



RESEARCH ARTICLE

MEMBRANES FOR GUIDED TISSUE REGENERATION –AN UPDATE

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Abstract

The ideal outcome of the periodontal therapy is periodontal regeneration with new attachment. However, complete regeneration of the lost periodontal tissue has not been reported yet. In the last 20 decades, guided tissue regeneration (GTR) has evolved as a potential alternative to drive the innate biologically determined regenerative capacity of the periodontal tissue. GTR therapy that uses membranes is based on the principle of cell exclusion and provides stability to the wound and isolates the space for in-growth of the new cells from the base of the lesion to restore the functionality of the periodontium. The concept of GTR has been applied with distinct levels of clinical success in periodontal regeneration. There is no single biomaterial that favours to restore different tissue types. This review gives an insight of different membranes developed till date and their impact on the experimental and clinical manageability of the periodontal defect. Furthermore, this review discusses the recent approach for periodontal regeneration by developing advanced bioactive scaffolds and controlled drug delivery system with the incorporation of nanotechnology, regenerative medicines and stem cell therapy.

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Introduction:-

Periodontal disease if left untreated leads to the progressive destruction of the periodontal tissue and is a major cause of tooth loss in adults. The conventional periodontal therapies has been successful in ameliorating the active disease but the outcomes are variable and results in several repair pattern that does not restore the normal function of the periodontal tissue.

It was Melcher in 1976, who postulated that, the type of cell which repopulates the root surface after periodontal surgery determines the nature of the attachment that will form [1]. Aukhil and colleagues (1988) showed that each cell type results in a specific type of repair or regeneration of gingival epithelium: LJE, bone ankylosis, root resorption, and PDL regeneration (bone, cementum, and PDL)[2].

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In periodontal situations, formation of a long junctional epithelium is the most common form of tissue repair and a typical outcome of traditional periodontal surgery [3]. While regeneration is an ideal outcome of periodontal surgery, the classical studies by Karring (1980, 1982) [4,5], Nyman (1980) [6], Polson & Caton (1982) [7] , Lindhe (1982)[8] & Warrer (1993) [9] further clarified that the cells from PDL have the potential for regeneration of the attachment apparatus of the tooth. Also, it was emphasized that the migration of the epithelium into the periodontal defect impedes the re-establishment of the normal connective tissue attachment. The necessity for exclusion of epithelial and connective tissue cells of the gingiva from the wound led to development and application of GTR membranes.

Biologic Concept of GTR:

Currently, GTR therapy has emerged as a crucial and accepted as superior to conventional procedures. Guided tissue regeneration with the use of barrier membranes works on the principle of cell exclusion to obtain a secluded, physically protected surgical niche to foster progenitor cell differentiation into osteoblasts, fibroblasts and cementoblasts [10].

These barrier membranes must exhibit certain essential physical and mechanical properties such as biocompatibility, cell-occlusiveness, tissue integration, space making and should be clinically manageable, thereby allowing the clinician to conduct the surgical procedure without any undue difficulty [11].

Membranes from different biomaterials have been developed and modified and several authors have reported considerable periodontal regeneration following the use of GTR strategies in well selected clinical cases. However, the variations observed in the results have been attributed to the differences in the case selection, surgical skill and post surgical factors [12].

The review is an attempt to analyze commercially available different biomaterials till date for the GTR and their clinical outcomes as evidenced by different studies.

GTR Biomaterials:

For the propaedeutic purpose, GTR membranes have been broadly classified into two major categories as non resorbable and resorbable membranes.

Guided tissue regeneration using Non Resorbable Barriers:

The non-resorbable GTR barriers, is represented by synthetic and metallic membranes processed in thin layers. These include expanded polytetrafluoroethylene (ePTFE), the dense PTFE (dPTFE) and titanium sheets or meshes.

The first material clinically used to test the hypothesis of GTR, was cellulose acetate laboratory filter (Millipore filter) and ePTFE (Gore- Tex) [13,14] which showed successful regeneration of the alveolar bone and new attachment of new cementum with inserting PDL fibres .

ePTFE is manufactured from polytetrafluoroethylene, when it is subjected to high tensile stress forming porous microstructure of solid nodes & fibrils. Owing to its inertness, biocompatibility, nonadhesiveness, mechanical strength and hydrophobicity, it has been accepted as gold standard for human and animal comparative studies. [15]

Numerous clinical research have evidenced the benefits of ePTFE membranes when used alone [16] or in combination with bone substitutes [17,18] including gain in clinical attachment and horizontal and vertical bone fill.

The high density PTFE (d-PTFE – TefGen) developed in 1993 from the pure medical grade & inert PTFE having a pore size of 0.3 μ , offers an improved alternative to ePTFE membranes as they are non expanded, non –permeable and can be removed with the gentle tug.

It prevents periodontopathogenic bacteria adhesion [19] and assures good bone regeneration even when the membrane is exposed to the oral cavity [20]. The limitation with d-PTFE membranes is their tendency to collapse into the defect.

The Gore-Tex ePTFE has been reinforced with titanium to overcome the problem caused by the compression or the displacement of the graft during the post-op period [21], to increase the mechanical strength and tent like effect for the defect morphology that does not create an adequate space.

They are often used to contain the autogenous and the allografts when the bone is damaged or can be used in staged /non staged approach with dental implants to gain bone volume. Recently, a clinical study reported that GTR with Ultra thin pure titanium mesh (Ultra-Ti) in intrabony defects, yielded significant RAL gain, PD reduction and bone fill when compared to OFD [22].

The disadvantage with these membranes is increased risk of exposure due to their stiffness. A recent case series study of 4 patients [23] reported that by partial removal of the exposed titanium mesh while leaving the remainder to continue the regenerative process, the bone volume reached a level that was adequate for dental implants. Yet another benefit of exposure removal is to create a hygienic space for the implant.

Regrettably, the non resorbable membranes requires second surgery for membrane retrieval further implicating discomfort and pain to the patient and also jeopardizing the maturation of the new and sensitive regenerated tissue. This led to the development of absorbable membranes.

Guided Tissue Regeneration Using Resorbable Barriers:

Recently, absorbable membrane are degraded in-vivo which starts immediately upon placement in the surgical site and the rate may vary considerably (between 4 weeks and several months) particularly for those materials that are degraded enzymatically[24]. Both the natural and the synthetic membranes have been tested for GTR.

Synthetic resorbable GTR membranes are organic aliphatic thermoplastic polymers, most commonly poly- α -hydroxy acids, which include polylactic (PLA), polyglycolic acid (PGA) and their copolymers [24]. The biodegradation of these membranes occurs by hydrolysis releasing lactic acid and glycolic acid which are eliminated through Krebs cycle as carbon dioxide and water.[25]. The degradation rate of these membranes can be tuned through the addition of lactides and glycols to the polymer chain [26].

To date a wide range of polymeric membranes have been used in the clinical settings and has proved their efficacy in various periodontal defects. Guidor, made of polylactic acid and a citric acid ester acetyl tributylcitrate was the first to appear in the market. The membrane was removed from the market possibly because of foreign body reaction following degradation.

Hybrid membranes like Resolute (copolymer of lactide and polyglycolide), Epiguide a poly(D,L-lactic acid) PDLLA asymmetric composite membrane containing the Bioglass [27], Atrisorb, only GTR membrane manufactured chairside, PCL membrane (polycaprolactone) or poly trimethylene carbonate (TMC) incorporating calcium carbonate nanofibres [28], PCL membranes with silica xerogel [29] and SPCL bilayered membranes (a blend of starch and PCL functionalized with Si) [30] have shown excellent cellular response in terms of proliferation and differentiation of pre-osteoblast cells and a much higher bone formation rate. Some of the shortcomings of these membranes include lack of tissue integration, inflammation at the flap margins and frequent recession during healing.

Natural biomaterials have been recently explored as GTR membranes. They can be collagen based, alginate/chitosan based or platelet based barrier membranes.

Collagen based membranes derived from human skin animal (porcine & bovine), tendons or intestines have gained increased popularity owing to its auspicious biological properties as low immunogenicity, chemotactic for fibroblasts, extensibility and high tensile strength [31]. Most of the commercially available collagen membranes are manufactured from Type I and Type II or a combination of both.

Taking into consideration their poor performance in vivo, in terms of controlling degradation rate, lack of mechanical strength and space making ability in the absence of adequate bone support [32], various physical/chemical cross linking methods have been proposed ensuring improved biomechanical property, matrix stability and degradation rate which is proportional to the number of crosslink [33].

Despite of their effectiveness, cross linking with glutaraldehyde has shown increased cytotoxicity owing to the release of glutaraldehyde during degradation [34].

Alginate based membranes have been developed for GBR in situ [35] and have shown significant new bone formation while avoiding growth of the soft tissue.

Similarly, chitosan a natural occurring biopolymer has been found to be processed into membranes owing to its biocompatibility and biodegradability. Various modifications were made to improve the bioactivity as chitosan based membrane with a coating of alginate [36], asymmetric porous membrane [37], chitosan membrane coated with calcium silicate [38], with bioactive glass [39] and novel zinc doped chitosan-HA [40].

Although the natural biomaterials have excellent biocompatibility with cellular binding sites but have low mechanical strength but synthetic biomaterials have tunable degradation rates and mechanical properties, but lack cellular binding motif [41].

Nanotechnology in GTR:

The critical drawbacks of the aforementioned biomaterials strongly emphasized the need for both bioactive and multilayered membranes that aim to not only meet the adequate mechanical properties and degradation rate but also to deliver the biomolecules (antimicrobials and growth factors) and/or stem cells in order to amplify the regenerative potential. With the introduction of the nanotechnologies, the traditional biomaterials are modified or combined with the nanobiomaterials to provide a suitable scaffold system for osteoconduction and osteointegration to induce functional periodontal tissue regeneration.

These nanobiomaterials offer a larger surface area to volume ratio, increased wettability and protein adsorption when compared to the conventional biomaterials [42]. Also, a well defined nanofibrous network and nanofibrous scaffold holds an enormous potential to drive the regeneration of periodontal tissue. For example, parallel nanogrooves or nanofibres are associated with the alignment and cell migration while nanopits direct cell morphology and differentiation [43]. Also, the imitation of fibre alignment is critical for PDL regeneration.

Electrospinning, discovered by Formals in 1938 is a simple and effective method to prepare a 3D nanofibrous matrix with a high surface area, improved hydrophilicity and wettability [44]. The nanofibrous scaffolds mimics the architecture of the ECM of PDL, positively encourages cell-extracellular matrix interactions, increase cell proliferation rate, maintain cell phenotype, support stem cell differentiation and activate cell-signaling pathways by providing physical and chemical stimuli to cells [45].

Since, electrospinning controls cell orientation on the surface of the matrix, channel containing scaffold [46] and 3D printing micro channel fibres guiding scaffolds [47] were developed to induce the partial alignment of the fibres, however, no in vivo results could confirm the guiding function of 3D printing scaffold.

Recently, a novel spatially designed and functionally graded bioactive periodontal membrane with a layered structure synthesized via sequential layer by layer process by spinning different polymers solution one at a time was introduced by Bottino et.al.2011 [48]. The functionally graded membrane (FGM) comprises of a core layer (CL) and two bioactive surface layer (SL) interfacing with bone containing nano-hydroxyapatite (n-HAp) to enhance osteoconductivity and epithelium containing metronidazole (MET) to inhibit the bacterial colonization. The CL comprises of poly (DL-lactide-co-ε-caprolactone) (PLCL) layer surrounded by two composite layers composed of a protein/polymer ternary blend (PLCL: PLA : GEL) [48].

Moonesi Rad R et.al [49] developed a novel FGM comprising of cellulose acetate layer prepared gelatin and 7% boron- modified bioactive glass (7B- BG) nanoparticles prepared by electrospinning when characterized by scanning electron microscopy (SEM) and spectroscopy revealed increased surface wettability, biodegradability, better attachment, spreading, and proliferation of human dental pulp stem cells, increase in Ca-P layer formation and higher alkaline phosphatase (ALP) enzyme activity of cells.

Drugs and Growth Factors Incorporating Membranes:

Since most of the therapeutic modalities are aimed at specific aspect of the periodontal disease and that the extended use of systemic antibiotics after GTR operation to prevent bacterial contamination poses significant limitations such as development of bacterial strain resistance.

With the tissue engineering approach, several investigators have developed a biodegradable polymer based scaffold incorporating bioactive (drugs and growth factors) molecules to provide signals for tissue regeneration, to stimulate the innate regenerative capacity and to reduce the risk of failure of regenerative procedure on exposure of the membrane to the oral cavity.

The release system can be classified into monodrug and multidrug delivery system based on the number of drugs to be delivered.

Various monodrug delivery system incorporating growth factors like stromal-cell-derived factor 1 (SDF-1) [50], rhGDF-5/beta TCP [51] and BMP-2 [52] has been developed to facilitate recruitment of host mesenchymal and hematopoietic stem cells and bone regeneration.

Since the bioactivity of the incorporated bioactive agents is often reduced after the fabrication process and the non conventional methods of incorporation could not control the burst release, a nanosphere encapsulated microsphere system for bone regeneration is developed where BMP-2 bound to heparin and was encapsulated into heparin conjugated gelatin nanospheres which were immobilized in nanofibrous microspheres [53].

Further, incorporation of antibacterial substances showed reduced biofilm formation, prevented bacterial contamination and increased attachment of periodontal ligament cells even in the presence of periodontal pathogens [54]. In a recent SEM analysis by Chen CF 2015 [55], showed that incorporating amoxicillin or tetracycline greatly reduces adhesion of *S. mutans* or *A. actinomycetemcomitans* on ePTFE, glycolide or collagen membranes. The proven efficacy of tetracyclines is due to its antibacterial and non-bacterial properties like anti-collagenase, anti-inflammatory, osteoclast inhibitory and fibroblast stimulation thereby prolonging the degradation time of collagen membranes which is desirable in clinical situations where membranes are to be retained for prolonged duration of time [56].

Further, incorporating 25% doxycycline and dexamethasone in the bilayered electrospun poly(lactic co-glycolic acid) (PLGA) membranes with nanofibres had shown significant osteogenic potential, increased ALP and osteocalcin activity, calcium deposition [57].

However, the bacteria found on GTR membranes includes a wide variety of Gram-positive and Gram-negative bacteria and is negatively associated with gingival recession and loss of clinical attachment. A multidrug delivery system was developed loaded with four different drugs (metronidazole, ketoprofen, doxycycline and simvastatin) separately in different layers of a slower eroding polymer system composed of cellulose acetate phthalate and Pluronic F-127 [58].

Another multiphase drug delivery carrier developed is lovastatin loaded into PLGA microspheres encapsulated in a tetracycline loaded chitosan which has been shown to control infection, inflammation and facilitate osteogenic potential [59].

A particulate based drug delivery system is developed where the bioactive reagents in particulates are incorporated into a scaffold. In this system, IGF-1 loaded in alginate microparticles and BMP-6 loaded in PLGA microparticles which were incorporated into a chitosan scaffold [60].

Amniotic membranes have also shown antibacterial properties in addition to its anti-inflammatory, antifibrotic, low immunogenicity and angiogenic properties. A recent study, by Ashraf H et.al 2019 reported that the antibacterial property of the amnion chorion membrane (Bio Xclude) may be beneficial in the early wound healing and is as bactericidal as paper discs inoculated with tetracycline at its minimum bactericidal concentration [61].

Immunomodulatory Biomaterials:

It is recently, that an immunomodulatory scaffold is developed for alveolar bone regeneration targeting the macrophages that play an important role in activation and the resolution of the inflammation. These macrophages are capable of switching from classically activated pro-inflammatory M1 phenotype to pro-healing M2 phenotype and IL-4 is an effective cytokine to bring about this change. In this system, since IL-4 has a binding domain for the heparin, the nanofibrous heparin modified gelatin microspheres were incorporated with IL-4 thereby prolonging the denaturation and degradation of IL-4 for its sustained release. This immunomodulatory biomaterial has been found to be promising in potentiating new bone formation [62].

GTR and Stem Cell Therapy:

As previous studies have clarified that the cells from the periodontal ligament have the potential to induce the formation of new tissue, the hybrid tissue engineering technology and GTR technique has pleaded the development of biomimetic scaffolds with the incorporation of the progenitor stem cells. Different types of cells, including bone marrow derived mesenchymal stem cells (BMSC's) [63], periodontal ligament stem cells (PDLSC's) [64] and dental pulp stem cells (DPSC's) for scaffold free and scaffold based approaches. Scaffold free approach had the limitation of diffusion of the cells out of the defect. However, in the scaffold based or cell sheet technique where the cells are entrapped in the ECM and is capable of inducing significant bone formation than cells suspension by preventing migration of the cells [65].

Conclusion and Future Perspective:-

Guided tissue regeneration has come a long way with the clear understanding of basic biology of periodontal regeneration. To overcome the structural and the biomechanical limitations of the biodegradable and non-biodegradable membranes, biomimetic scaffolds were developed. Although the regenerated periodontal tissue could simulate the natural periodontal tissue, but none of the scaffolding system could regenerate Sharpey's fibre without which the tooth will be unstable and will not be able to bear the brunt of the occlusal forces.

Also, there are only few studies that have reported the outcomes for the use of GTR combined with bone graft material or tissue engineering strategies on horizontal bone loss regeneration [66]. There is an urge for more clinical studies to further validate the use of the novel biomaterials to be able to completely restore the functionality of the damaged periodontium at micro and nanoscale level.

Despite these problems, GTR has emerged as predictable alternative for the treatment of the periodontal defects. With the hope of overcoming these challenges we will be able to provide promising health care to the patients.

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