REVIEW ARTICLE

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Biomaterial development for oral and maxillofacial bone regeneration

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Many oral and maxillofacial bone defects are not self-healing. Guided bone regeneration (GBR), which uses a barrier membrane to prevent the soft tissues from invading the defect to enable slower-growing bone cells to penetrate the area, was developed as a therapy in the 1980s. Although there has been some success with GBR in some clinical situations, better treatments are needed. This review discusses the concept of GBR focusing on bioactive membranes that incorporate osteoconductive materials, growth factors and cells for improved oral and maxillofacial bone regeneration.

Key words: Bone, Guided bone regeneration, Barrier membrane, Bone substitutes, Drug delivery, Stem cells

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I. Introduction

Facial trauma, bone resection due to cancer, periodontal disease, and bone atrophy after tooth extraction may leave non-self-healing oral and maxillofacial bone defects[1]. Although there is an inherent self-repair potential, a non-union remains for bone gaps greater than 25 mm or even only 500 μm, depending on the location, vascularization, and the mechanics[2]. The presence of infection or inflammation is another determining factor for bone regeneration capability at a given site. For the alveolar bone in particular, periodontitis, advanced periodontal disease that affects around 15% of adult humans, induces the destruction of the alveolar bone around teeth and can cause them to fall out[3,4]. Without the mechanical stimulus from teeth, alveolar bone naturally further degrades[4,5]. Interventions with tooth implants are additionally complicated since sufficient quantity and quality of the alveolar bone must be present to stabilize the implant[6]. Peri-implantitis, infection and inflammation around tooth implants, can have the same detrimental effects on the alveolar bone that periodontitis has on natural teeth.

As discussed by Mikos et al.[7], there are some unique challenging aspects of tissue regeneration in oral and maxillofacial tissues. The irregular architecture and necessary precision of positioning the biomaterial replacements suggest injectable or moldable substitutes. Implants need sufficient mechanical properties and appropriate resorption rates that coordinate with tissue ingrowth. Moreover, the environment of the oral cavity with its flora presents additional complications for alveolar bone engineering.

Despite these challenges, guided bone regeneration (GBR) was developed and has served as a treatment since the 1980s for restoring osseous maxillofacial tissues[8,9]. An excellent overview on this subject was provided by Buser[10]. Through the use of a barrier membrane, this therapeutic strategy physically excludes ineffective soft tissue cells to allow osteoprogenitor cells to populate the area. Since its initial conception, the GBR membranes have progressed from non-resorbable to resorbable to bioactive occlusive materials. GBR has been shown to reliably close critical size periodontal defects and can even support neo-osteogenesis, bone growth extending past the original boundaries[11]. Although there has been success with GBR in various clinical situations, others, particularly alveolar ridge augmentation combined with implant placement, require further research to improve outcomes[11]. Thus, research on advanced materials, growth factor incorporation, and the inclusion of cells is ongoing to improve GBR for oral and maxillofacial bone regeneration.

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II. Guided Bone Regeneration

1. Concept and requirements

GBR gained traction as a therapy in the 1980s, but in 1957 there was already an initial report of space creation with plastic cages for bone regeneration in femoral defects in dogs[12]. Other studies followed, including those focusing on the craniofacial area[13,14], but the mechanism of action was initially hypothesized to be the protection of the blood clot. In the early 1980s, the guided tissue regeneration principle was established, explaining that a specific tissue can be regenerated when cells with the restorative ability for that tissue type are able to occupy the wound space[9,15]. Through space maintenance and the preclusion of faster growing cells from the gingival connective tissue and oral mucosal epithelium, osteogenic cells are allowed to infiltrate and form bone[8,9,15-17].

By enabling new bone formation, GBR may obviate the use of autologous bone grafts. Although an excellent source, the use of autogenous tissue is not desirable due to the pain and morbidity associated with the graft harboring site[4,18]. To function properly, GBR membranes must meet certain requirements, including cell exclusion, space creation, scaffolding for progenitor cell in-growth, biocompatibility, host tissue integration, and clinical manageability[1,11,17].

2. Non-resorbable and resorbable membranes

The first generation of barrier membranes was non-resorbable, with expanded polytetrafluorothylene (ePTFE) (Teflon; Gore, Flagstaff, AZ, USA) membranes becoming the most frequently used[1,8,11]. With its high stability, non-immunogenicity, and reliably successful results, ePTFE membranes have been considered a gold standard for bone regeneration[11,19], despite the fact that ePTFE-membrane exposure to the oral cavity always resulted in a failure of the treatment.

Even with the favorable outcomes using non-resorbable ePTFE, the strong disadvantage of requiring a second surgery to remove the material encouraged the development of a second generation of GBR with degradable barrier membranes. Along with the morbidity associated with a second surgical procedure for non-resorbable membrane removal, there is a risk of tissue damage and disturbed healing[19]. Additionally, there are some post-surgical complications of membrane exposure with non-biodegradable membranes that lead to a high incidence of infection that can decrease bone regeneration[20,21]. Besides avoiding the second surgery, resorbable membranes are also advantageous because of improved soft tissue healing and lower bacterial contamination risk due to decreased exposure from the degrading membrane[19]. In addition to the requirements stated previously, there are also further properties that these barrier membranes must fulfill: biocompatible degradation products that don't interfere with bone regeneration, appropriate degradation profile to synchronize with new tissue growth, and sufficient persistent mechanical and physical properties to perform the barrier function and allow *in vivo* use[3].

Amongst the most common bioresorbable membranes are synthetic polyesters (poly(lactic acid), poly(glycolic acid), and poly(caprolactone) and their copolymers)[22-25] and tissue-derived collagen[21,26-29]. Polyester membranes display biocompatibility and possess a high degree of customization, with degradation rates and mechanical properties that can be adjusted based on polymer composition and concentration[30,31]. As a natural component of the extracellular matrix, collagen is biocompatible and cell adhesive. Although collagen isn't inherently mechanically stable, it can be modified through various means of crosslinking[3]. Poly(ethylene glycol) (PEG) is also known as a biodegradable and biocompatible polymer. Since many oral and maxillofacial defects require precise shapes, an injectable material is desirable, such as a PEG-based *in situ* forming gel for GBR that demonstrated effectiveness in a clinical trial[32-34].

In addition to the type of material, the physical form also plays a role in determining a material's properties, which can affect degradation rates and tissue integration. Porosity is one of the most important characteristics. With some materials, such as poly(lactide-co-glycolide), the porosity can be imposed by using porogens that form the pores and then are removed in a subsequent processing step[35]. Electrospinning is a manufacturing technique that creates elongated fibers with a degree of control over properties including fiber length, width, and orientation and overall porosity[3]. Researchers have explored these techniques for improving GBR membranes.

Although the existing bioresorbable membranes fulfill many requirements, most of them cannot maintain adequate space to act as a barrier membrane over an extended period of time[5,19]. However, in combination with a bone substitute material, these composite membranes have shown success[24].

III. Bioactive Membranes

In addition to providing mechanical support to resorbable

GBR membranes, the inclusion of bone substitute materials can render a membrane bioactive. Current research aims to develop a third generation of GBR membranes that are not only occlusive and degradable, but also contain bioactivity to biologically stimulate osteoprogenitor cells for enhanced bone growth. Using tissue engineering principles, advanced materials with the incorporation of bioactive molecules and cells are being explored for the development of the next generation of membranes. Instead of simply maintaining a space for osteoprogenitor ingrowth, the critical aspects of the natural environment are being recapitulated.

1. Bone graft materials

Effective bone graft materials can biologically stimulate bone growth through either osteoconduction by allowing cell growth through a scaffolding mechanism or osteoinduction by recruiting osteoprogenitors into the defect space[4]. Although cancellous autogenous bone grafts act as an osteoconductive material, the associated morbidity of the graft site restricts its use. Other natural sources include both allografts and xenografts, which are processed to reduce immunogenicity[36]. Demineralized bovine bone matrix retains type-1 collagen, non-collagenous proteins as well as a small amount of osteoinductive growth factors[37]. One of the most common commercially used products is deproteinized bovine bone matrix, Bio-Oss (Geistlich Biomaterials, Wolhusen, Switzerland), which is stripped of all organic elements by pyrolysis, a high temperature sintering, leaving hydroxyapatite (HA) as the main component[38]. Unfortunately, high temperature sintering also affects HA and alters it from a biological HA to a more synthetic one[39]. Despite positive outcomes of naturally-derived materials, there are still concerns regarding disease transmission and immunogenicity[4].

Synthetic graft materials supply various formulations of calcium phosphate without the limitations of the animal-derived products. Bone is composed of a majority of calcium phosphate and these synthetic substitutes are known to have biologically active surface chemistry for osseointegration[40]. Additionally, these biodegradable, inorganic materials have a crystallographic structure similar to bone and controllable porosity that is important for mimicking bone, making them effective substitutes[41,42]. HA has been utilized as granules[42,43], incorporated as nanoparticles[3], or as a coating[44]. Tricalcium phosphate (TCP), which resorbs faster than HA, is another commonly used inorganic bone substitute[41,45]. Bioactive glass is another inorganic bone graft material that displays osteoconduction and an ability to bond with bone through chemical linkages[46,47].

2. Growth factors

Membranes and graft materials can act as a barrier to fibrous tissue ingrowth and a scaffold to support bone and some even provide some osteoinductive activity, but a further critical role of biomaterials in improving oral and maxillofacial bone regeneration is delivering bioactive molecules. Growth factors are signaling proteins that regulate cellular growth, proliferation, and differentiation. Enamel matrix derivative and platelet rich plasma are both biologically-derived products that contain multiple growth factors and have demonstrated enhanced healing in periodontal tissues even though the mechanism of action is not understood[3,48,49]. Platelet rich plasma is comprised of various autologous growth factors that have individually been identified to enhance bone regeneration, including platelet-derived growth factor[50], fibroblast growth factor[51], and insulin-like growth factor[52], but its positive effect on regeneration processes appears to be restricted to soft tissue healing[49,53].

The class of bone morphogenetic proteins (BMPs), first identified to generate extraskeletal bone formation in bone extracts in 1965[54], have now been extensively studied and shown to be critical for the induction of bone[55]. The class of BMPs consists of 15 variants, with BMP-2, BMP-4, BMP-7, and BMP-12 shown to be particularly effective in bone regeneration[56]. BMP-2 and BMP-7 have been recombinantly produced for commercial use[45]. BMP-2 has shown impressive potential to regenerate bone in animal studies and the clinic, including in oral applications[57]. GBR in conjunction with BMP-2 delivery by a bone substitute material has been shown to be an effective strategy in humans as well[58]. However, milligram doses that are orders of magnitude higher than normal physiological levels are required[59]. An enhancer of BMP-2, such as N-methyl pyrrolidone (NMP), can possibly avoid the high cost and possible side effects and safety concerns of the large dose[60]. In a rabbit calvarial model, NMP enhanced bone regeneration over a polylactide membrane alone, emphasizing the importance of the bioactivity[61].

Besides enhancers, controlled release of growth factors can increase their efficiency. In fact, acting as a vehicle for local delivery of growth factors and protecting them from degradation and inactivation are major roles of biomaterials.

A slow, controlled release BMP-2 delivery system has been shown to induce and sustain bone formation[62]. Many of the same materials used as bone grafts have also been explored as delivery systems: collagen[63], calcium phosphates[64], and polyesters like polycaprolactone[65].

Growth factors can non-covalently bind or covalently attach to the carrier[66]. Non-covalent growth factor delivery systems can function through adsorption (e.g., collagen sponge[63] and deproteinized bovine bone matrix[38]), ion complexation with charged polymers (e.g., poly-L-ornithine and poly-L-arginine complexes[67]) or physical entrapment (e.g., polyesters[68] and PEG[38]). The materials that physically incorporate growth factors can take various forms from liposomes[69] to nanoparticles[70] to hydrogels[71]. In contrast to these modes of delivery, covalent systems retain growth factors at the site of action until cleaved off or the carrier is degraded, extending the residence time[66]. Additionally, immobilization enables spatial growth factor delivery, limits side effects by constraining the growth factors to the site of action, and mimics physiological matrix-bound situations[72]. Growth factors can be covalently tethered to a material directly[73,74] or through a linker[75,76]. Another strategy is genetically engineering fusion growth factors that include an attachment site outside the active protein sequence. BMP-2 was engineered to contain amino acid domains that enable both enzymatic covalent attachment to and release from fibrin-based substances, creating a system that mimics physiological binding and liberation[77]. Although it is possible that the growth factor may lose some activity through all of these covalent immobilization methods, this loss may be mitigated by the higher retention and other advantages.

3. Cells

In addition to allowing the delivery of bioactive molecules, biomaterials are also critical for enabling cell-based therapies. To encourage proliferation and differentiation, cells require an artificial extracellular matrix, which can be supplied through an appropriately designed biomaterial[36]. Including a cell source can further encourage oral and maxillofacial bone growth through direct tissue growth and bone repair as well as growth factor secretion from the cells[78]. Although this area has only been actively pursued relatively recently, due to advancements in biological cell research, there are some promising studies.

Mesenchymal stem cells (MSCs), multipotent adult stem cells that can be harvested from mesenchymal tissues such as bone marrow, have been suggested as a cell source for tissue engineering[56]. Since they are easily attained and expanded, bone marrow MSCs (BMSCs) are the most commonly explored MSCs[36]. A number of studies have demonstrated alveolar bone regeneration with BMSCs[79-81]. A study also showed the positive effect of combining stem cells with growth factor release through BMP-2 expressing BMSCs[82].

In addition to BMSCs, there are several other cell types that are being explored for oral and maxillofacial bone tissue engineering. Umbilical cord MSCs are another easily obtainable reservoir of stem cells and initial studies show promising results for bone regeneration with this source[83-86]. Adipose-derived stem cells are other extraoral and non-craniofacial cells that are easily accessible and were successfully used to regenerate bone[87,88]. The periodontal ligament and dental pulp are both sources of stem cells in the oral cavity that have been isolated and characterized[56,89]. Stem cells from both of these tissues have demonstrated bone regeneration capabilities[90-92]. However, these cells are more difficult to harvest[36].

With all of these stem cells sources, the biomaterial that supports them is a critical aspect for facilitating bone regeneration. Many of the same materials that have been developed as bone graft materials and bioactive molecule delivery vehicles have been explored as cell scaffolds. HA[93], collagen[94], fibrin[87], and poly(lactide-co-glycolide)[95] as well as composites such as HA/TCP[96], chitosan-gelatin[79], and calcium phosphate cement-chitosan-polyglactin[86] are amongst these scaffolds.

IV. Conclusion

The field of bone regeneration for oral and maxillofacial tissues has progressed dramatically from the first non-resorbable GBR membranes to bioactive materials. Guided by tissue engineering principles, there is a large amount of current research on designing membranes consisting of bioactive materials that can deliver growth factors and cells. Future improvements will require appropriate combinations of materials, growth factors and cells that permit temporal and spatial growth factor release, suitable degradation profiles that both allow tissue ingrowth and maintain sufficient occlusivity, and positive mimicking of the extracellular matrix to support and encourage cell proliferation and differentiation. Composite materials, multi-layered constructs, and varying physical forms are amongst the possible strategies in biomaterial development for this

ongoing body of research.

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