo* assays for testing specific aspects of biocompatibility. Cell culture cytotoxicity, subcutaneous implantation, blood compatibility, hemolysis, carcinogenesis, mutagenicity, pyrogenicity, short- and long-term histological tissue reaction are some of the assays used to evaluate biocompatibility (5-7).

Characteristics for GTR membranes have been described by several authors. They include biocompatibility, cell exclusion, space maintenance, tissue integration and ease of use. In the future, membranes should be biologically active. Cell exclusion property requires the membrane to separate gingival flap from the coagulum in the wound space. Although this is a generally recognised requirement, there are no studies investigating the influence of this principle on the outcome of GTR procedures. The next property is space maintenance for new alveolar bone, periodontal ligament and tooth cementum. Membranes should withstand masticatory forces, flap tissue tension and prevent collapse of soft tissues or reduction of the wound space (10-12). Tisuue integration property ensures wound stabilization and inhibition of epithelial migration, resulting in gain of attachment level (10, 13, 14). GTR membranes should be easy to use, thus allowing the clinician to conduct the surgical procedure without undue difficulty.

Nonresorbable membranes

Nonresorbable membranes retain their build and form in the tissues, requiring a second surgical procedure for removal, thus adding to the trauma of the periodontal tissues and to patient discomfort, as well as raising the costs and duration of therapy.

The first non-resorbable membranes approved for clinical use were made of expanded polytetrafluorethylene (ePTFE, Gore-Tex®). PTFE is a fluorocarbon polymer with exceptional inertness and biocompatibility, prevents tissue ingrowth and does not elicit foreign-body response after implantation, but is nonporous (15). ePTFE is chemically identical, causes minimal inflammatory reaction in different tissues, allows tissue ingrowth and has been used in vascular surgery for several decades (16-18). It is manufactured when PTFE is subjected to high tensile stress, forming porous microstructure of solid nodes and fibrils. Gore-Tex® ePTFE membrane consists of two parts. First, an open microstructure collar which promotes connective tissue ingrowth, positioned coronally (19), and prevents apical epithelial migration and ensures wound stability. This membrane part is 1mm thick and 90% porous (8). The other part is occlusive membrane 0.15 mm thick and 30% porous, serving as a space provider for regeneration, which possesses structural stability and serves as a barrier towards the gingival flap (10, 11).

Human histological samples have indicated that ePTFE membranes can lead to significant periodontal regeneration after a 3 months healing period (2). Six months after insertion of ePTFE membrane new cementum with inserting fibers was demonstrated (20). Effectiveness of ePTFE membranes was investigated in numerous clinical studies (21-24). Some studies did not find significant differences when the regenerative procedure was compared to conventional flap surgery with open debridement (25). Membrane insertion can cause minor complications such as pain, purulence and swelling, with an incidence somewhat higher than that reported for conventional periodontal surgery (26).

The Gore-Tex® ePTFE membrane has been modified by incorporation of titanium reinforcements, set between two ePTFE layers, resulting in heightened mechanical strength and better space maintenance (7, 11, 20). Animal studies revealed clinically relevant cementum and bone regeneration 2 months after insertion (11, 27), and clinical studies found no difference compared to non-modified membranes (21). Titanium reinforcement membranes also have their application in guided bone regeneration procedures (GBR) aimed at augmentation of toothless alveolar bone, in cases where implants are planned and insufficient alveolar bone mass is present.

Membrane made from dense non-porous PTFE-a (TefGen-FD®) was tested on rat calvarial defects showing results similar to ePTFE membrane application, but with limited tissue integration (28).

In the literature use of other nonresorbable materials for GTR membranes is described, like several case-reports of rubber-dam (29, 30) and glass ionomer (31). Although the number of investigations is limited, it seems that these materials do not fulfill all the mentioned requirements for GTR procedures.

Non-resorbable membrane made of knitted nylon fabric mechanically bonded onto a semipermeable silicone membrane and coated with collagen peptides has been developed. Shortcomings of this membrane are its low rigidity and limited regenerative response (32, 33).

Removal of membranes requires a second surgical procedure, jeopardises success and possibly interferes with healing by inflicting damage to new and sensitive regenerated tissue (34), and thus led to development of resorbable membranes.

Resorbable membranes

Resorbable membranes do not require additional surgery, reduce patient discomfort and costs,and eliminate potential surgical complications.

By their inherent nature, absorbable membranes disintegration is not possible to control. The disintegration starts immediately upon placement in the surgical site, and speed can vary considerably amongst individuals, particularly for materials requiring enzymatic degradation like collagen. Data on optimal persistance of membranes *in vivo*, vary between 4 weeks and several months (35, 36). Because of their biodegradation, resorbable membranes elicit tissue reactions which potentially influence wound healing and compromise regenerative outcome. Resorbable membranes can be natural or synthetic.

Natural materials

Collagen has been used in medicine and dentistry because of its biocompatibility and improvement of healing (37, 38). Collagen has many auspicious biological activities: it has low immunogenicity (39, 40), is hemostatic (41), attracts and activates periodontal ligament and ginigival fibroblast cells (42), potentially augments tissue thickness (43). During wound healing interactions between collagen and various cell types take place (44, 45).

Collagen is acquired from animal skin, tendons or intestines. After isolation and purification by means of enzymatic preparation or chemical extraction, it is further processed to various forms (46, 47). The most common chemical modification is crosslinking, usually aldehyde treatment (48), resulting in reduced water absorption, decreased solubility and increased tensile strentgth (46). Although theoretically danger of bovine spongiform encephalopaty transmission exist (BSE), FDA permitted collagen for human use, and collagen-based products are permitted on the EU market as well (Table 1).

Technological complexity of the manufacturing process can be illustrated on the example of Bio-Gide® membrane (Geistlich Biomaterials) production. Collagen is of porcine origin, production consists of several phases and includes formation of collagen bilayer (49). According to EU guidelines chemical elimination of viral and bacterial contamination is performed. Collagen antigenicty depends on two terminal peptide regions, which are removed. Lipid and protein remnants are removed using specific purification procedures. Then structural quality of the membranes is then controled by segment analysis. The final product consists of pure collagen fibers with no traces of organic or chemical residues. Lastly biocompatibility and sterility is checked.

Implanted collagen is enzymatically degraded by

macrophages and polymorphonuclear leukocytes and resorption velocity can vary greatly, depending on collagen source and modifications (50). Enzyme collagenase initiates membrane resorption at the specific site. Resulting fragments denaturate and become gelatine, which is then degraded to amino-acids by gelatinases and other enzymes. Some periodontal pathogens like Porphyromonas gingivalis produce collagenase. Since bacteria colonize the exposed membrane during healing, uncontrolled degradation can take place resulting in unfavourable outcome (51).

Locci and coworkers (52) compared collagen and PTFE biocompatibility and showed that PTFE inhibited gingival fibroblasts DNA synthesis, while collagen membrane stimulated proliferation of these cells. Besides, PTFE membrane significantly reduced extracellular matrix synthesis, so results stand in favour of collagen biocompatibility. Wang and coworkers (53) showed higher adherence of osteoblasts to surfaces of collagen than non-collagen membranes.

Data suggests that the period in which collagen membranes stay intact and prevent apical proliferation of epithelium suffices (54), since the critical epithelial proliferation time is approximately 14 days.

Pitaru et al (55) investigated the influence of fibronectin and heparan-sulphate on connective tissue root surface coverage after collagen membrane application and reported 30 % higher root surface coverage than with membrane alone.

Animal studies tested collagen membranes regenerative potential. A bovine collagen membrane (BioGide®) resorbed in 8 weeks, and a rat-tail collagen membrane resorbed in 4 weeks (56, 57). Chronic inflammatory infiltrate was present around the membrane, but completely disappeared after resorption. Both membranes led to periodontal regeneration.

A type I collagen GTR membrane is manufactured from bovine Achilles tendon (BioMend®). Membrane is semi-occlusive (pore size 0.004 μm) and resorbs in 4 to 8 weeks. Clinical studies revealed certain effectiveness, which seemingly depends on form and size of the defect (58-60), probably due to compromized space maintenance.

Another type I collagen membrane, derived from calf pericardium and cross-linked by diphenolphosphorylazide has been evaluated for GTR. Histologically significant inflammatory reaction was observed (61). Experiments on a canine model indicated weak regenerative potential (62), but clinical studies indicated effective GTR outcome (63, 64).

Histological evaluation of microfibrillar hemostatic collagen membrane derived from bovine corium (Avitene®) in humans was no more effective than a control group (65). Membrane was difficult to handle. Clinical evaluation of another hemostatic collagen material (Collistat®) also resulted in regeneration outcomes similar to control treatment. Histological evaluation indicated that the material was completely resorbed seven days after implantation (66).

Parodi et al (67) reported histologically verified periodontal ligament, cementum and alveolar bone regeneration, with no signs of inflammation, after insertion of collagen membrane enriched with chondroitin-sulphate (Paroguide®).

It seems that collagen membranes show limited value in GTR, probably because of insufficient toughness and lowered space maintenance. Meta-analysis of clinical GTR investigations showed equal effectiveness to nonresorbable (68).

Other natural products tested for GTR without success were dura mater (69, 70), oxydized cellulose (71, 72) and laminar bone (73).

Synthetic materials

Synthetic resorbable materials are usually organic aliphatic thermoplastic polymers. The materials most commonly used are poly-α-hydroxy acids, which include polylactic polyglycolic acid and their copolymers. One of the advantages of polyhydroxy acid is hydrolysis to final products water and carbon dioxide. Degradation time can vary, lengthened through the addition of lactides or glycols (74, 75).

Although high concentrations of degradation products can be toxic for cells, sufficient biocompatibility was reported *in vitro* (76). Significant foreign-body reactions to porous polylactide polymer implants, interfering with alveolar bone formation, have been observed (14).

A double-layered absorbable membrane (Guidor®) made of polylactic acid and a citric acid ester acetyl tributylcitrate was the first to appear on the market. The external layer of the membrane designed to allow integration of the overlying gingival flap has rectangular perforations (400-500/cm^2). It seems that such a surface design successfully promotes tissue integration, since only limited gingival recession after usage has been reported (77, 78). Between the internal and external layers are internal spacers creating space for tissue ingrowth. The internal layer has smaller circular perforations (4000-5000/cm^2) and outer spacers for maintaining the space between the membrane and the root surface. Histological animal studies showed complete resorption of membrane 6-12 months after implantation, and function maintenance for at least six weeks. Degradation process includes foreign-body reaction characterized by macrophages and multinuclear cells (79). Clinical studies have proved the membrane efficacy on various periodontal defects (80, 81). The membrane was removed from the market for unknown reasons.

Synthetic resorbable membrane Resolute® consists of an occlusive membrane of glycolide and lactic copolymer and a porous web of polyglycolide fiber. The occlusive membrane prevents cell ingrowth, and porous part promotes tissue integration. Histological studies showed effectiveness similar to non-resorbable membranes with mean clinical attachment gain of 2 mm, and with gain of 4 or more mm in more than 85% of the treated sites, structure retainment for 4 weeks and complete resorption 5-6 months after placement (82-84).

Fibers of polyglactin 910, a copolymer of glycolide and l-lactide form a tightly woven mesh (Vicryl Periodontal Mesh®). It seems that the membrane looses its structure after 2 weeks, and completely resorbs in 4 or more weeks (85, 86, 78). Although animal studies indicate lack of tissue integration and recession formation, clinical evaluation suggests effectiveness equal to that of other GTR membranes (87, 88).

Atrisorb® membrane is the only GTR membrane manufactured chairside. Polylactic polymer is present in flowable form, dissolved in N-methyl-2-pyrrolidone. An irregularly shaped membrane is formed after exposure of the polymer to 0.9% saline solution for 4-6 minutes in a special cassette. The desired shape is cut. Membrane thickness is 600-750 μm, with modest adherence properties, and is placed into the defect by applying gentle pressure. Histologically complete resorption was observed 6-12 months after implantation (89, 90). Clinical studies reported its efficacy in the treatment of periodontal defects (91, 92).

Epi-Guide® is membrane made of polylactic acid polymers, has three layers designed to stop and keep away epithelial cells and fibroblasts. It maintains its structure for 20 weeks, and is fully resorbed in 6-12 months.

Experimental Mempol® membrane manufactured from polydioxanon (PDS), a dioxanon polymer, is bilayered. The first layer is completely unpermeable, covered with PDS loops 200 μm long on the gingival side, intended for integration with connective tissue. Clinical efficacy is comparable to that of polylactic membranes (Guidor®), although the tested membrane resulted in more frequent recession during healing (93).

Besides the already mentioned polyester membranes, use of polyurethane for membrane production has been tested as well (71, 94, 95). Polyurethanes are organic polymers containing urethane group -NH-CO-O-, materials with diverse properties. Polyether urethanes are degraded through enzymatic and oxidative degradation (96, 97).

Animal experiments showed that polyurethane membranes tend to swell, and inflammation at the flap margins and recession were more pronounced than in polylactic membranes (98, 93). The membrane seems to be present in the tissue for at least 8 weeks after implantation (98).

Future perspectives for the GTR

One of the main shortcomings of all clinical regenerative procedures is relatively high variation and low predictability of clinical attachment and bone gain (99, 100). Since products should retain their biocompatibility, but have better efficacy, this could be accomplished through usage of new techniques developed in similar biomedical branches.

The condition for predictable tissue regeneration is stimulation of precursor cells with necessary mes-

senger molecules. Control of progenitor cells in periodontal healing process is complex and mostly unknown. It seems that various local factors play a role in attracting the cells to the wound space from bone marrow and periodontal ligament spaces.

A good example for new trends is membrane surface modification, especially incorporation of adhesion molecules which should be able to physiologically stimulate cell and tissue adhesion. (101). The next step could be application of specific adhesion molecules resulting in tissue selection on the membrane surface (102-104). There is enough evidence indicating the important role of adhesion molecules in periodontal health and disease (105-108).

In order to minimize detrimental microbial influence on the regenerative procedure, addition of antimicrobial substances has been investigated (109, 110). Antimicrobial action might beneficially influence early phases of wound healing and thus improve the outcome of the regenerative procedure. However, one clinical investigation found no advantage for metronidazole as an additive present in the tested resorbable membrane (111).

Addition of growth and differentiation factors has been investigated. There is enough evidence that certain growth factors and cell mediators can act on competent cells in the healing of periodontal wound space and regeneration of tissues such as cementum and bone (112-115). Such molecules applied locally in an adequate vehiculum seem to act on diferentiation and cell migration to the wound space. An example is development of combined polylactide and alginate membranes, with controlled TGF-β release (116). This combination might have important influence on the outcome of the GTR procedure (117-119).

Conclusion

The use of GTR membranes can lead to significant periodontal regeneration, and formation of cementum with inserting fibers, although complete regeneration has never been reported.

The advantage of resorbable membranes is unnecessary surgical removal, while collagen membranes have additional advantages related to biological properties of collagen itself.

Products used for GTR should maintain biocompatibility, but develop better efficacy, possibly using new techniques and technologies that have been developed and applied in neighbouring medical branches.

Application of specific adhesion molecules should lead to tissue selection on the membrane surface. Addition of antimicrobial substances might minimize the influence of microbial contamination on regenerative outcome, growth factor incorporation should stimulate regenerative biologic potential of bone and cementum. Combination of these molecules might lead to significant changes in the outcome of GTR procedures. Further investigations are needed to improve clinical outcome, because there is insufficient proof of the clinical efficacy of these concepts. Better understanding of factors influencing regenerative procedure will probably improve predictability of therapy of bone defects around natural teeth and implants.