

Resorbable Membranes for Guided Bone Regeneration: Critical Features, Potentials, and Limitations

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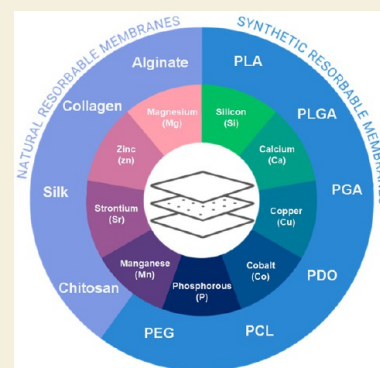
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ABSTRACT: Lack of horizontal and vertical bone at the site of an implant can lead to significant clinical problems that need to be addressed before implant treatment can take place. Guided bone regeneration (GBR) is a commonly used surgical procedure that employs a barrier membrane to encourage the growth of new bone tissue in areas where bone has been lost due to injury or disease. It is a promising approach to achieve desired repair in bone tissue and is widely accepted and used in approximately 40% of patients with bone defects. In this Review, we provide a comprehensive examination of recent advances in resorbable membranes for GBR including natural materials such as chitosan, collagen, silk fibroin, along with synthetic materials such as polyglycolic acid (PGA), polycaprolactone (PCL), polyethylene glycol (PEG), and their copolymers. In addition, the properties of these materials including foreign body reaction, mechanical stability, antibacterial property, and growth factor delivery performance will be compared and discussed. Finally, future directions for resorbable membrane development and potential clinical applications will be highlighted.

KEYWORDS: Guided bone regeneration, Barrier membranes, Tissue engineering, Biomaterials, Biocompatibility



INTRODUCTION

Aging, congenital diseases, cancer, trauma, tooth loss, and periodontal disease are considered as common causes of bone loss and disorders in our maxillofacial region. Lack of horizontal and vertical bone in the site of an implant may lead to major clinical problems and needs to be augmented prior to implant treatment.¹ To achieve desirable repair in bone tissue, methods such as guided bone regeneration (GBR), distraction osteogenesis, and split-crest surgery have been applied by clinicians. Among all methods, GBR has been widely accepted and used in approximately 40% of patients with bone defects.² GBR is a surgical procedure that uses a barrier membrane to encourage the growth of new bone tissue in areas where bone has been lost due to injury or disease (Figure 1a).³ The barrier membrane acts as a physical barrier to prevent the ingrowth of soft tissue, such as fibroblasts, while allowing bone cells to migrate into the area and form new bone. The goal is to block and exclude the fast migration of epithelial cells and fibroblasts to the defect site by placing a membrane between hard tissue and soft tissue.⁴ GBR is commonly used in dental implant surgery, where it is used to increase the amount of bone available to support the implant. It can also be used to repair bone defects caused by periodontal disease or traumatic injury. The procedure typically involves making a small incision in the soft tissue, creating a space for the barrier membrane and bone graft material and then suturing the tissue closed (Figure 1b).^{5,6}

Recovery time can vary depending on the extent of the bone loss and the specific procedure used.³

To ensure a successful GBR, four biologic principles must be achieved as follows: “primary wound closure” to establish uninterrupted wound healing, “exclusion of Epithelium and connective tissue” without interfering with angiogenesis to provide enough blood supply, “space maintenance” to provide suitable space for bone regeneration, and “stability” in the fibrin clot and implanted material to prevent disturbance in the healing process.⁷

Osseous regeneration, the process of growing new bone tissue to fill in areas of bone loss, relies on the migration of osteogenic cells to the defect region (Figure 1c).^{8,9} This can be achieved through the use of various techniques and materials including the usage of growth factors.¹⁰ Growth factors are substances that promote the growth and differentiation of cells such as bone cells. They can be derived from various sources, including human or animal tissue, or artificially created.¹¹ They are used to enhance the body’s natural healing process and accelerate the growth of new bone tissue. The GBR procedure aims to create an environment that is conducive to bone

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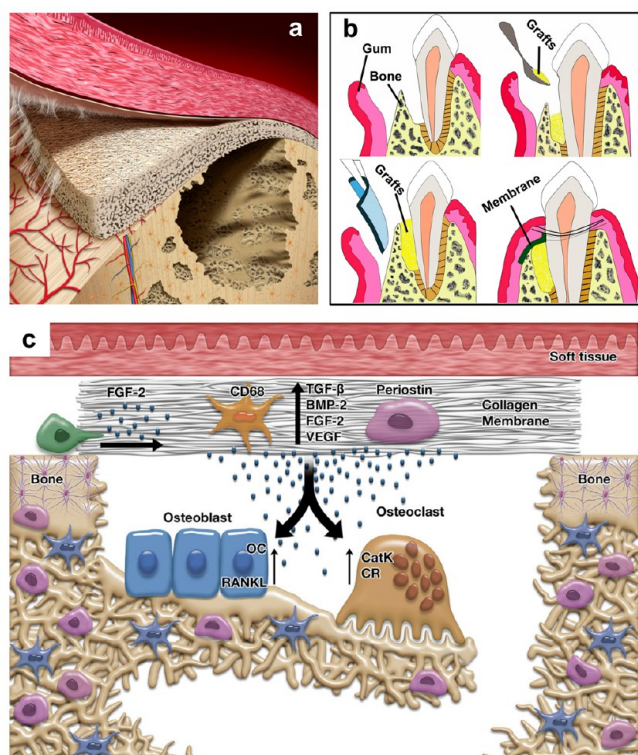


Figure 1. (a) Schematic illustrations of the principle of guided bone regeneration (GBR)²¹⁸ and (b) the combination of guided tissue regeneration (GTR) and bone graft techniques for periodontal defects management.²¹⁹ (c) Illustration of the cellular and molecular cascades occur during GBR process.²¹⁸ Reprinted with permission from ref 218. Copyright 2017 John Wiley & Sons. Adapted with permission from ref 219. Copyright 2010 Elsevier.

regeneration by isolating the bone defect from surrounding soft tissue and providing the necessary growth factors and bone graft materials.¹² Once the new bone tissue has formed, it integrates with the surrounding natural bone, providing a stable foundation for dental implants and other restorations.

After GBR surgery, a sequence of events leads to bone regeneration: Immediately after the surgery, the body's immune system responds by initiating an inflammatory response.¹³ This process is necessary for removing debris and bacteria and recruiting cells needed for healing. During the first 24 h, the grafted space would become filled with blood clots which are enriched by growth factors and chemoactive agents such as cytokines. After the blood clot absorption, cells

called osteoblasts and osteoclasts start to migrate to the bone defect site. Mesenchymal stem cells (MSCs) with osteogenic differentiation capability would be transported to this area via newly formed vessels and contribute to forming osteoid structures (Figure 1c).^{14–16} Osteoblasts and osteoclasts are pivotal cells involved in bone formation and resorption, respectively. Upon injury, these cells proliferate and begin to form a clot, which serves as a scaffold for new bone formation.

■ BARRIER MEMBRANES

Barrier membranes, which are used to separate the bone defect from surrounding soft tissue,^{17,18} are considered a key element in GBR as they create a physical barrier that prevents the ingrowth of unwanted cells such as gingival tissue (Figure 2a).¹⁹ They also serve as scaffolds for the migration of osteogenic cells, which are responsible for forming new bone. Meanwhile, according to recent research focused on the biological processes occurring within membranes, it has been suggested that these membranes may have the potential to actively participate in the regeneration of bone in areas where there are defects.²⁰ Five main criteria have been identified to describe an ideal barrier membrane intended for GBR:³ biocompatibility, space maintenance, occlusive functionality, easy handling, and bioactivity (Figure 2b).²¹ Several generations of barrier membranes have been developed for use in GBR procedures. The first-generation membranes are made of highly purified, synthetic materials such as polytetrafluoroethylene (PTFE), offering great resistance to displacement, excellent mechanical stability, and long-term stability. However, they are nonresorbable, which means a second step of surgery is required for membrane removal, adding extra cost and burden for the patient.²² Yet another constraint is the excessive rigidity of these materials, which may result in the separation of soft tissue and subsequent membrane exposure, potentially leading to complications.²³

Based on these facts, the second generation of barrier membranes, including naturally sourced and synthetic absorbable membranes, has been developed to overcome the shortcomings of nonabsorbable membranes.^{24,25} The former is fabricated from natural biodegradable biomaterials such as chitosan, collagen, gelatin, silk etc., as it is desirable for an implanted membrane to be gradually replaced by the patient's own tissue during the recovery phase.³ However, poor mechanical stability and limited time of barrier functionality due to the resorption process are their main drawbacks, restricting their real-world applications. The latter can be tailored to have specific mechanical and degradation proper-

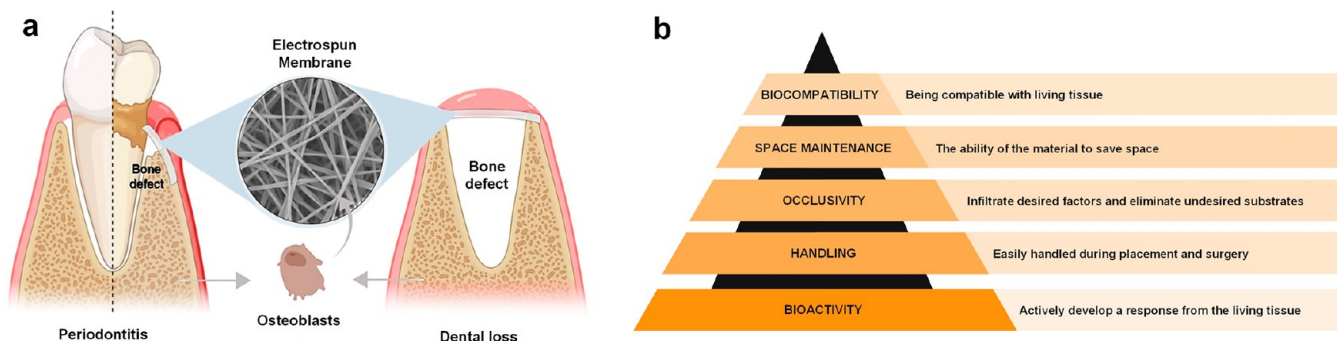


Figure 2. (a) Schematic illustration of the placement of an electrospun barrier membrane for GBR in scenarios involving periodontitis and dental loss. Reprinted with permission from ref 19. Copyright 2020 Elsevier. (b) Five main criteria to define practical barrier membranes for GBR.

ties, allowing customization to suit different tissue types and healing rates. They also eliminate the need for a second surgical procedure to remove the membrane, reducing patient discomfort and healthcare costs.

The third generation involves the use of membranes produced via tissue engineering, as previously proposed by Sam and Pillai²⁶ as multifunctional membranes that not only serve as barriers but also enable the controlled release of specific agents such as antibiotics, growth factors, and adhesion factors at the wound site, that could help facilitate and regulate the natural wound healing process in a more effective manner. Later, Lee et al.²⁷ emphasized on the need to move beyond the fundamental principles of GBR by introducing this generation of membranes that incorporate the release of growth factors and antibiotics. In fact, in the third generation, resorbable membranes and bioactive agents such as antibiotics, growth factors, and adhesion factors are combined to enhance cell migration, proliferation, differentiation, and extracellular matrix deposition, leading to faster and more efficient tissue regeneration.²⁸ It can also provide a controlled release of growth factors, which can reduce the required dose and minimize potential side effects. The growth factors can be either incorporated into the membrane matrix during the manufacturing process or loaded onto the membrane surface after fabrication.^{6,29–37} However, these newly developed membranes are relatively more expensive than other types of membranes.

NATURAL RESORBABLE MEMBRANES

Natural resorbable barrier membranes are biological materials designed to be used in medical procedures and dissolve or break down over time in the body. They are made from natural materials such as collagen, gelatin (which is derived from collagen), chitosan, silk, etc.³⁸ and are used in a variety of applications, including in wound healing, in tissue engineering, and as a barrier in GBR procedures, to separate the bone defect from the surrounding soft tissue and promote bone formation (Figure 3).³⁹

Collagen

Collagen's etymology originates from “colla”, meaning glue in Greek and “gene”, meaning producing or generating.⁴⁰ Collagens are the most abundant structural proteins in mammals (30% of total protein mass),⁴¹ comprising 28 types and at least 46 polypeptide chains in vertebrates.⁴² One-third of the total protein in humans and 75% of the dry weight of the skin is composed of collagen, making it the most ubiquitous component in the extracellular matrix (ECM).⁴³ The discovery of collagen dates back to the 1890s when Mall and Young produced gelatin by boiling reticular tissue in water. A decade later, Siegfried found that reticular tissue split up into gelatin,⁴⁴ and later, in 1904, Tebb proved that reticulin is a type of fiber in connective tissue artifact from collagen.⁴⁵

Collagen consists of three parallel left-handed polyproline type II polypeptide strands called α chains. These triple helix polypeptides stagger by one residue to form a unique right-handed triple helix structure,⁴⁶ presented in Figure 4a. ECM contains many classes of collagenous structures, including fibrils, beaded filaments, anchoring fibrils, and networks. Most collagen fibrils are heterotypic, consisting of at least two different fibrillar collagen types. In cartilage, collagen fibrils are largely made of collagen type II cross-linked with collagen types XI, IX, and III.⁴⁷ In the skin, the most abundant types of

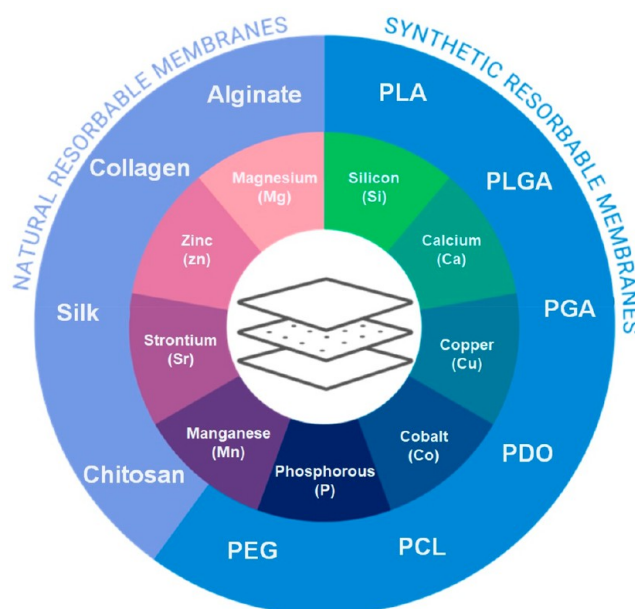


Figure 3. Classification of resorbable membranes based on their natural or synthetic origins in the outer layer. The inner layer displays the current substances used for bioactivity enhancement.

collagens are types I and III, while in the cornea they are types I and IV.⁴⁸ The main sources of collagen are the skin, tendon, and cartilage which could be extracted from different animals like calves, mice, fish, chickens, and birds.⁴⁹ Collagen-based membranes available on the market are biocompatible and biodegradable. However, they suffer from rapid loss of mechanical stability particularly in a liquid or high humidity environment due to their rapid degradation rate.⁵⁰ In addition, they are relatively expensive with limited supply sources, making it a challenge to apply them in bone regeneration applications.⁵¹ Different degradation patterns and rates are reported for the commercially available collagen membranes.⁵² The resorption rate is associated with collagenic tissue structure, which would be different based on collagen origin (porcine, bovine, etc.) and the organ from which it is derived (such as skin, tendon, pericardium, etc.). In addition, other characteristics, including porosity, thickness, and weight, could influence the resorption rate and degradation pattern.⁵³ Increasing numbers of studies are now focused on enhancing the bioactivity properties, loading the collagen membrane with active organic and inorganic compounds. Organic compounds include growth factors, cytokines, and anti-inflammatory agents. Inorganic compounds such as calcium phosphate substrates and bioceramics have shown promising results.⁵⁴

Collagen has gained significant attention for use in GBR. From a biological perspective, collagen is an important component of the ECM and provides a structural scaffold for cell attachment and proliferation. When used in GBR, collagen can promote cell adhesion and proliferation, as well as enhance tissue regeneration and bone formation.⁵⁵ Additionally, collagen can act as a carrier for various bioactive molecules, such as growth factors, which can further enhance bone regeneration.⁴³ From a material perspective, collagen possesses several desirable properties for GBR, including biocompatibility, biodegradability, and low immunogenicity. Moreover, it can be processed into various forms, including membranes, sponges, and gels, allowing for versatility in its use.⁴⁹

Chitosan

Chitosan (Figure 4b) is a cationic polymer that is not widely available in the environment but can be extracted from chitin through the process of deacetylation.⁵⁶ As a biocompatible, biodegradable, nontoxic, antioxidant, anticancer, anti-inflammation, and hemocompatible material chitosan has attracted extensive attention for biomedical applications including tissue regeneration. In addition, chitosan has a unique feature of attracting both negatively and positively charged molecules due to the presence of amino groups and a hydroxyl group in its chemical structure.⁵⁷ These functional groups can significantly enhance the mechanical and biological properties of chitosan, making it suitable for a wide range of biomedical and pharmaceutical applications. In addition, it could be modified with other bioactive molecules and made into different forms and shapes, including membranes, hydrogels, sponges, and fiber forms.⁵⁸ There are numerous techniques to produce chitosan-based biomaterials including solvent casting,^{59–61} compression molding,^{62,63} freeze-drying,^{64,65} electrospinning,^{66,67} dense gas foaming,^{68,69} and microwave assisted method.^{70,71} Despite recent advances in developing mechanically stable and biodegradable chitosan-based membranes,⁷² chitosan is not commonly used as a natural resorbable membrane for GBR yet as more research is needed to evaluate its efficacy and safety.

Chitosan has been reported as a suitable material for GBR applications, has been shown to promote cell adhesion and proliferation, and can stimulate bone formation and regeneration.⁶⁹ Chitosan also has antibacterial properties, which can be beneficial in preventing infection during the bone healing process.⁷³ From a material perspective, chitosan is biocompatible and biodegradable and has low immunogenicity. Additionally, chitosan can be processed into various forms, such as films, sponges, and microspheres, allowing for versatility in its use.⁷² However, some challenges associated with the use of chitosan in GBR include poor mechanical properties and limited control over degradation rate. Despite these challenges, chitosan-based materials have shown potential in promoting bone regeneration and continue to be an area of active research.⁶⁶

Silk Fibroin

Silk fibroin is a natural polymer derived from *Bombyx mori* silkworm (Figure 4c) and is considered as an excellent bioinspired material for tissue engineering and bone regeneration applications, both in vitro and in vivo environments.^{74,75} It has outstanding biocompatibility, biodegradability, oxygen and water vapor permeability, and mechanical and physical properties,^{76,77} and has been used as a biodegradable scaffold to induce osteogenesis, mineral resorption, and bone regeneration in vivo,⁷⁸ with minimum inflammatory reaction and great cellular response (comparable to collagen).⁷⁹ When it degrades, silk fibroin produces nontoxic amino acids which can be easily absorbed in vivo, with a tunable degradation time from a few days to several months.^{51,80,81} In vivo studies of silk fibroin scaffolds on Lewis rats demonstrated decomposition after 8 weeks and full resorption after 1 year,⁸² indicating promising biodegradability and bioresorbability for this natural polymer. However, the main disadvantage of using silk fibroin in GBR applications is that the material can become brittle when it dries.⁸³ This can make it difficult to handle and can also reduce its mechanical strength and durability.⁸⁴ Some strategies to overcome this

problem include adding plasticizers, increasing water content, and controlling the manufacturing process in order to eliminate the formation of rigid β -sheet structures.^{85,86} Additionally, silk fibroin membranes can be sensitive to changes in pH and temperature, which can also affect their mechanical properties.⁸⁷

Due to its ease of processing, silk fibroin has been fabricated in a wide range of forms and shapes, including hydrogels, mats, films, and sponges, making it an excellent material for GBR application in biomedical and pharmaceutical applications, particularly in dentistry.^{88,89} In addition, the active sites on the silk surface can act as potential bonding spots for bioactive molecules, such as growth factors and stem cells, facilitating bone regeneration and osteodifferentiation.⁹⁰ A wide range of manufacturing techniques, such as electrospinning, spin-coating, freeze-drying, micropatterning, and bioprinting, have been introduced to fabricate silk-based biomaterials for tissue engineering and bone regeneration.⁸⁰ The main challenge, however, is to enhance the osteogenic characteristics of the silk-based material's GBR application. To tackle this issue, silk-based composite materials made of polymers, hydroxyapatite,^{78,91,92} magnesium oxide,^{93,94} graphene,^{95,96} and antibacterial agents such as silver nanoparticles^{97,98} and curcumin nanoparticles^{99,100} were incorporated into silk. Using an electrospinning technique, Ghafouri et al.⁵¹ fabricated functionally graded membranes (FGMs) made of electrospun nanofibers of silk fibroin (extracted from sericin protein) and poly(ethylene oxide) (PEO), with fiber diameters ranging from 300 to 550 nm. The membrane layout consisted of two layers, which were mechanically pressed to each other to form an FGM. The loose layer was designed to guide osteogenic cell growth and bone regeneration, while the dense layer provided a better barrier effect, prohibiting epithelial tissue growth into bone defects. The fabricated membranes demonstrated excellent stability and degradability performance in artificial saliva solution, featuring a negligible mass loss of <0.003 g after 4 weeks, with retained bilayer structure after 8 weeks.⁵¹

■ SYNTHETIC RESORBABLE MEMBRANES

Synthetic resorbable membranes are designed to be biocompatible and to degrade over time, allowing the body to absorb them and promoting the regeneration of new bone tissue. Some examples of synthetic resorbable membranes for GBR include polylactic acid (PLA),^{101–103} polyglycolide (PGA),^{3,104} poly(lactic-co-glycolic) acid (PLGA),^{103,105} polydioxanone (PDO),^{106,107} polycaprolactone (PCL),^{108–110} and polyethylene glycol (PEG) membranes.^{111,112} However, synthetic bioresorbable membranes also suffer from lack of control over the resorption rate dictated by factors like local pH and lack of inertness because of unfavorable reactions during degradation (Figure 3).¹¹³

PLA

PLA is a biodegradable polymer used to synthesize barrier membranes for biomedical applications, particularly GBR. It is a synthetic polymer made from repeating units of lactic acid (Figure 5) and is a member of the polyester family. PLA membranes are suitable barriers for preventing the migration of cells from the surrounding soft tissue into the bone defect while allowing for the ingrowth of new bone cells from the surrounding tissue. PLA can be fabricated into different forms, including films, fibers, and meshes, and can be combined with other materials to improve its properties, such as chitosan,

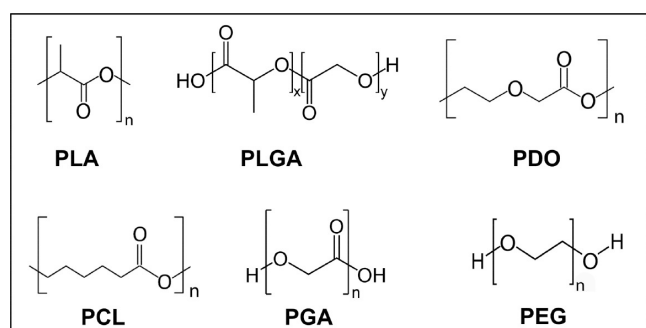


Figure 5. Chemical formulas of the classical synthesized polymers used as barrier membranes.

hydroxyapatite, or growth factors.¹¹⁴ Additionally, the physical and mechanical properties of PLA can be adjusted by varying the molecular weight and degree of crystallinity, which can improve the handling, stability, and barrier properties of the membrane.^{25,115} Despite its advantages, PLA has some disadvantages such as a slow degradation rate, which may result in long-term inflammation, and its acidic degradation products and increased risk of foreign body reaction.^{114,116} To overcome these limitations, studies focused on different modifications and blends of PLA, including copolymers and composite materials, to improve its degradation rate and biocompatibility for GBR applications.^{17,101}

PGA

PGA is a biodegradable polymer that has been extensively studied for its use in GBR as a barrier membrane.^{117,118} It is a synthetic polymer made from repeating units of glycolic acid (Figure 5) and is another member of the polyester family.¹¹⁸ PGA starts to degrade within a few weeks after implantation and is entirely absorbed by the body within a few months. This eliminates the need for removal of the implant and reduces the risk of infection and other complications associated with nondegradable materials.¹¹⁹ PGA has a high tensile strength, which makes it suitable for a wide range of medical applications such as sutures, surgical mesh, scaffolds for tissue engineering, and barrier membrane in GBR.¹⁰⁴ When used in combination with other materials, such as collagen or calcium phosphate (Figure 3), PGA can improve its mechanical properties and degradation rate. Studies have demonstrated that PGA-based membranes can promote new bone formation in GBR procedures, and their degradation products have been found to be biocompatible and nontoxic.¹¹⁹ Additionally, it has high thermal stability and a high melting point which makes it suitable for sterilization process.¹²⁰ However, PGA can cause an inflammatory response in vivo due to its acidic degradation products. To overcome this issue, various modifications of PGA have been explored, such as blending it with other polymers or surface-modifying with hydroxyapatite.^{118,119}

PLGA

PLGA is a biodegradable and biocompatible copolymer commonly used in the medical field for drug delivery and tissue engineering applications. It is composed of repeating units of lactic acid and glycolic acid (Figure 5), which are biodegraded by hydrolysis in the body over time.^{121,122} The rate of degradation can be controlled by adjusting the ratio of lactic acid to glycolic acid in the copolymer.¹²² PLGA has several desirable properties, such as good biocompatibility, controlled degradation, and tunable mechanical properties,

making it a popular choice for various biomedical applications. For example, its microspheres can be used for sustained drug delivery,^{123,124} whereas its scaffolds can be used for tissue engineering.^{125,126} It is a thermoplastic polymer, which means it can be melted and molded into various shapes,¹²⁷ making it appealing for a range of biomedical applications. In the context of GBR, PLGA has been used as a scaffold material to provide a temporary support structure for the growth of new bone tissue.^{125,126} The scaffolds serve as physical support and provide a surface for cell attachment and proliferation, which helps to promote the formation of new bone tissue.¹²⁵ They can be designed to have specific mechanical properties and pore sizes to optimize conditions for bone growth. The degradation rate of the PLGA scaffold can be adjusted to match the rate of new bone growth,¹²⁸ ensuring that the scaffold is present for the right amount of time to support the growth of new bone tissue.¹²⁹

PDO

PDO is a biocompatible, absorbable suture material that can also be used in GBR as a barrier membrane, which can be broken down by the body over time. It is a polymer made from repeating units of *p*-dioxanone (Figure 5) and is also a member of the polyester family.¹³⁰ PDO sutures are flexible and easy to handle, which makes them suitable for a wide range of surgical procedures, including general surgery, orthopedics, and ophthalmology. PDO sutures start to lose their strength within the first few days after surgery and can be completely absorbed by the body within 4–6 months. PDO is also known for its good handling properties and minimal tissue reaction.¹³¹ Additionally, PDO sutures are nonantigenic, which means that they are unlikely to cause an immune response and have minimal tissue reaction, which reduces the risk of infection and wound healing complications.¹³¹ PDO has a good balance of mechanical and degradation properties, meaning it has good strength while also breaking down at a suitable rate, making it a promising option for GBR.¹³² Despite its advantages, PDO has some disadvantages such as high cost and the fact that it can cause some tissue inflammation.¹³³

PCL

PCL is a biodegradable and biobased thermoplastic polymer that has a wide range of uses, including as a biomedical material for controlled drug delivery,^{134,135} as a bioresorbable suture,^{136,137} and as a component in 3D printing filaments.^{138,139} It has a linear, aliphatic polyester structure consisting repeating units of caprolactone, which is a six-carbon ester.¹⁴⁰ The chemical formula for PCL is $[-(C_6H_{10}O_2)_n-]$, where n represents the number of repeating units in the polymer chain (Figure 5). The repeating units are connected by ester bonds, and the resulting polymer chain has a flexible and amorphous structure. This structure gives PCL distinctive mechanical properties, such as good toughness, flexibility, and low melting temperature,¹⁴⁰ making it an attractive alternative to petroleum-based plastics. From a material perspective, PCL has desirable properties, such as slow degradation, ease of processing, biocompatibility, and biodegradability. The degradation rate of PCL can be controlled by modifying the molecular weight and blending it with other polymers.¹⁴¹ Additionally, it is resistant to deformation and can maintain its strength and shape even when bent or twisted. From a biological perspective, PCL has been used as a carrier for various bioactive agents including growth factors, antibiotics, and stem cells, which can further

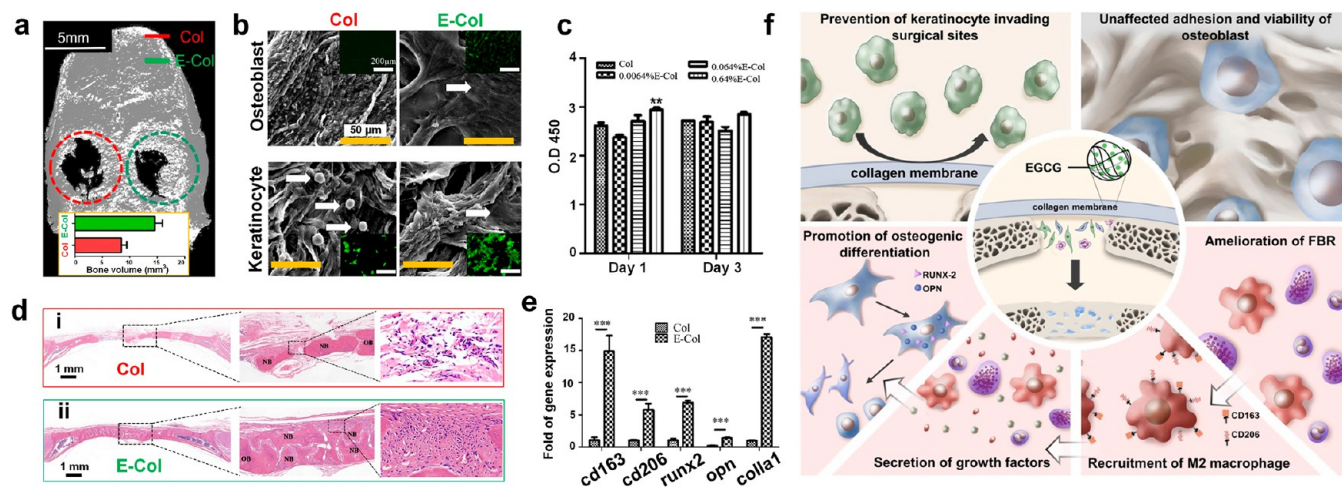


Figure 6. (a) In vivo promotion of bone regeneration after membrane implantation. Inset: the comparison of defect size with the bone volume using Micro-CT 4 weeks post membrane implantation of Col and E-Col. (b) SEM images of keratinocytes and osteoblast seeded on EGCG-modified collagen membranes with insets to show their corresponding live/dead staining. (c) Cell viability and (d) H&E staining of membrane sites 8 weeks post membrane implantation of Col (red rectangle circled) and E-Col (green rectangle circled), NB represents new regenerated bone. (e) Results of qPCR, which demonstrated that expression of CD206, CD163, RUNX2, OPN, and colla1 was significantly upregulated in E-Col compared to that of Col. (f) Schematic diagrams on the function of EGCG-modified collagen membrane. Adapted with permission from ref 32. Copyright 2019 Elsevier.

enhance the bone regeneration process.¹⁴² In the context of osteogenesis, PCL can be made into porous scaffolds, which provides a suitable environment for cell growth and helps to promote the formation of new bone tissue.^{143,144} PCL has a high resistance to water and most organic materials, making it suitable for biomedical, tissue engineering, and wound healing applications.^{145–147}

PEG

PEG is a bioresorbable polymer (Figure 5) with great biocompatibility, low toxicity, and ease of processing. This material could be efficiently used in composite membranes and blended with other polymers in making various new options for GBR membrane purpose.^{148,149} The effectiveness of PEG hydrogel as a barrier membrane for GBR in animal models, such as rabbits,¹⁵⁰ has been explored featuring the potential of PEG hydrogel to successfully promote bone regeneration in defect areas.¹⁴⁸ PEG-hydroxyapatite hybrid hydrogel has also been investigated as a scaffold for bone tissue engineering and has shown the ability to support bone cell growth and promote bone regeneration in vitro and in vivo.¹⁵¹ These findings suggest that the PEG hydrogel may have potential as an effective material for GBR and bone tissue engineering applications. Furthermore, PEG has been used as a plasticizer to enhance the material properties of other polymers including PCL.¹¹² This is done in order to decrease the hardness of PCL, lower the glass transition temperature, and reduce the amount of solvent required for solvent-involved manufacturing processes. In a multicentered randomized clinical trial by Jung et al.,¹¹¹ clinical outcomes of PEG versus native collagen membranes covering bony dehiscence type defects around bone level dental titanium implants were assessed. In this study, vertical bone fill was measured at baseline and after 6 months of healing as a primary parameter. Marginal bone level (MBL) and soft tissue conditions were recorded as secondary parameters after 18 months of loading. Results demonstrated that both PEG and collagen groups had comparable vertical bone fill (59% and 64%) and a change in defect height, and a

comparison between two groups did not show a statistical difference.¹¹¹

BIOACTIVE COMPOSITE MEMBRANES

Bioactive composite membranes refer to a type of material that is designed to have biological activity and can be used in various medical applications such as tissue engineering, drug delivery, and wound healing. These membranes typically combine biocompatible and biodegradable polymers with bioactive molecules such as growth factors, peptides, and proteins. The bioactive molecules incorporated into the composite membrane can interact with cells and tissues, promoting cell proliferation, differentiation, and migration. This can be especially useful in tissue engineering applications, where the goal is to create new tissues or organs by growing cells on a scaffold or membrane. Pal et al.¹⁵² developed a hydrogel composed of a collagen and elastin-like polypeptide interpenetrating network that encapsulates BMP-2, doxycycline, and 45S5 Bioglass, that enhanced human adipose-derived stem cell attachment, proliferation, and differentiation toward the osteogenic lineage. In vitro culture has revealed the deposition of hydroxyapatite particles within a collagenous fibrillar structure. When implanted in a rat cranial defect model, the hydrogel fosters the development of mature mineralized tissue that links with the surrounding bone, indicating significant potential for improved bone healing following cranioplasty.¹⁵² Bioactive composite membranes can also be used for drug delivery, as the bioactive molecules can be designed to release drugs or other therapeutic agents over time, providing sustained and localized delivery to the desired tissue. Here, we present a short review of the latest achievements and research directions on key requirements of bioactive composite membranes for GBR application:

BIOCOMPATIBILITY

The introduction of collagen material in the surgical site has been reported to provoke foreign body reaction (FBR),^{13,153} an inevitable immune response that occurs when a foreign

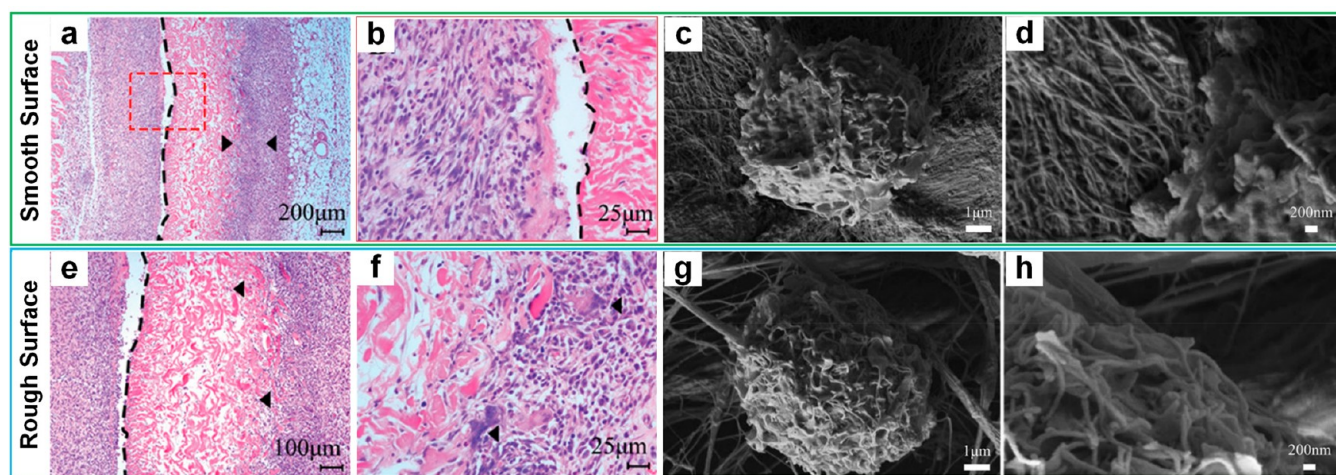


Figure 7. Host subcutaneous tissue response to collagen membranes 7 days post implantation by H&E staining and cell attachment of macrophages on (a–d) smooth and (e–h) rough sides of the collagen membranes. (a,b) The smooth surface prevents the cells from invading into the membranes, and (c,d) the macrophages grow on the smooth surface without migrating into the collagen network. (e,f) The cells migrate into the membrane scaffold on the rough side, generating a monocyte infiltration zone. (g) Macrophages migrate into the collagen network with (h) extended pseudopodia attach firmly on the collagen fibers. Adapted with permission from ref 168. Copyright 2018 Royal Society of Chemistry.

material (such as a membrane or a bone substitute) is placed in the body.¹⁵⁴ By recognizing the implanted material as a foreign body, the inflammatory response is activated, which can lead to polymorphonuclear leukocyte (PMN) recruitment.¹⁵⁵ The inflammatory response is a complex process that is mediated by various cells and molecules, including immune cells (such as neutrophils and monocytes) and inflammatory mediators (such as cytokines and chemokines).¹⁵⁴ During acute inflammation, the blood vessels in the area of injury or foreign material become more permeable, allowing immune cells and other inflammatory mediators to migrate to the site. The immune cells then begin to phagocytose and remove the foreign material, while the inflammatory mediators help to recruit more immune cells and promote tissue repair. The acute inflammation can last for a few days to a week and is followed by the resolution and the remodeling phase.¹⁵⁴

Monocytes and macrophages are closely related types of white blood cells that play important roles in the immune response and inflammation,¹⁵⁶ and could be polarized into proinflammatory (M1) or proregenerative (M2) phenotypes.¹⁵⁷ The efficient and timely switch from M1 to M2 phenotype is reported to be responsible for successful healing and regeneration,¹⁵⁸ while a prolonged M1 polarization can lead to an increase in fibrous encapsulation and bone regeneration failure.¹⁵⁹ To minimize FBR, biocompatible materials and techniques are used in GBR. Chu et al.^{32,160} demonstrated the potential of epigallocatechin-3-gallate (EGCG)-modified collagen membrane (E-Col membrane) as an excellent barrier membrane to minimize FBR and enhance bone regeneration in vivo (Figure 6a). EGCG is the most abundant catechin in green tea, has demonstrated remarkable anticancer, anti-inflammatory, antifibrosis, and pro-osteogenesis characteristics, and has been proposed to modulate the macrophage polarization in biomaterials.¹⁶¹ The results showed that the migration of keratinocytes was successfully prevented in EGCG-modified collagen membranes (Figure 6b), providing enough room for osteoblasts with no adverse effect on the viability and adhesion of osteoblasts. A significantly higher cell viability was observed in the modified membrane with cell seeds on day 1 compared to the

unmodified one (Figure 6c), with comparable live/dead staining results (Figure 6b, insets). Quantitative polymerase chain reaction (qPCR) analysis demonstrated that the expression of CD206, CD163, RUNX2, OPN, and colla1 markers significantly increased in E-Col membranes compared to that of Col (Figure 6e). The highest bone healing was observed in the modified membrane with EGCG with a thick and ordered bone formation and the closure of the bone defect, while the most fibrous tissue was found in the pure collagen membrane, which is unfavorable (Figure 6d). These findings demonstrate that the EGCG-modified collagen membranes are favorable for switching from M1 to M2 phenotype and can act as a suitable barrier against the invasion of keratinocytes to the bone defect, thus promoting in vivo bone regeneration for guided bone regeneration practice (Figure 6f).

The immune response is considered among the most critical aspects of biomaterial-mediated reconstruction of hard and soft tissue, which could be modulated by modification of the properties of biomaterial. It was previously believed that a biomaterial is better to be “inert”, but according to a paradigm shift in the nature of biomaterials, recent advances are more focused on “immunomodulatory” materials.^{162–165} There are several nutrient elements proved to be efficient in osteo-immunomodulation including calcium (Ca), zinc (Zn), and silicon (Si) (Figure 3).^{166,167} Using pulsed laser deposition (PLD) technique, Chen et al.¹⁶⁸ deposited a highly homogeneous, uniform layer of $\text{Ca}_2\text{ZnSi}_2\text{O}_7$ nanoparticles, as a bioactive glass, on a commonly used Bio-Gide collagen membrane with a rough surface on one side to manipulate the osteo-immunomodulatory property of the barrier collagen membrane. The other side, however, was made of a smooth surface of a dense collagen fiber network. As presented in Figure 7a,b, the smooth surface could successfully prevent the migration of soft tissue into the defect site, while cell migration into the membrane scaffold was observed in the rough surface (Figure 7e,f), forming a monocyte infiltration zone. The osteo-immunomodulatory properties of collagen membrane showed that the smooth surface acts like a foreign body reaction toward inert biomaterials, with macrophages from the M2

phenotype, and more anti-inflammatory cytokine profile was seen in the smooth surface (Figure 7c,d). The shift toward the M2 macrophage phenotype is reported to be a key component of fibro-encapsulation.¹⁵⁸ On the rough surface, the FBR was more like a bioactive material. Fibroblasts were declined with significantly lower fibro-encapsulation response on the rough surface (Figure 7g,h).¹⁶⁸ The dominant phenotype presented in situ was M1 which plays a key role in FBR. Moreover, a proinflammatory cytokine profile was observed in the rough surface with increased revascularization compared to a smooth surface profile.¹⁶⁸

Effective regulation in osteogenesis was reported by incorporating nutrient elements significantly enhancing osteogenic differentiation. Calcium ions have been found to trigger the calcium-sensing receptor (CaSR), a member of the G protein-coupled receptor superfamily and a key player in the maintenance of calcium homeostasis.¹⁶⁹ This leads to a reduction in Wnt5A protein and consequently suppresses inflammation.¹⁷⁰ Zinc ions, on the other hand, are responsible for the normal function and development of immune cells, while deficiency in Zn can lead to impairment in production and inflammatory cytokines signaling process.¹⁷¹ It is speculated that Si ions released from the membrane could activate the Wnt3 pathway, a signaling molecule that plays a crucial role in the regulation of bone formation and remodeling. These findings demonstrated that the next generation of membranes need a paradigm shift from just a physical barrier to a bioactive barrier with osteo-immunomodulation as an effective way to improve bone regeneration.¹⁶⁸

MECHANICAL STABILITY

Mechanical stability is one of the most critical characteristics of GBR barrier membranes as the amount of regenerated bone in the bone defect would be reduced if the membranes collapse into the defect space. So, the utilization of barrier membranes in biomedical and pharmaceutical applications is limited by their mechanical instability in aqueous environments. Generally, cross-linked membranes and scaffolds are less susceptible to rapid enzymatic degradation and show better mechanical stability compared to non-cross-linked biomaterials.^{172,173} However, many cross-linkers are toxic or immunogenic, hindering use in *in vivo* applications.¹⁷⁴ To address this issue, Acevedo et al.⁷³ proposed the application of UV radiation as a nontoxic cross-linking technique to upgrade the mechanical properties of a biodegradable gelatin-chitosan membrane (Figure 8a,b). The membranes were made of the biopolymers of gelatin-chitosan (56% gelatin, 42% chitosan) and a homogeneous distribution of titania (TiO₂, 1%) and hydroxyapatite (HAp, 1%) nanoparticles (Figure 8c,d). Gelatin is a naturally driven polymer obtained by incomplete denaturation of collagen. Compared to collagen, gelatin is less expensive with reduced antigenicity, and shows similar cell adhesion and proliferation. Bonded together, gelatin and chitosan form a polyelectrolyte complex with enhanced physical and mechanical properties, enabling the copolymer to be embedded with biological molecules such as growth factors. The results showed a significant enhancement in the membrane's stiffness upon increasing UV radiation doses, indicating efficient cross-linking of the material. The optimal mechanical property was reported to occur under an optimum UV dosage of 20 J·cm⁻² (Figure 8e), demonstrating the maximum cross-linking state. Similarly, an increment in cell adhesion, spreading, and growth velocity was observed on UV-

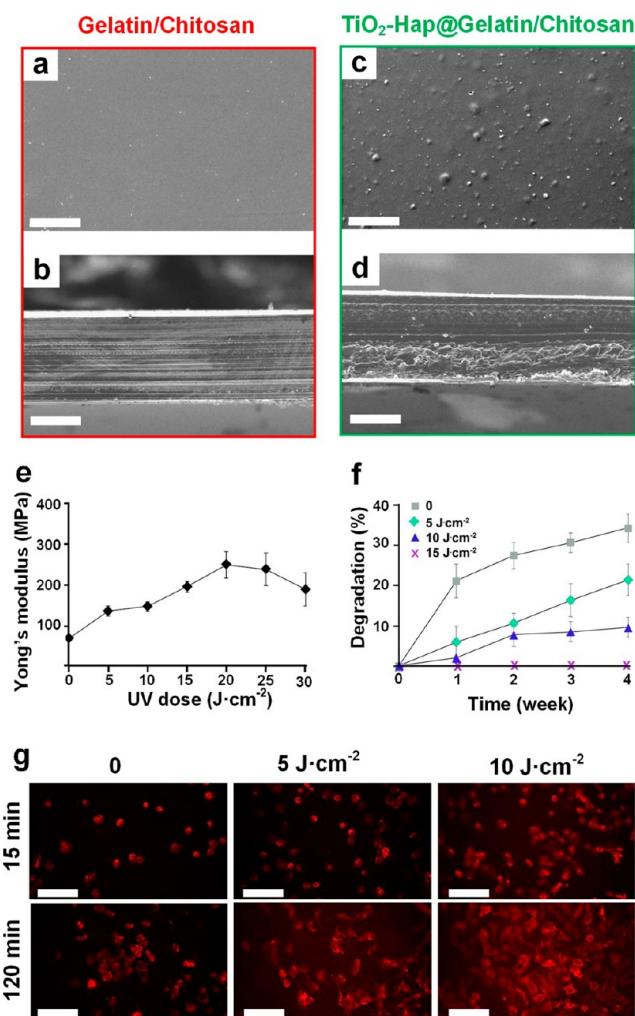


Figure 8. SEM images of the nanocomposed gelatin-chitosan membrane: (a) top view and (b) transversal section of the membrane without nanoparticles (red rectangle circled) and corresponding (c) top and (d) side views of membrane incorporated with nanoparticles (green rectangle circled). (e) Mechanical and (f) degradation behavior of the UV-irradiated nanocomposed gelatin-chitosan membranes. (g) Cell spreading on UV-irradiated nanocomposed gelatin-chitosan membranes at different time intervals. Scale bars are 100 μ m. MEF cells in suspension (2.5×10^5 cells/film) were allowed to attach and spread onto UV-irradiated membranes (0, 5, and 10 J·cm⁻²) for 15 and 120 min. Adapted with permission from ref 73. Copyright 2019 Elsevier.

irradiated membranes (Figure 8g), demonstrating the acceptable biocompatibility of the cross-linked membranes. However, the degradation percentage decreased to zero for the UV-treated membranes with more than 10 J·cm⁻² radiation doses (Figure 8f). In addition, serious concerns could be raised regarding nonresorbable titania nanoparticles, as they have been classified as a possible carcinogen to humans, according to the World Health Organization's International Agency for Research on Cancer (IARC).¹⁷⁵

In a similar approach, Ansarizadeh et al.¹⁷⁶ developed a novel barrier membrane for GBR application by combining collagen-chitosan and lyophilized advanced platelet rich fibrin (A-PRF), featuring excellent mechanical stability, cell viability, degradability, and reasonable osteogenic differentiation. Increasing the Chit/Col weight ratio from 0 to 4 and

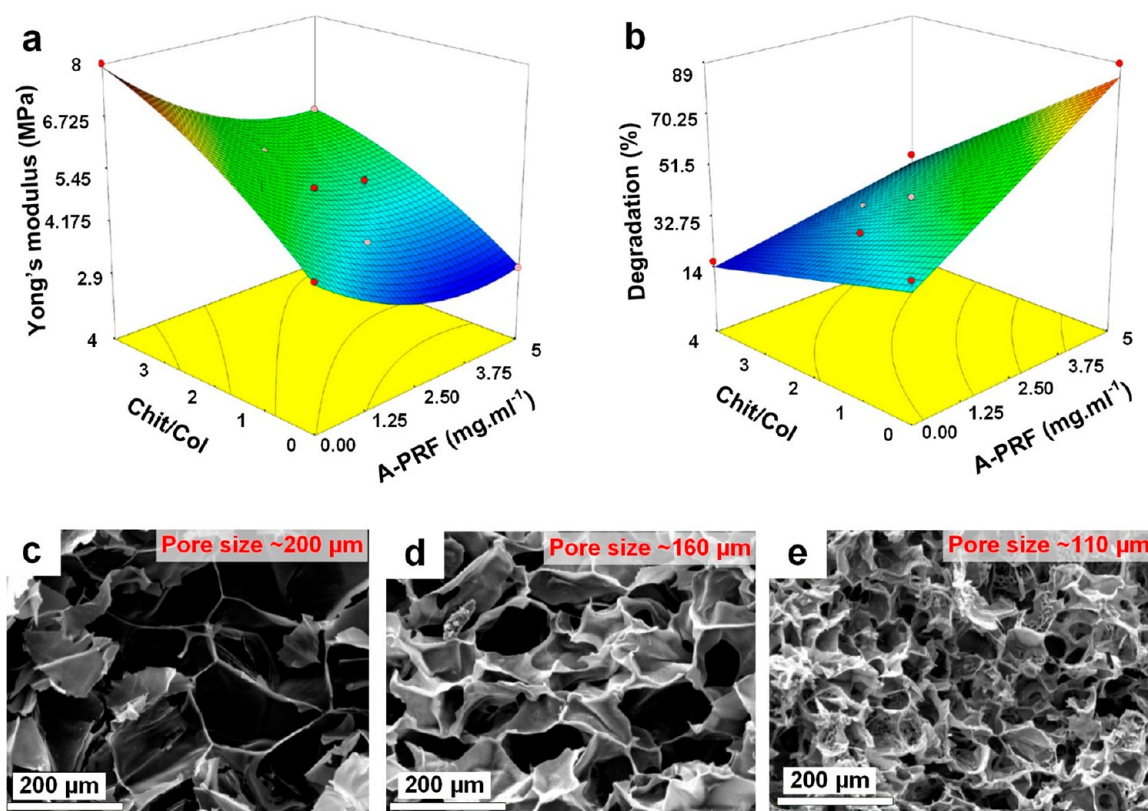


Figure 9. (a) Effect of Chit/Col weight ratio and A-PRF concentration on Young's modulus of the membrane. (b) Effect of Chit/Col weight ratio and A-PRF concentration on degradation rate of membrane. The cross-sectional SEM images of membranes made of (c) collagen, (d) collagen-chitosan (Chit/Col weight ratio = 1), and (e) collagen/chitosan (Chit/Col weight ratio = 4). Adapted with permission from ref 176. Copyright 2019 Elsevier.

decreasing the A-PRF concentration from 5 to 0 resulted in a significant enhancement in the Young's modulus of the fabricated membranes (from 3 to 8 MPa, Figure 9a), which peaked at 8 MPa for the membrane with a Chit/Col weight ratio and A-PRF concentration of 4 and 0.58 mg·mL⁻¹, respectively. This enhanced mechanical property could be ascribed to a higher cross-linking stage between collagen and chitosan chains caused by free amine group in chitosan leading to morphology deformation, pore size reduction, and greater interconnectivity within collagen-based membranes (Figure 9c–e). Enhanced mechanical properties, cell infiltration, nutrient diffusion, and consequently cell survival rate have been previously reported for membranes with smaller pore size.^{177,178} Cell viability of MSCs was also improved by both increasing the chit/col weight ratio and A-PRF concentration, which could be attributed to the higher number of amine groups in the membrane with a higher Chit-Col weight ratio, leading to a better cell adhesion property. In addition, increasing the A-PRF concentration resulted in an increase in the degradation rate of the fabricated membranes (Figure 9b), which is attributed to a higher degradability of fibrins, and consequently higher release rate of growth factors, compared to that of collagen and chitosan. Using a response surface methodology, a polynomial model was designed to mathematically explore the correlation between two independent variables and predict the optimum Col/Chit/A-PRF ratios.¹⁷⁶

Chen et al.¹⁷⁹ developed a new composite scaffold developed using a combination of elastin-like polypeptide (ELP) and silk fibroin (SF). The scaffold was created by using a dehydrothermal treatment that is both simple and environ-

mentally friendly. The resulting scaffold showed unique mechanical properties and was able to support the adhesion, proliferation, and differentiation of both bone mesenchymal stem cells and chondrocytes in vitro. The scaffold also demonstrated its ability to enhance cartilage and subchondral bone repair in vivo, making it a potential biomaterial for repairing tissues such as skin and for use in osteochondral repair.¹⁷⁹ Omar et al.¹⁸⁰ compared the efficacy of two resorbable collagen membranes for GBR in rat femurs. They found that elastin-collagen had a higher percentage of new bone formation in the central defect area compared to that of pure collagen. Molecular analysis also revealed differences in BMP2 expression, with higher expression observed in the collagen group and higher expression of BMP2 and remodeling activity observed in the elastin-collagen group.¹⁸⁰ These findings suggest that the healing process of GBR can be influenced by the type of resorbable collagen membrane used. Gurumurthy et al.¹⁸¹ reinforced collagen with ELP and optimized the composite composition using response surface methodology. The optimized composite exhibited an improved tensile strength, elastic modulus, and toughness. Physical property analysis demonstrated that ELP lowered the residual water content and interacted with collagen. The collagen-ELP composite demonstrated a dense microstructure, which improved the mechanical properties. These composites can be utilized to construct rigid, porous structures that are suited for tissue engineering applications.

Another approach to enhance the mechanical properties of barrier membranes is adding PCL, a nontoxic, nonallergenic, biodegradable, and biocompatible polymer. Several studies

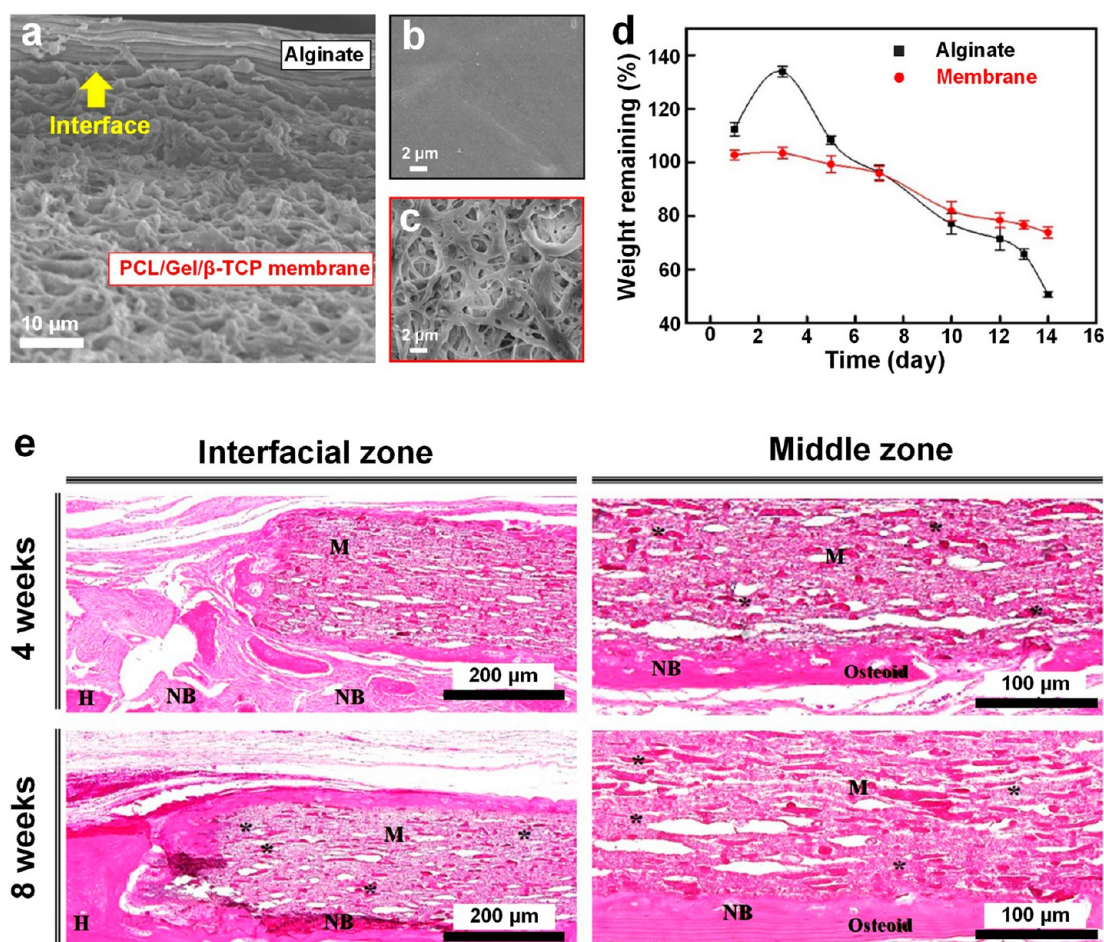


Figure 10. SEM images of (a) a dual-layered membrane made of (b) an alginate sheet and (c) the electrospun cross-linked PCL/Gel/ β -TCP membrane. The yellow arrow indicates the alginate-membrane interface. (d) Quantitative study of in vitro post degradation weight loss of electrospun membrane and casted alginate sheet. (e) H&E stained images of electrospun membrane after 4 and 8 weeks of implantation. M: membrane, H: host, NB: new bone formation. Asterisk (*) denotes the bone ingrowth via pores of the membrane. Adapted with permission from ref 186. Copyright 2022 IOP Publishing Ltd.

reported the development of natural-synthetic based polymers, using PCL, featuring enhanced mechanical stability and modulated degradation rate.^{109,182–184} However, one of the main drawbacks of PCL is its slow degradation rate, as previous studies reported a 24-month degradation time, which is not practical for GBR applications.¹⁸⁵ To improve the degradation profile of PCL-modified gelatin membranes, Joo et al.¹⁸⁶ fabricated dual-layered membrane embedding PCL and gelatin into alginate sheet and followed by an activation stage (Figure 10a–c) where beta-tricalcium phosphate (β -TCP) molecules were embedded into the membrane. β -TCP is a biocompatible ceramic material that has been used in a variety of medical applications, including as a bone substitute or scaffold for bone tissue engineering. It can also be used in dental implants and as a filler for bone defects.^{186,187}

Alginate-based materials have been used in GBR to protect the bone defect, but also to act as a source of growth factors, promoting bone formation and remodeling.¹⁸⁸ They can also improve bone repair and regeneration and reduce the risk of complications such as bacterial infection.^{189,190} Another unique aspect of alginate is its pH-dependent strength properties, so the membrane would immediately become firm within the initial stages after implantation upon being exposed to the acidic environment of the wound area.^{186,191} In vitro

degradation showed approximately 50% and 22% weight loss after 2 weeks with visible cracks and deformations observed on alginate and electrospun membranes, respectively (Figure 10d).¹⁸⁶ The combination of PCL and gelatin can enhance cell attachment to the membrane, and β -TCP is a substrate that could improve cell attachment in bone area and increase osteogenic differentiation and cell proliferation. In vivo histologic analysis results showed good space maintenance at the defect site with impressive new bone formation after 8 weeks (Figure 10e). At 8 weeks, the membranes maintained their shape and thickness with great integration into the surrounding tissues. No inflammatory cells were detected with minor penetration of soft tissue into the new bone area after 4 weeks of implantation (Figure 10e).¹⁸⁶

The main concern about PCL, however, is its poor cell adhesion and inadequate wettability properties, which could be attributed to the low surface energy and hydrophobicity of PCL, which makes it difficult for cells to adhere to the surface.¹⁹² To address this issue, β -TCP was included in the composite membrane due to its great osteo-conductivity and biocompatibility.¹⁹³ Additionally, β -TCP could act as a precursor for Ca^{2+} and PO_4^{3-} ions, encouraging bone regeneration in osteogenesis.¹⁹⁴ Using the electrospinning technique, Ezati et al.¹⁴¹ fabricated polymeric nanofibers with a

diameter range of 200–500 nm, resulting in highly porous membranes with efficient nutrient exchange and cell migration into the scaffold. The mechanical stability analysis showed significant improvement in the elastic modulus, elongation, and ultimate tensile strength (UTS) of the membranes upon addition of β -TCP, with the optimal mechanical properties peaking for the 5% β -TCP composite (200 MPa, 38% and 8.5 MP for modulus, elongation, and UTS, respectively). This could be attributed to cross-linking between polymer chains caused by β -TCP content and, consequently, pore size reduction, as previously reported by Tsuru et al.¹⁴² In addition to mechanical properties, higher wettability and roughness were observed in the fabricated membranes upon addition of β -TCP. The MTT assay demonstrated a higher cell affinity and proliferation rate for membranes with 3–5% β -TCP, with more cell contacts and extent of filopodia and lamellipodia compared to the control sample. This enhanced cell adhesion could be attributed to the higher hydrophilicity and surface roughness of the modified composite membranes. Gene expression tests were performed to examine two osteogenic markers: alkaline phosphatase (ALP) and type I collagen. The former is for early stage differentiation, and the latter is for later differentiation of MG63 cells. MG63 cells expressed more maturation markers and higher expression of type I collagen by increasing the β -TCP content up to 3% β -TCP, which could be due to the release of calcium ions from the composite membrane. Moreover, samples with a higher concentration of β -TCP showed greater biodegradation rate and weight loss (up to 67% after 4 weeks of incubation), which could be related to higher hydrophilicity of membranes with β -TCP content.¹⁹⁵

Silk fibroin is a protein found in silk that can be processed into various materials with different properties, including films, fibers, and hydrogels. One of the challenges in working with silk fibroin is that it becomes brittle when dried, resulting in high tensile strength but poor elongation. This is because the protein molecules lose their ability to interact with each other and form a stable network, which leads to a decrease in the mechanical properties. Various strategies have been developed to overcome this brittleness, such as cross-linking, blending with other polymers, or incorporating plasticizers.^{88,196} To tackle this problem, Geão et al.¹⁹⁷ designed a flexible SF membrane by adding glycerol (GLY) and polyvinyl alcohol (PVA) as plasticizers. GLY is a clear, colorless, and odorless liquid that is widely added to plastics to increase their flexibility and durability, and to personal skincare products as a moisturizer.¹⁹⁸ PVA is a water-soluble synthetic polymer that is commonly added to cement and plaster-based products to improve their flexibility and durability, and to adhesives and coatings to increase their viscosity and workability.¹⁹⁹ It is considered a nontoxic and environmentally friendly plasticizer, as it is water-soluble and can be easily removed during cleaning.²⁰⁰ Benefiting from GLY and PVA properties, Geão et al.¹⁹⁷ managed to decrease the membrane's UTS from 40 MPa in pure SF membrane to 22 and 12 MPa in membranes with 10% GLY and 10% PVA, respectively (Figure 11a). As expected, a significant enhancement in membrane's elongation was achieved upon the addition of 10% plasticizer from 147% for SF, to 228% and 249% for 10% GLY and PVA, respectively (Figure 11b). Higher surface roughness was also achieved by increasing the GLY and PVA contents (up to 30%) with an acceptable degree of transparency. The SEM images depicted that the control membrane had a smooth surface after undergoing thermal treatment (Figure 11c, i). However,

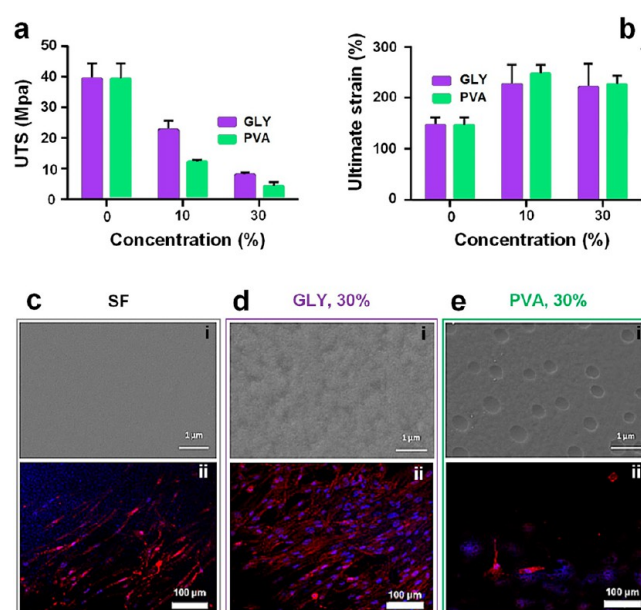


Figure 11. Representative (a) ultimate tensile stress (UTS) and (b) ultimate strain of membranes with different GLY and PVA content. (i) SEM micrographs and (ii) confocal images of the produced SF-based membranes: (c) pure SF, (d) 70SF:30GLY, and (e) 70SF:30PVA dried for 12 h. Adapted with permission from ref 197. Copyright 2019 Springer Nature.

when GLY was added to the membrane, a globular microstructure was formed, which was more pronounced at a concentration of 30% (Figure 11d,e, i). In vitro cellular studies showed enhanced human periodontal ligament cell (hPDLs) adhesion, viability, and proliferation in samples with SF and GLY, while no visible cells were observed on the membrane with PVA content (Figure 11c–e, ii). This higher cell viability properties of GLY could be attributed to the higher hydrophilicity of the surface caused by hydroxy (–OH) groups.²⁰¹

ANTIBACTERIAL ACTIVITY

Bacterial infection in GBR membranes is another major problem, leading to complications in the procedure. The membrane is used to cover the bone defect and protect it from bacterial contamination, but if it becomes infected, it can impede the healing process and lead to potential failure.^{202,203} The most common bacterial species that cause infection in GBR membranes are *Streptococcus* spp, *Staphylococcus* spp, and *Porphyromonas gingivalis*.^{204,205} One common solution reported is to directly dope the membrane with antibiotics to minimize the risk of infection.^{206,207} However, the uncontrollable continuous release of antibiotics was reported as the main drawback, hindering their real-world application as anti-infectious membranes.

Recently, He et al.²⁰⁸ designed a two-layered FGM with gelatin as the surface layer to provide biocompatibility and PCL as the core material (Figure 12a), to simultaneously enhance the mechanical properties of the fabricated membranes. A strong tensile strength of 6 MPa and strain at failure of 490% were achieved for the developed membrane, which was significantly higher compared to the ones for gelatin (2.6 MPa and 210%, respectively). However, a dramatic reduction in mechanical properties was observed after 5 weeks in the cell culture medium, which could be attributed to the breakdown

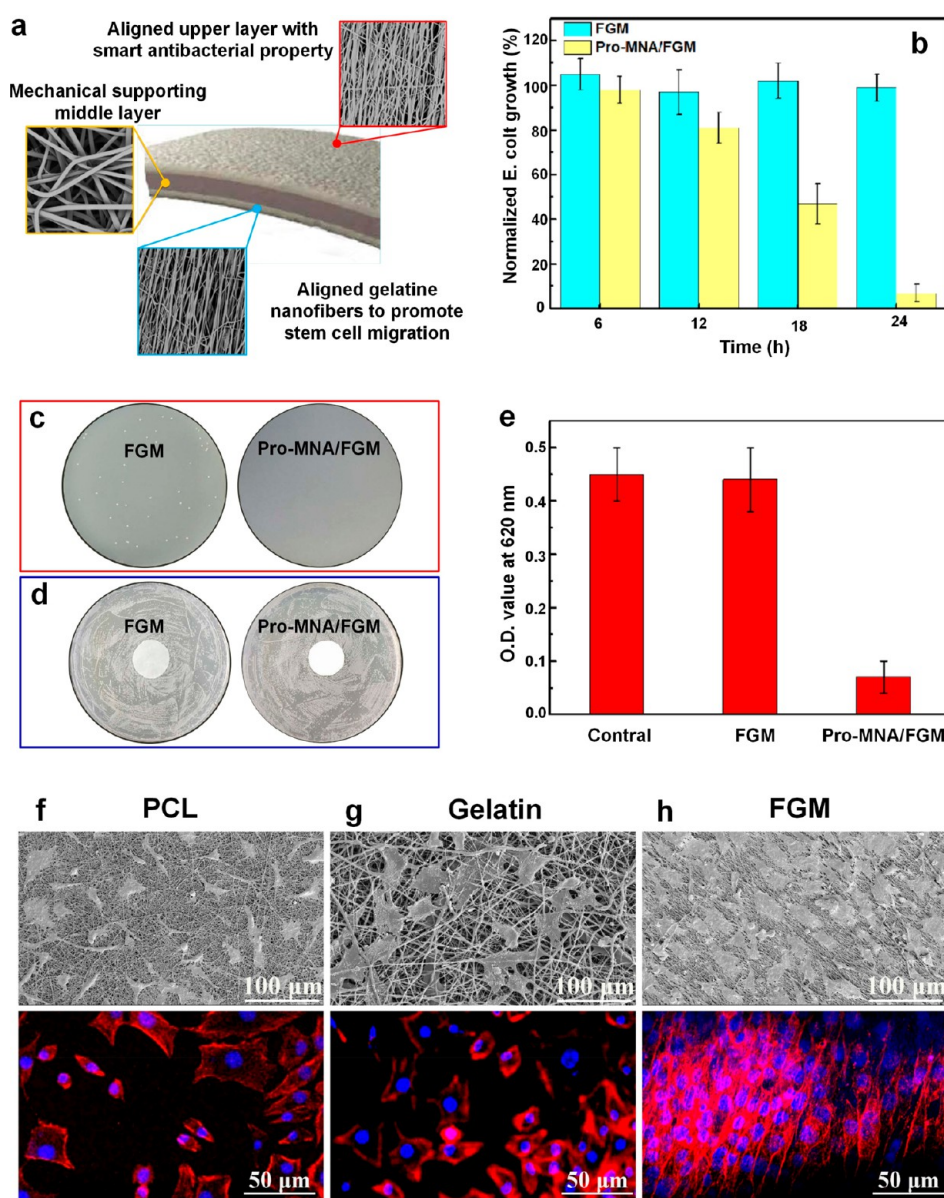


Figure 12. (a) Sandwich structured multifunctionalized graded membrane. (b) Normalized growth of *E. coli* incubated with the membranes for different durations. (c, left) Plate samples showing colonies of *S. aureus* and inhibition zone of the membrane: the sample incubated with FGM (without Pro-MNA loading) shows some bacterial colonies were formed. (c, right) No bacterial colonies formed for the sample incubated with Pro-MNA/FGM. (d) Nonetheless, both the control sample and Pro-MNA/FGM did not exhibit a consistently stable inhibition zone, which suggests that no MNA diffused from the membrane. (e) Quantitative study of the OD values in the bacteria-cell infection model to investigate the bacterial growth. (f-h) SEM micrographs (up) and confocal fluorescent images (bottom) of cells proliferated on R-PCL, R-gelatin, and FGM membranes for 7 days. Adapted with permission from ref 208. Copyright 2020 Elsevier.

of the PCL molecules.¹¹³ The interfacing space was loaded with pro-metronidazole (Pro-MNA), a hydrophobic and water-insoluble prodrug form of metronidazole, to develop a self-defensive membrane with controllable antibiotic release that has been used to treat a variety of bacterial and protozoal infections. In contrast to bacterial infection in the control sample, significantly restrained bacterial growth efficiency was observed for Pro-MNA/FGM within 24 h of incubation (Figure 12b). The mechanism of infection responsive release in Pro-MNA membrane showed a trigger in MNA release, due to the accumulation of esterase and decrease in pH value. The antibiotic effect reached the optimum level at 24 h after incubation, inhibiting the formation of any bacterial colonies in the Pro-MNA/FGM sample (Figure 12c,d). Pro-MNA/FGM

inhibited bacterial growth and prevented bacterial adhesion on cells compared to the control (Figure 12e), suggesting that Pro-MNA loaded FGM has an inherent self-defensive antibacterial property. Epithelial cell adhesion and proliferation increased continuously within 7 days of incubation, with a consistent ranking of FGM > R-gelatin > R-PCL (Figure 12f–h). This result could be attributed to the synergistic effect of chemical composition and fibers alignment on cell proliferation and adhesion.²⁰⁸

Abe et al.²⁰⁹ found that the PLCL bilayer membrane had reduced bacterial adhesion and blocked bacterial penetration, compared to the membranes of collagen and PLGA (Figure 13a), leading to a favorable outcome for regenerative treatments. Moreover, colony-forming units (CFU) counts of

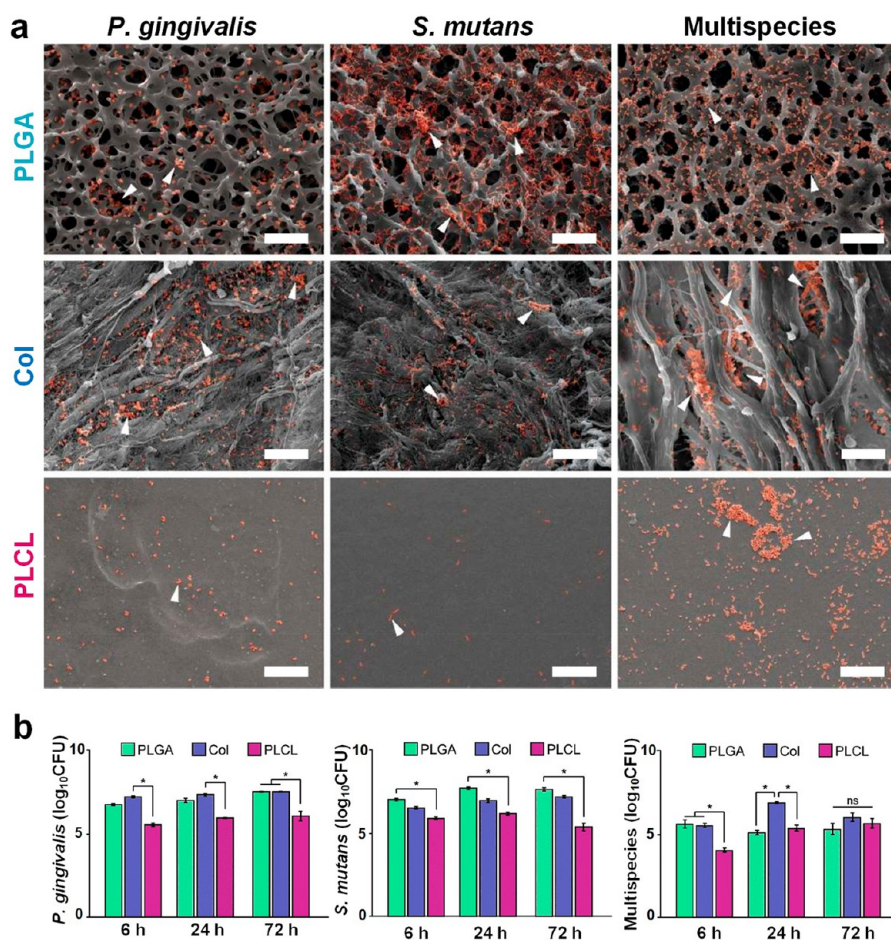


Figure 13. (a) SEM images of the *P. gingivalis*, *S. mutans*, and multiple bacteria obtained from human saliva cultured on PLGA, Col, and PLCL membranes after 24 h. White arrows indicate the bacterial cells (colored in orange for a clear vision). All scale bars are 10 μ m. (b) CFU counts of cultured *P. gingivalis*, *S. mutans*, and multiple bacteria obtained from human saliva on the three membranes at different time intervals. Adapted with permission from ref 209. Copyright 2022 John Wiley & Sons.

the incubated bacteria throughout the experimental period up to 72 h (Figure 13b), revealing that (1) *P. gingivalis* showed that the PLCL membrane had fewer adherent cells compared to the PLGA or Col membranes. While the PLCL membrane had significantly lower numbers of adherent *P. gingivalis* than the Col membrane, there was no statistical difference between the PLCL and PLGA membranes until after 72 h of incubation with *P. gingivalis*. In contrast, there were no significant differences in the adherence of *P. gingivalis* to PLGA and Col membranes at any time point. (2) For *Streptococcus mutans*, the PLCL membrane exhibited significantly fewer adherent cells than the PLGA membrane after 6, 24, and 72 h of incubation, and a lower number of adherent cells than the Col membrane, although no statistical significance was found. (3) CFU counts of membranes incubated with bacteria from human saliva displayed that the PLCL compact layer had significantly fewer CFUs than PLGA and Col at 6 h, and after 72 h of incubation there were no statistical differences in CFU counts between any of the groups (Figure 13b).²⁰⁹ The properties of the PLCL membrane, including reduced bacterial adhesion and penetration, may be beneficial for regenerative treatments, and in the case of exposure at surgical sites, efficient barrier function may facilitate the management of complications in guided tissue and bone regeneration.

CELL PROLIFERATION ENHANCEMENT

Growth factors are naturally occurring proteins that play a crucial role in stimulating and regulating bone growth and healing. In GBR, growth factors are usually delivered directly to the site of the defect via a carrier material, such as a collagen sponge or a synthetic scaffold, to stimulate the formation of new bone tissue, reduce the healing time, and improve the final outcomes of the procedure. The most common growth factors used in barrier membranes for GBR procedures in dentistry and oral surgery are typically obtained from either platelet-rich plasma (PRP) including Platelet-derived growth factor (PDGF), Growth/differentiation factor 5 (GDF-5) and Fibroblast growth factor-2 (FGF-2) or BMP including recombinant human bone morphogenetic protein-2 (rhBMP-2) and recombinant human bone morphogenetic protein-9 (rhBMP-9). In the field of regenerative medicine, these growth factors have been studied for their potential to promote tissue regeneration and repair including the regeneration of bone, connective tissues, and blood vessels. They have shown enormous potential to enhance tissue regeneration and repair, including the regeneration of bone, cartilage, and other connective tissues. They have been studied to stimulate the proliferation and differentiation of bone-forming cells (osteoblasts) and promote the formation of new blood vessels (angiogenesis), which are necessary for delivering oxygen and

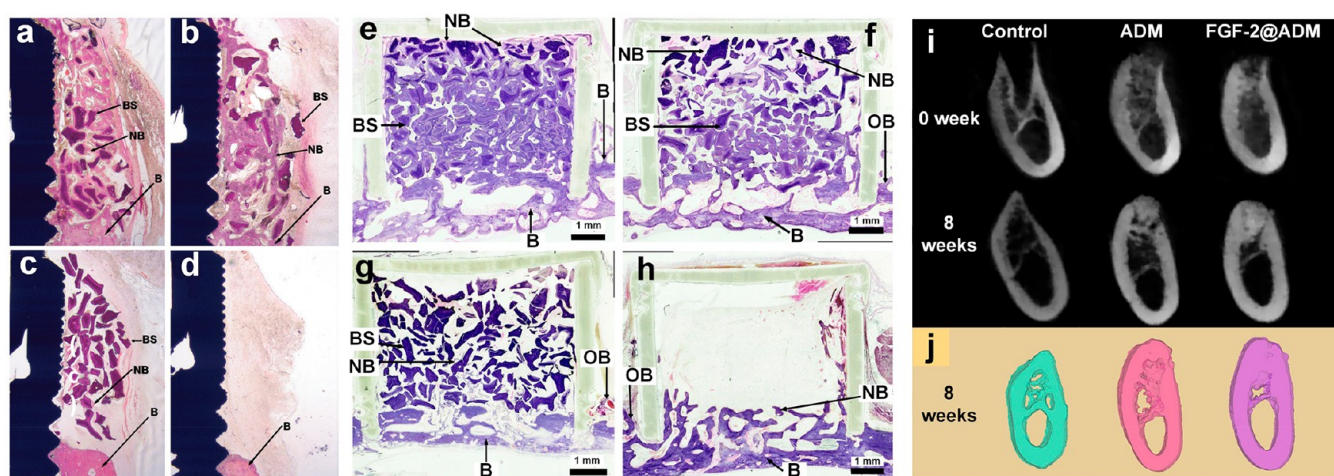


Figure 14. Histological bone growth at 8 weeks on different membrane groups: (a) DBBM/BMP-2, (b) DBBM/PDGF, (c) DBBM, and (d) control. Reprinted with permission from ref 211. Copyright 2016 John Wiley & Sons. H&E stained images of the histologic slide of different group cylinders after 8 weeks of healing: (e) DBBM/BMP-2, (f) DBBM/PDGF, (g) DBBM, and (h) empty control. Reprinted with permission from ref 212. Copyright 2017 John Wiley & Sons. (i) CBCT observation of extraction socket healing between control, ADM, and FGF-2@ADM immediately and 8 weeks after surgery. (j) 3D reconstruction images of the central region of extraction socket corresponding to the three groups mentioned above 8 weeks after surgery. Adapted with permission from ref 213. Copyright 2021 John Wiley & Sons.

nutrients to the repair sites. The main challenge, however, is dosing, as determining the optimal dose of growth factor to use in GTR and GBR procedures can be challenging, and there is a lack of consensus on the best approach. In addition, there is a lack of standardization in the production and preparation of growth factors for use in GTR and GBR procedures, which can affect their effectiveness and safety. Furthermore, using growth factors can significantly increase the cost of GTR and GBR procedures, which can be a burden to some patients.

Shirzaei Sani et al.²¹⁰ reported the potential use of hydrogel-based biomaterials in tissue engineering in promoting tissue regeneration in vivo due to their high-water content, permeability, and ability to create a supportive environment for cells. However, the clinical application of hydrogels has been limited by factors such as poor mechanical performance, low adhesive strength, uncontrolled degradation rates, and susceptibility to bacterial colonization. To tackle this issue, they introduced a new type of hydrogel made from methacrylated hyaluronic acid (MeHA) and an ELP, which is elastic, antimicrobial, and adhesive. This hydrogel can be rapidly photo-cross-linked in situ, making it suitable for the regeneration and repair of various tissues. Different physical properties of the hydrogels were engineered by varying the concentrations of MeHA and ELP. The MeHA/ELP hydrogels exhibited a higher adhesive strength to tissue than commercially available tissue adhesives. Zinc oxide nanoparticles were incorporated into the hydrogel to make it antimicrobial, and it was found to significantly inhibit the growth of methicillin-resistant *Staphylococcus aureus*. The hydrogel also supported the growth, spreading, and proliferation of mammalian cells in vitro. In vivo subcutaneous implantation demonstrated that MeHA/ELP hydrogels did not elicit a significant inflammatory response but could be efficiently biodegraded and promoted the integration of new autologous tissue.

Yamano et al.⁵⁵ evaluated the effect of using bioactive collagen membranes with PDGF and GDF-5 on bone regeneration in a preclinical animal model. The results showed that both PDGF and GDF-5 released from the bioactive

collagen membranes significantly increased bone regeneration compared to control groups without growth factor treatment, with GDF-5 demonstrating an accelerated yet dose-dependent bone regeneration behavior compared to PDGF. In addition, the collagen membranes loaded with GDF-5 showed significantly higher ALP and cell proliferation activities and osteogenic gene expression of transcription factors compared to the ones loaded with PDGF or without any growth factor. In another approach, Thoma and co-workers^{211,212} compared the bone regeneration performance of recombinant human PDGF-BB (rhPDGF-BB) and rhBMP-2, which are the synthesized versions of PDGF and BMP-2, respectively. In dental surgery, these growth factors are usually used in conjunction with other materials, such as bone substitute materials or barrier membranes, to promote new bone growth and improve the volume and quality of the surrounding bone. Using dental implants on the buccal sides of an edentulous ridge in ten dogs, they reported a significant increase in bone volume and density around the dental implants, leading to its higher stability and longevity. As presented in Figure 14a–d, the mean percentages of regenerated area within total defect area increased to 56.95% and 48.86% for deproteinized bovine bone mineral (DBBM) mixed with rhBMP-2 and a collagen membrane (CM) (DBBM/BMP-2) (Figure 14a) and DBBM mixed with rhPDGF-BB and CM (DBBM/PDGF) (Figure 14b), respectively, which is significantly higher compared to 33.44% (Figure 14c) and 1.59% (Figure 14d) for DBBM and control samples at 8 weeks, respectively. In another study, they investigated the use of rhBMP-2 and PDGF-BB for localized bone regeneration in rabbits and assessed the effectiveness of this treatment using histologic and radiographic methods. At 8 weeks, the area of bone regeneration (histology; AA_{Histo}) peaked at 96.29% for DBBM/BMP-2, in which bone formation filled the entire space between the DBBM particles, reaching every corner of the cylinders (Figure 14e). The cylinders were therefore densely packed with mineralized tissues. In DBBM/PDGF, bone formation partially reached the top of the cylinder with mean AA_{Histo} of 46.37% (Figure 14f), which was slightly higher than DBBM (Figure 14g) and control (Figure 14h)

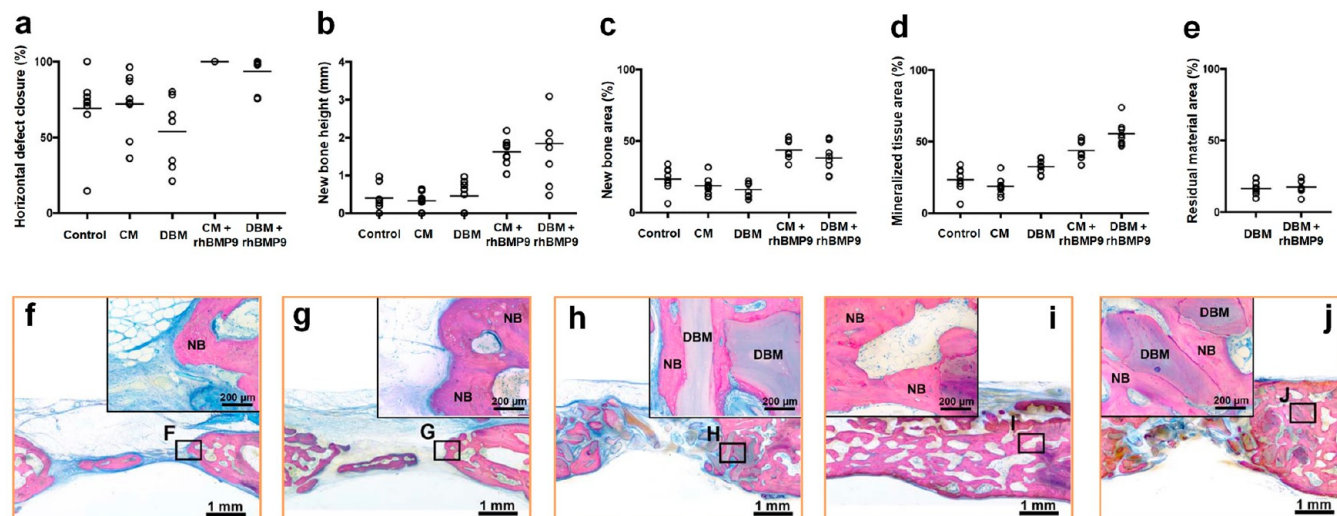


Figure 15. Histomorphometrical measurements of linear parameters: (a) percentage of horizontal defect closure in each group; (b) length of maximum new bone height in the middle area of defect; (c) percentage of new bone area, (d) mineralized tissue area, and (e) residual material area in DBM and DBM+rhBMP9 groups. Dot plots demonstrate data for all specimens and the bars indicate the average of the values in each group. Images of the toluidine blue and fuchsin staining in bone defects at 8 weeks in (f) control, (g) CM, (h) DBM, (i) CM+rhBMP9, and (j) DBM+rhBMP9 groups. Insets correspond to the rectangles accordingly. NB shows new bone, and DBM refers to residual materials. Adapted with permission from ref 214. Copyright 2017 John Wiley & Sons.

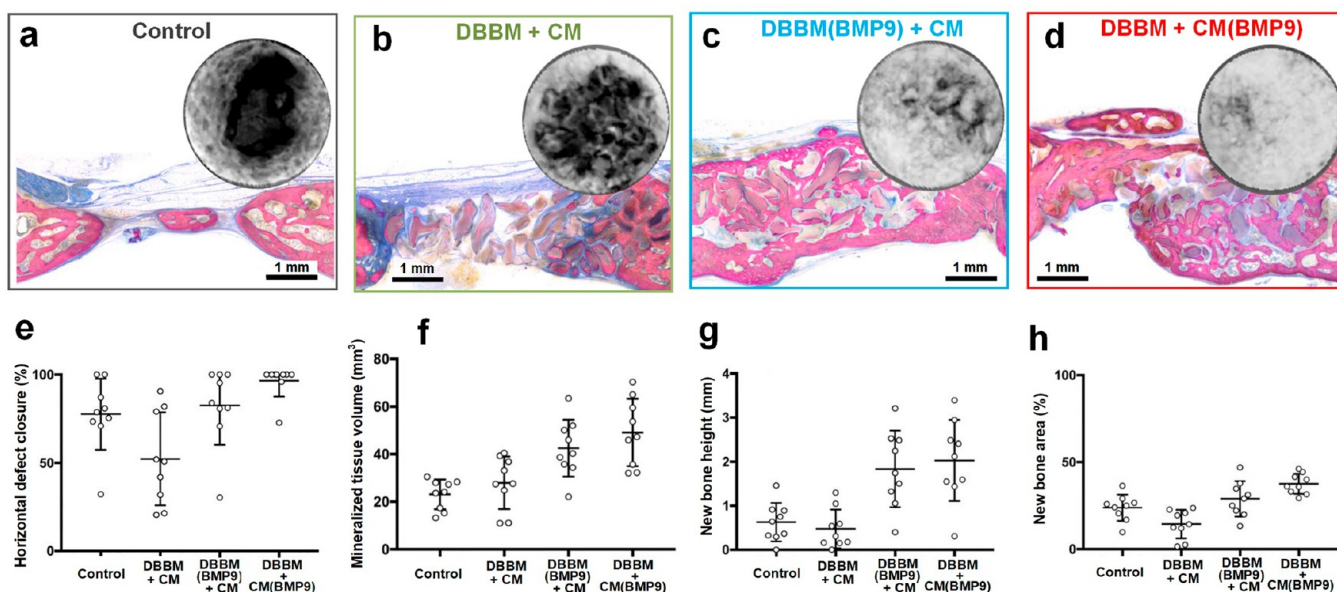


Figure 16. Toluidine blue and fuchsin staining on the middle sections in the bone defects at 8 weeks in (a) control, (b) DBBM/CM alone, (c) DBBM(BMP9)+CM, and (d) DBBM+CM(BMP9) groups. Insets: μ CT images in bone defects from the top view at 8 weeks. (e) Statistical study of the horizontal bone defect closure. (f) Mineralized tissue volume measured by μ CT, (g) length of new bone height in the middle area of the defect, and (h) new bone area. Dot plots show data for all specimens, and bars indicate the average and SD of the values in each group. Adapted with permission from ref 36. Copyright 2017 John Wiley & Sons.

samples with 39.66% and 35.98%, respectively. The highest bone regeneration was achieved for DBBM/BMP-2 (35.62%), with statistically significant differences compared to all other groups ($P < 0.05$). The results showed that the use of rhBMP-2 significantly enhanced bone regeneration compared to all other groups including those with rhPDGF-BB. In another approach, the effect of FGF-2 on the preservation of the alveolar ridge after tooth extraction was investigated using acellular dermal matrix (ADM) membranes. ADM membranes are semisynthetic membranes derived from natural sources (human or animal skin tissue) but undergo a process of

decellularization to remove cells and cellular components and potential modification with other materials such as growth factors. Using bovine xenografts as a bone substitute material, combined with FGF-2-loaded ADM membrane, Wang et al.²¹³ examined the changes in the width and height of alveolar ridges and extraction socket bone healing 8 weeks post-extraction. They reported a significant inhibitory effect on alveolar bone width reduction for the FGF-2-loaded membrane (Figure 14i,j) when compared with the unloaded membrane and empty control.

BMP-9 is another member of the BMP family of growth factors that plays a key role in the regulation of bone formation and has been shown to have a potent osteogenic effect in various animal models. To find out the best carrier system for BMP-9, Kobayashi et al.²¹⁴ studied the effect of two carrier systems, porcine-derived CM and DBBM on the effective delivery of BMP-9 on bone formation in a rabbit calvarial defect model (10 adult female New Zealand white rabbits). The results showed that the CM treated with rhBMP-9 led to complete resolution of the horizontal bone deficiency in all specimens (Figure 15a) with no signs of multinucleated giant cells (MNGC). New bone height and new bone area were both significantly increased in the CM-rhBMP9 and the DBBM-rhBMP9 groups when compared to the control, CM, and DBM groups (Figure 15b,c and f–j). When evaluating the overall amount of mineralized tissue in the treated bone sites, the combination of DBM-rhBMP-9 led to a significantly greater amount of mineralized tissue compared to just DBM (Figure 15c,d). There was no difference in the residual material area between the groups treated with DBM and DBM-rhBMP-9 (Figure 15e). These findings suggest that the use of CM, rather than DBM, in combination with rhBMP-9 may result in a more consistent stimulation of bone growth and complete coverage of the affected area. In a very similar work,³⁶ the team compared the bone regeneration performance of rhBMP-9 loaded on DBBM and CM in a rabbit model. They found that the CM/rhBMP-9 samples achieved close to complete closure of horizontal bone defects, as evidenced by the formation of new bone layers in the CM-implanted areas and the bone growth surrounding DBBM materials (Figure 16a–e). Of note, both DBBM/rhBMP-9 and CM/rhBMP-9 significantly enhanced new bone formation, as demonstrated by an increase in mineralized tissue volume (Figure 16f) and new bone height and area (Figure 16f–h). However, the combination of rhBMP9 with CM showed to be more effective in promoting wound closure compared to DBBM-rhBMP9, which could be attributed to the tendency of BMP9 to be absorbed by CM rather than other bone grafts, including HAp^{215,216} and ceramics.²¹⁷ However, further research is necessary, utilizing large animal studies and a variety of bone grafts and barrier membranes, to further explore these results.

SUMMARY AND OUTLOOK

The use of resorbable membranes for GBR has been a significant development in the field of dentistry and orthopedics. The ability to selectively prevent the growth of nonosteogenic cells and allow the infiltration of osteogenic cells has shown great promise in promoting bone regeneration. Natural barrier membranes, such as collagen-based membranes, are widely used due to their biocompatibility and ability to support cellular attachment and growth. However, their mechanical properties are often inferior to those of synthetic membranes. Synthetic barrier membranes, such as PLGA and PCL, have superior mechanical properties and can be tailored to meet specific requirements. Bioactive nanocomposite-based barrier membranes, such as natural or synthetic polymers reinforced with HAp and TCP, have been developed to enhance the osteogenic potential of membranes and improve bone regeneration. However, there is still a need to optimize the design and properties of these membranes to maximize their efficacy. While resorbable membranes have shown promise in promoting bone regeneration, their success depends on various factors,

including the membrane's degradation rate, mechanical properties, biocompatibility, and ability to support cellular attachment and growth.

There is a need for continued research to optimize the design and properties of resorbable membranes. One important aspect of membrane designs is the degradation rate. Resorbable membranes that degrade too quickly may not provide sufficient support for bone regeneration, while those that degrade too slowly will impede bone formation. Therefore, the development of membranes with controlled degradation rates could improve their effectiveness in promoting bone regeneration.

Another important aspect of membrane design is their mechanical properties. Resorbable membranes should have sufficient strength to withstand the forces of the surrounding tissue, while allowing for cellular infiltration. Furthermore, the incorporation of bioactive materials such as HAp, TCP, and growth factors into resorbable membranes could further enhance their osteogenic potential. The development of new materials and manufacturing techniques may also lead to improved resorbable membranes for GBR. For instance, 3D printing technology has been used to fabricate customized implants for bone regeneration, and this technology could potentially be used to create resorbable membranes with tailored properties. Additionally, clinical studies are needed to evaluate the long-term safety and efficacy of modified resorbable membranes in promoting bone regeneration. Such studies could provide valuable information about the optimal membrane design and properties for various clinical applications.

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administration (equal), supervision (equal), visualization (equal), writing-original draft (equal), writing-review & editing (equal).

Notes

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