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Membranes for Guided Bone Regeneration: A Road from Bench to Bedside

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Bone resorption can negatively influence the osseointegration of dental implants. Barrier membranes for guided bone regeneration (GBR) are used to exclude nonosteogenic tissues from influencing the bone healing process. In addition to the existing barrier membranes available on the market, a growing variety of membranes for GBR with tailorable physicochemical properties are under preclinical evaluation. Hence, the aim of this review is to provide a comprehensive description of materials used for GBR and to report the main industrial and regulatory aspects allowing the commercialization of these medical devices (MDs). In particular, a summary of the main attributes defining a GBR membrane is reported along with a description of commercially available and under development membranes. Finally, strategies for the scaling-up of the manufacturing process and the regulatory framework of the main MD producers (USA, EU, Japan, China, and India) are presented. The description of the regulatory approval process of GBR membranes is representative of the typical path that medium- to high-risk MDs have to follow for an effective medical translation, which is of fundamental importance to increase the impact of biomedical research on public health.

1. Introduction

Long-term integration for osteointegrated devices implanted in the alveolar ridge is a key goal in dental implantology. However, bone resorption or loss as a result of edentulism, traumas, and tumors can jeopardize the osseointegration of prosthetic-replaced teeth.[1,2] Different techniques are available to augment alveolar bone insufficiency such as, onlay and inlay grafting, free vascularized autografts, distraction osteogenesis, grafting of the maxillary sinus, ridge splitting, and guided bone regeneration (GBR).[3] However, among these strategies for alveolar ridge augmentation, GBR is the most documented with long-term follow-up studies yielding positive comparable outcomes.[3–5] Currently, GBR is one of the most common technique for horizontal and vertical defect augmentations, or to preserve alveolar sockets after tooth extraction.[6–9] Barrier membranes for GBR application play a key role in preventing the ingress of cells from the surrounding epithelium and connective tissue (**Figure 1**), to favor osteoprogenitor cells to proliferate and form new bone tissue in the site of implants.[9,10]

Although many different classes of GBR membranes are commercially available (discussed in Section 3), these can be generally divided into two categories: nonresorbable and resorbable membranes. Among the nonresorbable membranes, the expanded polytetrafluoroethylene (ePTFE) membranes have been accepted as the gold standard material for their mechanical stability, biocompatibility, and ability to facilitate bone regeneration in many clinical studies.[9] However, nonresorbable membranes suffer from various drawbacks, as their stiffness may cause soft tissue dehiscence, which can lead to the membrane exposure and subsequent infection.[11,12] Additionally, a second surgery is needed to remove nonresorbable membranes, resulting in patient discomfort and higher economic burden.[13,14] Synthetic and naturally derived resorbable membranes have been developed to avoid the need for a second surgical intervention and to reduce the risks of membrane exposure. However, the variability in resorption rate and poor mechanical properties of the resorbable membranes are significant limiting factors.[15] Therefore, to improve the clinical outcomes of the GBR approach, a new generation of active barrier membranes with enhanced regenerative properties has been proposed. In this context, a growing body of preclinical studies (described in Section 4) focuses on membrane's structural modifications and direct or indirect loading of active compounds (i.e., growth factors, cytokines, inorganic compounds, and anti-inflammatory agents). Moreover, recent work highlights how barrier membranes can be strategically combined to therapeutic approaches targeting bone healing disorders (i.e., osteoporosis) to meet patient-specific needs. Altogether, the preclinical studies herein reported aim to depict new GBR strategies employing classic and new generation GBR membranes and their impact on bone tissue engineering approaches.

The successful regeneration of bone defects in the alveolar ridge is a timely regulated process. It has been proposed that an ideal GBR membrane should preserve its barrier function for

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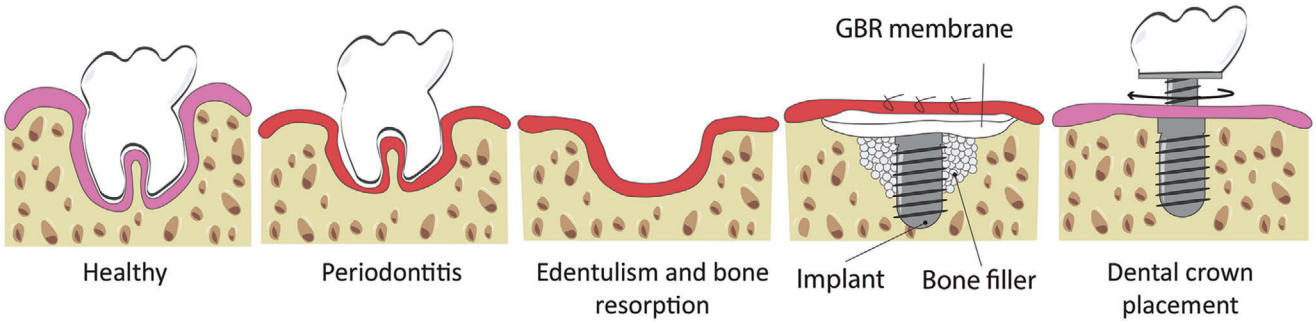

Figure 1. Schematic of GBR application through the use of bone filler and a barrier membrane.

16–24 weeks.[16] The regenerative timeline dictates the temporal window of action of the hypothetical medical devices (MDs) destined for GBR applications and determines material composition, manufacture process, and regulatory pathway. For example, the European classification of MDs takes into account not only potential hazards and risks of failure, but also the length of time the MDs will stay in contact with the body (hence, its degree of invasiveness) and whether the effect is local or systemic.[17] Although the process by which medium- to high-risk MDs find their way from bench to bedside is well-defined in most countries, the differences among them are not always well edited. Manufacturers are subjected to different regulations depending on the country, and these rules are evolving over the years. Although one must comply with a set of directives in continuous development, these are not the only aspects to be considered as limiting factors on the road for the commercialization. Indeed, an often-overlooked aspect is the industrialization of the manufacturing process. The repeatability and reliability of the production process need to be confirmed before transferring the design to the manufacturing process. The production process relies on multiple factors; quantity to be produced, surface finishing, post machining and cleaning process, and sterilization process. Finally, the packaging, instruction for use, and labeling printing requirements need to be carefully planned in order to produce the final product.[18]

The aim of this review is to present the latest advancement in GBR membranes and to describe the process leading to the industrial development of materials for such biomedical applications. Therefore, the focus is initially set on the description of the medical requirements to design functional GBR membranes. Then, commercially available membranes and those currently under preclinical evaluation, are described from both a medical and a regulatory point of view. Regulatory requirements to develop these MDs are listed for USA, Europe, Japan, China, and India. Finally, a discussion on MD production scale-up and industrialization that brings MDs from bench to bedside, is also presented. Understanding the commercialization process of medium-/high-risk MDs (according to the European MD classification; the details of the regulatory requirements in different countries are reported in Section 6) such as a membrane for GBR applications, would aid scientists to integrate this information into the product development process from an early stage. A strategic approach is key for an effective medical translation of MDs, which is fundamental to increase the public health impact of biomedical research.

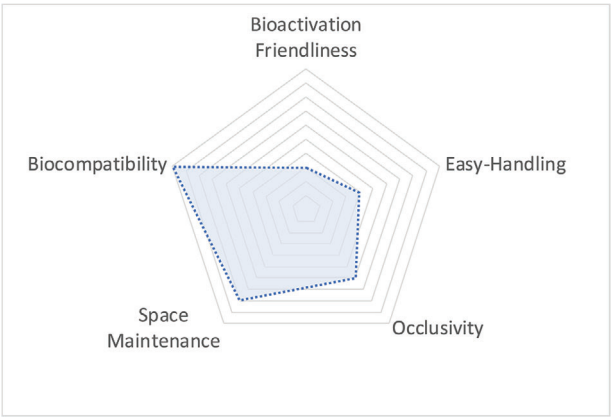

Figure 2. Schematic of the main criteria for the design of a barrier membrane for GBR applications.[19]

2. Medical Requirements for the Design of GBR Membranes

Diseases, trauma, and congenital defects can lead to tissue damage or loss, hence the need to replace missing form and function. GBR is a fundamental procedure in dental implantology and periodontology where barrier membranes have a crucial role in isolating soft tissue to favor bone tissue growth. Although the membranes employed for GBR greatly differ in their origin and structure, they should fulfill a number of criteria. Indeed, a meta-analysis on recently published works employing GBR membranes identified five main criteria and their order of importance (**Figure 2**).[19] A successful design of a functional product to deploy as GBR membrane should take into account the following characteristics: 1) biocompatibility, 2) space maintaining, 3) occlusive function, 4) easy handling, and 5) bioactivation friendly property.[1,19,20]

1) GBR membrane is defined as biocompatible when the interaction with the host does not impair the surrounding tissue, the healing process, or the safety of the patient. In particular, if the membrane is resorbable, it should either degrade or integrate with the host tissue.[16,21]
2) The space maintenance ability of a GBR membrane, which correlates to the mechanical stability of the product, is required to avoid collapse within the defect during the healing

Table 1. Membranes used in the clinical practice organized by material composition.

Family	Material	Commercial name	Characteristics	
Polytetrafluoroethylene (PTFE)	- Expanded PTFE - Dense PTFE - Dual textured expanded PTFE - Titanium-reinforced PTFE	- Gore-Tex - Cytoplast TXT-200 - NeoGen - Gore-Tex-Ti; Cytoplast Ti-250; NeoGen Ti-reinforced	Synthetic	Nonresorbable
Titanium	Titanium	Frios BoneShields; Ridge-Form Mesh	Metallic	
Collagen	- Type I collagen - Atelocollagen from type I collagen - Type I and III collagen - Type I, III, IV, VI collagen and other protein - Not specified type of collagen - Collagen and elastin - Cross-linked type I collagen - Cross-linked type I and type III collagen - Porcine pericardium	- CollaTape; Tutodent; Cova MAX; Parasorb Resodont - Koken Tissue Guide; Terudermis; - BioGide; Botiss Jason - DynaMatrix - Heal-All Biomembrane - Creos xenoprotect - BioMend; OSSIX PLUS; OsseoGuard - OsseoGuard Flex; EZ Cure; MatrixDerm EXT - Vitala Porcine Pericardium Collagen Membrane	Natural	Resorbable
Polyesters	- Poly-D,L-lactide-*co*-glycolide - D,D-L,L-polylactic acid - Poly-D,L-lactide and poly-L-lactide, blended with acetyl tri-*n*-butyl citrate - Polyglycolide, poly-D,L-lactide-*co*-glycosides, poly-L-lactide	- Gore Resolut adapt; GC Membrane - Epi-Guide; Atrisorb - Guidor - BioMesh-S; Tisseos; Vicryl; GORE RESOLUT ADAPT Regenerative Membrane	Synthetic	

process and must be able to protect the defect space for new bone formation.[22]

3) The barrier membrane needs to prevent the invasion of cells from the mucosa into the defect space, without compromising the oxygen and nutrient exchange (i.e., occlusivity).[23] Therefore, occlusivity is strongly linked to porosity, as such larger pore size will allow cells from the surrounding connective tissue to migrate and proliferate into the defect area inhibiting the activity of bone forming cells.[24] The overall dimension of the pores could influence cell adhesion. Small pores could limit cell migration and enhance collagen deposition, reducing the ability of blood vessels to infiltrate the area of interest.[25]
4) A GBR membrane would need to be easily handled during surgery (i.e., easy handling), without being excessively rigid, which could compromise tissue integration or lead to dehiscence of the soft tissues.[2]
5) Although the role of the membrane was initially intended as a passive barrier, this concept could be reconsidered in the context of the next-generation membranes. Indeed, a growing number of studies are developing new strategies for bone regeneration incorporating bioactive compounds into the membrane, giving it an active role in the regeneration process (i.e., bioactivation friendly property).[26,27]

3. Commercially Available Membranes

The history of membranes for GBR is linked to the development of membranes for guided tissue regeneration (GTR), which started in the late 1960s. One of the first attempts in the GTR field was reported by Cohen and Funakoshi in 1971 using a Millipore membrane.[28] This material was used preclinically[29] and clinically[30] in 1982 by Nyman et al. to evaluate bone regeneration mediated by periodontal ligament cells. Two years later, Gottlow et al.[31] confirmed the benefits of guided tissue regeneration in animals using a Millipore filter and ePTFE (Gore-Tex – W.L. Gore and ASSOC, Flagstaff, AZ, USA) membrane. In 1986, Gottlow et al. performed a clinical study with 10 patients implanted with PTFE (Teflon).[32] The first membranes proposed for GBR were conceived to act solely as occlusive membranes. They were placed between the patient's bone or the bone filler and the gum to provide a mechanical barrier to avoid soft tissue growth into the osseous defect. Since then, four families of materials are mainly used for guided regeneration in the clinical practice. 1) PTFE derivatives, including ePTFE and high-density PTFE, used alone or reinforced with titanium;[33] 2) titanium membranes; 3) collagen resorbable natural membranes; 4) synthetic resorbable membranes made of polyesters. **Table 1** outlines, according to their composition, many of the membranes already used clinically. In this section, the main characteristics of those membranes will be presented following the chronological order of development.

The choice of the material is determined by the characteristics of the bone defect. Commercial membranes for GBR are intended for use during the process of GBR and guided tissue regeneration and the indications for use may be numerous, such as bone augmentation around implants placed in immediate or/and delayed extraction sockets, localized ridge augmentation for later implantation, alveolar ridge reconstruction for prosthetic treatment, alveolar ridge preservation consequent to tooth extraction, filling of bone defects after root resection, cystectomy, removal or retained teeth, etc. Critical-size defects require long structural integrity of the membrane for the entire healing period.

As such, both resorbable[34] and nonresorbable[35] membranes have been employed for vertical and/or horizontal critical-size defects. Particularly in the case of the nonresorbable membranes, ePTFE porosity is believed to enhance regeneration by improving wound stability. However, the stiffness of this material is often responsible of soft tissue dehiscence, causing membrane exposure and early bacterial infection.[36] In case of soft tissue dehiscence, bacteria from the oral cavity can penetrate into the defect site, impairing the regenerative outcomes. For example, Lang et al.[37] reported a study in which 6 patients out of 19 had infections that led to the removal of the implant after only 3–5 months. Consequently, bone regeneration observed in these patients was variable, between 0% and 60%.[37] By contrast, the low porosity of high-density PTFE is known to prevent cell and bacteria adhesion and therefore is generally associated with a reduced risk of infection.[11] However, a clinical trial conducted by Ronda et al.[38] did not show any difference between ePTFE and high-density PTFE membranes. Incorporating flexible titanium in PTFE membranes allows the surgeon to shape the membrane to adapt it to the implant area. When compared to PTFE alone, titanium-reinforced PTFE could provide superior stability of the material for some types of bone defects, such as in supracrestal bone defects and in sites with buccal dehiscence.[25,39] It has also been proposed as an alternative to PTFE in cases involving advanced bone loss, due to increased provision of space.[40]

The main drawback of the nonresorbable membranes is the need of a secondary removal surgery. Apart from the cost and inconvenience for the patient, extreme care should be used during the surgery to prevent damaging the underlying new granular tissue. Concerning the clinical outcome using different PTFE, some clinical trials have demonstrated similar results for several indications, such as vertical ridge augmentation around dental implants.[38]

Collagen is the only material from animal origin used as main component of GBR membranes (Table 1).[15] Among the different collagens, type I is the most prevalent (>90%) and the best described and used, followed by type III collagen. Less common is the use of collagen type IV and VI. Collagen used for clinical membranes is frequently of bovine or porcine origin, and in only few cases from equine origin. BioMend Membrane (Zimmer Biomet Dental, Palm Beach Gardens, FL, USA) was released in 1995 in the United States, it was the first collagen-based membrane produced for guided tissue and bone regeneration application.[41] Natural collagen is obtained by decellularization process followed by "cleaning" steps to remove antigenic components. This helps to preserve the native structure of collagen leading to membranes easy to handle and with capacity to adapt to the application area. An advantage of collagen versus nonresorbable materials is the biodegradation of the implant through local collagenases and proteases. Degradation time of these membranes is generally considered as short, but this depends on the tissue origin and manufacture process. For example, Collatape (Integra LifeSciences Corp., Plainsboro, NJ, USA) made of bovine collagen presents a barrier effect of 1–2 weeks, whereas Botiss Jason (Botiss Biomaterials GmbH, Zossen, Germany) made of porcine pericardium and Copios Extend (Zimmer Biomet Dental, Palm Beach Gardens, FL, USA) made of porcine dermis present a barrier effect of 8–12 and 24–36 weeks, respectively.[15] Degradation time is important to provide a barrier that lasts during the whole healing process and this is why strategies to retard collagen degradation after implantation are proposed, such as cross-linking or larger and thicker membranes.[42] Different ways to cross-link collagen are based on physical (UV irradiation),[43] chemical (glutaraldehyde,[44] hexamethylene diisocyanate,[45] diphenylphosphorylazide[46]), and enzymatic (ribose)[47] interactions. Increasing cross-linking ratio causes longer degradation times but clinical trials and meta-analysis have demonstrated similar efficacy in terms of tissue regeneration compared to non-cross-linked membranes. On the contrary, natural membranes showed better tissue integration, less postoperative complications, and better biocompatibility.[42]

Synthetic resorbable membranes appear as an alternative to collagen membranes and synthetic nonresorbable membranes.[48] Their advantage lies in the ability to adjust the chemistry and the preparation method to control physicochemical properties of the membranes, i.e., size, shape, porosity, mechanical properties, degradability.[49] Almost all the commercially available membranes of this type are composed of aliphatic polyesters polylactic acid (PLA), polyglycolide acid (PGA), polycaprolactone (PCL), and their copolymers, widely used in sutures. PLA can be used alone or copolymerized with PGA, for example. The rational beyond this is that polyester degradation is mainly due to hydrolysis and therefore dependent on the hydrophobicity of the polymer.[50,51] PLA being more hydrophobic than PGA, degradation time is much longer. Indeed, in vivo degradation of PLA lasts for more than 4 years but copolymerization with PGA or PCL reduces resorption time to less than 1 year, which is better for GBR.[52] As an example, Resolute Adapt (W.L. Gore and ASSOC, Flagstaff, AZ, USA) membrane is made of polylactic-*co*-glycolic acid (PLGA) and resorption time is between 5 and 6 months. One should not confound resorption time and persistence of barrier effect, that is always at least 2 times shorter.[48] Most membranes provide good barrier effect during at least 6 weeks and up to 24 weeks, like in the case of PLGA membrane Resolut Adapt LT (W.L. Gore and ASSOC, Flagstaff, AZ, USA), whose degradation time is 3 times longer than the barrier effect. Other material properties with an impact on hydrolysis are molecular weight, crystallinity, and material processing. This is why two membranes made of PLGA, in the case of Resolut Adapt and Resolut Adapt LT (W.L. Gore and ASSOC, Flagstaff, AZ, USA), present, respectively, 8–10 and 16–34 weeks barrier effect.

Processing of the material is a parameter to control tissue regeneration and many strategies are proposed by manufacturers.[52] Combination of several layers with different pore sizes to prepare multiphasic scaffolds is frequently proposed: less porous side acts as a barrier, preventing epithelial cell infiltration, and the other side in contact with bone defect allows tissue integration.[53] This approach is used in Guidor Matrix Barrier and Resolut (Sunstar Americas, Inc., Schaumburg, IL, USA) and Resolut (W.L. Gore and ASSOC, Flagstaff, AZ, USA). Membranes can also be constituted by woven fibers,[54] like in the case of Vicryl Periodontal Mesh (Ethicon, Inc., Somerville, NJ, USA), or by a polymer solution to be dissolved during the surgical procedure and molded in a cassette to form the Atrisorb membrane (Atrix Laboratories, Inc., Fort Collins, CO, USA).[55] The small number of clinical studies carried out with this membranes makes it difficult to conclude about their advantages compared to other membranes.

The large number of clinically available resorbable synthetic membranes offers surgeons a wide range of physicochemical properties that can impact tissue regeneration.[49] Even if clinical trials have been conducted to compare the outcomes of some of the materials,[56,57] results do not allow to predict which membrane would be the best option for each patient. Clinical trials often differ in important aspects such as, the selection criteria of the patients, the type of defect to be treated, the way of executing the treatment, the duration, and the evaluation criteria. The clinical outcomes would depend not only on the type of membrane but also on the surgeon's experience and expertise, the patient's clinical history and life habits, such as smoking and oral hygiene. Therefore, any indirect comparison of clinical trial outcomes should be established through rigorous metadata analysis.[58]

To conclude for the choice of materials, when comparing collagen membranes to nonresorbable membranes, apart from the unique surgery previously mentioned, it has been extensively shown that collagen membranes actively participate in the regeneration process: they attract cells that secrete factors involved in bone formation and remodeling and they retain growth factors. This is also true when comparing to resorbable synthetic membranes. Numerous studies demonstrate low immunogenicity of natural resorbable membranes, consequently, more than three quarters of the membranes used in the dental field are collagen membranes.[59] There is a large number of marketed collagen membranes with different physicochemical properties depending on the cross-linking, but also on the collagen origin and the manufacturing process. These parameters have to be examined to choose the best membrane for each indication. Current preclinical studies aim to improve bioactivity and biocompatibility of membranes following strategies where materials are combined with bioactive molecules to overcome these limitations. Advancements in this field are described in the next section.

4. GBR Strategies and New Membranes under Preclinical Evaluation

4.1. In Vivo Evaluation of GBR Membranes in Combination with Biological Cues, Natural Elements, and Synthetic Active Materials

Many strategies have been developed to enhance the efficacy of GBR membranes, which involve structural modifications and/or the incorporation of biological cues to elicit bone regeneration. In particular, this section describes how the regenerative potential of the GBR approach can be improved by either indirect (i.e., at the defect site) or direct (i.e., functionalized membrane) loading of active compounds. Examples of in vivo investigations employing such approaches are reported in **Table 2**.

4.1.1. Conditioned Media

Transplanted stem cells are promoters of tissue regeneration for their ability to release cytokines and growth factors. However their use for tissue regeneration is heavily regulated, and has several limitations associated to high costs and strict requirements for safety and quality management.[60,61] Therapies using mesenchymal stem cells (MSCs), known as mesenchymal stromal or multipotent stromal cells, are a heterogeneous class of cellular treatments. Indeed, the immunomodulatory and/or immunosuppressive properties associated to stromal cell populations are not yet fully understood, as well as how the interactions between cellular niches drive cell fate.[62–66] To overcome the limitations associated with the direct use of these cell populations, it has been proposed the use of the conditioned medium from bone-marrow-derived MSCs to enhance bone and periodontal tissue regeneration.[67–69] In particular, Katagiri et al.[70] reported a first-in-human study on the use of conditioned media from human MSCs for bone augmentation prior to dental implant placement. In this work, MSC-conditioned media was loaded into a scaffold made of beta-tricalcium phosphate (β-TCP, Osferion, Olympus Terumo Biomaterials, Tokyo, Japan) and covered with a PLGA membrane (GC Membrane, GC, Tokyo, Japan). After six months, some remnants of β-TCP and newly formed bone were found covering the lateral window almost completely. Moreover, radiographic analysis showed early bone formation in all the eight patients, five of which were GBR cases. Despite this study showing the safety of the conditioned media application, the efficacy of this approach would need to be evaluated in a separate clinical trial. Although the application of the conditioned media was directed to the scaffold placed into the bone defect, such approach could be also directly applied to the barrier membrane for the development of third generation GBR membranes, opening new avenues for bone regeneration strategies.

4.1.2. Cytokines and Growth Factors

Cytokine and growth factor delivery for bone healing has been tested not only when incorporated into membranes, but also when administered locally to the defect by injection or in combination with biocompatible carriers (i.e., bone fillers). In the vast majority of the studies, the combined effect of growth factors and bone filler increases to a greater extent in presence of a barrier membrane that protects the defect area from soft tissue ingrowth. A growing body of literature shows that bone regeneration could be enhanced by exposing the treated area to a number of different growth factors, such as platelet-derived growth factor (PDGF),[71] platelet-rich growth factor (PRGF),[72] transforming growth factor beta-1 (TGF-β1),[73] and fibroblast growth factor-2 (FGF-2).[74] The regeneration of bone defects depends on the recruitment of progenitor cells from bone marrow or present at the defect site, which subsequently differentiate to form mature bone tissue.[75] The osteogenic growth peptide (OGP) and its C-terminal pentapeptide OGP(10–14) stimulate the differentiation, proliferation, alkaline phosphatase activity, and matrix mineralization of osteoblastic cells.[76] A biopolymer-based membrane made of bacterial cellulose and collagen functionalized with OGP(10–14) showed high osteoinductive properties in a rat femoral defect.[77] In the context of active compounds delivery, the chemical link between the active compound and the biomaterial used for GBR membranes is of fundamental importance for in situ tissue regeneration. Indeed, the correlation between

Table 2. Preclinical studies exploring the effect of GBR membranes on bone healing. Abbreviations: rhBMP-2, recombinant human bone morphogenetic protein 2; rhTGF-β1, recombinant human transforming growth factor beta 1; FGF-2, fibroblast growth factor; DBBM, deproteinized bovine bone mineral; Zn, zinc; Ti, titanium; Sr, strontium; CM, collagen membrane; HA, hydroxyapatite; BCP, biphasic calcium phosphate; rhBMP-9, recombinant human bone morphogenetic protein9; β-TCP, beta tricalcium phosphate; PRGF, platelet-rich growth factor; SDF-1α, stromal cell-derived factor-1 alpha; L-PRF, Leukocyte- and platelet-rich fibrin; PGS, polyglycerol sebacate; cmRNA, chemically modified RNA; $scCO_2$, supercritical CO_2; mSIS, multilaminate small intestinal submucosa; hAM, human amniotic membrane; PBSGL, copolyester—poly (butylene succinate-*co*-glycolate); PLA95, poly-5D/95L-lactide; EGCG, epigallocatechin-3-gallate; SIS, small intestinal submucosa; OGP, osteogenic growth peptide.

Characteristics	Animal models	Experimental groups	Main findings	Ref.
Loading of growth factors into the defect	Canine Alveolar ridge augmentation	- Collagen sponge soaked in rhBMP-2, covered with CM - Collagen sponge soaked in DBBM, covered with CM - Collagen sponge alone, covered with CM	Collagen sponge soaked in rhBMP-2 provided the greatest bone fill among the three treatment procedures.	[81] Similarly: using titanium mesh or ePTFE[82,83]
	Canine Alveolar ridge augmentation	- PRGF adsorbed in β-TCP and covered with a CM - PRGF adsorbed in β-TCP	The presence of the CM did not affect bone regeneration nor implant osseointegration.	[72]
	Canine Dehiscence defects	- Sham - ePTFE membrane (MEM) - Biphasic calcium phosphate (BCP) - Cyanoacrylate-combined calcium phosphate (CCP) - BCP + MEM - CCP + MEM	All the MEM groups showed more bone formation. BCP + MEM and CCP + MEM showed greater bone formation within the defect and on top of the implant; the bone regeneration heights averaged 3.96 ± 2.86 and 5.45 ± 0.25 mm for the BCP + MEM, CCP + MEM, respectively.	[203]
	Canine Alveolar ridge augmentation	- Deproteinized bovine bone block in combination with a collagen barrier membrane - Deproteinized bovine bone block infused with recombinant human platelet-derived growth factor (rhPDGF-BB) - Deproteinized bovine bone block infused with rhPDGF-BB, plus a collagen resorbable barrier membrane	rhPDGF-BB combined with deproteinized bovine block without barrier membrane showed to regenerate significant amounts of new bone in severe mandibular ridge defects.	[71]
	Canine Alveolar ridge augmentation	- Calcium carbonate and hydroxyethyl starch loaded with 2.5 µg mL^{-1} rhTGF-β1 covered or not with ePTFE membrane - Calcium carbonate and hydroxyethyl starch loaded with 25 µg mL^{-1} rhTGF-β1 covered or not with ePTFE membrane	rhTGF-β1 + barrier membrane greatly enhanced bone regeneration in osseous oral defects	[73]
	Canine Alveolar ridge augmentation	- Collagen minipellets containing FGF-2 covered with ePTFE membrane - Collagen minipellets covered with ePTFE membrane	Controlled application of FGF-2 accelerates bone regeneration in membrane-protected bone defects	[74]
Loading of growth factors or cytokines into the membrane	Rat Calvarial defects	- Bio-Oss + CM with SDF-1α physically adsorbed - Bio-Oss + CM with SDF-1α chemically cross-linked - Bio-Oss + bone marrow stem cells (BMSCs) - Bio-Oss + CM	Bio-Oss + CM chemically conjugated with SDF-1α promoted new ectopic bone and microvessels formation compared to SDF-1α physically adsorbed and showed similar effects on new bone formation compared to Bio-Oss + BMSC group.	[78] Similar:[80]
	Rat Femural defect	- Bacterial cellulose - Bacterial cellulose + OGP - Bacterial cellulose + collagen - Bacterial cellulose + collagen + OGP - Empty defect	All the groups promoted higher levels of bone regeneration than the control group. The bacterial cellulose + collagen + OGP group showed more bone tissue in the repaired area at 2 and 4 weeks than other membranes.	[77]
	Rat Calvarial defect	- Perforated CM (Bio-Gide) + pDNA for BMP-9 - Perforated CM + cmRNA for BMP-9 - Empty defect	The CM could be used to deliver functional pDNA and cmRNA in vitro and in vivo. After 4 weeks in vivo, CM + pDNA and CM + cmRNA resulted in higher bone volume to tissue volume ratio when compared to empty defect.	[87]

(*Continued*)

Table 2. Continued.

Characteristics	Animal models	Experimental groups	Main findings	Ref.
	Rabbit Calvarial defect	- CM (Bio-Gide) - DBBM (Bio-Oss) - CM loaded with rhBMP-9 - DBBM loaded with rhBMP-9 - Empty defect	CM + rhBMP-9 induced a greater volume of native host bone and a complete horizontal bone defect closure in absence of multinucleated giant cells.	[86]
Loading of inorganic compounds into the membrane	Canine Dehiscence defect	- CM (GENOSS) with DBBM (Bio-Oss) - PCL/PLGA/β-TCP membrane with DBBM (Bio-Oss)	In vitro and in vivo studies demonstrated that PCL/PLGA/β-TCP membranes have similar levels of biocompatibility and bone regeneration as CM. The 3D printed membrane showed higher tensile strength both in wet and dry states, the tensile property of collagen was reduced by 99% under wet conditions.	[98]
	Rabbit Calvarial defect	- Chitosan–magnesium membrane - CM (Heal All) - Empty control	No significant differences between the chitosan–magnesium membrane and Heal-All groups were observed.	[111]
	Rat Calvarial defect	- Gelatin membrane coated with Zn–HA powder - Koken Tissue Guide - Empty control	The Zn–HA gelatin membrane group yielded significantly greater bone formation compared to the collagen membrane and the unfilled control group.	[110]
	Rabbit Calvarial defect	- BCP + strontium (Sr) HA-containing collagen membrane - Bio-Oss + Sr - Bio-Oss + Bio-Guide	Mineralized new bone significantly increased in Sr/BCP from 12 to 24 weeks, with less residual grafting material	[109]
Membrane loaded with anti-inflammatory	Rat Calvarial defect	- EGCG–Collagen membrane- CM	The highest bone-healing efficacy was observed in EGCG–Collagen membrane group after 8 weeks of implantation. EGCG–Collagen membrane in vivo induced the recruitment of M2 macrophages, promoting secretion of growth factors (VEGF and BMP-2) and the expression of osteogenic markers (RUNX-2 and OPN).	[127]
Membranes structurally modified	Dog Alveolar ridge augmentation	- HA bone graft covered with microporous Ti mesh (50 μm pore diameter) - HA bone graft covered with macroporous Ti mesh (1700 μm pore diameter)	The microporous Ti mesh overall showed a higher degree of new bone volume in the defect site.	[153]
	Rat Calvarial critical-size defect	- Zn membrane without pores - Zn membrane with 300 μm pores - Zn membrane with 1000 μm pores - Ti membrane without pores as control	Zn membrane with 300 μm pores showed to drive better osteogenesis.	[150]
	RabbitTibia defect	- PGS membrane with 25 μm pores - PGS membrane with 53 μm pores - CM (Bio-Gide) - Empty defect	After 4 weeks in vivo, the PGS membrane with 25 μm pores was showing the highest bone volume to tissue volume ratio, and after 12 weeks, it was almost completely covered by new bone.	[152]
	RabbitCalvarial defect	- Electrospun PBSGL of increasing glycolate ratio: 0, 10, 20, 40	PBSGL membrane with higher glycolate ratio support more new bone formation, with no adverse inflammatory response.	[149]
	Pig Alveolar ridge augmentation	- Electrospun PLA95/β-TCP - PLA membrane (Epi-Guide) - Empty defect	Increased cementum and bone height were observed between empty control and the ES PLA95/β-TCP membrane.	[204]
	RatCalvarial defect	- CM - Mineralized-CM-48 h - Empty defect	Mineralized-CM with controllable surface stiffness promoted the adhesion, proliferation, and osteogenic differentiation of mesenchymal stem cells. In vivo, the defect was almost completely covered by the new bone tissue in the Mineralized-CM-48 group.	[148]

(*Continued*)

Table 2. Continued.

Characteristics	Animal models	Experimental groups	Main findings	Ref.
	RabbitCalvarial defect	3D printed resorbable PCL membranes with: - 130 µm pore size + Bone grafting material (Bio-C) - 300 µm pore size + Bone grafting material (Bio-C) - 700 µm pore size + Bone grafting material (Bio-C) - Bone grafting material (Bio-C)	The 130 µm pore size group showed a significantly high level of new bone formation.	[151]
	RatCalvarial defect	- Bilayered PLGA membrane - Empty defect	The solid layer inhibited connective tissue invasion, while the inner layer promoted proliferation, osteogenic differentiation, and bone regeneration in vivo.	[154]
Naturally derived membranes	RabbitCalvarial defect	- Gore-Tex - Autologous pedicle periosteum layer	After 12 weeks, defects covered either by the periosteum or by the membrane were almost completely closed. Pedicle periosteum enhances regeneration by acting as a barrier and source of osteogenic components.	[139]
	Dog Alveolar ridge augmentation	- Nonresorbable membrane with DBBM - Nonresorbable membrane with β-TCP - Resorbable membrane with DBBM - Resorbable membrane with β-TCP - L-PRF with DBBM - L-PRF with β-TCP	The L-PRF with DBBM showed a higher amount of new bone formation. L-PRF demonstrated to be more efficient than resorbable and nonresorbable membranes.	[140]
	RabbitMandibular defect	- DBBM (Bio-Oss) - DBBM + mSIS - DBBM + CM (Bio-Gide) - Empty defect	mSIS showed longer degradation time (3 months). The overall volume and maturation of new bone production were similar to the commercial CM.	[141]
	MouseCalvarial defect	First in vivo study: - hAM fresh - hAM cryopreserved - CM (Bio-Gide) Second in vivo: - Defect filled with HA - Defect filled with HA + BMP-2 - Defect filled with HA and covered with cryo-hAM - Defect filled with HA + BMP-2 and covered with cryo-hAM - Defect filled with HA and covered with CM - Defect filled with HA + BMP-2 and covered CM	Cryo-hAM was linked to more bone formation when the mesenchymal side of the tissue was covering the defect site, however when compared to CM in vivo, hAM showed limited bone regeneration properties in presence of bone substitute.	[142]
Effect of sterilization on membrane	Dog Class III furcation defect	- CM (Vitala) - CM + $scCO_2$ - Empty defect	The supplementary $scCO_2$ treatment on the CM did not impact the biocompatibility, allowing for the infiltration of cells and degradation over time. In vivo, the CM + $scCO_2$ presented similar performance in GTR toCM.	[144]
Membranes in osteoporotic models	Diabetic rats Calvarial subcutaneous	Hyaluronic acid adsorbed CM from porcine pericardium	Induced diabetes significantly reduced the thickness of the CM, while hyaluronic acid delays membrane degradation in uncontrolled diabetic compared with normoglycemic rats.	[164]
	Osteoporotic ratsCalvarial defects	- Heparinized mineralized SIS loaded with BMP2-related peptide P28 (mSIS/P28) - mSIS- SIS	mSIS-heparin-P28 greatly enhanced osteoporotic bone regeneration	[162]

(*Continued*)

Table 2. Continued.

Characteristics	Animal models	Experimental groups	Main findings	Ref.
	Osteoporotic rats Calvarial critical-size defects	Defect treated with dPTFE membrane. Osteoporosis was induced by ovariectomy (OVX) and calcium-deficient diet: - healthy control - sham operated - OVX rats treated with a single dose of zolendronic acid - OVX rats with no treatment	d-PTFE membranes favored bone regeneration in osteoporotic and healthy rats.	[161]
	Rat Alveolar ridge augmentation	A Ti microimplant covered with a Ti-reinforced ePTFE membrane was implanted in three experimental groups: - streptozotocin-induced diabetes - insulin-controlled diabetes- healthy	Significant de novo bone formation can be achieved via GBR treatment in presence of uncontrolled diabetes. Insulin-mediated metabolic control may reverse these adverse effects.	[165]
	Diabetic miceCalvarial defect	- FGF-2-loaded polyglycolate:polylactide membranes - Membrane alone	Membranes containing FGF-2 enhanced bone formation in diabetic animals to near normal levels.	[166]

active compounds' release dynamics and their paracrine activity needs to be evaluated accordingly to the biological activity intended for the defect model in use. In this context, a collagen membrane chemically conjugated to stromal-cell-derived factor-1 alpha (SDF-1α) and combined with deproteinized bovine bone mineral (DBBM, Bio-Oss, Geistlich, Wolhusen, Switzerland) was able to significantly increase new bone production and microvessel sprouting than a collagen membrane physically adsorbed with SDF-1α.[78] SDF-1α is a strong chemoattractant often used for regenerative medicine applications.[79] In another study, a sixfold increase in newly formed bone volume was observed when SDF-1α was loaded onto a PCL/gelatin electrospun membrane and implanted with a titanium scaffold into a 5 mm skull defect in nude rats.[80] A group of growth factors that is widely used for GBR applications is represented by the bone morphogenetic proteins (BMPs). The local administration of bone morphogenetic protein-2 (BMP-2) was able to enhance the osseointegration of dental implants.[81] In a canine model of saddle-type ridge defect, a collagen sponge previously soaked with BMP-2 was placed into the damaged area and covered with a collagen membrane, this surgical procedure provided a higher degree of bone regeneration when compared to the same collagen sponge loaded with DBBM or without supplements. After 12 weeks from the surgery, the bone fill percentage ranged from 79% to 92.5% in defects treated with BMP-2 from different manufacturers, Medtronic and Osstem, respectively. The analysis of the osseointegration of functionally loaded implants indicated a higher bone-to-implant contact and bone density in BMP-2-treated groups without differences among manufacturers.[81]

In other studies, the use of BMP-2 combined with barrier membranes made of titanium mesh[82] or ePTFE[83] demonstrated to be beneficial for the underlying bone healing. Fujioka-Kobayashi et al.[84,85] reported the effect of BMP-9 on bone-marrow-derived MSCs loaded into a DBBM scaffold (Bio-Oss, Geistlich, Wolhusen, Switzerland) or into a collagen membrane (Bio-Gide, Geistlich, Wolhusen, Switzerland). In both cases, BMP-9 positively influenced the expression of osteoblastic genes, alkaline phosphatase (ALP) activity, and calcium deposits.[84,85] In a model of rabbit calvarial defect, the direct delivery of BMP-9 into the DBBM scaffold or into the collagen membrane increased the regeneration of new bone and mineralization. However, only by loading BMP-9 into the collagen membrane caused a complete horizontal defect closure, therefore giving a more predictable bone induction outcome.[86] In an alternative delivery approach, nanoplexes of polyethyleneimine loaded with either plasmid DNA (pDNA) or chemically modified RNA (cmRNA) encoding for BMP-9 were loaded into perforated collagen membranes to heal rat's calvarial bone defects, both cases demonstrated to induce high bone volume to tissue volume ratio.[87] Although the positive outcomes of these studies, it is important to note that BMP class of growth factors are commonly applied in supraphysiological doses with the consequent high costs and associated side effects.[88–90] Thus, there is increasing interest in cytokine- and growth-factor-free and cell-free biomaterial systems that could support the endogenous healing capacity through the recruitment of host endogenous stem or progenitor cells to the injury site.[91–93]

4.1.3. Inorganic Compounds

Synthetic calcium phosphates, including hydroxyapatite (HA), β-TCP, and their combination into biphasic calcium phosphates (BCPs), are largely used for orthopedic and dental applications because of their osteoconductive and osteoinductive properties. Such class of synthetic ceramics, like other bioactive bone graft materials (bioglass, bone-derived or coral-derived HA) allow vascular ingress, cellular infiltration and attachment, cartilage formation, and calcified tissue deposition.[94–97] The effect on bone repair of a 3D printed membrane composed of β-TCP, PLGA, and PCL was compared to a collagen membrane (GENOSS, Suwon, South Korea) in a canine implant model.[98] Eight weeks after the surgery, the histologic and histomorphometric analysis showed that the PCL/PLGA/β-TCP group did not differ significantly from the collagen group. However, the new bone area and bone-to-implant contact of the PCL/PLGA/β-TCP group were

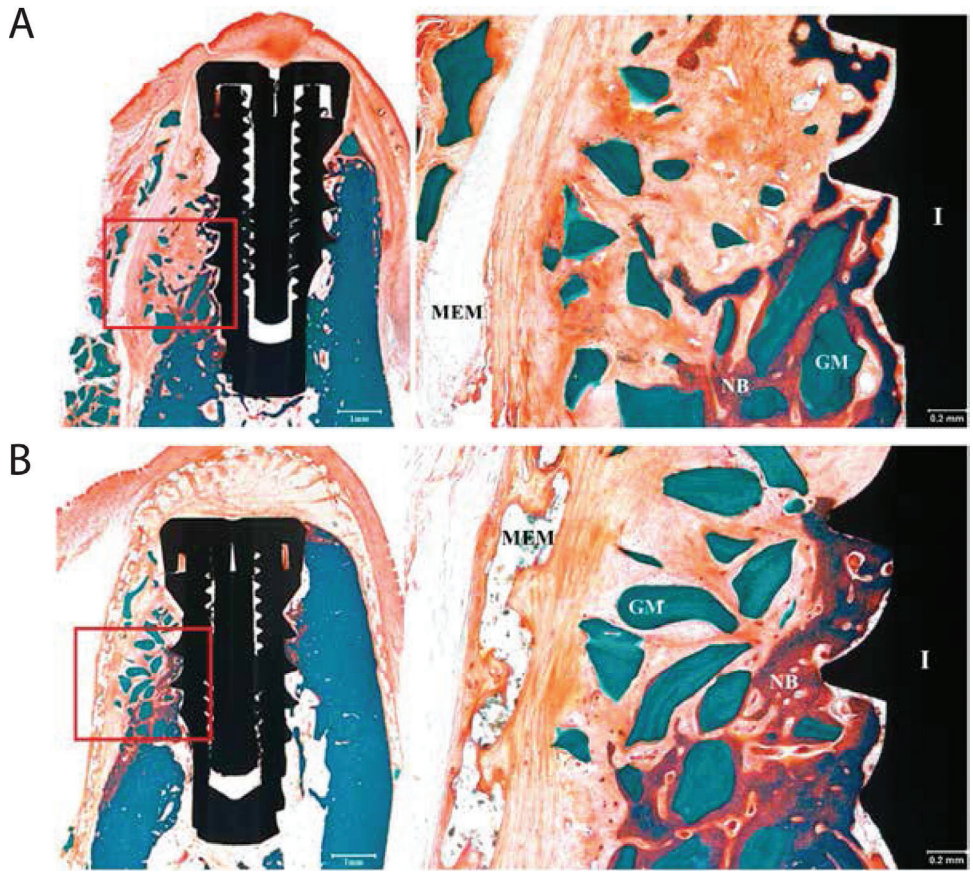

Figure 3. Histological analysis of implant osseointegration. Red box: selection of the region of interest from the implant shoulder. NB, new bone; GM, grafting material; MEM, membrane; I, implant (Goldner Trichrome stain; magnification ×12.5 and ×40. Analysis of A) the collagen group and B) PCL/PLGA/β-TCP group. Reproduced under the terms of the CC-BY 3.0 license.[98] Copyright 2016, the Authors. Published by IOP Publishing.

significantly higher than those of the collagen group (**Figure 3**).[98] In some GBR membranes, synthetic ceramics have been combined with other inorganic compounds, such as strontium (Sr), zinc (Zn), or magnesium (Mg).[99] Sr is involved in bone mineral metabolism by inducing the expression of osteogenic-related genes, differentiation markers, proliferation, and reducing apoptosis levels.[100–102] Pasqualetti et al.[103] have demonstrated that Sr also affects bone mineralization during skeletal development in zebrafish embryos. On the other hand, Zn is involved in the preservation of bone mass by stimulating bone formation by osteoblasts and inhibition of bone resorption by osteoclasts.[104] Application of Mg on the surface of dental implants significantly increases the bone–implant fixation in vivo. Indeed, Mg is strongly involved in bone metabolism, stimulating osteoblast proliferation and protecting from excessive bone resorption.[105–108] Rabbit calvarial defects filled with BCP, covered with a Sr–HA–collagen membrane and allowed to heal for 24 weeks, yielded increased levels of mineralized new bone and lower amount of residual grafting material when compared to a nonmodified collagen membrane (Bio-Gide, Geistlich, Wolhusen, Switzerland).[109] Similarly, the use of a gelatin membrane mixed with Zn–HA led to higher levels of new bone formation in a model of rat skull defect.[110] A chitosan–Mg membrane able to stimulate ALP activity in vitro in MG63 cells, showed similar levels of efficacy in vivo when compared to a collagen membrane (Heal All, Yantai Zhenghai Biotechnology Co., Shandong, China).[111] Taken together, these results suggest that membranes loaded with inorganic compounds could improve implant integration and new bone regeneration. However, more studies are needed to clarify the potential of these compounds, whose action can be influenced by several factors such as dose, loading technique, and type of membrane associated.

4.1.4. Antimicrobial Drugs

The use of polymer-based MDs which incorporate antimicrobial drugs are becoming an increasingly routine way of preventing chronic infection and device failure.[112] Although the incorporation into GBR membrane of antimicrobial agents such as silver ions,[113–116] tetracyclines,[117–119] metronidazole,[120–122] and azithromycin[123] may inhibit bacterial infection, it is difficult to determine which antimicrobial agent provides the most appropriate infection control.[20] Systems employed for the long-term release of prophylactic inhibitory or subinhibitory amounts of antibiotics, in absence of strict harmonized guidelines, raise concerns for their weakly proved efficacy and for their possible contribution to enhancing bacterial biofilm formation and selecting

resistant mutants.[112] In the context of controlling the foreign body response, a valid approach is to engineer biomaterials to immunomodulate the host response to the implant and maximize the regenerative capacity of progenitor cells.[124] In a model of rat calvarial defect, epigallocatechin-3-gallate (EGCG) – an extract from green tea with proven anti-inflammatory effects[125,126] – was able to induce bone healing by recruiting pro-regenerative M2 macrophages, promoting secretion of growth factors (Vascular Endothelial Growth Factor,VEGF and BMP-2) and the expression of osteogenic genes (Runt-related transcription factor-2, RUNX-2 and Osteopontin, OPN).[127,128]

4.1.5. Extracellular-Matrix-Based Membranes

The extracellular matrix (ECM) is a complex network of proteins and polysaccharides surrounding cells in tissues. The ECM composition and resulting mechanical and biochemical properties vary considerably between different tissue types. In addition to providing structural support, it influences various cellular processes, such as metabolic activity, proliferation, and differentiation. The ECM accomplishes these functions by acting as a substrate for cellular adhesion, polarization, and migration.[129] Biologic materials composed of mammalian ECM have been effectively used for the repair and reconstruction of a variety of tissues, including skeletal muscle,[130–132] esophagus,[133–135] and heart,[136–138] among others. Barrier membranes for GBR applications have been successfully fabricated using various biological sources, such as pedicle periosteum,[139] leukocyte- and platelet-rich fibrin (L-PRF),[140] and small intestinal submucosa (SIS). In particular, multilaminate small intestinal submucosa (mSIS)[141] consisted in a stack of eight lyophilized layers of acellular porcine SIS.[141] When implanted in subcutaneous mice pockets, mSIS lost 74% of the initial mass after 12 weeks, while the collagen membrane (Bio-Gide, Geistlich, Wolhusen, Switzerland) control completely degraded. In a rabbit critical-size mandible defect model, while the amount of bone regenerated in presence of mSIS was comparable to the control (collagen membrane), the preparation process was affected by the variability of the donors, which directly influenced the final mSIS efficacy. The human amniotic membrane (hAM) is an abundant and readily available human tissue used in regenerative medicine for its biological and mechanical properties. Histologically, hAM is composed of a layer of epithelial cells and a layer of MSC, this characteristic makes hAM an interesting candidate for GBR membranes. The regenerative properties of the two layers of freshly prepared hAM versus cryopreserved hAM were compared in a model of mice critical-size calvarial defect.[142] While no difference was found between the bone regeneration ability of fresh and cryopreserved hAM, a greater response was given by covering the defect with the mesenchymal side of cryo-hAM compared to its epithelial side. However, bone formation was mainly restricted to the periphery of the defect and only few small bone nodules were found inside the defect area. The introduction of HA particles and BMP-2 into the defect increased bone regeneration in both the presence of cryo-hAM (MSC side) or of a collagen membrane (Bio-Gide, Geistlich, Wolhusen, Switzerland). Not all studies in which biologically sourced materials have been used report a positive healing response. These alternative outcomes have typically been attributed to variations in manufacturing methods and/or the variability of the biological source.[129]

4.2. GBR Membranes with Tailored Physical Properties to Enhance Bone Regeneration

The membrane for GBR is responsible for providing a timely mechanical support at the implant site until the new bone is able to withstand mechanical load. The membrane material should be sufficiently robust to resist cell traction forces and wound contraction forces during tissue healing in vivo,[143] therefore it is necessary to carefully balance the biomechanical properties with the degradation kinetics. It is well accepted that sterilization protocols may potentially alter the structure and characteristics of biomaterials through degradation and or cross-linking of the naturally derived collagen matrix.[144] Sterilization of commercial GBR membranes is made mostly by gamma irradiation or ethylene oxide (ETO) treatment.[145] However, sterilization of collagen through irradiation can damage the structure of the material, while sterilization by ETO introduces the potential complication of ETO residuals that must be monitored and controlled. An alternative sterilization method is the use of supercritical CO_2 ($scCO_2$), this method could result in improvements in biological tissue processing/cleaning/safety with little to no disruption of the native collagen structure.[146] Tovar et al.[144] showed the effect of $scCO_2$ sterilization method on a collagen membrane (Vitala, Osteogenics Biomedical, Inc., Lubbock, TX, USA) used for a canine class III furcation lesion. After $scCO_2$ treatment, the gross fibrous structure was maintained without significant mass loss. Both $scCO_2$-treated and nontreated membranes showed partial resorption after 6 weeks, vascularized new bone and periodontal regeneration.

Biomimetic approaches to biomaterial design enable molecular, structural, and biological compatibility similar to that of the tissue being replaced to facilitate the regeneration of complex tissues.[147] Different approaches to fabricate materials with properties and features analogous to native bone tissue have also been investigated. For example, the surface of a collagen membrane has been mineralized intrafibrillary by deposition of amorphous calcium phosphate (ACP) stabilized with carboxymethyl chitosan to increase the stiffness and degradation time.[148] The biomimetic mineralization process, which consisted in the transformation of ACP nanoparticles into HA, was completed in 48 h. Within this time frame, the ACP nanoparticles could penetrate into the grooves of the collagen fibers and react to form plate-like HA crystals. In this case, the stiffness was directly proportional to the degree of the HA crystal size, and therefore to the overall mineralization reaction time. When implanted subcutaneously, such mineralized membrane seeded with MSCs gave rise to vascularized new bone tissue, while in a model of critical-size rat calvarial defect, the membrane was able to almost completely repair the defect in 12 weeks. In another study, Pajoumshariati et al.[149] developed an electrospun membrane with tunable mechanical properties based on poly(butylene succinate) and PGA with varying glycolate ratios. In vitro, the increase in the glycolate ratio correlated with increased mechanical properties, osteogenic gene expression, ALP activity, and calcium content. Similarly, in vivo tests on rabbit calvarial defects confirmed more

bone formation associated to membranes with higher glycolate ratio.[149]

Membrane porosity is a fundamental design consideration as it directly determines the surface area available for the adhesion and growth of cells both in vitro and in vivo contexts. Therefore, the pore network of the biomaterial is linked to the potential for host tissue ingrowth to penetrate into the central regions, allowing membrane remodeling and degradation. In assessing the significance of porosity, several in vivo studies have been conducted utilizing hard scaffold materials such as Zn,[150] 3D printed resorbable PCL,[151] polyglycerol sebacate,[152] or titanium[153] with defined pore dimensions. Although the pore sizes assessed in these studies varies from 25 to 1000 µm according to the fabrication process, the authors agree that smaller-sized pores are beneficial for in vivo new bone formation. Gradients of porosity in GBR membranes have also been achieved with multiple material layers. For instance, a PLGA membrane composed of an external solid layer to shield from connective tissue invasion, and a porous inner layer to allow cell penetration and differentiation, demonstrated improved vertical new bone formation eight weeks postimplantation in a rat skull defect.[154] When implanted separately, the solid layer could prevent connective tissue infiltration, while the porous layer collapsed partially upon cell infiltration, validating the distinct functions of the two architectures.

4.3. GBR Membranes in Pathological Contexts: Preclinical Studies

GBR is a generally accepted therapeutic application to achieve bone regeneration and it has been proposed as a promising strategy to cure osteoporotic fractures. Although several studies have investigated the regenerative ability of the GBR approach in healthy bone, only few reports have explored the efficacy of GBR strategy in osteoporotic bone. Osteoporosis is a metabolic disease characterized by reduced bone mass and changes in bone microarchitecture, resulting in increased risk for fractures.[155] It is reported that 27.6 million people are suffering from osteoporosis in Europe in 2010, a higher prevalence is represented by postmenopausal Caucasian women,[156] elderly,[157] and diabetic patients.[158] Strategies to enhance GBR-driven bone regeneration in osteoporotic patients include the use of drugs, growth factors, or modification of the membrane architecture.[159] Bisphosphonates (e.g., zolendronic acid (ZA)) are among the most widely prescribed drugs for the treatment of osteoporosis since they are able to inhibit osteoclast activity and bone resorption.[160] It has been reported that a single low dose injection of ZA combined with a dense PTFE (dPTFE) membrane (Cytoplast, Osteogenics Biomedical, Inc., Lubbok, TX, USA), successfully enhanced new bone formation in rat critical-size calvarial defects.[161] In this study, osteoporosis was induced in 6-month-old female rats by bilateral ovariectomy and the administration of a calcium-deficient diet. Similarly, heparinized and mineralized ECM derived from small intestinal submucosa loaded with BMP-2-related peptide P28 (SIS/P28), has been proposed for guided osteoporotic bone regeneration.[162] In vivo, SIS/P28 greatly enhanced the formation of new bone and accelerated the healing of critical-sized bone defects in the osteoporosis model. Several clinical and experimental studies have associated type 1 diabetes mellitus with suppressed bone formation potentially due to decreased osteoblastic recruitment and activity. Diabetes can be chemically induced by injection of streptozotocin. In vivo studies indicate that osseointegration can be successfully achieved in chemically induced diabetes, while systemic insulin treatment can reverse the detrimental effect on the osseointegration process.[163] Since GBR collagen membranes undergo rapid degradation when implanted in subjects affected by uncontrolled diabetes, a possible strategy to delay the degradation over time, is represented by the introduction of cross-linked hyaluronic acid within the collagen membrane architecture.[164] Alternatively, a titanium-reinforced ePTFE membrane has been employed in a model of chemically induced diabetic rat for alveolar ridge augmentation.[165] While de novo bone formation could be achieved even in the presence of uncontrolled diabetes, the predictability of the surgery outcome was significantly lower when compared to healthy subjects, such that an insulin-mediated metabolic control could benefit surgery's efficacy and implant osteointegration. In the context of growth factor's release to mitigate the effect of diabetes on bone regeneration, Santana and Trackman[166] evaluated the influence of FGF-2 in bone healing of mice calvarial defect. FGF-2-loaded polyglycolate:polylactide membranes significantly stimulated bone formation in diabetic animals to levels close to the healthy controls. Those are only few preclinical examples showing the versatility of the GBR approach for bone healing. Indeed, GBR membranes can be combined to therapeutic approaches aimed to mitigate or correct the effect of bone healing disorders, leading to new paths for bone tissue regeneration strategies.

5. Industrialization Process

Despite large investments, the number of new tissue engineering products achieving clinical application and market release is low.[167] Product development involves numerous changes before reaching the final product design. Early stage design can take up to 70% of the total product life cycle, influencing 70–85% of the total product cost.[168] Therefore, it is during the early development stages of the product that efforts focused to reduce and control development risks should be expended.[169] A collaborative network of engineers, researchers, industrial designers, entrepreneurs, and regulatory experts could help in validating an early prototype into an industrial product ready for serial production with improved replicability and user-friendliness.[170] An integrated approach to the industrialization process not only would allow to comply with the fundamental requirements in terms of traceability, reproducibility, efficiency, and safety of the manufacturing process, but also highlights solutions to be implemented for enhancing characteristics that could be overlooked, such as aesthetics and packaging among others. A valid approach to minimize risks and help identify key factors for a successful design of experiments is represented by the Quality By Design (QbD) strategy, which implements a statistical method to investigate the effect of multidimensional combinations and interactions of different parameters on the desired outputs.[171–173]

5.1. Quality by Design

In the 1990s, Dr. J. Juran published his book on the concept of QbD.[174] His aim was to advance product and process quality in the manufacturing industry, particularly in the automotive industry.[174] QbD was later adopted by the Food and Drug Administration (FDA) and the International Conference on Harmonisation as a risk-based approach for drug engineering.[175] Although the QbD approach is more often seen in industrial context, it can be introduced in research projects. It represents an opportunity to deeply understand the product characteristics, ensure higher quality standards, and reduce costs. In particular, the specific parameters to be applied to the development of GBR membranes are strongly dependent on the selected biomaterials. As such, the material attributes, the fabrication, and scale-up process can differ greatly among products with same intended final use. Although we cannot define standard parameters to apply specifically for GBR membrane's development, QbD can describe a set of rules and priorities that will help defining a risk-free manufacturing process.

The QbD approach aims to provide a deep understanding of the product and its manufacturing, hence the identification and control of all the variables is necessary to ensure the desired quality. In our experience, a new project implementing the QbD approach begins with a draft of the target product profile (TPP) document. The TPP plays a key role in ensuring a clear and efficient product development. Indeed, the TPP document provides a summary of the product to be developed, its features, a set of studies that must be done to demonstrate product's efficacy, safety, and quality. If used properly, the draft of the TPP document allows to address issues (e.g., scaling-up of the manufacturing process, preclinical and clinical studies) early in the product development process, preventing late-stage failures. Moreover, this document should be revisited multiple times over the course of the development of the product.

Next, a set of variables determining product efficiency and patient safety need to be assigned and ranked according to their critical role. Such variables are known as critical quality attributes (CQAs). These variables need to be kept within a defined range to ensure the expected quality of the product. Hence, a risk assessment needs to be generated to establish the severity and the impact of uncertainty on efficacy and safety. Factors influencing variability of CQA are critical manufacturing attributes (CMAs) and critical process parameters (CPPs), which are associated with formulation and production parameters, respectively. A risk assessment analysis at this stage is fundamental to identify those parameters. When the effect of the attributes is not known, their criticality may be estimated using the design of experiments (DoE).

DoE is a set of statistical approaches to help defining experiment design and analysis. Generally, when experiments are planned in a research laboratory, only one factor at a time is varied, while all others are held constant. Conversely, in DoE, all the factors of interest can be examined in a single experiment, minimizing the number of total experiments required and providing information on key process interactions. This type of information on interactions between factors cannot be easily obtained by investigating the effect of each factor separately. The multidimensional relationship of CMA and CPP able to ensure quality of the product and reliability of the process will define the design space (DS) (**Figure 4**). Therefore, the use of parameters belonging to the DS will still generate the same product, while using parameters outside the DS would lead to a different product, hence it would initiate a regulatory postapproval-change process. Finally, a process analytical technology will measure in real time the critical attributes and will help control whether the quality attributes are kept within the previously assigned DS and to monitor the operating conditions (Figure 4). QbD approach not only allows an efficient use of resources, but also gives detailed analysis and information on reproducibility and errors. The application of QbD reduces the size and hence the cost of process validation trials. The implementation of the QbD approach would provide a strong framework for guiding the different steps of product development, but also it would contribute to establishing a collaborative network of experts in paving the way to ensure the production and commercialization of error-free MDs.

5.2. Legal Obligations of Manufacturers

Regulatory bodies in different countries establish the obligations that manufacturers of MDs should comply with to ensure that all the MDs produced and placed on the market remain in conformity with current regulations. In USA, first, manufacturers must be registered with the FDA.[176] Besides, the Code of Federal Regulation Part 820 establishes Quality System Regulation, also known as current good manufacturing practices, (International Organization for Standardization (ISO) 9001:2015 "Quality Management Systems – Requirements").[177,178] Similarly, the European Union approved new Regulation 2017/745 on medical devices (European Union Medical Device Regulation, EU MDR) obliges manufacturers to register in the Unique Device Identifier database (for more details, see Section 7). A quality management system and a postmarket surveillance system are mandatory for all manufacturers, which is appropriate to the risk class and the type of device. In addition, a system for risk management and to report incidents must be established. The quality management system has to be kept updated and continuously improved.[177] Concerning the risk management system, the EU MDR aligns closely with the EN ISO 14971:2019.[179] A risk management plan for each device should be completed, identifying possible hazards associated to each device and estimating the risk associated with the intended use and misuse. Finally, assessment of production and postmarket information on the documented risk assessment are required, as well as changes to control measures based on the assessment of production and postmarket information.[180]

6. Regulatory Requirements

Membranes for GBR are identified as MDs by health authorities. In this section, we address important considerations about USA and EU's new regulation requirements and pathways for MD approval. In particular, we focus on classification and the regulatory requirements of GBR membranes undergoing clinical and preclinical evaluation. Moreover, **Table 3** compiles classification and approval requirements of MDs not only in EU and USA, but also in three other big MD markets: Japan, China, and India.

Table 3. Summary of MD classification and regulatory requirements in the five largest markets. Information refers to manufacture MDs for sale or for distribution; importation of MDs already approved by foreign countries may follow different approval pathways and fulfill other requirements. Abbreviations: QMS: quality management system that complies with GMP; RCB: registered certification body; PMDA: Pharmaceuticals and Medical DeviceAgency; NMPA: National Medical Products Administration; CMDE: Center for Medical Device Evaluation.

USA				EU				Japan		
No explicit rules for classification that is based on i) substantial equivalence, ii) risk of illness or injury, iii) safety and efficacy		Product	Company	Classification rules based on: duration of use, invasiveness, contact with the body, biological effect, supply of energy	I	Conformity assessment without intervention of external parties	• Essential requirements (mandatory for all countries) • European requirements (voluntary) • Conformity assessment • CE marking	Classification based on the risk to patients in the event of malfunction	General medical device (Almost negligible risk)	Class I. notification/self-declaration ("Todokede")
	I Low risk	FDA product registration	QMS with general controls							
	II Medium risk	Premarket notification (501(k))	QMS with special controls		IIa	Conformity assessment by notified bodies			Controlled medical device (relatively low risk)	Class II. Certification: the device is reviewed by a registered certification body (RCB) ("Ninsho") Certain class II MDs require PMDA's approval ("Shonin")
	III High risk	Premarket approval (PMA)	QMS with special controls and PMA		IIb				Specially Controlled device (relative high risk)	Class III. With certification standards: reviewed by the RCB ("Ninsho") Without certification standards: PMDA's approval process ("Shonin")
					III					Class IV. PMDA's approval process ("Shonin")

(*Continued*)

Table 3. Continued.

China			India		
Classification based on safety and effectiveness	I The safety and effectiveness can be ensured through routine administration	Notification	Classification rules based on: duration of use, invasiveness, contact with the body, biological effect, supply of energy, composition, use	A Low risk	Quality management system and technical review requirements confirmed by a notified body assessed by the State Licensing Authority after license (within 120 days)
	II Further control is required to ensure the safety and effectiveness	Registration: NMPA approval and CMDE technical review		B Low–moderate risk	Quality management system and technical review requirements confirmed by a notified body assessed by the State Licensing Authority prior license
	III The device is implanted into the human body; used for life support or sustenance; or pose potential risk to the human body, and thus must be strictly controlled in respect to safety and effectiveness	Registration: NMPS approval and CMDE technical review		C Moderate–high risk	Quality management system and technical review requirements confirmed by Central Licensing Authority prior license
				D High risk	

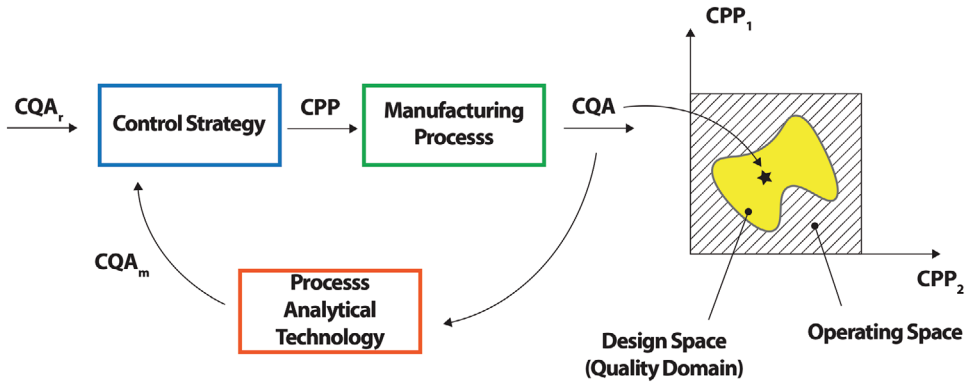

Figure 4. Schematic of the critical quality attributes within the design space. CQA_r and CQA_m stand for CQA reference and CQA measures, respectively.[175]

6.1. USA Regulation

MD regulation in the US began in 1976 when the Medical Device Regulation Act or Medical Device Amendments of 1976 were approved.[181] The FDA office responsible for MD evaluation and approval is the Center for Devices and Radiological Health (CDRH) (**Table 4**). The classification of implants being recognized as MDs depends on the intended use of the device and the subsequent indications for use, already mentioned in Section 4. When developing a membrane for GBR, it is essential to identify the mechanism by which the primary intended purpose is achieved, as this determines its classification as a MD or drug. If the membrane is classified as a drug, the documentation and the regulatory pathway are much more demanding and complicated, and the process can be significantly prolonged over time. To avoid this, it must be demonstrated that the desired effect of the membrane is not achieved through chemical action. This can be controversial and sometimes is at the origin of debates, since cellular and tissue regeneration processes are always the result of chemical or biochemical reactions.[2]

6.1.1. GBR Membranes: MD Classification

FDA marketing authorizations are public.[182,183] Membranes for GBR are in general considered as class II medium risk and, taking into consideration administrative and documentation requirements, the clearance is obtained by proceeding to the premarket notification 510(k).[184] The aim of this premarket submission is to demonstrate that is "substantially equivalent" to a "predicate;" this means, that the device is at least as safe and effective as a legally marketed device. In the case of GBR membranes, this is the most common path to obtain market approval. Nevertheless, FDA approval path includes not only a product component, but also a company component, meaning that MD companies are required to have a quality management system that complies with GMP, as explained in Section 5.2.

6.1.2. GBR Membranes from Animal Origin

Many of the GBR membranes in the market and in clinical or preclinical studies are made of collagen from animal sources, introducing several risks, mainly related to the transmission of diseases. In light of this, the FDA Department of Health and Human Services released a "Medical devices utilizing animal tissues and their derivatives" in 2019, that provides recommendations to select and handle animal tissues and to evaluate the risk of pathogen contamination. FDA's advice is to use ISO 22442 guidance but it clearly states that alternative approaches can be used if they satisfy the requirement of the regulations.[184] When producing GBR membranes with compounds from animal origin, extra documentation must be provided in the premarket submission (510(k), premarket approval (PMA)), including i) data about the control of animal tissue collection, ii) Information about the manufacturing controls for animal tissue components, iii) data about sterilization procedures, taking into consideration ISO 11135, 17665-1, 11137-1 and -2, 14160, 14937, iv) relevant information about transmissible spongiform encephalopathy-specific issues. In conclusion, GBR membranes from animal origin demand extra documentation to reduce the risk of pathogen transmission. Even if the FDA does not impose specific tests, following ISO 22442 Part 1, 2, and 3, as well as indications compiled in the guidance for Industry and FDA Staff published in 2019 seems the best way to comply with current regulation.

6.1.3. GBR Membranes Containing Drugs/Biologicals

The mechanism by which a MD exerts its principal effect, through chemical reaction or being metabolized, is key to classification as a MD. This is particularly important when developing a combination MD like GBR membranes containing drugs or biologicals, such as growth factors. Since 2002, the Office of Combination Products serves as a focal point for combination product issues and for medical product classification and assignment issues for agency staff and industry. The first step is to determine which are the regulatory pathway and the competent authority for approval based on the primary mode of action (PMOA), (Section 503(g)). Since the CDRH that is responsible of MD approval is considered the easiest regulatory path through the FDA, it is frequently suggested to argue that the combination MD PMOA is mechanical. In the case of GBR membranes containing drugs or biological products, it is not difficult to demonstrate that the device has a principal function other than delivering the active

Table 4. Regulatory authorities and legislation currently applied. Market size data in US$[195].

Country	US (140 billion $)	EU (130 billion $)	Japan (27 billion $)	China (20 billion $)	India (3.5 billion $)
Institution	FDA Center for Devices and Radiological Health	National competent authorities and European Medicines Agency (EMA)	Japan's Ministry of Health, Labor and Welfare (MHLW) Pharmaceutical and Medical Device Agency (PMDA)	NMPA: National Medical Products Administration (ex-CFDA, China Food and Drug Administration)	India's Ministry of Health and Family Welfare (MoHFW) and the Central Drugs Standard Control Organization (CDSCO)
Regulation	Medical Device Regulation Act or Medical Device Amendments of 1976. Food and Drug Administration Modernization Act of 1997 Title 21 Code of federal regulations subchapter H: Medical Devices	Medical Devices Regulation 2017/745	The Act on Pharmaceuticals and Medical Devices (Act on Securing Quality, Efficacy and Safety of Pharmaceuticals, Medical Devices, Regenerative and Cellular Therapy Products, Gene Therapy Products, and Cosmetics) 2014	Regulations on Supervisory Management of Medical Devices 2014	Medical Device Rules 2018

compound. Nevertheless, even if a combination MD is assigned to the CDRH, additional requirements are demanded, according to The Code of Federal Regulations 21 Part 4 Regulation of Combination Products, referred to GMP for Combination Products (Subpart A) and Postmarketing Safety Reporting for Combination Products (Subpart B).[185]

6.1.4. General Consideration for Synthetic GBR Membranes and Manufacturing Processes

Although the regulatory requirements for synthetic GBR membranes can vary dependently on the material used and manufacturing technique, there are some general considerations that can be taken into account. Specification standards are available in order to guarantee the reproducibility of the material characteristics, for instance: the ASTM F2579-18 standard focuses on the specification for amorphous PLA and PGA resins for surgical implants, while ASTM F754 – 08(2015) is specific for implantable PTFE. However, these specifications address material characteristics, some of them may be altered by the manufacturing technique required for the production of the MDs. As such, the properties of fabricated forms of these materials should be analyzed independently with appropriate test methods in order to guarantee safety and efficacy. In the context of the manufacturing technique, there is a broad range of different approaches available, however we will limit our focus to the additive manufacturing process, which is a rapidly growing technology often used for product research and development in many industries. A guidance document on "Technical considerations for additive manufactured medical devices"[186] is available to outline technical considerations associated with additive manufacturing processes, and suggestions for testing and characterization for devices fabricated with this method. In particular, with the additive manufacturing process, the starting material may undergo substantial physical and/or chemical changes and this must be taken into account when it comes to biocompatibility testing. The starting material can be exposed to melting and solidification cycles, which may result in undesired material chemistry for some polymers. In this case, if the biocompatibility is not analyzed as described by ISO-10993 "Biological Evaluation of Medical Devices Part 1: Evaluation and Testing within a Risk Management Process" or if the test identifies a problem, additional material chemistry information may be needed. Additionally, based on the specifics of the material/machine type used, it might be necessary to provide information or testing for polymers to guarantee that there are not undesired or unintentionally formed chemical entities that could pose at risk to patient health.

6.2. EU Regulation

In 2017, the EU approved the new Regulation 2017/745 on medical devices, amending Directive 2001/83/EC, Regulation (EC) No 178/2002, and Regulation (EC) No 1223/2009 and repealing Council Directives 90/385/EEC and 93/42/EEC.[187] In particular, the new EU MDR was expected to come into full application on May 2020.[17,188–190] However, the date of application has been postponed by one year due to the COVID-19 crisis.[191] Several changes have now been introduced, mainly related to assess-

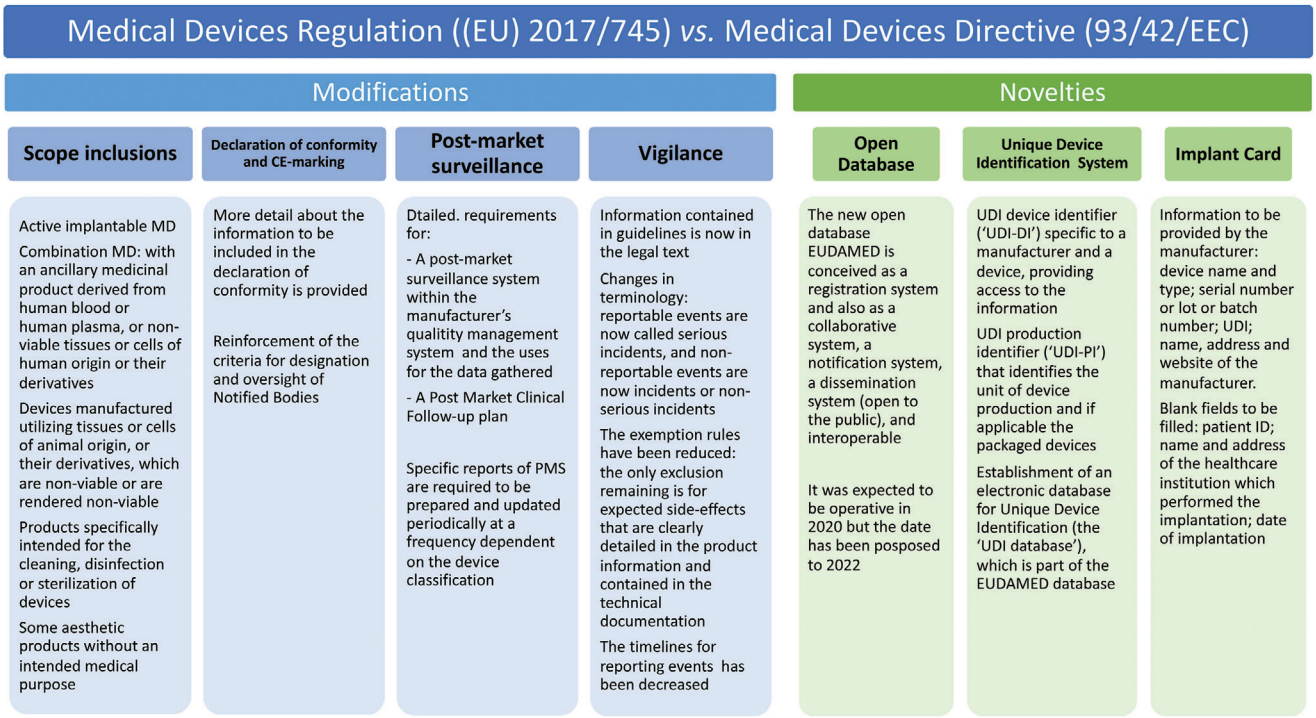

Figure 5. Schematic showing the main modifications and novelties included in the MDR compared to the medical device directive.[207]

ment of safety and performance, transparency of information to patients on the benefits and risks, trading between EU member states, and the responsibilities of Notified Bodies, that need to be designated under the EU MDR (**Figure 5**).[207] Considering the novelty of EU MDR, **Figure 6** summarizes the main steps to follow for CE marking of GBR membranes and the following subsections compile the main requirements of the new Regulation 2017/745 that refer to GBR membranes.[206] Similar to USA rules, the EU Regulation 2017/745 defines a GBR membrane as a MD according to its intended mechanism of action. EU regulation, nevertheless, is more precise, and refers to pharmacological, immunological, or metabolic means, and not only chemical action.

6.2.1. GBR Membranes: MD Classification

Taking into consideration EU classification, membranes for GBR are classified as IIb or III, according to Rule 8: "all implantable devices and long-term surgically invasive devices are classified as class IIb unless they are active implantable devices or their accessories, in which cases they are classified as class III." Of note, Regulation defines "long-term" as continuous use for more than 30 days. In practice, most GBR membranes in the market are considered as medium to high risk, equivalent to class IIb. Requirements are in all cases conformity assessment based on a quality management system and on assessment of technical documentation after a notified body evaluation (major steps are listed in Figure 6).

6.2.2. GBR Membranes from Animal Origin

Membranes derived from animal origin follow under the scope of Regulation 2017/745, that states "Regulation does apply to devices manufactured utilizing tissues or cells of animal origin, or their derivatives, which are nonviable or are rendered nonviable." Requirements in this case include document information about the sourcing animals, such as geographical origin and veterinary controls. Besides, tissue extraction and manipulation must be done so as to assure safety for humans entering in contact with the device, including safety with regard to viruses and other transmissible agents. In this context, particular attention has to be paid to viral inactivation, except when the use of such methods would lead to unacceptable degradation compromising the clinical benefit of the device. Moreover, when membranes for GBR derived from bovine, ovine, and caprine species, which is generally the case, Regulation 722/2012 concerning particular requirements with respect to active implantable MDs and MDs manufactured utilizing tissues of animal origin apply.[192] In practice, extra requirements are demanded: including a) a risk analysis and risk management process from the manufacturer, b) a justification for the use of animal derivatives or tissues, having taken into account synthetic alternatives or lower risk tissues, c) the results of elimination and inactivation studies or results of the analysis of relevant literature, d) a quality control of the sources of raw materials, finished products, production process, testing, and subcontractors, e) the need to audit matters related to the sourcing and processing of animal tissues and derivatives, processes to eliminate or inactivate pathogens, including those activities carried out by suppliers.

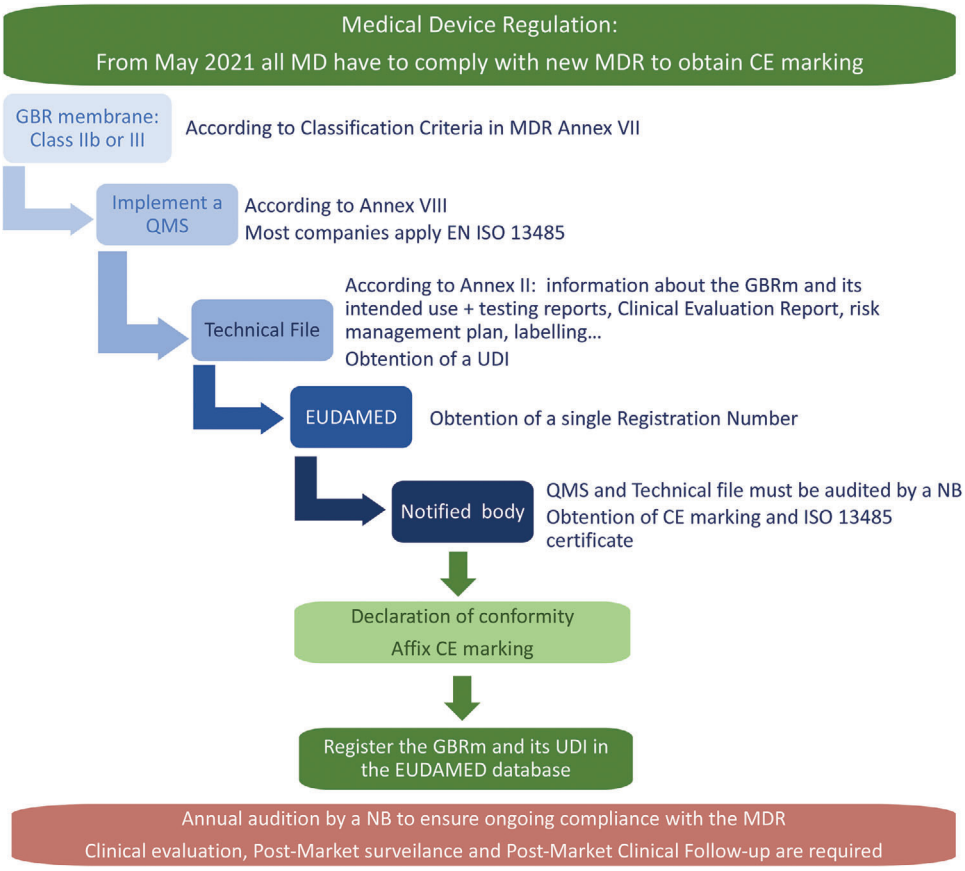

Figure 6. CE Marking regulatory process for GBR membranes according to MDR 2017/745.[206]

6.2.3. GBR Membranes Containing Drugs/Biologicals

In the case of combination products, the European Commission states, similarly to the FDA, that if the action of the medicinal substance is ancillary to support the proper functioning of the device, this one falls under MDR. Besides, the notified body shall seek a scientific opinion from one of the competent authorities on the quality and safety of the ancillary substance before it can issue a CE certificate. This can cause important delays in the procedure since the consulted authority has 150–210 days to provide its opinion. If the action of the medicinal substance is considered nonancillary, combination products are regulated by Directive 2001/83/EC.[193] The Committee for Medicinal Products for Human Use has recently published a Guideline on the quality requirements for drug–device combinations.[194]

6.3. Regulation in Asian Countries

The Asian market of MDs is exponentially growing.[195] As a consequence, MD policies have been recently modified and changes will be introduced in the coming years. The three most relevant MD markets in Asia nowadays are Japan, China, and India.[196]

In Japan, the authority responsible of MD regulation is the Ministry of Health, Labor and Welfare together with the Pharmaceutical and Medical Device Agency. The Act of Pharmaceuticals and Medical Devices was released in 2014 and introduced a classification based on the risk to patients in the event of malfunction (Table 3).[197] According to this classification, GBR membranes may be considered Specially Controlled Devices associated to relative high risk. In the case of devices with an associate certification standard, the MD is reviewed by a registered certification body (this pathway is known as "Ninsho"). In the absence of certification standards, the MD has to follow the more complex Pharmaceutical and Medical Devices Agency approval process (pathway "Shonin").[198]

In 2014, the Regulations on Supervisory Management of Medical Devices were approved in China. According to this new regulation, one of the critical points with GBR membranes that are considered class II or III MD, is that they must have local clinical trials in a certified center. Since most of the manufacturers of GBR membranes come from countries other than China, this is a great challenge that makes approval for marketing longer and tougher. China's State Council proposed in 2017 new regulations for drugs and devices. Although these regulations have not yet taken effect as laws, they expect modifications to be introduced in the coming years. One of the novelties is the acceptance of foreign clinical trials that must however meet the standards of the National Medical Products Administration (NMPA).[199]

Since 2018, MDs in India are regulated by the Medical Device Rules. According to this, GBR membranes are classified as class

B (low moderate risk) or class C (moderate high risk) devices and therefore approval is done by Central Government.[200] The new regulation states that the only requirement to import and market MDs is to apply for an import license (in the past, a registration license was also demanded); licenses granted under the Medical Devices Rules are perpetual. Moreover, for GBR membranes manufactured in regulated jurisdictions, that is, USA, EU, Australia, Canada, and Japan, they do not require prior official inspection, contrary to MDs from Unregulated Jurisdictions, simplifying the process and reducing the time for approval.

7. Conclusion

The application of a barrier membrane to shield the bone defect from soft tissue ingrowth is an established strategy that has been successfully implemented clinically. However, the costs associated with the development of this high-risk MD and the complexity of a dynamic regulatory framework prevent new barrier membranes to reach the clinical stages and finally the market. The aim of this review was to provide a comprehensive overview of commercially available GBR membranes and the latest preclinical advancements in the context of the regulatory frameworks of different countries, in order to shorten the path that brings research from bench to bedside. The advantages of a QbD approach are reported to highlight the benefits of an integrated and risk-free approach to the industrialization process. A multidisciplinary network of experts would not only inspire future investigations, but also aid the translation of scientific knowledge and rise the impact of biomedical research on the public healthcare system.

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Conflict of Interest

D.L. has shares in SILTISS company that acquired from INSERM four patents related to the production of polysaccharide materials for tissue regeneration.

Keywords

biomaterials, bone tissue engineering, medical device regulations, preclinical studies, quality by design

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[1] I. Elgali, O. Omar, C. Dahlin, P. Thomsen, *Eur. J. Oral Sci.* **2017**, *125*, 315.
[2] F. Zivic, S. Affatato, T. Miroslav, M. Schnabelrauch, N. Grujovic, K. L. Choy, *Biomaterials in Clinical Practice*, Springer International Publishing, Cham **2018**.
[3] T. L. Aghaloo, P. K. Moy, Which Hard Tissue Augmentation Techniques Are the Most Successful in Furnishing Bony Support for Implant Placement?, **2007**.
[4] R. Dimitriou, G. I. Mataliotakis, G. M. Calori, P. V. Giannoudis, *BMC Med.* **2012**, *10*, 81.
[5] C. H. F. Hämmerle, R. E. Jung, A. Feloutzis, *J. Clin. Periodontol.* **2002**, *29*, 226.
[6] O. Moses, S. Pitaru, Z. Artzi, C. E. Nemcovsky, *Clin. Oral Implants Res.* **2005**, *16*, 210.
[7] J. Y. Lee, J. Lee, Y. K. Kim, *J. Dent. Sci.* **2013**, *8*, 281.
[8] I. Beitlitum, Z. Artzi, C. E. Nemcovsky, *Clin. Oral Implants Res.* **2010**, *21*, 1242.
[9] M. Retzepi, N. Donos, *Clin. Oral Implants Res.* **2010**, *21*, 567.
[10] J. Gottlow, *J. Periodontol.* **1993**, *64*, 1157.
[11] P. Ghensi, W. Stablum, E. Bettio, M. C. Soldini, T. R. Tripi, C. Soldini, *Oral Implantol.* **2017**, *10*, 335.
[12] A. S. Almutairi, *F1000Research* **2018**, *7*, 685.
[13] M. Chiapasco, M. Zaniboni, *Clin. Oral Implants Res.* **2009**, *20*, 113.
[14] B. S. McAllister, K. Haghighat, *J. Periodontol.* **2007**, *78*, 377.
[15] L. Sbricoli, R. Guazzo, M. Annunziata, L. Gobbato, E. Bressan, L. Nastri, *Materials* **2020**, *13*, 786.
[16] A. Hoornaert, C. D'Arros, M.-F. Heymann, P. Layrolle, *Biomed. Mater.* **2016**, *11*, 045012.
[17] I. C. Santos, G. S. Gazelle, L. A. Rocha, J. M. R. S. Tavares, *Expert Rev. Med. Devices* **2012**, *9*, 299.
[18] G. Aitchison, D. Hukins, J. Parry, D. Shepherd, S. Trotman, *Open Biomed. Eng. J.* **2009**, *3*, 21.
[19] J. Caballe-Serrano, A. Munar-Frau, O. Ortiz-Puigpelat, D. Soto-Penaloza, M. Penarrocha, F. Hernandez-Alfaro, *J. Clin. Exp. Dent.* **2018**, *10*, 0.
[20] O. Omar, I. Elgali, C. Dahlin, P. Thomsen, *J. Clin. Periodontol.* **2019**, *46*, 103.
[21] M. Weshler, I. V. Antoniac, *Handbook of Bioceramics and Biocomposites*, Springer, Berlin **2014**. https://www.springer.com/gp/book/9783319124599.
[22] C. H. F. Hämmerle, R. E. Jung, *Periodontol. 2000* **2003**, *33*, 36.
[23] S. H. Oh, J. H. Kim, J. M. Kim, J. H. Lee, *J. Biomater. Sci., Polym. Ed.* **2006**, *17*, 1375.
[24] G. Zellin, A. Linde, *Biomaterials* **1996**, *17*, 695.
[25] L. Rispoli, F. Fontana, M. Beretta, C. E. Poggio, C. Maiorana, *J. Otolaryngol. Rhinol.* **2015**, *1*, 8.
[26] G. Sam, B. R. M. Pillai, *J. Clin. Diagn. Res.* **2014**, *8*, ZE14.
[27] M. C. Bottino, V. Thomas, G. Schmidt, Y. K. Vohra, T. M. G. Chu, M. J. Kowolik, G. M. Janowski, *Dent. Mater.* **2012**, *28*, 703.
[28] E. S. Cohen, E. Funakoshi, *Guided Tissue Regeneration*, People's Medical Publishing House, Shelton, CT **2007**.
[29] S. Nyman, J. Gottlow, T. Karring, J. Lindhe, *J. Clin. Periodontol.* **1982**, *9*, 257.
[30] S. Nyman, J. Lindhe, T. Karring, H. Rylander, *J. Clin. Periodontol.* **1982**, *9*, 290.
[31] J. Gottlow, S. Nyman, T. Karring, J. Lindhe, *J. Clin. Periodontol.* **1984**, *11*, 494.
[32] J. Gottlow, S. Nyman, J. Lindhe, T. Karring, J. Wennstrom, *J. Clin. Periodontol.* **1986**, *13*, 604.
[33] Z. Sheikh, N. Hamdan, Y. Ikeda, M. Grynpas, B. Ganss, M. Glogauer, *Biomater. Res.* **2017**, *21*, 9.
[34] S. M. Meloni, S. A. Jovanovic, I. Urban, E. Baldoni, M. Pisano, M. Tallarico, *Clin. Implant Dent. Relat. Res.* **2019**, *21*, 669.

[35] J. F. E. Hamrick, in *Horizontal Alveolar Ridge Augmentation in Implant Dentistry: A Surgical Manual* (Ed: L. Tolstunov), Wiley-Blackwell, Hoboken **2016**, pp. 107–119.
[36] M. G. Vroom, L. J. M. M. Grundemann, *Tandartspraktijk* **2014**, *35*, 8.
[37] N. P. Lang, C. H. F. Hämmerle, U. Brägger, B. Lehmann, S. R. Nyman, *Clin. Oral Implants Res.* **1994**, *5*, 92.
[38] M. Ronda, A. Rebaudi, L. Torelli, C. Stacchi, *Clin. Oral Implants Res.* **2014**, *25*, 859.
[39] S. Jovanovic, M. Nevins, *Implant Dent.* **1995**, *4*, 293.
[40] M. G. Vroom, L. Gründemann, *Inspyred - Altern. EAO Voice* **2018**, *6*, 8.
[41] S.-T. Li, H.-C. Chen, N. S. Lee, R. Ringshia, D. Yuen, **2013**. https://www.zimmerbiometdental.com/wps/wcm/connect/dental/93d16bb8-bd49-40b6-a9df-2afd090cf4f9/Biomend+White+Paper_ZD1218.pdf?MOD=AJPERES&CACHEID=ROOTWORKSPACE.Z18_1O041O02L8PAF0A9JPRUH520H793d16bb8-bd49-40b6-a9df-2afd090cf4f9 ZD1218, Rev. 05/13 (accessed: August 2020).
[42] J. Jiménez Garcia, S. Berghezan, J. M. M. Caramês, M. M. Dard, D. N. S. Marques, *J. Periodontal Res.* **2017**, *52*, 955.
[43] K. S. Weadock, E. J. Miller, L. D. Bellincampi, J. P. Zawadsky, M. G. Dunn, *J. Biomed. Mater. Res.* **1995**, *29*, 1373.
[44] L. H. H. Olde Damink, P. J. Dijkstra, M. J. A. Van Luyn, P. B. Van Wachem, P. Nieuwenhuis, J. Feijen, *J. Mater. Sci.: Mater. Med.* **1995**, *6*, 460.
[45] L. H. H. Olde Damink, P. J. Dijkstra, M. J. A. Van Luyn, P. B. Van Wachem, P. Nieuwenhuis, J. Feijen, *J. Mater. Sci.: Mater. Med.* **1995**, *6*, 429.
[46] H. Petite, V. Frei, A. Huc, D. Herbage, *J. Biomed. Mater. Res.* **1994**, *28*, 159.
[47] B. Brodsky, R. A. Berg, G. Avigad, E. Eikenberry, M. Jain, S. Tanaka, *US4971954A*, **1990**.
[48] J. Wang, L. Wang, Z. Zhou, H. Lai, P. Xu, L. Liao, J. Wei, *Polymers* **2016**, *8*, 115.
[49] E. Milella, P. A. Ramires, E. Brescia, G. La Sala, L. Di Paola, V. Bruno, *J. Biomed. Mater. Res.* **2001**, *58*, 427.
[50] L. N. Woodard, M. A. Grunlan, *ACS Macro Lett.* **2018**, *7*, 976.
[51] M. Vert, S. M. Li, G. Spenlehauer, P. Guerin, *J. Mater. Sci.: Mater. Med.* **1992**, *3*, 432.
[52] P. Gentile, V. Chiono, C. Tonda-Turo, A. M. Ferreira, G. Ciardelli, *Biotechnol. J.* **2011**, *6*, 1187.
[53] S. Ivanovski, C. Vaquette, S. Gronthos, D. W. Hutmacher, P. M. Bartold, *J. Dent. Res.* **2014**, *93*, 1212.
[54] A. H. Gager, A. J. Schultz, *J. Periodontol.* **1991**, *62*, 276.
[55] D. Kim, T. Kang, D. Gober, C. Orlich, *ISRN Dent.* **2011**, *2011*, 1.
[56] U. Sakallioglu, U. Yavuz, M. Lütfioglu, I. Keskiner, G. Açikgöz, *Int. J. Periodontics Restor. Dent.* **2007**, *27*, 79.
[57] A. R. Vernino, H. L. Wang, J. Rapley, S. J. Nechamkin, T. A. Ringeisen, M. Derhalli, J. Brekke, *Int. J. Periodontics Restor. Dent.* **1999**, *19*, 56.
[58] R. DerSimonian, N. Laird, *Contemp. Clin. Trials* **2015**, *45*, 139.
[59] I. Rodriguez, G. Selders, A. Fetz, C. Gehrmann, S. Stein, J. Evensky, M. Gree, G. Bowlin, *Mouth Teeth* **2018**, *2*.
[60] B. Parekkadan, D. Van Poll, K. Suganuma, E. A. Carter, F. Berthiaume, A. W. Tilles, M. L. Yarmush, *PLoS One* **2007**, *2*, e941.
[61] J. Lee, H. Jin, S. Endo, E. Schuchman, J. Carter, J. Bae, *Stem Cells* **2010**, *28*, 329.
[62] D. J. Prockop, *Mol. Ther.* **2009**, *17*, 939.
[63] J. Galipeau, M. Krampera, *Cytotherapy* **2015**, *17*, 125.
[64] I. A. Jones, R. Togashi, M. L. Wilson, N. Heckmann, C. T. Vangsness, *Nat. Rev. Rheumatol.* **2019**, *15*, 77.
[65] P. Bianco, X. Cao, P. S. Frenette, J. J. Mao, P. G. Robey, P. J. Simmons, C.-Y. Wang, *Nat. Med.* **2013**, *19*, 35.
[66] A. I. Caplan, *Stem Cells Transl. Med.* **2017**, *6*, 1747.
[67] M. Osugi, W. Katagiri, R. Yoshimi, T. Inukai, H. Hibi, M. Ueda, *Tissue Eng., Part A* **2012**, *18*, 1479.
[68] T. Inukai, W. Katagiri, R. Yoshimi, M. Osugi, T. Kawai, H. Hibi, M. Ueda, *Biochem. Biophys. Res. Commun.* **2013**, *430*, 763.
[69] T. Kawai, W. Katagiri, M. Osugi, Y. Sugimura, H. Hibi, M. Ueda, *Cytotherapy* **2015**, *17*, 369.
[70] W. Katagiri, M. Osugi, T. Kawai, H. Hibi, *Head Face Med.* **2016**, *12*, 5.
[71] M. Simion, I. Rocchietta, D. Kim, M. Nevins, J. Fiorellini, *Int. J. Periodontics Restor. Dent.* **2006**, *26*, 415.
[72] L. Batas, A. Stavropoulos, S. Papadimitriou, J. R. Nyengaard, A. Konstantinidis, *Clin. Oral Implants Res.* **2016**, *27*, 981.
[73] J. D. Ruskin, R. Hardwick, D. Buser, C. Dahlin, R. K. Schenk, *Clin. Oral Implants Res.* **2000**, *11*, 107.
[74] R. Hosokawa, K. Kikuzaki, T. Kimoto, T. Matsuura, D. Chiba, M. Wadamoto, Y. Sato, M. Maeda, A. Sano, Y. Akagawa, *Clin. Oral Implants Res.* **2000**, *11*, 345.
[75] F. M. Chen, L. A. Wu, M. Zhang, R. Zhang, H. H. Sun, *Biomaterials* **2011**, *32*, 3189.
[76] S. C. Pigossi, M. C. Medeiros, S. Saska, J. A. Cirelli, R. M. Scarel-Caminaga, *Int. J. Mol. Sci.* **2016**, *17*, 1885.
[77] S. Saska, S. C. Pigossi, G. J. P. L. Oliveira, L. N. Teixeira, M. V. Capela, A. Gonçalves, P. T. de Oliveira, Y. Messaddeq, S. J. L. Ribeiro, A. M. M. Gaspar, R. Marchetto, *Biomed. Mater.* **2018**, *13*, 035009.
[78] X. Yu, H. Sun, J. Yang, Y. Liu, Z. Zhang, J. Wang, F. Deng, *Biomed. Mater.* **2019**, *15*, 015009.
[79] T. Kitaori, H. Ito, E. M. Schwarz, R. Tsutsumi, H. Yoshitomi, S. Oishi, M. Nakano, N. Fujii, T. Nagasawa, T. Nakamura, *Arthritis Rheum.* **2009**, *60*, 813.
[80] W. Ji, F. Yang, J. Ma, M. J. Bouma, O. C. Boerman, Z. Chen, J. J. J. P. van den Beucken, J. A. Jansen, *Biomaterials* **2013**, *34*, 735.
[81] S. A. Jovanovic, D. R. Hunt, G. W. Bernard, H. Spiekermann, R. Nishimura, J. M. Wozney, U. M. E. Wikesjö, *Clin. Oral Implants Res.* **2003**, *14*, 793.
[82] Y.-T. Hsu, K. Al-Hezaimi, P. Galindo-Moreno, F. O'Valle, A. Al-Rasheed, H.-L. Wang, *J. Periodontol.* **2017**, *88*, 896.
[83] A. A. Jones, D. Buser, R. Schenk, J. Wozney, D. L. Cochran, *J. Periodontol.* **2006**, *77*, 1184.
[84] M. Fujioka-Kobayashi, K. Sawada, E. Kobayashi, B. Schaller, Y. Zhang, R. J. Miron, *Clin. Oral Implants Res.* **2017**, *28*, 381.
[85] M. Fujioka-Kobayashi, K. Sawada, E. Kobayashi, B. Schaller, Y. Zhang, R. J. Miron, *J. Periodontol.* **2016**, *87*, e101.
[86] M. Fujioka-Kobayashi, E. Kobayashi, B. Schaller, M. Mottini, R. J. Miron, N. Saulacic, *J. Biomed. Mater. Res., Part A* **2017**, *105*, 2655.
[87] B. Khorsand, S. Elangovan, L. Hong, M. S. D. Kormann, A. K. Salem, *J. Biomed. Mater. Res., Part B* **2019**, *107*, 1824.
[88] A. S. Herford, *Chin. J. Traumatol. (Engl. Ed.)* **2017**, *20*, 1.
[89] O. P. Gautschi, S. P. Frey, R. Zellweger, *ANZ J. Surg.* **2007**, *77*, 626.
[90] M. J. Danesh-Meyer, *Ann. R. Australas. Coll. Dent. Surg.* **2000**, *15*, 144.
[91] W. S. Vanden Berg-Foels, *Tissue Eng., Part B* **2014**, *20*, 28.
[92] M. Mehta, K. Schmidt-Bleek, G. N. Duda, D. J. Mooney, *Adv. Drug Delivery Rev.* **2012**, *64*, 1257.
[93] I. K. Ko, S. J. Lee, A. Atala, J. J. Yoo, *Exp. Mol. Med.* **2013**, *45*, e57.
[94] Z. Tang, X. Li, Y. Tan, H. Fan, X. Zhang, *Regener. Biomater.* **2018**, *5*, 43.
[95] R. Z. LeGeros, S. Lin, R. Rohanizadeh, D. Mijares, J. P. LeGeros, *J. Mater. Sci.: Mater. Med.* **2003**, *14*, 201.
[96] H. Yuan, H. Fernandes, P. Habibovic, J. De Boer, A. M. C. Barradas, A. De Ruiter, W. R. Walsh, C. A. Van Blitterswijk, J. D. De Bruijn, *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 13614.
[97] H. Yuan, H. Fernandes, P. Habibovic, J. De Boer, A. M. C. Barradas, A. De Ruiter, W. R. Walsh, C. A. Van Blitterswijk, J. D. De Bruijn, *Nat. Rev. Rheumatol.* **2011**, *7*, 1.
[98] J. Y. Won, C. Y. Park, J. H. Bae, G. Ahn, C. Kim, D. H. Lim, D. W. Cho, W. S. Yun, J. H. Shim, J. B. Huh, *Biomed. Mater.* **2016**, *11*, 055013.

[99] M. Jiménez, C. Abradelo, J. San Román, L. Rojo, *J. Mater. Chem. B* **2019**, *7*, 1974.
[100] J. M. Fernández, M. S. Molinuevo, A. D. McCarthy, A. M. Cortizo, *BioMetals* **2014**, *27*, 601.
[101] W. Querido, A. L. Rossi, M. Farina, *Micron* **2016**, *80*, 122.
[102] C. Ehret, R. Aid-Launais, T. Sagardoy, R. Siadous, R. Bareille, S. Rey, S. Pechev, L. Etienne, J. Kalisky, E. De Mones, D. Letourneur, J. A. Vilamitjana, *PLoS One* **2017**, *12*, e0184663.
[103] S. Pasqualetti, G. Banfi, M. Mariotti, *J. Trace Elem. Med. Biol.* **2013**, *27*, 375.
[104] N. M. Lowe, W. D. Fraser, M. J. Jackson, *Proc. Nutr. Soc.* **2002**, *61*, 181.
[105] K. M. Pang, J. W. Lee, J. Y. Lee, J. B. Lee, S. M. Kim, M. J. Kim, J. H. Lee, *Clin. Oral Implants Res.* **2014**, *25*, 616.
[106] J. W. Park, C. H. An, S. H. Jeong, J. Y. Suh, *Clin. Oral Implants Res.* **2012**, *23*, 294.
[107] Y.-T. Sul, P. Johansson, B.-S. Chang, E.-S. Byon, Y. Jeong, *J. Appl. Biomater. Biomech.* **2005**, *3*, 18.
[108] C. Janning, E. Willbold, C. Vogt, J. Nellesen, A. Meyer-Lindenberg, H. Windhagen, F. Thorey, F. Witte, *Acta Biomater.* **2010**, *6*, 1861.
[109] S. Kitayama, L. O. Wong, L. Ma, J. Hao, S. Kasugai, N. P. Lang, N. Mattheos, *Clin. Oral Implants Res.* **2016**, *27*, e206.
[110] J. Chou, M. Komuro, J. Hao, S. Kuroda, Y. Hattori, B. Ben-Nissan, B. Milthorpe, M. Otsuka, *Clin. Oral Implants Res.* **2016**, *27*, 354.
[111] Y. Guo, Y. Yu, L. Han, S. Ma, J. Zhao, H. Chen, Z. Yang, F. Zhang, Y. Xia, Y. Zhou, *Mater. Sci. Eng., C* **2019**, *100*, 226.
[112] D. Campoccia, L. Montanaro, P. Speziale, C. R. Arciola, *Biomaterials* **2010**, *31*, 6363.
[113] J. Li, Y. Zuo, Y. Man, A. Mo, C. Huang, M. Liu, J. A. Jansen, Y. Li, *J. Biomater. Sci., Polym. Ed.* **2012**, *23*, 81.
[114] S. Tokuda, A. Obata, T. Kasuga, *Acta Biomater.* **2009**, *5*, 1163.
[115] J. Ye, Q. Yao, A. Mo, J. Nie, W. Liu, C. Ye, X. Chen, *Int. J. Nanomed.* **2011**, *6*, 1853.
[116] J. Zhang, Q. Xu, C. Huang, A. Mo, J. Li, Y. Zuo, *Clin. Oral Implants Res.* **2010**, *21*, 321.
[117] E. Kütan, G. Duygu-Çapar, C. Özçakir-Tomruk, O. C. Dilek, F. Özen, Ö. Erdoıan, I. Özdemir, M. Kroachi, A. Gürel, *Arch. Oral Biol.* **2016**, *63*, 15.
[118] Y. J. Park, Y. M. Lee, S. N. Park, J. Y. Lee, Y. Ku, C. P. Chung, S. J. Lee, *J. Biomed. Mater. Res.* **2000**, *51*, 391.
[119] M. Lian, B. Sun, Z. Qiao, K. Zhao, X. Zhou, Q. Zhang, D. Zou, C. He, X. Zhang, *Colloids Surf., B* **2019**, *176*, 219.
[120] J. Xue, M. He, Y. Niu, H. Liu, A. Crawford, P. Coates, D. Chen, R. Shi, L. Zhang, *Int. J. Pharm.* **2014**, *475*, 566.
[121] R. Shi, J. Ye, W. Li, J. Zhang, J. Li, C. Wu, J. Xue, L. Zhang, *Mater. Sci. Eng., C* **2019**, *100*, 523.
[122] Y. Wang, Y. Jiang, Y. Zhang, S. Wen, Y. Wang, H. Zhang, *Mater. Sci. Eng., C* **2019**, *98*, 134.
[123] A. Mathew, C. Vaquette, S. Hashimi, I. Rathnayake, F. Huygens, D. W. Hutmacher, S. Ivanovski, *Adv. Healthcare Mater.* **2017**, *6*, 1601345.
[124] O. R. Mahon, D. C. Browe, T. Gonzalez-Fernandez, P. Pitacco, I. T. Whelan, S. Von Euw, C. Hobbs, V. Nicolosi, K. T. Cunningham, K. H. G. Mills, D. J. Kelly, A. Dunne, *Biomaterials* **2020**, *239*, 119833.
[125] Y. Zhong, Y. S. Chiou, M. H. Pan, F. Shahidi, *Food Chem.* **2012**, *134*, 742.
[126] S. Riegsecker, D. Wiczynski, M. J. Kaplan, S. Ahmed, *Life Sci.* **2013**, *93*, 307.
[127] C. Chu, Y. Wang, Y. Wang, R. Yang, L. Liu, S. Rung, L. Xiang, Y. Wu, S. Du, Y. Man, Y. Qu, *Mater. Sci. Eng., C* **2019**, *99*, 73.
[128] C. Chenyu, J. Deng, Y. Hou, L. Xiang, Y. Wu, Y. Qu, Y. Man, *Mater. Sci. Eng., C* **2017**, *76*, 31.
[129] M. C. Cramer, S. F. Badylak, *Ann. Biomed. Eng.* **2019**, *48*, 2132.
[130] B. Perniconi, D. Coletti, P. Aulino, A. Costa, P. Aprile, L. Santacroce, E. Chiaravalloti, L. Coquelin, N. Chevallier, L. Teodori, S. Adamo, M. Marrelli, M. Tatullo, *Front. Physiol.* **2014**, *5*, 354.
[131] V. J. Mase, J. R. Hsu, S. E. Wolf, J. C. Wenke, D. G. Baer, J. Owens, S. F. Badylak, T. J. Walters, *Orthopedics* **2010**, *33*, 511.
[132] J. Dziki, S. Badylak, M. Yabroudi, B. Sicari, F. Ambrosio, K. Stearns, N. Turner, A. Wyse, M. L. Boninger, E. H. P. Brown, J. P. Rubin, *npj Regener. Med.* **2016**, *1*, 16008.
[133] A. Nieponice, F. F. Ciotola, F. Nachman, B. A. Jobe, T. Hoppo, R. Londono, S. Badylak, A. E. Badaloni, *Ann. Thorac. Surg.* **2014**, *97*, 283.
[134] T. Hoppo, S. F. Badylak, B. A. Jobe, *World J. Surg.* **2012**, *36*, 2390.
[135] S. F. Badylak, T. Hoppo, A. Nieponice, T. W. Gilbert, J. M. Davison, B. A. Jobe, *Tissue Eng., Part A* **2011**, *17*, 1643.
[136] A. Quarti, S. Nardone, M. Colaneri, G. Santoro, M. Pozzi, *Interact. Cardiovasc. Thorac. Surg.* **2011**, *13*, 569.
[137] P. V. Kochupura, E. U. Azeloglu, D. J. Kelly, S. V. Doronin, S. F. Badylak, I. B. Krukenkamp, I. S. Cohen, G. R. Gaudette, *Circulation* **2005**, *112*, I144.
[138] S. F. Badylak, P. V. Kochupura, I. S. Cohen, S. V. Doronin, A. E. Saltman, T. W. Gilbert, D. J. Kelly, R. A. Ignotz, G. R. Gaudette, *Cell Transplant.* **2006**, *15*, S29.
[139] A. Hasuike, H. Ujiie, M. Senoo, M. Furuhata, M. Kishida, H. Akutagawa, S. Sato, *In Vivo* **2019**, *33*, 717.
[140] J. B. Lee, J. T. Lee, S. Hwang, J. Y. Choi, I. C. Rhyu, I. S. L. Yeo, *J. Periodontol.* **2020**, *91*, 120.
[141] W. Wu, B. Li, Y. Liu, X. Wang, L. Tang, *J. Biomed. Res. Int.* **2018**, *2018*, 3270293.
[142] M. Fénelon, O. Chassande, J. Kalisky, F. Gindraux, S. Brun, R. Bareille, Z. Ivanovic, J. C. Fricain, C. Boiziau, *J. Mater. Sci.: Mater. Med.* **2018**, *29*, 78.
[143] D. W. Hutmacher, J. T. Schantz, C. X. F. Lam, K. C. Tan, T. C. Lim, *J. Tissue Eng. Regener. Med.* **2007**, *1*, 245.
[144] N. Tovar, L. Witek, R. Neiva, H. F. Marão, L. F. Gil, P. Atria, R. Jimbo, E. A. Caceres, P. G. Coelho, *J. Biomed. Mater. Res., Part B* **2019**, *107*, 1320.
[145] L. Schorn, J. Handschel, J. Lommen, F. P. Von Beck, R. Depprich, N. Kübler, H. Holtmann, *In Vivo* **2019**, *33*, 1447.
[146] J. L. Wehmeyer, S. Natesan, R. J. Christy, *Tissue Eng., Part C* **2015**, *21*, 649.
[147] J. W. Nichol, A. Khademhosseini, *Soft Matter* **2009**, *5*, 1312.
[148] Y. Wang, Y. Hua, Q. Zhang, J. Yang, H. Li, Y. Li, M. Cao, Q. Cai, X. Yang, X. Zhang, C. Li, *J. Tissue Eng. Regener. Med.* **2018**, *12*, 1545.
[149] S. Pajoumshariati, H. Shirali, S. K. Yavari, S. N. Sheikholeslami, G. Lotfi, F. Mashhadi Abbas, A. Abbaspourrad, *Sci. Rep.* **2018**, *8*, 7513.
[150] H. Guo, D. Xia, Y. Zheng, Y. Zhu, Y. Liu, Y. Zhou, *Acta Biomater.* **2020**, *106*, 396.
[151] J.-H. Shim, J. Jeong, J.-Y. Won, J.-H. Bae, G. Ahn, H. Jeon, W.-S. Yun, E.-B. Bae, J.-W. Choi, S.-H. Lee, C.-M. Jeong, H. Y. Chung, J.-B. Huh, *Biomed. Mater.* **2018**, *13*, 015014.
[152] B. Jian, W. Wu, Y. Song, N. Tan, C. Ma, *Int. J. Nanomed.* **2019**, *14*, 2683.
[153] Y. D. Rakhmatia, Y. Ayukawa, Y. Jinno, A. Furuhashi, K. Koyano, *Odontology* **2017**, *105*, 408.
[154] I. Yoshimoto, J. I. Sasaki, R. Tsuboi, S. Yamaguchi, H. Kitagawa, S. Imazato, *Dent. Mater.* **2018**, *34*, 538.
[155] R. L. Jilka, *Med. Pediatr. Oncol.* **2003**, *41*, 182.
[156] E. Hernlund, A. Svedbom, M. Ivergård, J. Compston, C. Cooper, J. Stenmark, E. V. McCloskey, B. Jönsson, J. A. Kanis, *Arch. Osteoporosis* **2013**, *8*, 136.
[157] W. Becker, P. Hujoel, B. E. Becker, P. Wohrle, *Clin. Implant Dent. Relat. Res.* **2016**, *18*, 473.
[158] U. Stumpf, P. Hadji, L. van den Boom, W. Böcker, K. Kostev, *Osteoporosis Int.* **2020**, *31*, 1315.

[159] Ö. Erdoğan, D. M. Shafer, P. Taxel, M. A. Freilich, *Oral Surg., Oral Med., Oral Pathol., Oral Radiol., Endodontol.* **2007**, *104*, 738.e1.
[160] E. F. Eriksen, A. Díez-Pérez, S. Boonen, *Bone* **2014**, *58*, 126.
[161] N. Mardas, J. Busetti, J. A. P. de Figueiredo, L. A. Mezzomo, R. K. Scarparo, N. Donos, *Clin. Oral Implants Res.* **2017**, *28*, 362.
[162] T. Sun, M. Liu, S. Yao, Y. Ji, L. Shi, K. Tang, Z. Xiong, F. Yang, K. Chen, X. Guo, *Int. J. Nanomed.* **2018**, *13*, 791.
[163] J. T. Siqueira, S. C. Cavalher-Machado, V. E. Arana-Chavez, P. Sannomiya, *Implant Dent.* **2003**, *12*, 242.
[164] M. Eliezer, A. Sculean, R. J. Miron, C. Nemcovsky, E. Weinberg, M. Weinreb, H. Zoabi, D. D. Bosshardt, M. Fujioka-Kobayashi, O. Moses, *J. Periodontal Res.* **2019**, *54*, 644.
[165] M. Retzepi, M. P. Lewis, N. Donos, *Clin. Oral Implants Res.* **2010**, *21*, 71.
[166] R. B. Santana, P. C. Trackman, *Int. J. Oral Maxillofac. Implants* **2006**, *21*, 711.
[167] S. J. Hollister, *Biofabrication* **2009**, *1*, 012001.
[168] Y. Asiedu, P. Gu, *Int. J. Prod. Res.* **1998**, *36*, 883.
[169] E. W. Larson, C. F. Gray, *Project Management: The Managerial Process*, McGraw-Hill, New York **2011**. https://www.mheducation.com/highered/product/project-management-managerial-process-larson-gray/M9781259666094.html.
[170] G. Talò, C. Turrisi, C. Arrigoni, C. Recordati, I. Gerges, M. Tamplenizza, A. Cappelluti, S. A. Riboldi, M. Moretti, *J. Tissue Eng. Regener. Med.* **2018**, *12*, 405.
[171] P. Lenas, M. Moos, F. P. Luyten, *Tissue Eng., Part B* **2009**, *15*, 395.
[172] I. Papantoniou, M. Sonnaert, L. Geris, F. P. Luyten, J. Schrooten, G. Kerckhofs, *Tissue Eng., Part C* **2014**, *20*, 177.
[173] D. Martinez-Marquez, A. Mirnajafizadeh, C. P. Carty, R. A. Stewart, *PLoS One* **2018**, *13*, e0195291.
[174] J. Juran, *Juran on Quality by Design: The New Steps for Planning Quality into Goods and Services*, Simon and Schuster, New York **1992**. https://www.simonandschuster.com/books/Juran-on-Quality-by-Design/J-M-Juran/9780029166833.
[175] T. Bastogne, *Nanomedicine* **2017**, *13*, 2151.
[176] Food and Drug Administration (FDA), "Overview of Device Regulation," **2018**.
[177] International Organization for Standardization (ISO), "ISO 9001:2015(en) Quality management systems — Requirements," https://www.iso.org/obp/ui/#iso:std:iso:9001:ed-5:v1:en (accessed: April 2020).
[178] Food and Drug Administration (FDA), "Quality System (QS) Regulation/Medical Device Good Manufacturing Practices," **2018**.
[179] International Organization for Standardization (ISO), "ISO 14971:2019(en) Medical devices — Application of risk management to medical devices," https://www.iso.org/obp/ui/#iso:std:iso:14971:ed-3:v1:en (accessed: April 2020).
[180] B. Brosseau, "Risk Management Under the New EU Medical Device Regulation," **2018**.
[181] J. A. Johnson, *FDA Regulation of Medical Devices*, Congressional Research Service, Washington, D.C., USA **2016**. https://crsreports.congress.gov/product/pdf/R/R42130.
[182] Food and Drug Administration (FDA), "Medical Device Databases," https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/medical-device-databases (accessed: April 2020).
[183] Food and Drug Administration (FDA), "Product Classification Database," https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpcd/classification.cfm (accessed: April 2020).
[184] Food and Drug Administration (FDA), Medical Devices Containing Materials Derived from Animal Sources (Except for In Vitro Diagnostic Devices) Guidance for Industry and Food and Drug Administration Staff, **2019**.
[185] Food and Drug Administration (FDA), "Regulation of Combination Products," https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?cfrpart=4 (accessed: April 2020).
[186] Food and Drug Administration (FDA), Technical Considerations for Additive Manufactured Medical Devices Guidance for Industry and Food and Drug Administration Staff Preface Public Comment, **2017**.
[187] The European Parliament, The Council of the European Union, Official Journal of the European Union: REGULATION (EU) 2017/745, **2017**.
[188] P. Sikder, S. B. Bhaduri, *Biomaterials in Translational Medicine*, Matthiew Deans, UK **2019**. Regulatory Aspects of Medical Devices and Biomaterials.
[189] H. J. Marcus, C. J. Payne, A. Hughes-Hallett, A. P. Marcus, G.-Z. Yang, A. Darzi, D. Nandi, *BMJ* **2016**, *353*, i2587.
[190] K. Imagawa, Y. Mizukami, S. Miyazaki, *Expert Rev. Med. Devices* **2018**, *15*, 497.
[191] European Commission, Amending Regulation (EU) 2017/745 on Medical Devices, as Regards the Dates of Application of Certain of Its Provisions, **2020**.
[192] The European Parliament, The Council of the European Union, Official Journal of the European Union: REGULATION (EU) 722/2012, **2012**.
[193] The European Parliament, The Council of the European Union, Official Journal of the European Communities: DIRECTIVE 2001/83/EC, **2001**.
[194] European Medicine Agency: Committee for Medicinal Products for Human Use, Guideline on the Quality Requirements for Drug-Device Combinations: Draft, **2019**.
[195] Pacific Bridge Medical, Sourcing and Manufacturing Medical Devices in Asia, **2014**.
[196] Pacific Bridge Medical, A. Gross, Asia Medical Markets Overview, **2015**.
[197] Japan Government, "Act on Securing Quality, efficacy and Safety of Products Including Pharmaceuticals And Medical Devices," **2015**.
[198] Pacific Bridge Medical, A. Gross, Japan Medical Device Regulatory Updates and Recent Revisions, **2014**.
[199] Pacific Bridge Medical, "China CFDA (now NMPA) Medical Device and Pharmaceutical Regulations," **2018**.
[200] Central Government of India, Medical Devices Rules, **2017**.
[201] J. Caballé-Serrano, A. Munar-Frau, O. Ortiz-Puigpelat, D. Soto-Penaloza, M. Peñarrocha, F. Hernández-Alfaro, *J. Clin. Exp. Dent.* **2018**, *10*, e477.
[202] H. Amara, J.-W. Lee, J.-J. Kim, Y.-M. Kang, E.-J. Kang, K.-T. Koo, *Int. J. Oral Maxillofac. Implants* **2017**, *32*, e265.
[203] J. S. Lee, S. H. Ko, Y. T. Kim, U. W. Jung, S. H. Choi, *J. Oral Maxillofac. Surg.* **2012**, *70*, 2070.
[204] C.-C. Chen, S.-Y. Lee, N.-C. Teng, H.-T. Hu, P.-C. Huang, J.-C. Yang, *Nanomaterials* **2019**, *9*, 599.
[205] W. Wu, B. Li, Y. Liu, X. Wang, L. Tang, *Biomed Res. Int.* **2018**, *2018*, 1.
[206] Regulatory Affairs Management Suite (EMERGO by UL), "Europe CE Marking Regulatory Process for Medical Devices," **2019**.
[207] E. Hoxey, Comparison of the Articles of the European Medical Devices Directive (93/42/EEC) and the Medical Devices Regulation ((EU)2017/745), **2017**.

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