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Review article

Recent advances in biofunctional guided bone regeneration materials for repairing defective alveolar and maxillofacial bone: A review☆

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ABSTRACT

The anatomy of the oral and maxillofacial sites is complex, and bone defects caused by trauma, tumors, and inflammation in these zones are extremely difficult to repair. Among the most effective and reliable methods to attain osteogenesis, the guided bone regeneration (GBR) technique is extensively applied in defective oral and maxillofacial GBR. Furthermore, endowing biofunctions is crucial for GBR materials applied in repairing defective alveolar and maxillofacial bones. In this review, recent advances in designing and fabricating GBR materials applied in oral and maxillofacial sites are classified and discussed according to their biofunctions, including maintaining space for bone growth; facilitating the adhesion, migration, and proliferation of osteoblasts; facilitating the migration and differentiation of progenitor cells; promoting vascularization; providing immunoregulation to induce osteogenesis; suppressing infection; and effectively mimicking natural tissues using graded biomimetic materials. In addition, new processing strategies (e.g., 3D printing) and new design concepts (e.g., developing bone mimetic extracellular matrix niches and preparing scaffolds to suppress connective tissue to actively acquire space for bone regeneration), are particularly worthy of further study. In the future, GBR materials with richer biological functions are expected to be developed based on an in-depth understanding of the mechanism of bone-GBR-material interactions.

Contents

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1. Introduction

Alveolar and maxillofacial bone defects caused by trauma, tumors, and inflammation are common [1–4]. Although bone has healing capacities, bone defects larger than a critical size together with nonunion fractures are still challenging to repair in clinical practice [5]. Autografts are considered the standard of care for repairing large periodontal bone defects, but their use is limited due to donor scarcity and site morbidity [6,7]. Allografts, on the other hand, can impart immunogenic responses due to host-foreign-tissue interactions and can be a source of disease transmission [8,9]. Among the most effective and reliable methods to attain osteogenesis, the guided bone regeneration (GBR) technique is extensively used in periodontal, alveolar, and implant surgery.

GBR technique was firstly proposed in 1988 by Dahlin, they applied grafts and barriers to mechanically exclude soft tissue from osseous defect to reconstruct defected bone [10]. Until now GBR techniques are always combined with bone grafting and other bone-substitute implantation therapies for bone regeneration [11,12]. Initially, the concept of GBR technique was to apply barrier materials to create secluded anatomic site to promote healing of bone [10]. With the development of medical and material science and technology especially the proposing of tissue engineering route in early 1990s, more and more elements such as scaffold or barrier materials, associated regenerative cells or stem cells, and cytokines or growth factor have been introduced into GBR techniques [13]. Among those elements, applications of GBR materials or materials-cell composites are the central elements of the GBR technique [14]. The basic function of GBR materials is to resist the invasion of connective tissue and maintain sufficient space for bone regeneration [15,16]. In addition, the ideal biomaterials for bone regeneration should have multiple biological functions to facilitate the self-healing capabilities of bone. These functions include: (i) providing the main structural, compositional, and biochemical cues for the formation of new tissue; (ii) promoting the recruitment, proliferation, and differentiation of progenitor cells; (iii) engaging the host's resident immune cells in the regenerative response; (iv) recovering an adequate local blood supply to support healing and remodeling; and (v) providing anti-infective function in non-sterile environments, such as bone absorption caused by periodontitis [8,17,18].

In this review, advances in GBR materials applied in the oral and maxillofacial regions in recent two years are presented. These materials are classified based on their biological functions. Functionalization strategies designed to overcome the negative properties, and facilitate space maintenance, osteoblast proliferation and differentiation, progenitor cell migration and differentiation, mineralization, vascularization, immunoregulation-induced osteogenesis, and anti-infective functionality have been described (Table 1). Finally, the latest advances in graded GBR materials with multiple functions are summarized. The aim of this review was to elucidate the effects of different biological functions of GBR materials on the regeneration of defective bones and to provide assistance to GBR materials researchers.

2. Space-maintaining materials

Providing column stability that preserves the space of the bone defect is a main function of GBR materials in bone-defect repair, especially in alveolar bone repair, to prevent blood clots and isolate the defect from the outlying connective tissue. It has been shown that new bone formation in a maintained space is enhanced by complete isolation of the periosteum using barrier materials [19]. To achieve this, the GBR materials need to meet certain criteria in terms of their mechanical properties. These space-maintaining materials applied in bone defect repair can be divided into three categories: those strengthen collagen membranes; biocompatible metal barrier materials; and other natural or synthetic biocompatible materials.

2.1. Strengthening collagen membranes

Collagen is a major structural protein of the extracellular matrix (ECM), and collagen-based materials can support the growth of various tissues owing to their superior biocompatibility and biodegradability. However, conventional collagen membranes can be prone to collapse or deformation within the defect under load due to their insufficient mechanical strength, which limits their capacity to

Table 1
Classification of biofunctional GBR materials.

Biofunctions of GBR materials	References
Facilitate space maintaining	[19–30]
Facilitate adhesion, migration, and proliferation of osteoblasts	[31–43]
Facilitate migration and differentiation of progenitor cells	[44–60,62–66,68–70]
Induce mineralization	[71–74,81–83,86–89]
Promote vascularization	[90,91]
Regulate immune behavior to induce osteogenesis	[93–95]
Suppress infection	[97–100]
Graded biomimetic membranes	[103–107]

act as a space-maintaining barrier. Various methods have been reported recently for strengthening collagen-based materials to enhance their mechanical properties.

Yu et al. reported that for biomineralization, collagen-based materials and intrafibrillar mineralized collagen materials demonstrated clear advantages, such as enhanced mechanical properties, and were more suitable for use in hard tissue repair than intermineralized collagen materials [20]. Neto et al. compared the biocompatibility and biodegradation of a bovine collagen membrane (Lyostypt®) and porcine collagen membrane (Bio-Gide®), which were applied as GBR membranes by implanting them in the subcutaneous tissue of mice [21]. Compared to the bovine collagen membrane, the porcine one resulted in less irritant reactions and a prolonged degradation period and was more favorable for guiding bone regeneration. Friedmann et al. prepared ribose-crosslinked collagen membranes, which resulted in sufficient bone volume for implant placement for repairing extraction sockets with or without alveolar ridge resorption [22]. In addition, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) was applied as a crosslinking agent to obtain crosslinked collagen membranes [23]. Compared to non-crosslinked collagen membranes, crosslinked collagen membranes have higher tensile strength and more appropriate mechanical properties, enabling membranes to survive in vivo and to supply the stress needed to encourage early tissues to differentiate into pre-osteoblasts. In addition, crosslinked collagen membranes showed excellent enzymatic degradation resistance and human gingival fibroblast proliferation support and in vivo bone defect repair effects. Therefore, EDC crosslinking of collagen membranes is considered a means of improving the physical characteristics of the membrane for guided bone generation.

2.2. Biocompatible metal barrier materials

Multidimensional bone defects require load-bearing membranes with higher strength than collagen-based membranes to achieve volume stability. For these cases, emerging technologies based on titanium mesh combined with resorbable or non-resorbable membranes have been proposed.

Chio et al. evaluated the results of GBR using three-dimensional preformed titanium mesh (3D-PFTM) with or without crosslinking or non-crosslinking collagen membranes covering the Ti mesh for non-contained horizontal defects in 100 patients [24]. The results showed that bone gain was enhanced using a 3D-PFTM on the buccal side of the implant simultaneously with implant placement. The application of resorbable collagen membranes, regardless of whether they were crosslinked, increased the hard tissue gain rate by reducing the exposure of the Ti mesh and inhibiting soft tissue penetration. In addition, the bone regeneration effect of customized Ti meshes manufactured with a digital workflow combined with autologous and heterologous bone implants were evaluated for achieving bone regeneration at future implant sites of five patients [25]. Satisfactory results were obtained in both horizontal and vertical defects, which showed that custom 3D Ti meshes manufactured using CAD-CAM (computer aided design-computer aided manufacturing) technology are promising as a safe and predictable alternative for the regeneration of various bone defects. In particular, a novel fully automated shape-memory-based bone generation device combined with a Ni/Ti strip sandwiched between two layers of silicone sheet for rabbit calvaria bone defect re pair was evaluated [19]. This device was able to augment bone vertically, enabled by its expanding and anti-invasive effects on the overlying periosteum and soft tissue. This device could enable a new generation of smart GBR membranes that can remember the original dimensions of resorbed bone areas.

In addition to Ti-based materials, Mg and various alloys have been investigated as GBR materials because of their biodegradability and excellent mechanical strength. However, the premature degradation of untreated Mg mesh can result in the uncontrolled release of H_2 to form a gas cavity, which can damage the tissues around the material. Therefore, different coating strategies have been developed. Steigmann et al. prepared a passivated Mg membrane by ion implantation under an argon atmosphere followed by physical vapor deposition treatment using a specially constructed coating system. Then, they evaluated the in vivo biocompatibility by using a subcutaneous implantation method in mice. However, their results showed that coating did not have a positive influence on gas cavity formation or improve the immune response compared to the uncoated membrane [26]. Another study developed an HF-treated mesh in a native collagen membrane for rabbit calvarial defect repair [27]. Compared with untreated Mg mesh, the HF-treated Mg mesh showed higher cytocompatibility. HF treatment prolonged the degradation period of the Mg mesh and prevented gas cavities, and the HF-treated Mg mesh could be degraded by mononuclear cells via phagocytosis for up to 12 weeks. The facilitation of bone regeneration by the HF-treated Mg mesh reached the same level as that of collagen membranes.

2.3. Other natural or synthesized biocompatible materials

Biocompatible materials with excellent mechanical properties have also been investigated as GBR materials in recent years. Dubus et al. prepared a novel membrane derived from human umbilical cord by freeze-drying after tissue stripping [28]. The mechanical strength was comparable to that of the Bio-Gide collagen membrane. This membrane is composed of collagen fibers and glycosaminoglycan, especially hyaluronic acid and chondroitin sulfate, and has a mechanical strength similar to that of commercial GBR collagen membranes, and thus, has the potential to be used for bone repair applications. Jung et al. developed a gellan gum/tuna skin gelatin film and evaluated its effectiveness in rabbit calvaria bone regeneration combined with tri-calcium phosphate (TCP) implantation [29]. The repair effect was comparable with that of a commercial collagen membrane. Apart from these natural materials, synthetic scaffolds have also been developed as GBR materials because they can provide an array of unique structures with various physicochemical properties and the ability to promote biological activity. Zhang et al. prepared a chitosan/polycaprolactone/gelatin sandwich-like structure by electrospinning and lyophilization [30]. This scaffold had appropriate physical structures, hydrophilicity, degradation period, and especially mechanical stability. In addition, this scaffold had excellent biocompatibility and achieved effective hemostasis. The scaffolds had strong cell-barrier effects and provided protection from external cell invasion, as indicated by the rat subcutaneous implantation experiments. The authors pointed out that this scaffold has the potential to serve as a platform for improving GBR designs for periodontal regeneration.

3. Materials to facilitate adhesion, migration, and proliferation of osteoblasts

Bone is a dynamic tissue that couples bone formation and bone resorption. Correspondingly, osteoblasts and osteoclasts are representative of bone formation and resorption, respectively. Osteoblasts adhere to the ECM by integrins and also act as secretory cells. Bone matrices, known as osteoids, are secreted by osteoblasts and then mineralized to form bone. Once the secretion ends, osteoblasts become ECM-trapped quiescent cells called osteocytes. Several strategies focus on improving the performance of GBR materials for guiding the adhesion, migration, and proliferation of osteoblasts or their precursor cells. These materials can be divided into four categories: (i) natural macromolecular materials for osteoblast adhesion; (ii) phosphate compounds incorporated with calcium

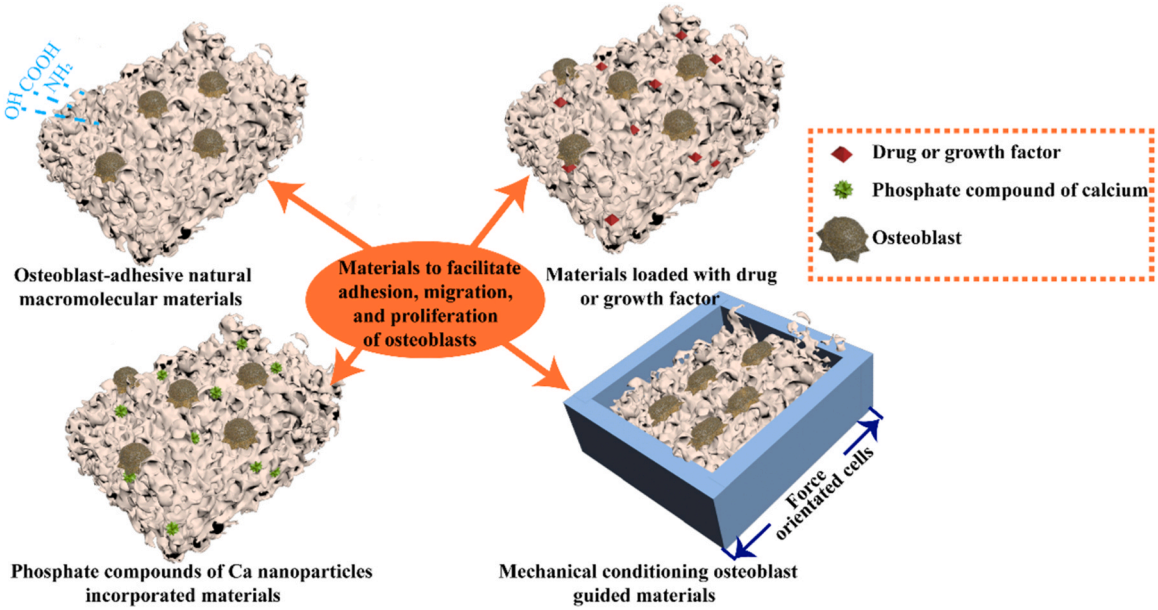

Fig. 1. Classification of adhesive mechanisms for natural macromolecular materials on osteoblast.

nanoparticles; (iii) drug- or growth-factor-loaded materials; and (iv) mechanical-conditioning osteoblast-guided materials.

3.1. Osteoblast-adhesive natural macromolecular materials

Natural macromolecules or their derivatives are often used to synthesize GBR materials because of their good affinity for cells. The hydroxyl, carboxyl, amino, aldehyde groups, or functional peptides of natural macromolecules could react with associated membrane proteins or chelate Ca ions to enhance the affinity between osteoblasts and materials. In addition, some natural materials are bone components that promote adhesion, migration, and proliferation of osteoblasts (Fig. 1).

Zhou et al. prepared a PLGA/PCL electrospun membrane as a basic material and then coated it with collagen I and then Ca-chelated polydopamine [31]. Coating of collagen on the PLGA/PCL membrane enhanced the hydrophilicity, supported cell adhesion, and assisted cell infiltration. Ca-chelated polydopamine enhanced cell–material interactions with higher expression of integrins and promoted proliferation and osteogenic differentiation of osteoblasts. The PLGA/PCL/collagen I-Ca-chelated polydopamine scaffolds met the criteria for bone repair procedures with attractive osteogenic properties, good biocompatibility, and biomimetic functionality.

Lin et al. fabricated a functionalized hydrogel-based barrier membrane with calcium-form poly-γ-glutamic acid (γ-PGA), glycerol, and gellan gum [32]. Calcium aggregates were successfully manufactured by the coulombic interaction between the negative charges of γ-PGA and the positive charges of Ca ions. The physical crosslinking effect of γ-PGA enhanced the mechanical properties and delayed the degradation of the fabricated membranes. Calcium aggregates on the surface of the membrane altered the roughness and mechanical stress, and subsequently affected protein absorption, cell adhesion, proliferation, and differentiation. The γ-PGA-Ca hydrogel-based membranes provided excellent performance for osteoblast proliferation and osteoblastic responses.

Boda et al. manufactured a bilayer membrane containing a chitosan nanofiber layer for hard tissue, and antimicrobial peptides (AMP) incorporating an oxidized pectin-coated chitosan nanofiber layer as a mucoadhesive [33]. The oxidized pectin coating adhered with adhesion proteins by hydrogen bonding and interaction between the aldehyde groups of the oxidized pectin and the amino groups of adhesion protein to enhance the affinity between the membrane and mucosa. In addition, AMP can be released in an acidic environment as ester bonds of pectin and be dissociated. The NH_3^+ of chitosan attracts PO_4^{3-} in hydroxyapatite (HA) to enhance the osteoblastic response of the hard-tissue layer.

Moe et al. fabricated layer-by-layer silk fibroin (SF)/poly(vinyl alcohol) (PVA)/SF membranes by particle deposition [34]. Dissolution of PVA resulted in the infiltration of osteoblasts into the membrane, and the amino acids on the surface of the SF layer stimulated osteoblast proliferation, alkaline phosphatase activity, protein secretion, and calcium deposition.

He et al. prepared acellular sheep periosteum, which had a similar structure to ECM and various promoting effects on the adhesion, proliferation, and osteogenic differentiation of osteoblasts in vitro [35]. No obvious immuno-inflammatory response occurred when this acellular sheep periosteum was subcutaneously implanted into the backs of Sprague Dawley rats.

3.2. Phosphate compounds with incorporated Ca nanoparticles materials

Bioactive ceramics such as hydroxyapatite (HA) granules and beta tri-calcium phosphate (β-TCP) are usually incorporated into GBR materials for their effects in enhancing the hydrophilicity, mechanical properties, and topographies of those materials to facilitate their biocompatibility, osteoblast affinity and proliferation, and mineralization. In addition, these ceramics enhance the osteoinductivity and osteoconductivity of GBR materials.

A series of PLGA electrospun membranes with HA and β-TCP were prepared in several studies [36–38]. The HA-β-TCP membrane could neutralize the acid degradation product of PLGA, enhance the mechanical properties and degradation rate, and increase the biocompatibility compared to pure PLGA membrane. The proliferation and migration of osteoblasts cultured on PLGA- HA-β-TCP and PLGA-β-TCP membranes were significantly improved when compared with those of pure PLGA membrane. The incorporation of these bioactive ceramics enhanced the osteoblastic response of these membranes.

3.3. Materials loaded with drugs or growth factors

To obtain additional biofunctions, local drug delivery systems have been developed for many bioactive molecules, such as drugs and growth factors. The delivery of these agents helps control the complex and self-regenerative phases of the host bone and periodontal tissue, inducing a specific cellular response or differentiation.

Bone morphogenic protein 2 (BMP-2) shows the highest osteogenesis inductivity among BMPs and can regulate the essential components required for the osteogenesis induction process of bone regeneration. A series of injectable BMP-2 agents containing cross-linking gelatin hydrogel-loaded Mg pins was synthesized [39]. BMP-2 in the hydrogel could be sustained for 25–40 days with hydrogel degradation. When the hydrogels were used in the cannulated screw, they delayed biodegradation inside the screw, induced uniform corrosion, and induced precipitation of bioactive compounds on the surface of the screw, while the BMP-2 in the hydrogel improved osteoblast proliferation and differentiation. The improvements were closely correlated with the concentration of BMP-2 in the hydrogels.

Alkindi et al. used a β-TCP and equine bone powder filler with platelet derived growth factor (PDGF)-impregnated collagen membranes to repair critical femoral defects in rats [40], which resulted in a higher volume of new bone compared with the same treatment with pure collagen membranes. The bone repair effect of this system was comparable to that of an autologous bone implanted group due to the potency of PDGF in imparting chemotactic and osteoproliferative functions through cell-surface tyrosine kinase receptors.

Federico et al. prepared hyaluronic acid with pendant aliphatic tails (HA-C_x) at different lengths, and dexamethasone (Dex)-loaded hyaluronic acid membranes were obtained by electrospinning using polyvinyl alcohol and 2-hydroxypropyl-cyclodextrin as flexible polymeric carriers and rheological modifiers [41]. The aliphatic tails modified the hydrophilicity and degradation of the final membranes and made them suitable for application in the GBR area. HA-C_x/Dex membranes were favorable in terms of the cytocompatibility and osteoblast proliferation, because the osteo-stimulatory drug Dex was sustainably released with the degradation of HA-C_x/Dex membranes. Osteogenic differentiation and mineralization of cultured cells on these membranes was promoted.

Öz et al. developed a thermosensitive gel/membrane combined system for GBR [42]. The membranes were fabricated by the solution casting method and loaded with alendronate sodium as an anti-osteoporosis drug. As a potent inhibitor of bone resorption, alendronate sodium can inhibit osteoclastic bone resorption via GTP-associated pathways. Gels prevents soft tissue migration to the defect site and prolonged the residence time of the nanoparticles loaded in membranes. The combined system was combined with a membrane to enhance bone regeneration activity in rabbit tibial defects.

3.4. Mechanical conditioning of osteoblast growth

In addition to applying various chemical reactions, inorganic particles, drugs, and growth factors to stimulate osteoblast production with GBR materials, researchers have focused on the mechanical conditioning of osteoblasts by GBR materials. Bone is a load-bearing tissue that has a unique hierarchical structure, where the cortical bone is composed of repeating osteons in which aligned mineralized collagen fibrils are aligned in certain directions. The surface topography of bone scaffolds has been widely used to regulate cell adhesion, proliferation, and differentiation. This is the design foundation for mechanical-conditioning GBR materials.

Yu et al. prepared multilayer collagen constructs with an angle-ply structure [43]. Aligned osteoblasts formed on the micropatterned collagen membranes produced by a templating method (Fig. 2). The anisotropic microgrooved collagen membranes effectively aligned the cells and promoted osteogenic differentiation. In addition to the microstructure, appropriate mechanical stimulation plays a key role in enhancing osteoblast proliferation and differentiation. Recent research has shown that, compared to compressive loading, tensile loading more effectively facilitates the proliferation and osteogenic differentiation of bone marrow mesenchymal stem cells (BMSCs) and enhances the expression of bone matrix proteins. In addition, they prepared mechanically conditioned multilayer-cell-anisotropic microgroove collagen structures through cyclic stretching. Compared to the same structure without mechanical loading, the mechanical-conditioning collagen promoted the differentiation and ECM production of osteoblasts and showed an excellent effect in repairing critical-sized mouse calvarial defects.

4. Materials to facilitate migration and differentiation of progenitor cells

Stem cells are a class of long-term self-renewing cells that can differentiate into a variety of cells under certain conditions. Transplanted stem cells promote tissue regeneration because of their ability to release cytokines and growth factors. Therapies using materials that promote the growth of human mesenchymal stem cells (MSCs), human bone mesenchymal stem cells (hBMSCs), human adipose-derived mesenchymal stem cells (hASCs), human endometrial stem cells (hEnSCs), dental-pulp derived mesenchymal stem cells (DPSCs), and human periodontal ligament stem cells (hPDLSCs) or stem-cell-material composites to enhance bone and periodontal tissue regeneration have been widely investigated in recent years. From the functionalization perspective of GBR materials on the biological properties of stem cells and progenitor cells, these materials can be divided into four categories (Fig. 3): (i) topography-modified materials to facilitate the adhesion of stem cells; (ii) biofunctional protein-modified materials to facilitate proliferation and osteogenesis differentiation of stem cells; (iii) osteogenesis-inducing metal-ion-incorporated materials to facilitate proliferation and osteogenic differentiation of stem cells; and (iv) drug-loaded materials to facilitate proliferation and osteogenic differentiation of stem cells.

4.1. Topography-modified materials to facilitate stem-cell adhesion

Specific groups, such as functional peptides contained in certain natural polymer materials, such as silk proteins, show some affinity to cells. In addition to the composition of the GBR material, its physical properties, such as stiffness, porosity, pore geometry, and topographical characteristics, affect biological properties such as cell adhesion and proliferation, and osteogenic differentiation of stem cells and progenitor cells.

Sartika et al. fabricated a 3D silk fibroin (SF) scaffold, which was biocompatible and provided sufficient pore sizes and distribution with fine interconnectivity for nutrient and oxygen transport to cells, which enabled effective growth and attachment of HASCs. In addition, osteogenic differentiation of HASCs was promoted after seeding of the SF scaffold. The HASC-incorporated 3D SF scaffolds effectively promoted bone regeneration in critical rat calvarial defects in vivo [44]. PCL-based fibrous scaffold embedded with decellularized bone ECM (bdECM) was fabricated using electrospinning [45]. The incorporation of bdECM enhanced the physical properties of the PCL scaffold, including the wettability, water uptake ability, and roughness, and provided an appropriate environment to facilitate the adhesion and osteogenic differentiation of hMSCs. In another study, poly(L-lactic acid/caprolactone) (PLCL) bilayer membranes were developed for GBR applications composed with loose layer and compact layer via a two-step freezing and lyophilization process [46]. The suitable topography and roughness of the loose layer made this

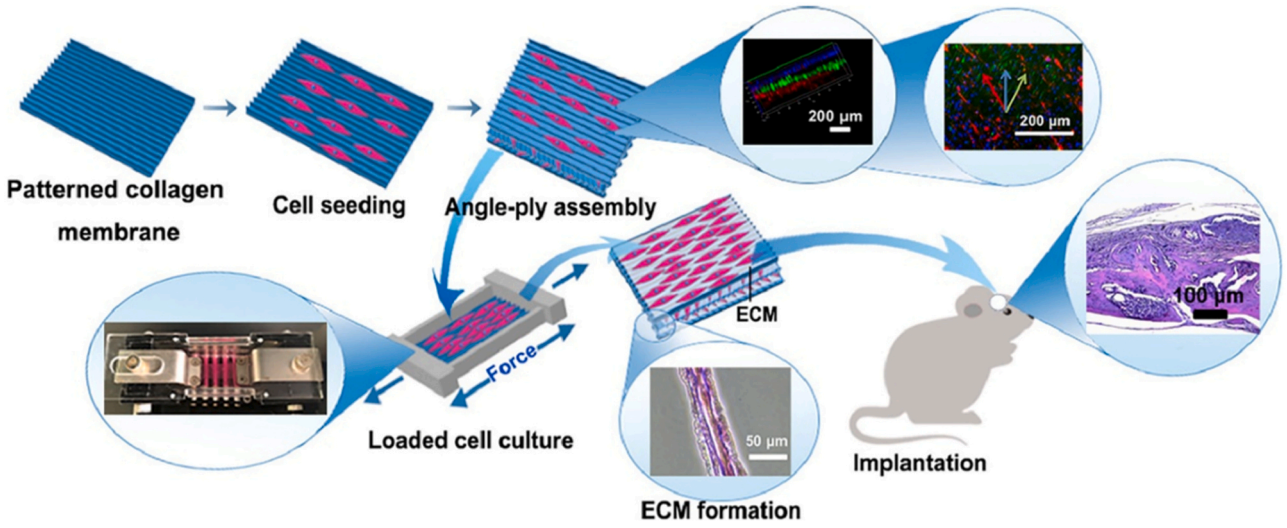

Fig. 2. Schematic illustration of the preparation of a laminated cell-collagen construct with angle-ply structure and its implantation in a mouse calvarial defect model [43].

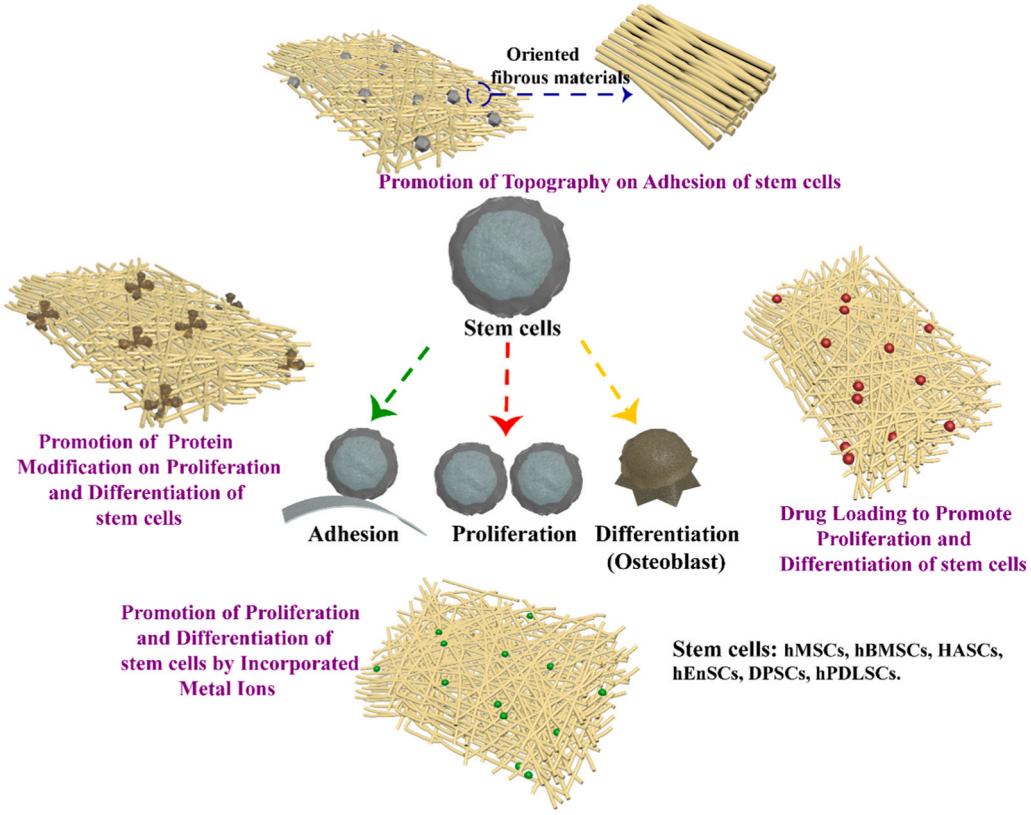

Fig. 3. Schematic classifications of GBR materials to facilitate migration and differentiation of progenitor cells.

scaffold compatible with the mineralized ECM secretion of hBMCs. The proliferation of human gingival epithelium progenitor cells (HGEPs) on the compact layer indicates a biocompatible surface that can promote cell attachment, which can later enable the attachment of epithelia.

In addition to applying the intrinsic properties and proper processing methods to develop suitable materials, the topography and physical properties of GBR materials are often modified by incorporating calcium phosphate nanoparticles. For example, methacrylate gelatin/nano-HA/PLGA membranes, polydopamine-polyacrylamide/hydroxyapatite hydrogel, and poly(3-hydroxybutyrateco-4-hydroxybutyrate) octacalcium phosphate nanofibrous membranes have been fabricated. All of these membranes with incorporated calcium-phosphate nanoparticles showed excellent cell affinity for hMSCs and hBMSCs, and the proliferation and osteogenesis of stem cells were enhanced. In vivo bone-defect repair was also promoted using such membranes [47–49].

Previous studies have noted that aligned structures can enhance cell adhesion to the surface and have a synergistic effect with the physicochemical properties of the material. For example, Lim et al. fabricated aligned equine bone-derived nano-hydroxyapatite (EBNH)/PCL membranes by electrospinning with a high-speed rotating drum collector [50]. The alignment of PCL fibers and bioceramics may have a synergistic effect in enhancing cell affinity,

viability, and other related functions. The osteogenic differentiation of DPSCs was also promoted by the aligned EBNH/PCL scaffold.

4.2. Biofunctional protein-modified materials to facilitate proliferation and osteogenesis differentiation of stem cells

Biofunctional proteins are capable of stimulating cell proliferation, differentiation, and preventing apoptosis, and include growth factors, cytokines (inflammatory molecules), or other proteins that bind to specific receptors on the cell membrane of their targets. Biofunctional proteins regulate cell adhesion, migration, proliferation, and stem cell differentiation. Biofunctional proteins that stimulate the proliferation and osteogenic differentiation of stem cells, such as bone morphogenetic protein (BMP) and recombinant human osteopontin (p-rhOPN), have been used in bone regeneration. Lactoferrin and multicomponent collagen-elastin-like polypeptide (ELP)-modified scaffolds were also investigated [51].

Rameshbabu et al. fabricated a porous hybrid placental ECM sponge (PIMS) using SF/placental-derived ECM [52]. Several intrinsic growth factors, such as transforming growth factor-β1, vascular endothelial growth factor (VEGF), and BMP-4 from the placental-derived ECM were present in the sponge, which provided a unique bioactive scaffolding to human amniotic mesenchymal stem cells, which enhanced proliferation and osteogenic differentiation. This functional scaffold provided sufficient angiogenesis and bone regeneration in a rabbit critical tibial defect model.

Some polypeptides also have an osteogenesis-inducing effect on stem cells, such as ELP. An ELP-bioglass scaffold was prepared and then combined with rat mesenchymal stem cells (rMSCs) and collagen solution to form a hydrogel. In vitro osteogenic differentiation of rMSCs was observed, and the ability of the hydrogel to heal critical-sized cranial bone defects was evaluated in a rat bilateral cranial critical-sized defect model. The rMSC/ELP/bioglass/collagen hydrogel increased the formation of high-quality mature bone in the cellular group, while the acellular scaffold/collagen hydrogel had immature bone and organized connective tissue. These results suggested that rMSC-seeded collagen/ELP/bioglass composite scaffolds can aid bone healing [53].

Lactoferrin, which has functions in the modulation of bone regeneration and inflammation, was also investigated for application as an osteogenic differentiation-modified protein in the fabrication of GBR materials. For example, poly dopamine (PDA)-coated PLLA/PCL electrospun membranes were developed, and then lactoferrin was immobilized onto the PDA coating [54]. The osteogenic differentiation of hASC seeds on the lactoferrin-modified scaffold was significantly enhanced. Furthermore, lactoferrin reduced the inflammatory response of macrophages. The implantation of these scaffolds in vivo to repair mouse calvarial defects resulted in satisfactory bone formation and reduced inflammatory reactions.

Osteopontin (OPN) is abundantly found in the mineral/tissue interface of bone, and structurally, OPN contains two essential amino acid sequences (RGD and SVVYGLR) that mainly interact with various αv integrin receptors on the surface of certain cells. Its major functions include cell attachment, bone formation, and wound healing, which vary among the cell lineages. For example, plant-derived recombinant human osteopontin (p-rhOPN) was combined with bacterial cellulose membrane (BCM) by chemical reaction of carboxyl groups on poly (acrylic acid)-grafted BCM with p-rhOPN [55]. P-rhOPN-BCM enhanced the osteogenic differentiation of hPDLSCs, indicating its potential for use as a GBR membrane to promote bone tissue regeneration.

As BMP-2 is a growth factor for bone regeneration, several investigations of BMP-2-modified GBR materials have been conducted. Some examples include PCL-based asymmetric porous membranes loaded with pDNA (encoding for BMP-2);[56] BMP-2 immobilized PCL/HAP electrospun membranes;[57] and BMP-2 and antibiotic-loaded mesoporous silica nanoparticles (MSN)-embedded PVA-core/PCL-shell electrospun membranes [58]. pDNA incorporated into the PCL membrane significantly enhanced the expression of BMP-2 of hBMSCs cultured on the membrane. All of these BMP-2 modified membranes enhanced the proliferation and osteogenic differentiation of hMSCs and hBMSCs and are promising GBR materials.

In addition to the direct immobilization of functional proteins onto materials, indirect modification of materials is another strategy to enhance the protein absorption ability. Du et al. compared the osteogenic ability of multiwalled carbon nanotubes (MCNTs) and nano-HA [59]. The MCNTs induced osteogenic differentiation of HASCs better than nHA because they could concentrate more proteins, including specific bone-inducing ones. Moreover, MCNTs can induce ectopic bone formation in vivo. Xie et al. fabricated an electrospun attapulgite (ATT)-doped poly (lactic-co-glycolic acid) (PLGA) scaffold for GBR applications [60]. They noted that ATT crystals could improve protein adsorption for a particular 3D nanostructure that was responsible for the adsorption capability. The BMSCs could attach to the PLGA/ATT scaffold more readily and spread better than on the commercial Bio-Gide membrane, and osteogenic differentiation of cells cultured on the PLGA/ATT scaffold was also promoted. Finally, the PLGA/ATT scaffolds also improved regeneration for repairing V-shaped buccal dehiscence on a dog tooth root.

4.3. Materials with osteogenesis-inductive metal ions to facilitate proliferation and osteogenesis differentiation of stem cells

In some GBR membranes, metal ions of metals such as Ca, Sr, Zn, Mg, or Cu have been added. The effect of Ca^{2+} on bone regeneration is clear. In addition, Sr^{2+} also affects bone mineralization during skeletal development, and Zn stimulates bone formation by regulating the balance between osteoblasts and osteoclasts. Mg is strongly involved in bone metabolism, stimulating osteoblast proliferation, and protecting against excessive bone resorption. Moreover, copper-doped scaffolds showed versatile functionality, including osteogenesis, angiogenesis, and antibacterial effects [11,61].

As demonstrated in Fig. 4, Xu et al. prepared an injectable sodium alginate hydrogel composite (CTP–SA) doped with cubic cuprous oxide (Cu_2O) and polydopamine-coated titanium dioxide (TiO_2 @ PDA) nanoparticles for guiding alveolar bone regeneration [62]. TiO_2 @PDA responded to visible-light illumination. TiO_2 @PDA produced reactive oxygen species (ROS) that have antibacterial effects under blue light (BL) irradiation, while Cu^+ from Cu_2O were oxidized to Cu^{2+} by the released ROS under BL. Cu^{2+} ions are conducive to the proliferation and osteogenic differentiation of hBMSCs and are beneficial for bone regeneration. Furthermore, CTP-SA had satisfactory antibacterial activity to achieve early debridement and induce the oxidation of Cu^+ to Cu^{2+} simultaneously. The enhanced release of Cu^{2+} combined with the photothermal effect of TiO_2 @PDA under near-infrared irradiation endowed CTP-SA with osteogenesis performance in a rat infective alveolar bone-defect model.

Lian et al. fabricated a composite PG-Cu@MSN fibrous scaffold by incorporating copper-loaded MSN (Cu@MSN) into a poly (lactic-co-glycolic acid)/gelatin (PLGA/Gel, denoted as PG) fiber matrix. Cu ions were released in a controlled manner and provided the PG-Cu@MSN fibrous scaffold with antibacterial activity, while the scaffold could also enhance the osteogenesis differentiation of hBMSCs. Therefore, this scaffold provided sufficient bone regeneration efficacy in a rat periodontal defect model [61].

Dubey et al. reported a fiber-reinforced hydrogel composed of highly porous poly(ε-caprolactone) fibrous mesh immersed in bioactive amorphous magnesium phosphate (AMP)-laden gelatin methacryloyl hydrogels [63]. The presence of PCL mesh and AMP improved the mechanical performance and hBMC proliferative rate of the gelatin methacryloyl hydrogel. As Mg^{2+} and HPO_4^{2-} could be

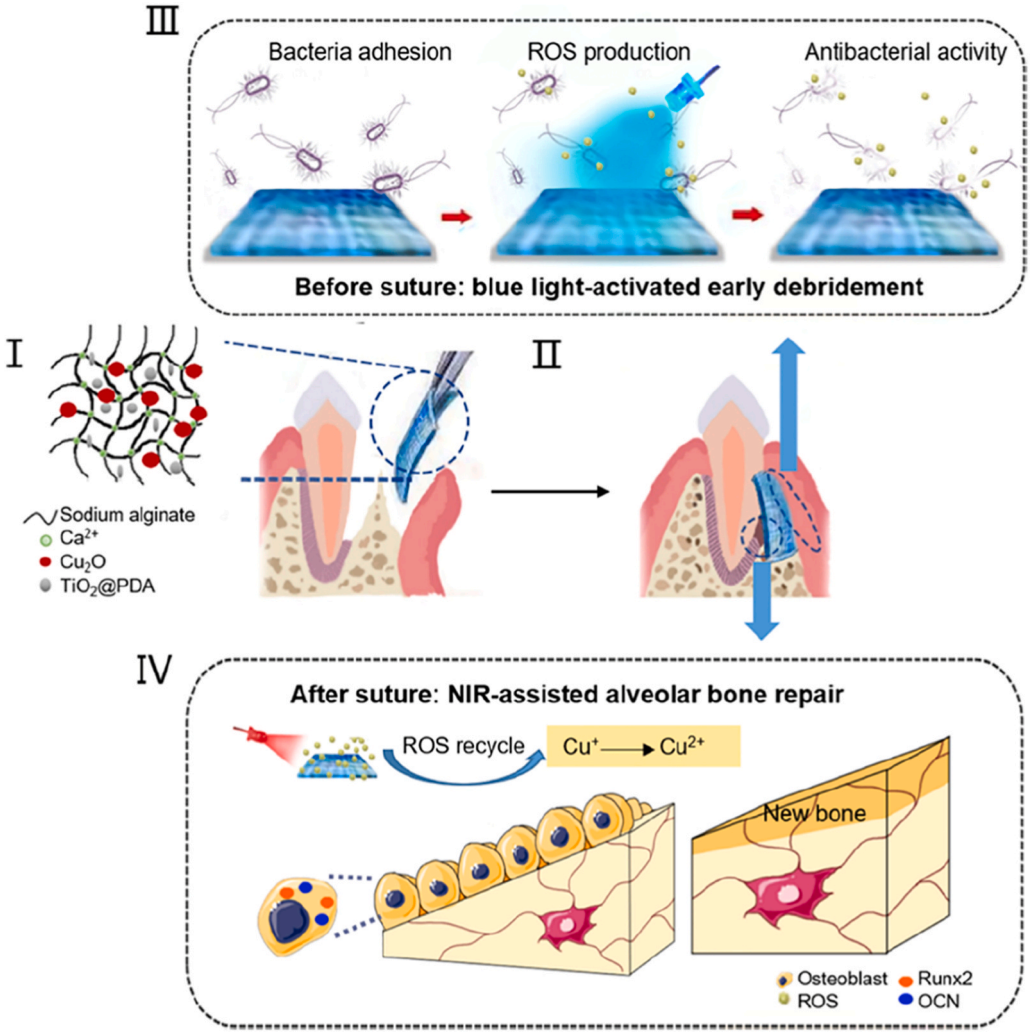

Fig. 4. Schematic diagram of the construction of CTP-SA and GTR surgery [62].

sustainably released by the degradation of the scaffold, and the osteogenic differentiation of hBMCs cultured with the scaffold was also enhanced. The fiber-reinforced scaffold showed favorable cellular responses, significantly higher rates of mineralization, and osteogenic gene expression in rat critical-size bilateral calvarial defect repair.

Metal-organic frameworks (MOFs)(Fig. 5), fabricated by bridging metal ions or clusters with organic ligands, provide a promising platform for biomedical applications. Zeolitic imidazolate frameworks (ZIFs) are a type of MOF that have attracted intense interest because of their impressive stability, and their high porosity and surface area. ZIFs are generally composed of tetrahedrally coordinated metal ions (e.g., Zn^{2+}, Co^{2+}, Cd^{2+}, and Mg^{2+}) and imidazolate derivatives (Im). Among these coordinated metal ions, Zn^{2+} is considered to have the potential to promote osteogenesis and angiogenesis [64].

Frassica et al. synthesized two macromers with different phosphonate pendant group concentrations: poly(diethyl(2-(propylthio) ethyl)-phosphonate methylsiloxane) diacrylate (PPMS-DA) and 25%-phosphonated analog (PPMS-DA 25%) [65]. Thereafter, microporous templated scaffolds were fabricated by crosslinking these macromers with poly-(ethylene glycol) diacrylate (PEG-DA) in various proportions. They investigated the effect of a phosphonated siloxane macromer in enhancing the osteogenic potential of templated PEG-DA scaffolds for bone regeneration. The introduction of phosphonated groups into the scaffolds promoted HAp deposition. The osteoinductive response of hBMSCs increased with the phosphonate content incorporated into the siloxane for increased calcium deposition.

As native bone has piezoelectric responsiveness, electroactive piezoelectric materials could trigger the electrical response of cells and promote the osteogenic differentiation of stem cells or osteoblasts. Zhao et al. reported a periosteum-mimicking scaffold composed of a bioactive collagen/bioglass micro-nanoparticles (BGM) nanofibrous membrane and piezoelectric responsive poly(vinylidene fluoride-trifluoroethylene) (PVFT) membrane for critical-sized bone regeneration (Fig. 6). The biomimetic scaffolds remarkably enhanced the proliferation, migration, and osteogenic differentiation of mouse bone-marrow-derived mesenchymal stem cells (mBMSCs). PVFT–BGM nanofibrous scaffolds also promoted the in vivo bone regeneration of critical-sized bone defects in rats. They proposed that the osteogenesis promoting mechanism of PVFT–BGM could be related to the negative pole of PVFT accumulating positive Ca^{2+} from BGM and activating the CaSR of osteoblasts and further promote osteogenesis [66].

4.4. Drug-loaded materials to facilitate proliferation and osteogenesis differentiation of stem cells

Previous studies have suggested that the incorporation of small-molecule drugs into composite materials can endow biomaterials with novel or better performance in BGR [67]. The most successful

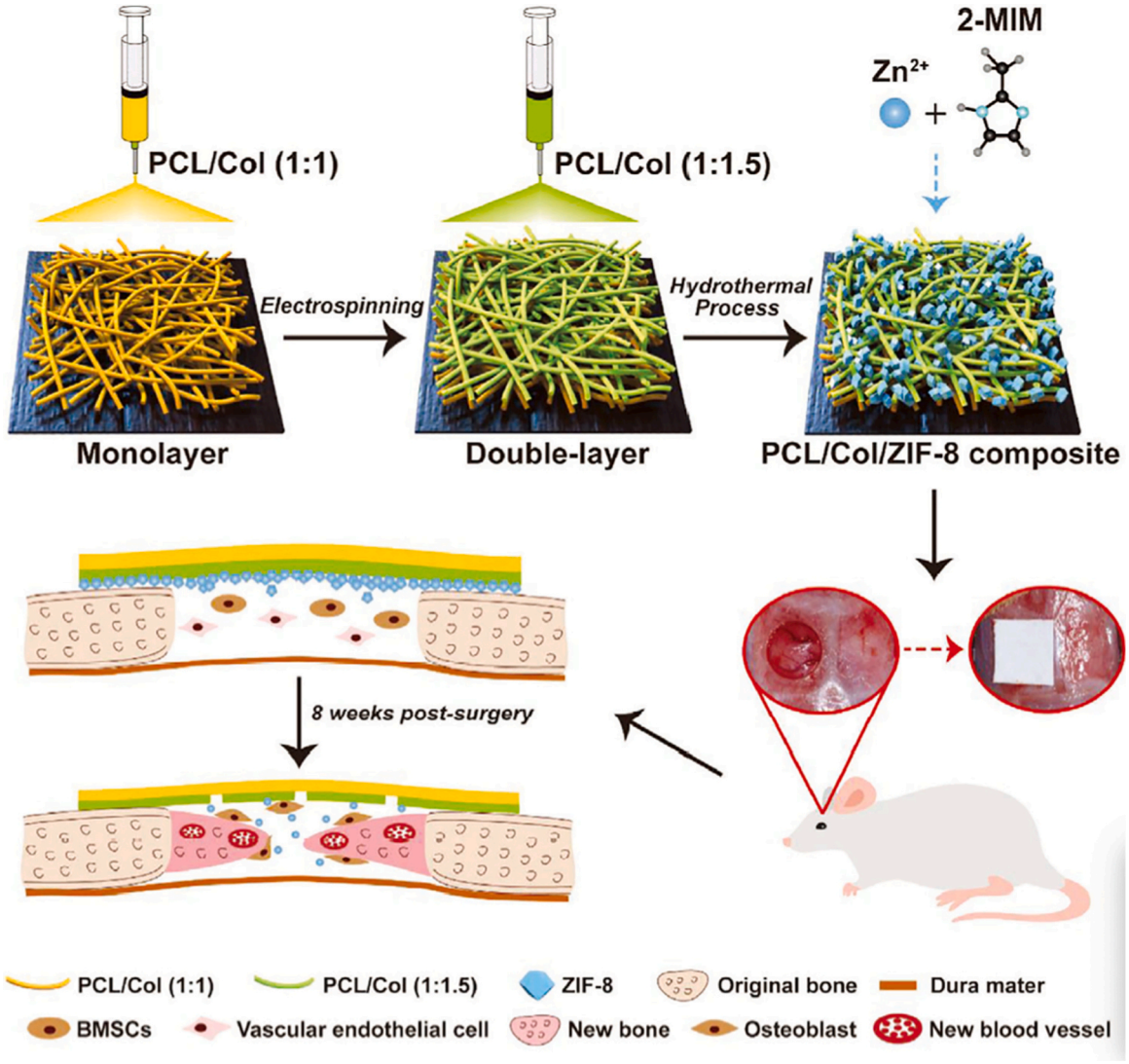

Fig. 5. Schematic of fabrication of the PCL/Col/ZIF-8 composite membrane for guided bone regeneration [64].

drug incorporation methods are based on encapsulating drugs in degradable polymeric networks, which can gradually release the drugs into the defect site. Among these encapsulation methods, electrospinning is one of the most frequently used as the resulting microstructures are similar to that of ECMs. Therefore, electrospun biodegradable polymeric membranes incorporating small-molecule drugs for the induction of osteogenic differentiation of stem cells have been investigated in recent years.

Metformin (Met) has a stimulating effect on the differentiation of various types of cells into osteogenic cells. Biodegradable polymeric electrospun membranes incorporating Met as a GBR material to induce osteogenetic differentiation have been investigated. Met-loaded poly(ε-caprolactone)-poly(vinyl alcohol) (PCL/PVA) fibrous scaffolds and crosslinking PCL/chitosan (CS)/MET (c-PCL/CS/MET) scaffolds were fabricated [68]. Both of these studies showed that the encapsulation of Met promoted the osteogenic differentiation of corresponding hEnSCs and BMSCs in vitro and critical bone defect regeneration in vivo.

Marteli et al. designed and synthesized a series of β-lactam-based integrin agonists [69]. These β-lactam derivatives were incorporated into PLLA electrospun GBR membranes. These scaffolds showed excellent affinity with integrins of cell membranes, and the adhesion of hBMSCs and expression of specific adhesion proteins and proliferation of cells seeded on this scaffold were significantly enhanced. The incorporation of β-lactam into PLLA scaffolds stimulated specific adhesion pathways, thus promoting the establishment of strong cell attachment that in turn activated hBMSC proliferation. These results showed the promising potential of this β-lactam-derivate-encapsulated scaffold in bone regeneration.

Wang et al. prepared a polydopamine-coated PCL electrospun scaffold coating with exosomes derived from mesenchymal stem cells (MSCs) and S-nitrosoglutathione (GSNO) for bone defect repair [70]. Exosomes have important immunoregulatory potential, and S-nitrosoglutathione has therapeutic potential for bone regeneration. MSC-derived exosomes were internalized by macrophages and hBMSCs, and the expression of proinflammatory genes in macrophages decreased, and the differentiation of hBMSCs was enhanced when co-cultured with PDA coating GSNO/exosome incorporated PCL scaffolds. They concluded that such scaffolds have the potential to serve as an important barrier membrane for osteogenesis and tissue regeneration.

5. Materials to induce mineralization

To obtain sufficient healing of the defective bone, there should be sufficient minerals deposited in the defected sites. Synthetic calcium phosphates and ceramics combined with other metallic elements, such as Sr, Zn, Mg, and Ta, are widely used for GBR in orthopedic and

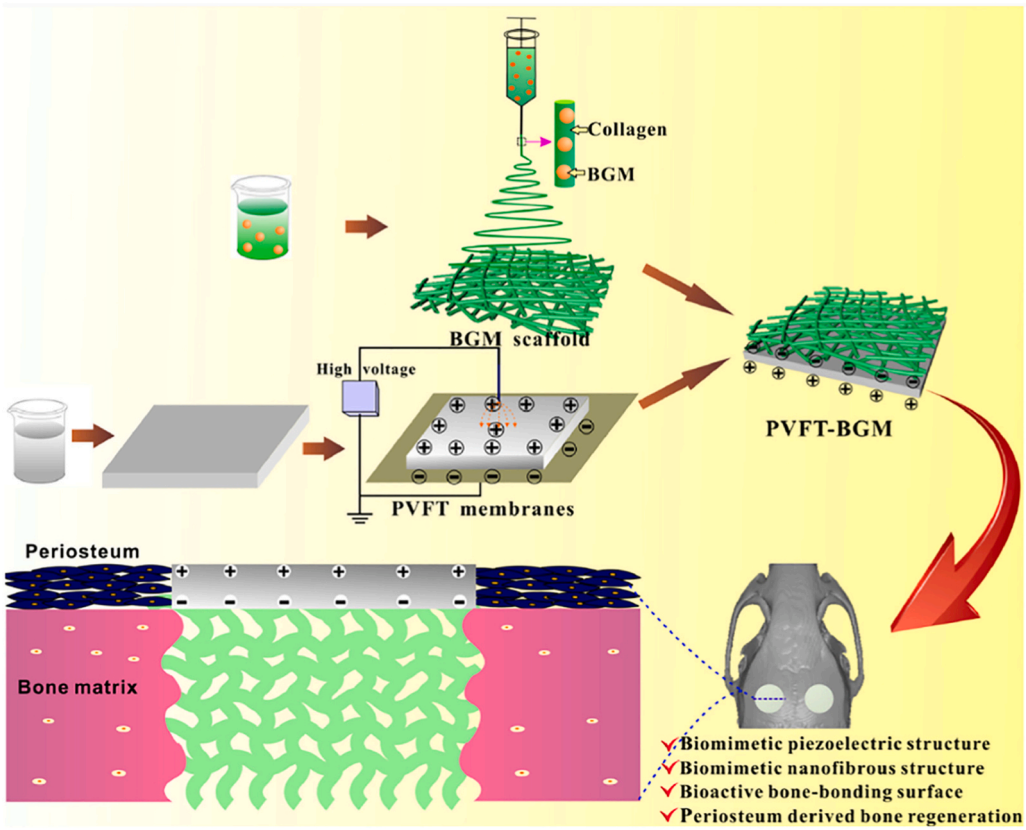

Fig. 6. Schematic illustration of the fabrication process and potential advantages in bone regeneration of PVFT-BGM scaffolds [66].

dental fields. Osteoconductivity and osteoinductivity are typical properties of these ceramics. The incorporation of these ceramics into GBR materials could induce rapid mineralization of the defect sites.

5.1. Calcium-doped GBR materials

Hydroxyapatite (HA) and bioactive glass (BG) are the most frequently applied calcium-containing components, which are incorporated into GBR materials by solution casting, 3D printing, and melt stretching multilayer deposition (MSMD) methods. The Ca-containing ceramics modulate osteogenesis by assisting angiogenesis, forming Ca-containing matrix vesicles, facilitating mineral crystallization, and providing sufficient stiffness to direct cells to differentiate into the osteogenic lineage.

Maura et al. incorporated BG and carbon nanotubes (CNTs) into porous PLA membranes [71]. The presence of BG induced HA deposition onto composite membranes, and the CNTs endowed the composite membrane with antimicrobial activity. Chang et al. prepared an HA/PLA hyperplastic scaffold using 3D printing and obtained satisfactory repair of osseous defects on the mandibular ramus of rats [72]. Mora-Boza et al. synthesized a type of glycerylphytate (G_3Phy)-crosslinked chitosan membrane and found that the chelating capacity of G_3Phy promoted in vitro mineralization of this membrane and subsequent direct bone-material bonding, while facilitating the osteogenic differentiation of hMSCs [73]. A series of PCL–HA scaffolds with or without BMP-2 dripping were also fabricated using the MSMD method. HA particles exposed on the surface of the PCL/HA scaffold changed the morphology of the scaffold, increasing the bioactive charges of calcium phosphate crystals. Then, the PCL/HA scaffolds can bond growth factors through surface energy and serve as suitable carriers for BMP-2, which induced excellent regeneration effect on rat calvarial defects when combined with collagen membranes [74].

5.2. Strontium-doped GBR materials

In addition to calcium, strontium is a bone mineral element. The Sr^{2+} ions have a cellular transport pathway similar to that of Ca^{2+} and can bond to the bone matrix during mineralization, followed by substitution in the HA structure instead of Ca^{2+}.[75] Sr promotes osteogenesis by stimulating osteoblasts, while decreasing bone resorption by inhibiting osteoclasts [75–77]. In addition, Sr is involved in bone-mineral metabolism by promoting the expression of osteogenic-related genes, differentiation markers, and proliferation, while reducing apoptosis [78–80]. Then, Sr ceramics were applied alone or incorporated into a polymer matrix, which were applied as bone-regeneration materials.

Etemadi et al. fabricated PCL/SF/$SrCO_3$ membranes by electrospinning, where the sustainable release of Sr^{2+} from the membrane and the presence of SF improved the adhesion, migration, and proliferation of osteoblasts cultured on the membranes [81]. Osteogenic differentiation and mineral deposition were also promoted. The PCL/SF/$SrCO_3$ membrane was thought to have potential as a GBR material. A series of Sr-incorporated bacterial cellulose (BC) and oxidized bacterial cellulose (OxBC) membranes, including Sr-substituted HA-incorporated BC, OxBC membranes, and Sr-apatite (SrAp)-incorporated BC and OxBC membranes were prepared [82]. These Sr-incorporated membranes induced calcification and deposition of other important elements, and OxBC/SrAp was the most promising for application as a GBR membrane. Tovani et al. fabricated collagen-free Sr-substituted CaP nanotubes by a track-etching method to mimic mineralized collagen fibrils. The synergistic effect of Sr^{2+} stabilized the Sr-substituted CaP nanotubes [83]. The release of Sr^{2+} supported the proliferation of osteoblasts and reduced the activity

and differentiation of osteoclasts. They pointed out that this new multi-functional Sr-substituted HA ceramic nanotube could induce biomimetic mineralization and balance the activity of osteoblasts and osteoclasts.

5.3. Zn-, Mg-, and Ta-doped GBR materials

Magnesium is involved in bone metabolism; both Zn and Mg can enhance bone formation and mineralization by stimulating the proliferation of osteoblasts and protecting bone from resorption by inhibiting the activity of osteoclasts [84,85]. Recently it was reported that Ta could enhance osteoconductivity by promoting the formation of Ca mineralization on the Ta-coating surfaces. To promote mineralization, and osteogenic and osteoconductive characteristics, these inorganic ceramics were successfully applied as additives in the fabrication of GBR materials. Both a Zn^{2+}-coated polymethylmethacrylate electrospun membrane and porous bulk Zn mesh satisfactorily achieved repair of rabbit and rat cranial defects as the Zn^{2+} ions induced mineralization and inhibited osteoclasts [86,87]. Ahmadi et al. fabricated Si and Mg co-doped fluorapatite nanoparticles (Si-Mg/FA)-incorporated polycaprolactone fumarate (PCLF)/gelatin-based membranes by electrospinning. The incorporation of Mg-containing nanoparticles altered the morphology and enhanced the mechanical strength of PCLF/gelatin membranes. The mineralization and cell-adhesion properties of these membranes were also promoted by the incorporation of Mg-containing nanoparticles, which indicates that this co-doped membrane is promising as a GBR material [88]. Furthermore, Ta/PLA electrospun membranes were prepared by the Ta sputtering method. The Ta coating enhanced the mechanical stability of the PLA membrane and promoted the proliferation and osteogenic differentiation of pre-osteoblasts due to the osteoconductivity of Ta [89].

6. Materials that promote vascularization

Vascularization is the first step in the repair of defective bone tissues. The optical blood supply is essential for osteogenic cells to survive, for which oxygen, nutrients, and metabolites of osteogenic cells need to be transported by blood circulation. Zhao et al. reported a tissue-engineered periosteum derived from decellularized submucosa of porcine small intestine [90]. They then used this membrane to repair irregular defects in the rabbit scapula and obtained satisfactory reparative effects. In particular, the vascularity of the defect point was sufficient to support the latter stage of bone regeneration because this membrane retains many bioactive components such as vascular endothelial growth factor (VEGF). Stromal cell-derived factor-1 alpha (SDF-1α)-conjugated collagen membrane was also fabricated [91]. This membrane was used to repair defects in rat crania, and sufficiently promoted the regeneration of the microvasculature and bone at defective sites. The SDF-1α enhanced the recruitment and osteogenic differentiation of MSCs to support bone regeneration. Furthermore, SDF-1α mobilized endothelial progenitor cells from the bone marrow to quickly enter the peripheral blood and mediate their migration in the peripheral blood to local wounds to participate in angiogenesis. The SDF-1α-conjugated collagen membrane promoted vascularization in defective sites, which is an essential factor for bone regeneration.

7. Immunoregulatory materials to induce osteogenesis

As exogenous implants, the application of GBR materials induces immune responses such as recruitment of macrophages. Macrophages play important roles in wound healing; in particular, M2-polarized macrophages have been recently reported to favor angiogenesis and osteogenesis. Therefore, many studies on GBR materials have focused on the inductive effects of biomaterials on M2 polarization and M1–M2 transition of macrophages. Common strategies include cytokine loading, topology modification, drug loading, and surface modification [92]. Yang et al. fabricated a series of strontium-substituted nanohydroxyapatite (SrHA)-incorporated and/or IFN-γ-coated small intestinal submucosa (SIS) membranes to repair rat cranial defects [93]. Among the SrHA/SIS, IFN-γ/SIS, and IFN-γ/SrHA/SIS membranes, the reparative effect of the IFN-γ/SrHA/SIS membrane was the best. At the initial stage of implantation, burst release of IFN-γ stimulated transient M1 macrophage polarization to direct the migration of vascular endothelial cells and osteoblasts, guide angiogenesis, and recruit more macrophages. The subsequent release of Sr^{2+} in the latter stage from SrHA induced M2 polarization of recruited macrophages and the M1 to M2 macrophage transition. M2 macrophages promoted angiogenesis and osteogenesis at the defective sites. Tanshinone IIA (Tan IIA)-loaded aligned-PCL electrospun membranes have also been reported to upregulate anti-inflammatory gene and protein expression and downregulate pro-inflammatory gene and protein expression in macrophages cultured on them [94]. The culture medium of these macrophages promoted the proliferation and migration of BMSCs and vascular endothelial cells. This was because the orientated topology of the Tan-IIA-loaded PCL membrane favored the recruitment of MSCs and the M1 to M2 macrophage transition. The latter subcutaneous implantation of Tan-IIA-loaded PCL membranes in rats demonstrated that orientated topology at an early stage and release of Tan IIA at the later stage could recruit MSCs, promote the M1 to M2 transition, and facilitate vascularization. Surface modification of GBR materials can also induce M2 polarization of macrophages and affect osteogenesis. Toyama et al. reported the application of an atmospheric-pressure plasma-treated titanium (APP-Ti) membrane in rat calvaria defect repair [95]. Compared to the untreated Ti membrane (N-Ti), more M1 and M2 polarization occurred in macrophages cultured on the APP-Ti membrane. Osteogenic gene expression in BMSCs co-cultured with APP-Ti macrophages was significantly enhanced compared to that in coculture with N-Ti macrophages. However, macrophages cultured with N-Ti secreted more plasminogen activator inhibitor-1 (PAI-1) and Syndecan-2, which could induce osteogenesis. The in vivo regenerated volume of new bone in rats treated with APP-Ti and N-Ti membranes was considerable. Although the expression of early osteogenic genes in rats treated with the APP-Ti membrane was significantly higher than that in rats treated with the N-Ti membrane, these early osteogenesis genes were not transformed into mineralized bone. On the contrary, the upregulation of secreted proteins PAI-1 and Syndecan-2 in rats treated with Ni-Ti membranes had a more significant effect on promoting osteogenesis.

8. Materials to suppress infection

The anatomical structures of alveolar and maxillofacial bones are complex, and there are multiple zones that can act as reservoirs of potentially pathogenic bacterial organisms. Furthermore, many pathological processes, such as periodontitis, which cause bone absorption and damage at these sites, are associated with pathogenic bacterial organisms [96]. Therefore, anti-infective function is an important characteristic of GBR materials intended for application in the alveolar and maxillofacial zones. An asymmetric membrane composed of a collagen–curcumin layer and a collagen–aspirin-loaded PLGA nanoparticle layer was developed to guide the regeneration of defected bone in the jaw of a dog [97]. The collagen/curcumin layer showed excellent antibacterial effects against *S. aureus*, *E. faecalis*, and *E. coli*. Both layers of the asymmetric membrane upregulated the expression of early osteogenic genes in dental pulp stem cell (DPSC) culture, and the aspirin layer prevented the soft tissue from occupying bone defects. The prepared asymmetric membrane successfully induced osteogenesis at defective sites in

Table 2
GBR materials applied in clinical trial or pilot study.

GBR Material	No. of patient	category of defected bone	Graft materials and covering	Bone regeneration outcomes	Biofunction
Platelet-rich fibrin membrane [108]	27	Staged lateral bone block	Grafted with autogenous bone combined with bovine bone mineral	Higher peri-implant marginal bone level and dental implant placement compared to resorbable collagen	Space maintaining, promote proliferation, adhesion, migration of cells and promote vascular regeneration
Injectable form of platelet rich fibrin (PRF) [109]	18	Horizontal bone defects	Grafted with mixture of particulate autogenous and exogenous and covering with collagen membrane and leukocyte PRF membrane	Promoting vertical and horizontal bone augmentation in maxillary and mandibular regions, permitting sufficient bone gain to future implant placement	Space maintaining, promote proliferation, adhesion, migration of cells and promote vascular regeneration
Three-dimensional performed titanium mesh [110]	100	Non-contained horizontal defects	Covering crosslinking collagen membrane	The mean hard tissue gain was 84.2 ± 21.5% after 6 months	Space maintaining
Digital customized titanium mesh [111]	5	Horizontal, vertical or combined defects	Grafted with heterologous bone and autologous bone (50:50) Covering collagen membrane	An average horizontal gain of 3.6 ± 0.8 mm and a vertical gain of 5.2 ± 1.1 mm 8 months after surgery	Space maintaining
Titanium membrane [112]	4	Alveolar ridge augmentation	Grafted with sulfate powder with normal saline, covering autogenous bone grafts	Possessing the ability to induce and guide new bone formation	Space maintaining
Ti-reinforced PTFE membranes [113]	40	Vertical defects during dental implantation	Dental implants	Hard and soft tissue were stable after 1 year of follow-up, with a peri-implant bone loss less than 1.0 mm in the first year	Space maintaining
Ribose cross-linked collagen membranes (RCLC) [114]	18	contained and non-contained extraction sockets	For contained sockets, RCLC membranes were positioned across extraction sockets and for non-contained sockets, bones substitute was used to support the membrane	20 extraction sockets healed uneventfully and all sites receivedstandard-diameter implants (4.1, 4.8, or 5.0 mm) without additional bone augmentation	Space maintaining
Mineralized collagen membrane combined with concentrated growth factor (prepared from fresh venous blood of patients) [115]	29	Alveolar ridge augmentation		Showing positive impact on reducing postoperative discomforts and new bone formation	Space maintaining, providing powerful biological scaffold with and acts as an integrated reservoir to emit growth factors for accelerating tissue regeneration
Bilayer porous polyethylene membrane (B-PPM) [116]	30	Alveolar ridge preservation	Grafted with synthetic bone graft	No significant difference in dimensional changes of alveolar ridge, new bone formation, connective tissue content, and residual bone grafts, and implant stability between B-PPM and e-PTFE membranes	Space maintaining
Polyethylene glycol (PEG) Membrane [117]	117	Posterior maxilla or mandible with expected buccal bony dehiscence	Grafted with synthetic bone	Supporting bone regeneration at dehiscence-type defects and obtained vertical bone fill with a relative change in defect height of 59.7%	Space maintaining
Synthetic PEG membrane [118]	30	Alveolar ridge preservation	Grafted with bi-phasic calcium phosphate particulate bone substitute	The mean percentage loss at the labial plate and at the coronal part of sockets was significantly lower when compared with porcine-derived collagen membrane	Space maintaining

vivo. Amoxicillin (AMX)-loaded PDLLA was also fabricated by electrospinning [98]. This membrane suppressed the proliferation of *S. sanguinis* and *P. gingivalis* in vitro and promoted the adherence and migration of periodontal ligament (PDL) cells. Finally, the reparative effect of this membrane on periodontal bone was evaluated in a rat periodontitis model. Wound dehiscence and sulcular inflammation were reduced in rats treated with the AMX–PDLLA membrane, and accelerated deposition of collagen fiber matrix was observed in the wounds. The regeneration condition of periodontal bone treated with the AMX–PDLLA membrane was comparable to that treated with a commercial collagen membrane. Furthermore, an Ag^+ dynamic crosslinking injectable 4-arm-polyethylene glycol-thiol (4-arm-PEG-SH) hydrogel incorporated with liposome calcium phosphate nanoparticles (Lip#CaP) was reported as a comprehensive reparative platform to guide bone regeneration with osteogenesis, angiogenesis, inductive, and antibacterial functions [99]. This hydrogel suppressed the proliferation of *E. coli*, *S. aureus*, and *S. epidermis*, promoted the proliferation of human umbilical vein endothelial cells, and enhanced alkaline phosphatase activity and ECM mineralization of MC3T3-E1 cells. Finally, the in vivo bone-guided regeneration effect of this hydrogel was evaluated in a rat calvarial critical-size defect model, and significantly enhanced osteogenesis and angiogenesis were observed in hydrogel-treated rats. Liu et al. fabricated a membrane with magnesium oxide nanoparticles (nMgO) incorporated into PLA/gelatin by electrospinning [100]. The incorporation of nMgO elevated the tensile strength to maintain structural stability and adjusted the degradation rate to match periodontal regeneration. This membrane promoted the osteogenic differentiation of BMSCs and suppressed the growth of *E. coli* and *S. aureus* in vitro. The nMgO-incorporated membrane effectively guided periodontal tissue regeneration in a rat periodontal defect model.

9. Graded biomimetic membranes

The microarchitecture and microenvironment of the dental ligament and alveolar bone tissues have a heterogeneous structure and graded mechanical and compositional properties. The design and fabrication of GBR materials with continuously graded properties to physiologically mimic the architecture of native periodontium tissues are crucial [101,102]. Luo et al. presented a structurally and chemically graded ginsenoside (Rg1)-loaded SF-poly(ε-caprolactone) (SF-PCL) nanofibrous and PCL microfibrous mesh by co-electrospinning [103]. The graded scaffolds showed a better osteogenic differentiation for MC3T3-E1 cells compared to pristine nanofibrous mesh. Incorporating Rg1 enhanced the proliferation of HUVECs, indicating that the graded Rg1-incorporated mesh was a promising candidate for GBR. Wang et al. prepared a Janus GBR membrane by sequential fractional electrospinning [104], which was composed of a random HA-loaded gelatin interlayer and gradually moved to aligned poly (methacryloxyethyltrimethyl ammonium chloride-*co*-2-Aminoethyl-2-methylacrylate hydrochloride) (P(DMC-AMA)) containing a PCL outer layer. In addition, there were topological and compositional gradients along the hierarchical direction, where the interlayer faces the defective bone to guide osteogenesis, and the outer layer faces the connective tissue to resist epithelial invasion and bacterial infection. In vitro measurements demonstrated that the inner layer enhanced the expression of osteogenic genes and mineralization, and the outer layer suppressed the proliferation of *S. aureus* and *E. coli*. Furthermore, the outer layer induced M2 polarization of macrophages, which provided a facilitative environment for osteogenesis. Rabbit cranial defect experiments confirmed that the Janus membrane achieved superior bone regeneration performance in vivo. Furthermore, graded nanofibrous membranes with tunable composition and bioactivity were fabricated using a sequential electrospinning technique [105]. This membrane was composed of two aligned gelation layers and one core random PCL layer, which benefits the proliferation and migration of osteoblasts, and the metronidazole-loaded outer layer regulated epithelial cell spread instead of infiltration and resisted bacterial infection. The core layer provided mechanical stability to the graded membrane and facilitated the maintenance of space for bone regeneration. The in vivo GBR effect was evaluated in a rabbit-skull defect model and obtained a better GBR effect than commercial collagen membrane. Jiang et al. prepared an electrospun membrane with a BMP-2/nano-HA distribution gradient and structural gradient from disorder to aligned fibers [106]. This graded membrane guided zonal expression of osteogenic genes and proteins that meet the demands of mimicking the natural structure of a soft-to-hard interface. Wu et al. fabricated a hierarchical micro/nanostructure by micro-sol electrospinning composed of VEGF-encapsulated hyaluronan/PLLA core-shell microfibers and self-assembled collagen nanofibers [107]. This hierarchical structure promoted the adhesion, proliferation, and in vitro osteogenesis of HUVECs and promoted the osteogenic differentiation of BMSCs, which mimicked the natural process of intramembranous ossification. Finally, the in vivo GBR effects of this hierarchical membrane were examined in rat cranial bone and periosteum defects, which were repaired in a uniform and rapid manner by an inherent periosteal ossific mechanism.

10. Materials in clinical trial or pilot study

Various GBR materials with different biofunctions have been summarized above, but almost all of them are still in laboratory research stage. In order to provide more references to clinicians, GBR materials applied in clinical trial or pilot study reported in recent were listed in Table 2. It could be seen from Table 2 that most of those GBR materials were applied by accompanying with bone or bone substitute grafting. It may indicate that those GBR materials don't have sufficient osteogenic induction ability independently. Parts of GBR materials listed in Table 2 could provide satisfactory bone augmentation effect but there were still some materials could not achieve superior bone inductive effect when compared to commercial collagen membranes. From the perspective of biofunctions, those GBR materials only maintained space maintenance function except two platelet rich fibrin contained strategies. Compared to GBR materials in research stage mentioned above GBR materials listed in Table 2 were lack of positive bone induction ability. From the perspective of material, GBR materials in clinical trial were almost derived Ti or collagen meshes. However, Ti meshes needs to be removed in most cases and degradation of collagen membranes are difficult to handle [119]. In contrast, abundant new synthetic materials and processing technologies have been applied in fabricating GBR materials those in research stage [14]. New design philosophies and technologies made those GBR materials to be more compatible and suitable ones to adapt complex sceneries in oral and maxillofacial regions.

11. Perspective

Although alveolar and maxillofacial bones related GBR materials have been developed rapidly in recently, few have been investigated in depth. Majority of studies focused on functioning GBR materials by loading them with one or multiple kinds of growth factors or cytokines. As a result, biological behaviors including adhesion, migration, proliferation, and differentiation of specific monotype of cells such as osteoblasts, osteo-progenitor cells, or vascular endothelial cells could be modulated or regulated. As healing of defected bone is a complex process and need coordinative performances of various types of cells, regulating interactions between different types of cells in molecular level are also crucial to successful bone regenerative strategies [120]. Take Receptor

activator of NFκB ligand (RANKL) signaling pathway as an example. This pathway was involved in the interactions of osteoblast and immune cells to osteoclast. RANKL induces precursors into mature osteoclasts by binding to RANK on the surface of osteoclast precursor cells [121]. RANKL is expressed by osteoblasts predominantly. But at the same time osteoblasts could also express soluble decoy receptor form of RANK, which could also bind to RANK and block the formation of osteoclasts [5]. Further, RANKL could also be produced by activated T cells and B cells, and proinflammatory cytokines such as IL-1, IL-6, TNF, and other member of TNF superfamily such as TNF receptor apoptosis inducing ligand (TRAIL) can enhance osteoclast formation by enhancing the sensitivity of osteoclasts to RANKL [122,123]. Given the necessary role for osteoclasts in bone remodeling it could be anticipated that GBR strategy targeting RANK pathway, may be expected to have detrimental effects on bone defect regenerating. However, few studies about GBR materials mentioned above have investigated intracellular interactions and osteogenesis mechanism with the depth of molecular level.

Besides, few GBR materials showed effectiveness in animal models that approximates oral and maxillofacial sites, where are complexly topological and mostly accompanied with bacterial. For example, periodontal disease such as periodontitis often cause alveolar bone resorption [124]. An important challenge for GBR materials applied in periodontitis is infection for that periodontitis is infectious diseases. Furthermore, application of GBR materials also could cause implant associated infections, which would exacerbate pre-existing infections and lead to progression of periodontitis [125]. GBR materials designed to be applied in periodontitis must be evaluated in more simulative conditions rather than been tested in frequently applied subcutaneous implanted model and cranial defect model. Therefore, efficacy of GBR materials without in vivo evaluation or tested in insufficient animal models should be questioned. The specificities such as infective and mechanical cues for specific disease and location at alveolar and maxillofacial sites should be taken into consideration in choosing evaluation methodology.

Beside classified biofunctional GBR materials, some new processing methods and new design concepts are worthy of further study. New processing methods such as 3D printing, and 3D printing combined with porogen leaching have been used to fabricate GBR scaffolds with characteristics that are much closer to natural ECM [126,127]. New design concepts such as fabricating coculture systems to engineer bone mimetic ECM niches and preparing scaffolds that suppress connective-tissue growth to actively acquire more space for bone regeneration also have been reported [128,129].

12. Conclusion

Enabling biofunctions is a crucial principle in the design and implementation of GBR materials used to repair defective alveolar and maxillofacial bones for that bone defects at these sites are usually accompanied by complex pathological processes. Here, we reviewed biofunctional GBR material research over the last two years for the regeneration of alveolar and maxillofacial bones. In the future, GBR materials with richer biological functions are expected to be designed and prepared based on an in-depth understanding of the mechanisms of bone-material interactions.

Conflicts of Interest

All authors declare no conflict of interest relative to the study.

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