Review Article

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Dentistry Section

Evolution of Barrier Membranes in Periodontal Regeneration-"Are the third Generation Membranes really here?"

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ABSTRACT

In the last decades, Guide Tissue Regeneration (GTR) technique has been applied for the treatment of various periodontal defects such as intrabony defects, furcation involvements and localized gingival recession defects. From early days of using membranes with the simple aim of minimizing toxic response in the host, membranes have come a long way. Third generation membranes not only act as barriers but also as delivery devices to release specific agents. Many clinical trials have focused on using membranes as delivery devices for antibiotics and growth factors. In this article we take a brief look at the evolution of barrier membranes and future avenues with regard to third generation membranes.

Keywords: Barrier membranes, Collagen membranes, Guided tissue regeneration, Growth factors

INTRODUCTION

Periodontitis is a globally prevalent inflammatory condition that leads to a progressive destruction of periodontal tissues, namely alveolar bone, periodontal ligament, and root cementum, and is a major cause of tooth loss in adults. Besides affecting the supporting tissues of the teeth, current evidence also suggests that chronic periodontitis has an associated risk of systemic complications. Periodontal regeneration is defined as the reproduction or reconstruction of lost or injured tissue so that the form and function of the lost structures are restored. However, periodontal regeneration thus far has not been entirely successful in humans. The poor innate ability of damaged periodontal tissues to regenerate and the constant microbial challenge in the oral cavity demonstrate the need for developing clinically effective procedures to regenerate healthy periodontal tissues.

There is a broad range of treatment options that are available, such as barrier membranes, autografts, demineralized freeze-dried bone allografts, bovine-derived xenografts, and combinations of membranes and fillers. Conventional periodontal therapies, such as open flap debridement (OFD), provide critical access to evaluate and detoxify root surfaces and establish improved periodontal form and architecture. Therefore, the disease process is arrested and conditions are created that favors tissue regrowth. However, periodontal defects, if left empty after OFD, fill with the first cells to reach the area, i.e., epithelial cells and fibroblasts, after cell proliferation, which generates a core of fibro-epithelial tissues that attach to the root surface. Unfortunately, the attachment does not allow time for the bone and periodontal ligament (PDL) cells to refill the pocket, so the defect persists. This traditional healing process, known as periodontal 'repair', ultimately prevents orderly and sequential regeneration of true hybrid periodontal tissues.

In 1976 Melcher suggested that, under physiological conditions, only cells from periodontal ligament can synthesise and secrete cementum to attach newly-synthesised collagen fibres of periodontal ligament or lamina propria of gingiva to tooth [1]. Guide Tissue Regeneration (GTR) employs a barrier membrane around the periodontal defect to prevent epithelial downgrowth and fibroblast transgrowth into the wound space, thereby maintaining a space for true periodontal tissue regeneration. As such, this procedure has been, and still is, widely employed in periodontal clinics and established as a basic technique in periodontal regenerative medicine.

Criterion Essential For Barrier Membrane

In order for a barrier material to function optimally, it has to meet certain essential design criteria [2].

Bio-compatibility-The material should not elicit an immune response, sensitization or chronic inflammation which may interfere with healing and present a hazard to the patient.

Cell-occlusiveness-The material should act as a barrier to exclude undesirable cell types from entering the secluded space adjacent to the root surface.

Tissue integration- The goal of tissue integration is to prevent rapid epithelial downgrowth on the outer surface of the material or encapsulation of the material, and to provide stability to the overlying flap.

Space-making- Barrier material is capable of creating and maintaining a space adjacent to the root surface. This will allow the ingrowth of tissue from the periodontal ligament.

Clinical manageability- It should be provided in configurations which are easy to trim and to place.

Types of Barrier Membranes

The barrier membranes used for GTR can be broadly divided into three generations of membranes [3].

First Generation Membranes

The first generation of barrier membranes developed in the 60s and 70s aimed to achieve a suitable combination of physical properties to match those of the replaced tissue with a minimal toxic response in the host. In the first GTR attempts, a bacterial filter produced from cellulose acetate (Millipore) was used as an occlusive membrane by Nyman et al., in 1982 [4]. Although this type of membrane served its purpose, it was not ideal for clinical application. Later studies have utilized membranes of expanded polytetrafluoroethylene (e-PTFE) specially designed for periodontal regeneration (Gore Tex Periodontal Material) [5]. Other non-resorbable membranes are titanium reinforced ePTFE, high-density-PTFE, or titanium mesh [6]. Studies have revealed that titanium reinforcement of high-density PTFE membranes lead to superior regenerative capacity when compared to traditional expanded PTFE membranes mainly due to the additional mechanical support provided by the titanium frame against the compressive forces exerted by the overlying soft tissue [7]. The major drawback is the need for second surgery for the removal of the membrane.

Second Generation Membranes

The second generation of barrier membranes was designed to be resorbable to avoid the need for surgical removal. There are two broad categories of bioresorbable membranes: the natural and the synthetic membranes. Advantages of scaffolds derived from natural matrices include a presentation of physiological cues for the induction and maintenance of cell machinery components and an ability to enzymatically degrade along natural pathways [8]. Natural membranes are made of collagen or chitosan. Successful treatment following the use of such barrier materials have been demonstrated, but the results of studies vary [9].

Several complications, such as early degradation, epithelial downgrowth along the material, premature loss of material, were reported following the use of collagen membranes. Although probably very minimal, there is a risk that infectious agents from animal products can be transmitted to humans, and autoimmunization has also been mentioned as risk. Synthetic barrier materials made of polyesters (e.g., poly(glycolic acid) (PGA),poly(lactic acid) (PLA), poly(-caprolactone) (PCL), and their copolymers) were evaluated in animal and human studies and are commonly used. These materials are biocompatible, but by definition they are not inert since some tissue reactions may be expected during degradation. There is also variability and lack of control over the rate of membrane resorption, which is influenced by factors such as the local pH and material composition [10]. Efforts have been made throughout the years to overcome the limitations of current barrier membranes. Biomechanical properties and collagen matrix stability can be enhanced by means of physical/chemical crosslinking, by ultraviolet (UV) radiation, genipin (Gp), glutaraldehyde, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) [11].

Third Generation Membranes

As the concept of tissue engineering has developed, third-generation membranes have evolved, which not only act as barriers but also as delivery devices to release specific agents such as antibiotics, growth factors, adhesion factors, etc., at the wound site on a time or need basis in order to orchestrate and direct natural wound healing in a better way [3]. Briefly they may be considered into the following sub divisions:

i) Barrier membranes with Antimicrobial activity- Bacterial contamination of the regenerating wound represents the most significant factor leading to a compromised outcome. Bacterial species, bacteria count, and the area of bacterial contamination present on the GTR membrane are some of the factors that may affect GTR outcome [12]. The bacteria found on GTR membranes include various Gram-positive bacteria as well as periodontal pathogens. Membrane bacterial count is positively associated with gingival recession and is negatively associated with clinical attachment gain [13]. A systemic antibiotic is usually prescribed after a GTR operation to reduce bacterial contamination and to prevent wound infection. However, the results are not predictable.

It was demonstrated that incorporation of amoxicillin or tetracycline into various GTR membranes may enhance the attachment of periodontal ligament cells in the presence of the oral pathogens streptococcus mutans and aggregatibacter actinomycetemcomitans [14]. Tetracyclines have been advocated as useful adjuncts in periodontal treatment. Incorporation of 25% doxycycline into a GTR membrane, which was composed of polyglycolic acid and polylactic acid, would seem to have a beneficial effect on periodontal bone regeneration in dogs [15]. When applied clinically, tetracycline-loaded expanded polytetrafluoroethylene (ePTFE) membranes reduced bacterial contamination and increased clinical attachment gain [16]. This proven efficacy may be related not only to their antimicrobial actions but also to their recently recognized non antibacterial properties, which include the anti-collagenolytic, anti inflammatory, osteoclast inhibitory, fibroblast stimulatory properties. Tetracylines thus prolonged the degradation time of collagen membranes, this property can be made to use in certain clinical situations where it is desirable to retain the membrane for a prolonged duration of time.

ii) Barrier membranes with Bioactive Calcium Phosphate incorporation- Many research groups have studied the effect of nanosized hydroxyapatite (HA) particles in electrospun matrices for bone tissue regeneration in vitro. Studies on the membrane prepared by Liao et al., demonstrated that the addition of nano-carbonated hydroxyapatite (nCHAC) improved both the biocompatibility and the osteoconductivity of the membrane [17]. This three-layered membrane had a porous side (to allow cell in growth) which contained nano-carbonated hydroxyapatite/collagen/PLGA, a pure PLGA non-porous side (to discourage cell adhesion), and a transitional layer consisting of nCHAC/PLGA. The authors demonstrated that the incorporation of nano-apatite played a significant role in terms of improving membrane bioactivity and facilitating early cell differentiation.

iii) Barrier membranes with Growth Factor release- Growth factors or morphogens modulate the cellular activity and provide stimuli to cells to differentiate and produce matrix toward the developing tissue. Growth factors have an essential role in the healing process and tissue formation. They influence tissue repair and disease, including angiogenesis, chemotaxis and cell proliferation; and control the synthesis and degradation of extracellular matrix proteins. Their mode of action is to bind to the extracellular domain of a target growth-factor-receptor that in turn, activates the intracellular signal-transduction pathways. Several bioactive molecules have demonstrated strong effects in promoting periodontal wound repair in preclinical and clinical studies. These bioactive molecules include PDGF, IGFI, basic fibroblast growth factor (FGF-2) , TGF-1 , BMP-2, -4, -7 and -12, and enamel matrix derivative (EMD) that have shown positive results in stimulating periodontal regeneration [18].

It was found that PDGF-BB loaded PLLA membrane might potentially enhance guided tissue regenerative efficacy in rat calvarial defects [19]. In another study Porous polysulfone coated PDGF-BB stimulated proliferation of human periodontal ligament fibroblasts adherent to Porous polysulfone [20]. Controlled release of basic fibroblast growth factor(b-FGF) from a sandwich membrane made of collagen sponge scaffold and gelatin microspheres induced successful regeneration of the periodontal tissues in a short period of time in beagle dogs [21]. Following the preparation of a system constituting of a poly(L-lactide) acid (PLLA) asymmetric membrane combined with an alginate film, it was found that growth factors such as TGF-beta can be incorporated into alginate membranes which functioned as drug delivery vehicle. This system was found to have retained the biological activity when tested in an in vitro model system [22]. A hybrid alginate/nanofiber mesh system with recombinant bone morphogenetic protein-2 (rhBMP-2) delivery system was found to be effective in repair of critical sized segmental defect in rat model [23].

Despite a long history of preclinical evaluation with promising results, the routine use of growth factors as therapeutic agents for periodontal regeneration is not a reality yet [24]. Limiting factors in the current efforts are related to both the mode of growth factor delivery and the requirements for multiple signals to drive the regeneration process. It is highly unlikely that a single exogenous agent can mediate, effectively, all aspects needed for tissue repair. Thus, delivery of a wide range of biological mediators is required if complete tissue regeneration is to be achieved. Furthermore, the way these growth factors are made available is of paramount importance. Ideally, they should be delivered locally, following specific and distinct kinetics, to mimic, as far as possible, the requirements of the injured tissue during the different regeneration phases in situ [25].

OTHER DEVELOPMENTS

Electrospinning (e-spinning) for membrane

The e-spinning technique has demonstrated great potential for processing membranes for periodontal regeneration. Recently, numerous research groups have explored its use to generate fibrous scaffolds for tissue regeneration. E –spinning produces a biocompatible and degradable natural or synthetic polymers that normally resembles the arrangement of the native extracellular matrix (ECM). Li et al., have cultured different cells such as fibroblasts, cartilage cells, and mesenchymal stem cells on PLGA and PCL nanofibrous e-spun scaffolds and demonstrated the ability of the nanofiber structure to support cell attachment and proliferation [26]. Systematic reviews on the e-spinning process and applications of these nanofibers in tissue engineering are available [27,28].

Functionally Graded Multilayered membranes

Use of multilayered barrier membranes was proposed to utilize a graded-structure with compositional and structural gradients that meet the local functional requirements by enhancing bone growth while preventing the gingival tissue down-growth. With this in mind, fabrication of a functionally graded three-layered membrane from PLGA, collagen and nano-hydroxyapatite by a layer-by-layer casting method was reported earlier [17]. The membrane was designed with one side constituted by 8% nano-carbonated hydroxyapatite/collagen/poly (lactic-co-glycolic) acid porous membrane allowing cell adhesion, and the opposite face with a smooth PLGA nonporous film. A novel functionally graded membrane (FGM) was designed and fabricated via multilayeringe-spinning [29]. The FGM consists of a core-layer (CL) and two functional surface-layers (SL) interfacing bone (nano-hydroxyapatite, n-HAp) and epithelial (metronidazole, MET) tissues. The CL comprises a neat poly(d,l-lactide-co-caprolactone) (PLCL) layer surrounded by two composite layers composed of a gelatin/polymer ternary blend (PLCL:PLA:GEL)

Platelet-Rich Fibrin membrane—An Autologous membrane

PRF was first developed in France by Choukroun et al., for specific use in oral and maxillofacial surgery [30]. The PRF protocol is very simple: A blood sample is taken without anticoagulant in 10-mL tubes which are immediately centrifuged at 3000 rpm for 10 min. A fibrin clot is then obtained in the middle of the tube, just between the red corpuscles at the bottom and acellular plasma at the top. Platelets are theoretically trapped massively in the fibrin meshes. By driving out the fluids trapped in the fibrin matrix, practitioners will obtain very resistant autologous fibrin membranes. The scientific rationale behind the use of these preparations lies in the fact that the platelet α granules are a reservoir of many growth factors (GFs) that are known to play a crucial role in hard and soft tissue repair mechanism. Gassling et al., claimed superior results when PRF membrane was used as a scaffold for human periosteal cell proliferation compared to collagen [31]. When compared to commercially available membranes, PRF membrane offers a pleasant alternative with its cost effectiveness and relative safety because of its autologous nature.

CONCLUSION

GTR procedure has been, and still is, widely employed in periodontal practice and established as a basic technique in periodontal regenerative medicine. Although the indications of GTR membrane in periodontal regeneration are limited to three wall and class II furcation defects, research efforts are pushing the limits to include more advanced periodontal defects with a predictable outcome. It seems likely that a combination of several techniques (such as GTR in association with bone grafts) may offer more chances for a beneficial outcome, although substantial evidence is still lacking. Third generation barrier membranes with additional antimicrobial action and calcium phosphate incorporation or as a source of growth factors offers exciting possibilities to the overall usefulness to the membrane. It is clear that the "ideal" membrane for use in periodontal regenerative therapy has yet to be developed. Based on a graded-biomaterials approach, it is hypothesized that a biologically active and spatially designed and functionally graded nanofibrous material that mimics closely the native ECM could succeed as the next-generation of GTR/GBR membranes for periodontal tissue regeneration.

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