Novel membrane for guided bone regeneration

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ABSTRACT: Membranes have been clinically used for guided tissue and bone regeneration for decades, but their use in everyday clinical practice is rather limited. We developed a biodegradable membrane (InionGTR™) composed of polylactide, polyglycolide and trimethylene carbonate aiming to improve the properties of membrane. Before application the membrane is treated with N-methylpyrrolidone (NMP) to achieve a rubber like consistency, to allow easy handling and manageability in the clinical setting. After placing the membrane NMP diffuses out from the polymer phase into the water phase. The loss of NMP in the polymer stiffens the membrane up and allows space maintenance in the defect area. In addition the influx and efflux of NMP creates a porous surface on the membrane leading to an improved integration of tissues into the porous surface layers of the InionGTR™ membrane. Therefore, the use of NMP improves the handling in the clinical setting, and allows tissue integration and space maintenance, both important for the outcome of the treatment. (Int J Artif Organs 2006; 29: 834-40)

KEY WORDS: Bone regeneration, Membrane, Dental, Guided tissue regeneration, Guided bone regeneration

INTRODUCTION

The principle of guided tissue regeneration (GTR) using a barrier membrane has been used in treatment of periodontal defects for almost 3 decades (1). The protocol employs barrier membranes to regenerate periodontal ligament, cementum and bone by excluding the faster growing soft tissue cells from the defect space. Clinical studies have widely demonstrated superiority of this treatment modality over traditional open flap debridement techniques (2, 3). More recently the principle of GTR has also been used in guided bone regeneration (GBR) in various other indications, for example to treat bony defects in dental implantology and also in other areas in the skeleton (4). A variety of membranes are available in the marketplace, starting from non-resorbable polytetrafluoroethylene (PTFE) membranes to resorbable collagen and polyactide (PLA) polyglycolic acid (PGA)-based membranes, all of which exhibit satisfactory clinical outcomes (5-7). Tatakis et al have referred to a set of design requirements that need to be met for a periodontal membrane to be effective (8). In short, these are biocompatibility, cell exclusion, space maintenance, tissue integration, ease of use and biological activity. The design criterion for optimal GTR/GBR membranes is slightly controversial since the membrane has to be malleable so that it can be placed onto the contoured 3D surfaces. But it also needs to shield the defect space, so that the blood clot under the membrane is stable, to allow optimal healing. Until now the optimal design criteria for GTR/GBR membrane has not been achieved. One of the criteria needed for barrier membrane is its ability to allow tissue ingrowth into the membrane. But at the same time the membrane has to act as a barrier to avoid soft tissue ingrowth through the membrane. The aim of this study was to develop a membrane which would be malleable enough to enable easy clinical application of membrane, but at the same time would fulfill the criteria of space
maintenance. Another aim was to characterize the surface
topography of the developed membrane and to
investigate the tissue integration performance of the
membrane when implanted into a rabbit calvarium.

MATERIALS AND METHODS

Tensile test

The polymeric film, composed of polylactide,
polyglycolide and trimethylene carbonate (PLA/PGA/TMC)
was first extruded and further compression moulded into a
thickness of 0.2 mm. Film specimens, with a width of 10
mm, and a length of 50 mm were cut from the compressed
film. In order to make the film malleable, the films were
plasticized by a “dipping” method which was developed
within this study. In this “dipping” method the plastic
specimen is first immersed totally into medical grade N-
methyl-pyrrolidone (NMP) (Pharmasolve®, ISP International
Specialty Products, New Jersey, USA) and thereafter the
specimen is removed and it is allowed to stabilize in dry air.
Several experiments were performed to find optimal
immersion time in order to obtain optimal characteristics in
the membrane, as too short an immersion time did not fully
plastizise the specimen and with a too long immersion time
the mechanical properties of membrane can be
compromised. The optimal properties were obtained with
specimens who were immersed into plastiziser for 15-35
seconds and left to stabilize for approximately 15-30
minutes. For this test series a total of 24 film specimens
were dipped into NMP solution for 25 seconds and
thereafter specimens were allowed to stabilize for 30
minutes. The treated specimens were further divided into 6
groups, from which one group was tested right after
stabilization in room temperature to model the conditions in
clinical setting before implantation (initial), and the 5 other
test groups were immersed into phosphate buffer solution
at +37°C, for 1, 5, 10, 30 and 60 minutes prior testing to
model the conditions after membrane is implanted and is in
contact with saliva, blood and other watery fluids.

The tensile testing was performed using a Zwick
Z020/TH2A (Zwick GmbH & Co., Ulm, Germany) universal
materials testing machine. Gauge length of 30 mm and
tensile speed of 20 mm/min were used with all test
samples. The samples which were immersed into
phosphate buffer solution were tested in a water bath at
+37°C after a specified immersion time.

Structural analysis

The structural analysis was performed for three test
specimens: 1) a PLA/PGA/TMC film without NMP
treatment (coded as E1M-11), 2) a PLA/PGA/TMC film
 treated with NMP (coded as E1M-11NMP), and 3) GORE
OsseoQuest membrane (W.L.Gore& Associates, Inc).
Scanning electron microscopy (SEM) analysis was
performed (Jeol T100, Japan) to obtain images of the
structure of film and membranes.

In vivo testing

In vivo testing was performed in rabbits on non-critical
size defects (6 mm) in the skull. Ethical Committee approval
was obtained from the local authorities (Zurich,
Switzerland). Rabbits were sedated by ketamin and further
anesthetized by a halothan-N2O inhalation method. The
surgical area was clipped and prepared with iodine for
aseptic surgery. A linear incision was made from the nasal
bone to the midsagital crest. The soft tissues were
reflected and the periosteum was dissected from the site
(occipital, frontal, and parietal bones). Four 6 mm
 craniotomy defect were created (2 in the parietal and 2 in
the frontal bone) with a 6 mm trephine in a dental hand
piece. The surgical area was flushed with saline to remove
bone debris. To avoiding any dural perforation the defects
marked half way through the bone by the trephine were
finally made by a round blurr creating a 6 mm defect with a
small overhang at the dural side. This enabled us to seal
the defects from the dural side by a 6 mm in diameter
membrane which was poked through the calvarial bone.
This membrane was fixed by the pressure from the dura.
Another rectangular membrane of 10x10mm was placed
above the defect. The 4 membranes of the top were
sutured together and the entire unit sutured to the lateral
remaining periost. The soft tissues were closed with
sutures. After the operation analgesia was provided by
injection of novalgin (50 mg/kg). Four weeks after operation
the rabbits were sacrificed after sedation with barbiturates
by a overdose of ketamin and the calvarial bone excised.

Specimens were radiographed using a dental
radiography unit with ultra speed dental films (Eastman
Kodak Company, NY, USA). The radiographs were
photographed, scanned and later used to localize the
middle section of the defects. After radiography the
samples were first prepared with a sequential water
substitution process that included 48 hours in 40%
ethanol, 72 hours in 70% ethanol (changed every 24 hrs), 72 hrs in 96% ethanol and finally 72 hrs in 100% ethanol. Samples were placed in xylene for 72 hrs for defatting the recovered bone (changed every 24 hrs). Next, infiltration was performed by placing the samples in methyl methacrylate (MMA) for 72 hrs (Fluka 64200) followed by three days in 100 mL MMA + 2 g di-benzoylperoxid (Fluka 38581, dehydrated in exicator) at 4°C. Samples were embedded by placing them in 100 mL MMA + 3 g di-benzoylperoxid + 10 mL plastoid N or dibuthylphtalat (Merck 800 19.25) and allowing polymerization to occur at 37°C in a air tight water bath. 4.5 µm sections were prepared from the midst of the defects and stained with Goldner Trichrome. Digital images were taken and processed with an image analysis program (Adobe Photoshop). From the images obtained the tissue integration into the membrane and the effect of the different membranes on the cellular level were analysed.

RESULTS

Tensile test

The results of tensile test of membrane strips are seen as force/strain graphs in Figure 1. With the developed “dipping” method stiff polymeric films were transformed rubbery and easily mouldable, as seen in force/strain graph (marked as “initial”). As soon as the plasticized film was in contact with watery solution the rubbery films started to become stiff again. Even after one minute immersion into phosphate buffer solution there is significant change in rigidity of film specimen due to diffusion of the NMP-plastiziser from the polymeric film into the watery solution. Stiffening effect is dependent on the immersion time and increasing immersion time increases the specimen stiffness up to 30 minutes immersion time. After 30 minutes the maximum stiffness is reached, as 60 minutes immersion time does not increase the rigidity of the sample any further.

Membrane structure

Without NMP treatment PLA/PGA/TMC film is dense with a smooth surface as seen in Figure 2. After the NMP treatment the surface becomes porous having open pores approximately 10 microns in diameter as seen in Figure 3. The porous layers form on both sides of film leaving the dense layer in the middle of the film, the thickness ratios of layers being approximately 1:1:1 (porous:dense:porous). In Figure 4 the cross sectional view of NMP treated InionGTR™ membrane with porous-dense-porous structure is seen. GORE OsseoQuest has a fibrous outer structure. In the middle of the membrane is an occlusive film into which the fibrous outer layers are partly attached (Fig. 5).
In the following Figures 6-8 representative examples for Goldner-trichrome stained histological sections of defects highlighting specific aspects are shown. Goldner-trichrome methodology stains bone dark green and osteoid, which is the zone of new bone formation red. The defect in all figures is located in the upper part.

In Figure 6, the histological section of plain PLA/PGA/TMC (E1M-11) membrane shows that membrane is not encapsulated by a thick fibrous tissue. Normal formation of a trabecular bone has occurred. The presence of the osteoid in the lower part of the picture demonstrates that bone formation is also present in regions very close to the membrane. It is also noticed that the smooth film does not allow tissue integration into the film.

In Figure 7, an example from InionGTR membrane (E1M-11NMP) is given. Due to the fact that also with this membrane no adverse body reactions were seen the identification of the membranes in the sections was very difficult. Intensive in-growth of cells can be observed and are shown in Figure 7. Osteoid and with it new bone formation occurs also in close proximity to the NMP treated membrane.

Histology of an OsseoQuest-treated defect is shown in Figure 8. Obvious is the in-growth of cells in almost all histosections from OsseoQuest treated defect. At some isolated small regions bone formation has already occurred in the membrane.

With both porous membranes, namely with InionGTR™ membrane and GORE OsseoQuest membrane soft tissue ingrowth and even bone formation can be detected inside membrane. When in contact with bone, the membrane surface performs as a scaffold, which enables bone integration into the membrane.
DISCUSSION

A membrane that features optimal space maintenance has to be stiff, so that it will not collapse into the defect. This is particularly important in the case where no graft materials are used. If a membrane collapses into the defect space, the potentially regenerated volume is reduced, the blood clot disturbed and will compromise the clinical outcome. The membrane should be stiff enough to withstand the pressures exerted by the overlying flaps and to withstand external forces like mastication, until the blood clot building underneath the membrane has matured enough to support it. This stiffness, on the other hand, would not allow good clinical manageability or ease of use, as a stiff membrane cannot be contoured easily to a three dimensional defect. Therefore an ideal membrane should exist in 2 stages. In the first one the membrane should be malleable to allow perfect clinical applicability. In the second, the healing phase, the membrane should be space maintaining and therefore stiff. In the developed InionGTR™ membrane these 2 distinct phases are achieved by using the plasticising agent, medical grade N-Methyl pyrrolidone (NMP), Pharmasolve®. Pharmasolve® is generally used to improve the solubility of poorly soluble drugs in pharmaceutical formulations. Pharmasolve® is also used as solvent in biodegradable periodontal implant devices, in Atrisorb® and Atridox™, which have been widely used clinically (9, 10). NMP softens the membrane temporarily and after leaching out the membrane material stiffens up again. During the insertion phase the material is soft and a little rubbery and this facilitates the safe and precise trimming of the membrane, easy insertion and the formation of a good seal between the root of the affected tooth and the membrane, hindering the intrusion of soft tissue cells into the space. In the soft stage the membrane shows adequate contourability and the shape given is ‘conserved’ when the membrane stiffens up.

The need for tissue integration dictates the incorporation of structural elements in the device promoting tissue ingrowth, while concurrently achieving cell exclusion. Flap stabilization through tissue integration of the membrane prevents wound failure and subsequent epithelialization (formation of a long junctional epithelium) of the ruptured/exposed tooth-gingival flap interface during the early healing sequence. This minimizes gingival
recession and device exposure. At the same time the formation of a pocket outside the device is prevented, which in turn reduces the risk for infection. Another benefit of these features is the maintenance of esthetics during and after the wound healing (11). One of the prime requirements for guided tissue regeneration barriers is occlusivity. It means that the barrier membrane has to be impermeable for epithelial cells; otherwise, these faster growing cells would populate the wound space and inhibit the regeneration of the periodontium. This inhibition of epithelial migration should be provided for the time span the periodontal defect needs to heal. It is advantageous to use biodegradable barrier as biodegradable membranes start to disintegrate and resorb gradually, so the removal operation that is needed with non-degradable barrier membranes e.g. teflon membranes can be avoided. Effective exclusion of epithelial cells will occur if a tight seal is formed between the membrane and bony borders of the defect or the tooth root. Otherwise, epithelial cells can migrate through the membrane–bone gap into the wound space and compromise the clinical result. In addition, the membrane outer surface must allow for soft tissue integration so that retraction of soft tissue over the membrane (membrane exposure) can be avoided. The ‘bioseal’ forming between the membrane and the full thickness soft tissue flap also reduces the chances of bacterial infection and wound failure. Equally important, the inside of the membrane must incorporate structural elements to allow the blood clot forming underneath the membrane to be stabilised (1, 12, 13).

The InionGTR™ membrane exhibits a surface structure that allows epithelial cells as well as blood cells to adhere. It needs to be noted that the roughness is not through and through but limited to the surface layer. In vivo tests in a cranial rabbit model showed close incorporation of epithelial cells into the membrane surface after four weeks. The incorporation with no signs of exfoliation was determined by stained histological cross-sections.

CONCLUSIONS

By the newly developed method a polymeric film, composed of polylactide, polyglycolide and trimethylene carbonate polylactide, co-polymer film was treated with N-Methyl pyrrolidone (NMP) and a novel functional barrier membrane was developed. Initially the membrane is malleable and enables optimal placement. When brought in contact with body fluids, the membrane stiffens up forming a shield over the defect. Using NMP treatment porous layers form on both surfaces of the polymeric membrane. The in vivo results show good integration of cells into the porous layers of this membrane.

REFERENCES


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Membrane for guided bone regeneration