Available online at www.sciencedirect.com

ScienceDirect

Ciência & Tecnologia dos Materiais 26 (2014) 108–117

Ciência & Tecnologia dos MATERIAIS

http://ees.elsevier.com/ctmat

Special Issue on Biomaterials

Membranes for periodontal tissues regeneration

Pedro S. Babo[a,b,+], Ricardo Leandro Pires[a,b,+], Rui L. Reis[a,b], Manuela E. Gomes[a,b,*]

[a] *3B's Research Group - Biomaterials, Biodegradables and Biomimetics, University of Minho, Portugal*
[b] *ICVS/3B's - PT Government Associate Laboratory, Portugal*
+ Authors have equally contributed to this paper

Abstract

The complexity of the tissues and the healing process of the periodontal wound turn the development of therapies for the predictable regeneration of functional periodontal tissues, a challenging exercise. In the last decades, the guided tissue regeneration (GTR) emerged as a strategy to drive the innate regenerative properties of the tissues involved in the periodontal apparatus (gingiva, tooth root, periodontal ligament and alveolar bone). GTR therapies have shown that the use of membrane barriers that are occlusive for gingival cell migration and that provide wound stability and space provision for the first intentional repair promoted by cells originated from periodontal tissues can partially restore the primitive anatomy and function of the periodontium. This paper describes examples of the variety of non-degradable and degradable membrane barriers developed for GTR approaches. During the last 20 years, the research has evolved to develop GTR barriers that avoid the recurrent problems related with the early membrane exposure and the recurrent infections. Furthermore, the association of the GTR to tissue engineering principles, such as the inclusion of biochemical cues and new architectures as well as the association with stem cells of different niches, has given rise to new materials with improved properties and biological performance.

Keywords: guided tissue regeneration; guided bone regeneration; biodegradable/non-biodegradable membranes; periodontal wound healing; periodontal defects.

1. Introduction

The periodontium is a complex structure that anchors the teeth to the mandibular or jaw bone, while withstands the forces originated by the masticatory process [1]. It comprises the cementum, a functionally oriented periodontal ligament (PDL), alveolar bone and gingiva. Its anatomy and function can be compromised by traumatic and/or pathological events, such as the gingival recession [2], periodontitis [1,3] or gingivitis [1,3].

Several therapies have been used to restore the lost alveolar bone and ligament tissue, such as scalling and root planning, open flap debridment, autogenous bone grafting, implantation of biomaterials including bone derivatives and bone substitutes, guided tissue regeneration (GTR) procedures, and implantation of biologic factors including enamel matrix proteins and platelet derivatives.

The outcomes of the conventional therapeutics for the treatment of the lost periodontal tissues are variable and very often result in several repair patterns that do not restore the normal function of the periodontal tissues (table 1).

Generally, in a periodontal wound, the fast growing epithelial and gingival tissues usually repopulate the empty space of the periodontal wound, resulting in long junctional epithelium. Although the formation of a new epithelial attachment may be compatible with an acceptable clinical outcome and clinical health, it doesn't regenerate the primitive PDL function [2].

The use of membranes for guided tissue regeneration (GTR) or for guided bone regeneration (GBR) was introduced into clinical dental practice in the mid-1980's, after the discovery of the intrinsic healing potential of the alveolar [5] and periodontal ligament origin cells [6,7]. It was hypothesized then that if cells derived from the PDL and alveolar bone were the first to repopulate tooth root surface, instead of the fast growing gingival epithelial and connective cells, the formation of a long junctional epithelium would be avoid, and the regeneration of a functional periodontium could be possible [8].

* Corresponding author.
E-mail address: megomes@dep.uminho.pt (M. Gomes)

http://dx.doi.org/10.1016/j.ctmat.2015.03.007

Table. 1 Different patterns of periodontal healing/regeneration (adapted from Chen et al., 2010 [1], and Polimeni et al., 2006 [4])

Pattern	Characterization
Long junctional epithelium (epithelial attachment)	Thin epithelial attachment extended apically along the instrumented root surface, which is formed by keratinocytes that migrate into the pocket from the crevicular epithelium
Connective tissue repair	Healing of periodontal defect by collagen fibers oriented parallel or perpendicularly to a instrumented root surface previously exposed to periodontal disease or otherwise deprived of its periodontal attachment
Bone and/or bone-like tissue repair (ankylosis)	Healing of periodontal defect by bone or bone-like tissue formation without specific PDL and/or acellular extrinsic fiber cementum regeneration
Periodontal tissue regeneration	Healing of the periodontal defect by regeneration of tooth cementum, a functionally oriented PDL, alveolar bone, and gingiva in periodontal defect

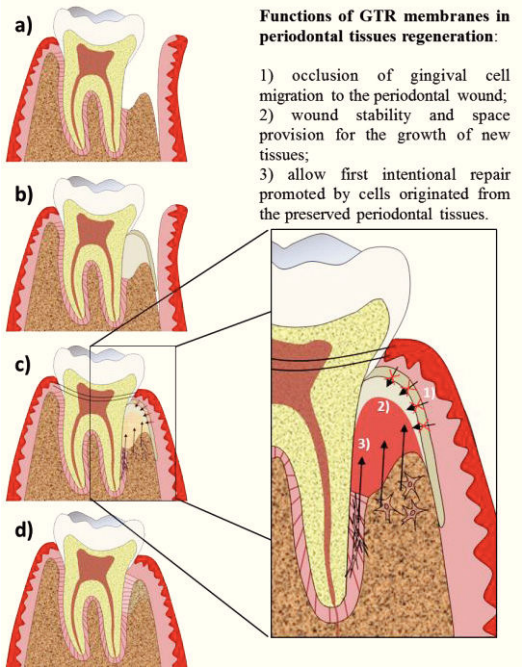

Fig 1. Schematic representation of the application and operating principle of a GTR membrane: a) reflection of gingiva and debridement of periodontal wound with loss of periodontal tissues; b) placement and stabilization of the GTR membrane providing the space necessary for the new tissues ingrowth; c) clot formation and colonization of the periodontal space with progenitor cells from the periodontal ligament and alveolar bone; d) reestablishment of most of the periodontal apparatus, with gain of periodontal function (based on Tal et al. [13], and Polimeni et al., 2006 [4]).

Accordingly, the function of the GTR and GBR (figure 1) is based on a tissue barrier, typically a membrane, which acts avoiding the apical migration of epithelium and gingival connective tissue while promoting cellular growth from the preserved periodontal tissues [9,10].

In 1984, Gottlow tested the effect of Millipore filter (Millipore filter; Micro Filtration System, MA, USA) or Gore-Tex membranes (W. L. Gore & Associates, Inc., AZ, USA) placed over scaled and planed root surfaces of monkeys in order to avoid the formation of granulation tissue from the gingiva flaps. Higher amount of new cementum with inserting collagen fibers was observed on the previously exposed roots covered with the membranes, proving the ability of the membranes to avoid the formation of long junctional epithelium and the innate potential of the cells from the PDL to regenerate periodontal tissues [8].

Later, in 1994, Hardwick et al. [11] summarized the main properties that GTR materials should present, namely biocompatibility, cell-occlusiveness, space maintenance, tissue integration, and clinical manageability [11,12].

The dogma for the development of new GTR and GBR barriers was then established. While the biocompatibility, tissue integration and clinical manageability are concepts transversal to all the biomaterials, the cell occlusiveness and space provision were the basis of this new technique. During the last decades, these principles have been refined and the weight of each one of them in a predictable regeneration of the lost periodontal tissues has been systematically tested [4]. It is not clear whether the occlusiveness to the gingival tissues has influence in the quality and amount of new tissues formed [14]. Some studies show no significant differences between occlusive and macroporous membranes [14]. On the other hand, it is well-known the importance of space provision for an effective regeneration of bone to a physiological form [4, 14]. Furthermore, the wound stabilization is essential for root colonization by periodontal cells [4]. The disturbing of the root surface-adhering fibrin clot will compromise its function as preventive measure to apical migration of the gingival epithelium [15].

There are also other staple factors that influence the success or failure of GTR/GBR, such as the time frame of the membrane functionality, ample blood fill of the area for regeneration, enhanced access of bone and bone marrow-derived cells, and prevention of soft tissue dehiscence over the membranes.

The GTR membranes can be roughly classified into non-resorbable and resorbable, both presenting advantages and disadvantages, as summarized in table 2.

2. Membranes for GTR/GBR

The first group, the non-resorbable GTR barriers, is represented by synthetic and metallic membranes processed in thin layers. Examples of non-resorbable materials used as GTR barriers are the expanded polytetrafluoroethylene (ePTFE), the most popular and earliest commercialized membranes for GTR [17,18], the dense PTFE (dPTFE) and titanium sheets or meshes.

A recurrent complication related with the non-resorbable membranes is the risk of membrane exposure, which results in graft failure and recurrent infection [4,14]. The occlusive non-resorbable membranes can interrupt the adequate blood supply to the gingiva, causing ischemia, followed by soft tissue dehiscence and subsequent membrane exposure. Furthermore, non-resorbable membranes require a second surgery for their removal, increasing the risk of infection and site morbidity.

Table. 2 Summary of the advantages and disadvantages of resorbable and non-resorbable GTR/GBR barriers, based on Polimeni, et al. [4] and Zhang et al. [16].

	Advantages	Disadvantages
Non-resorbable	-Stability; -Biocompatibility; -Give often predictable outcomes	-Additional surgery required for membrane retrieval potentially causing infection and site morbidity; -Membrane exposure is a recurrent complication resulting in graft failure,
Resorbable	-Reduced post-operative scarring and site morbidity (only one surgery is required); -The rapid resorbability reduce the probability of recurrent infections; -Lower rate of complications than non-resorbable membranes	-Generally have weak mechanical properties; -Some cases of tissue inflammation reported in the tissues adjacent to the implantation site

Several studies reported no significant differences between the treatment with the non-resorbable and resorbable GTR barriers [19,20]. However, the resorbable barriers offer many advantages over the non-resorbable materials, namely reduced post-operative scarring and site morbidity, since only one surgery is required, improved soft tissue healing [20,21], and reduced probability of recurrent infections [20]. Given those advantages, resorbable materials have largely replaced the gold standard e-PTFE non-resorbable membranes, becoming the standard for most clinical situations.

This review provides a general overview of the GTR technologies available, regarding the materials used, their main properties/characteristic and outcomes reported in the literature. The table 3 summarizes the membranes described in the current review.

2.1. Non-resorbable membranes

2.1.1. PTFE membranes

PTFE is chemically stable and biologically inactive, which make it highly resilient to the attack by microorganisms and enzymes. ePTFE membrane prevents soft tissue ingrowth into the injury site, providing the necessary space for bone regeneration [22]. These membranes have been accepted as the gold standard for human and animal comparative studies. The high-density PTFE (d-PTFE) is made of pure medical-grade and inert PTFE, which is non-expanded and non-permeable. Some of these membranes do not require a second surgery for removal of the membrane because they allow no primary closure and can be removed with a gentle tug [22]. Thus, d-PTFE offer an improved alternative to e-PTFE membranes [23]. Moreover, d-PTFE membranes have a porosity of up to one hundred times lower and are thinner (0.2-0.3mm) than the e-PTFE (around 1mm) membranes [16].

The PTFE membranes are commercially available in different shapes and textures. They can also be reinforced with titanium to improve their mechanical stability.

Since the PTFE membranes were the first commercial GTR membranes available, they are also the most extensively studied. They have been shown to increase the alveolar bone volume. In 1995, Bartee and Carr [24] tested the effect of d-PTFE membranes (TefGen-FD) in jaw bone defects in rats. For the two time points tested, 6 and 10 weeks, the bone defects were filled with hard tissue showing an improvement in the 10 weeks compared to 6 weeks. The d-PTFE membranes were shown to be easy to remove. A clinical case by Bartee [23] where it was used a d-PTFE membrane (TefGen, LifeCore Biomedical Inc.) with microporous hydroxyapatite for 21 days reported that by the end of the treatment, the graft was consolidated into osteoid-like matrix.

The d-PTFE membranes were also shown to prevent periodontopathic bacteria adhesion. Sela and co-workers [25] tested the ability of adhesion of three periodontopathic bacteria (*Actinobacillus actinomycetecomitans, Treponema denticola* and *Porphyromonas gingivalis*) to Collagen (Biomend), d-PTFE (TefGen, LifeCore Biomedical Inc.) and e-PTFE membranes (Gore-Tex). In the end of the study more bacteria were adhered to the collagen membranes than to the PTFE membranes.

The d-PTFE (TefGen, LifeCore Biomedical Inc.) membranes were found to be much easier to remove than e-PTFE membranes in a study performed in calvarial bone defects in rabbits, by Marouf and El-Guindi [22]

that also, paradoxically, concluded that e-PTFE membranes were more efficient than d-PTFE in promoting bone regeneration.
In 2010 [26] Monteiro et al. tested the ability of d-PTFE (Tecnoflon & Brasflon) membranes for GBR in subcutaneous and connective tissue in Wistar rats. The d-PTFE membranes presented good biocompatibility but induced the formation of an initial inflammatory infiltrated with oedema.
The ability of d-PTFE membranes to support bone remodelling in order to enable fixation of an implant, after tooth extraction, was tested in 2011 by Park et al. [10]. The authors used Mongrel dogs to assess the difference in using the d-PTFE (TefGen, LifeCore Biomedical Inc.) membranes with bone tacks in immediate implants without dehiscence. This study showed that in these cases, only the presence of a barrier membrane could prevent the loss of buccal bone following bone remodelling after tooth extraction at the sites with no dehiscence.

2.1.2. Titanium meshes

Titanium meshes (Friatec Titanium, Mannheim, Germany) were introduced to eliminate the problems caused by compression or displacement of the graft during the post-op period [27]. They are often used to contain the autogenous and alloplastic grafts, when the bone is damaged.
A recurrent problem with titanium meshes GTR barriers is the exposure of the mesh, which represents a major surgical complication of this reconstructive technique [28]. Patrick J. Louis and co-workers showed that porous titanium meshes were a reliable containment system for the reconstruction of the maxilla and mandible, and that this material produced well tolerated exposure, giving predictable results [28].

2.2. Resorbable membranes
2.2.1. Natural-origin materials

2.2.1.1. Collagen

Collagen is the major structural macromolecule in the human body and can be easily processed as a membrane. There are two major types of collagen used in the manufacture of membranes for GTR, type I and type III, usually from bovine or porcine origin [29].
Usually the collagen membranes are thicker (values vary with the collagen origin, type and crosslinking strategy used, but are usually around 1mm [30]) than the synthetic resorbable membranes, which will be addressed later. The main manufacturing process of these membranes is based on the extrusion-coagulation of diluted solutions of collagen. However, the collagen in its native form is degraded in a few days, and therefore, these membranes are not stable and cannot maintain the space needed for cell proliferation in the absence of bone support [31] .
To overcome these problems, various crosslinking techniques have been developed. This process involves the multiplication of natural occurring connections between the collagen molecules [32]. The process of crosslinking makes the membranes more rigid and decelerates the process of enzymatic degradation. The membrane rigidity and duration of degradation is proportional to the number of crosslinks of collagen [33, 34].
Both crosslinked and non-crosslinked collagen membranes are commercially available. Examples of commercially available crosslinked collagen membranes are the BioMendExtends, that use glutaraldehyde crosslinked bovine type I collagen and Ossixs membranes produced from enzymatic-crosslinked bovine type I collagen. Other membranes such as BioGides and TutoDent are produced with non-crosslinked type I and III collagens or bovine type I collagen respectively [30].
Despite the effectiveness of the crosslinking of collagen with glutaraldehyde, these membranes showed *in vitro* [35] and *in vivo* [13] increase of cytotoxicity that has been attribute to glutaraldehyde release during the degradation of collagen, inhibiting cell proliferation [35].
The process of crosslinking of collagen with glutaraldehyde and the amount of glutaraldehyde used, has been improved to withdraw its cytotoxicity [35]. Verissimo et al. performed a study about progressive crosslinking with glutaraldehyde in 2010 [36]. A polyanionic collagen membrane was prepared with consecutive cycles of mineralization with hydroxyapatite. The membranes were treated, at room temperature, with progressive concentrations of 0.01% (for 1h) and 0.05% (for 7 h) glutaraldehyde. Thus creating collagen membranes progressively crosslinked with glutaraldehyde. Verissimo et al. verified that the progressive glutaraldehyde crosslinking of polyanionic collagen membranes provided an increase of the biodegradation rate of membranes subcutaneously implanted in rats, combined with a low immune response, probably due to the low concentration of glutaraldehyde used in the crosslinking process [36]
To study the biocompatibility of different commercialized collagen membranes, Rothamel et al. in 2004 [37] performed an *in vitro* study using differently cross-linked collagen membranes (BioGides, BioMends, Ossixs and TutoDents) seeded/cultured with human PDL fibroblasts and human osteoblast-like cells. After 7 days the cell density and cell morphology was evaluated and the conclusion was that BioGides, TutoDents and Ossixs promoted the attachment and proliferation of human PDL fibroblasts and human osteoblast-like cells while

BioMends, which is crosslinked with glutaradehyde, had an inhibitory effect on the same cells [37].
In addition to the effects of the crosslinking on the biological behaviour of the membranes, topography also has a great effect on cell proliferation [38]. Furthermore, the source of the collagen used was also found to affect the properties of the membranes for guided bone regeneration [39,40].
Despite being widely used, the collagen membranes can trigger immune and inflammatory reactions, as any protein-based material. The type and source of collagen used seems to be the most important variable in the antigenic response, and tendon derived collagen seems relatively inert [32].
Other biomaterials used for tissue engineering purposes have been tested in the development of membranes for GTR applications. Materials such as alginate, chitosan, polycaprolactone or poly (trimethylene carbonate) have been used in recent studies [41-50].

2.2.1.2. Alginate and Chitosan based barriers

Alginate is a natural polysaccharide that has a slow degradation and thus alginate based membranes may last several months upon implantation. In 1998, Ishikawa and co-workers [51] developed an alginate membrane for GBR that can be formed *in situ*. The bone defect is filled with a Na-alginate solution that is ionically crosslinked in the surface by dropping a $CaCl_2$ solution. Then, an alginate membrane is formed, keeping the inside of the bone defect filled with unreacted Na-alginate solution, providing space for new bone ingrowth, while avoids the growing of soft tissues. Four weeks after implantation in rats, the bone defect was reconstructed with new bone [51].
Chitosan is a linear polysaccharide derived from chitin, the second most abundant natural occurring biopolymer and it can be processed into membranes with potential for several biomedical applications, such as wound dressing [52]. The biocompatible and biodegradable characteristics of chitosan membranes, [53] make this material suitable to be used in GTR techniques [54]. For example, Chen and collaborators [55] developed a chitosan-based membrane with an alginate coating to improve the mechanical properties.
Hua Hong in 2007 [47], developed an asymmetric porous chitosan membrane that can maintain the structure integrity for 5-6 weeks in lysosyme solution. This membrane was designed to be porous in the side of the lesion and non-porous in the opposite side. *In vivo* study with rabbits showed that this asymmetric membrane is capable of preventing apical migration of gingival epithelial cells and promoting growth of periodontal ligament cells in periodontal therapy. The asymmetric structure showed bioactivity both guiding and inducing tissue regeneration when combined with growth factors and other bioactive factors.
This osteoconductivity and osteoinductivity are staple features for GBR membranes [16]. The coating or incorporation of calcium salts such as calcium silicate [48] or calcium carbonate ($CaCO_3$) [50], or bioactive glass [46,49], have contributed to improve the osteoconductive and osteoinductive properties of GBR membranes. Fraga et al. [48] produced a chitosan membrane coated with calcium silicate that showed to induce the formation of a low crystallinity hydroxyapatite layer similar to the human bone *in vitro*, when immersed in a SBF solution.
A chitosan membrane combined with bioactive glass was developed by Mota et al. [46]. These membranes displayed a lower mechanical potential comparing with pure chitosan membranes, but improved bioactivity as they were able to induce the precipitation in vitro of a bone-like apatite layer. *In vitro* tests performed using human periodontal ligament cells and human bone marrow stromal cells showed that the composite membranes promoted cell metabolic activity and mineralization [46].

2.2.1.3. Platelet – based membranes

The Platelets are a natural source of growth factors and cytokines involved in the triggering of the wound healing cascade. Membranes based in platelet concentrate derivatives have been developed either using Platelets-rich fibrin (PRF) [56] or Platelets lysates (PL) [57].
Volker Gassling et al. [56] performed *in vitro* studies using human periosteal cells with the aim of comparing the functionality of the commercial collagen membrane Bio-Gide with a PRF membrane [58]. In summary, the PRF membranes showed slightly inferior biocompatibility (assessed by the LDH test), comparing with the collagen membranes BioGide, but a higher metabolic activity (assessed by MTT and WST tests) and proliferation level (BrdU test).
Babo et al., developed PL-based membranes cross-linked with genipin [57] thatshowed viscoelastic behaviour properties as well as appropriate mechanical properties for applications envisioning the regeneration of mechanically active tissues. The developed membranes demonstrate a positive response towards Human Adipose derived Stem Cells, concerning adhesion, proliferation and metabolic activity [57]. In addition, these membranes were able to promote the sustained release of PL proteins, namely the basic fibroblast growth factor (b-FGF) [57], a mitogenic growth factor that has been shown to be effective in the enhancement of angiogenesis [59] and new bone formation [60].

2.2.2. Synthetic origin membranes

The production of synthetic resorbable GTR membranes based on different variants of PLA – *poly(lactic acid)* and PGA – *poly(glycolic acid)* has gained interest in the last years. The degradation rate of the membranes produced with this synthetic polymers can be tuned just by lengthening of the polymer chain through the addition of lactides or glycols [29]. The biodegradation of these membranes occurs by the breakdown of polymeric chain by hydrolysis, releasing lactic acid and glycolic acid. These are natural metabolites of the body, which are eliminated through the Krebs cycle as carbon dioxide and water [61]

Most of the clinical studies comparing the performance of the synthetic-origin membranes with collagen membranes for GTR and GBR show that synthetic membranes have better performance [61,62]. However, if in on one hand, the biodegradable synthetic membranes limit the problem of reactivity to collagen proteins, on the other hand the degradation products of the synthetic-origin membranes may eventually trigger an inflammatory response [63].

Leal et al. (2013) developed a poly(D,L-lactic acid) (PDLLA) asymmetric composite membrane containing the Bioglass® [49]. PDLLA membranes were prepared by a solvent casting method that promoted a non-uniform distribution of the inorganic component along the membrane thickness. The incorporation of the Bioglass® microparticles enhanced the mechanical properties of the PDLLA membrane, and provided higher osteoinductive properties to the membrane side incorporating higher particle concentration. Furthermore, *in vitro* tests using human bone marrow stromal cells and human periodontal ligament cells showed that BioGlass enhanced cell proliferation and differentiation. The results obtained by Leal et al. suggest the positive effect of adding the bioactive microparticles in the PDLLA membranes [49].

Table. 3 Different membranes in GTR

Membranes		Comercial Name	Manufacturer	References
Non-Resorbable	e-PTFE	GORE-TEX®	W. L. Gore & Associates, Inc., AZ, USA	[18, 29, 65]
	Titanium	FRIOS® BoneShield	Friatec, Mannheim, Germany	[29]
	d-PTFE	Cytoplast™ Regentex GBR-200	Osteogenics Biomedical, Inc., TX, USA	[66]
		TefGen-FD®	American Custom Medical Inc, Luboock, TX, USA	[22]
Natural-Resorbable	Collagen/silica	Non Comercial		[41]
	Collagen type I and III, porcine	Bio-Gide® ED	Geistlich SHONE AG, Wolhusen, Swiss	[30, 37, 67]
	Collagen type I, bovine	Ossix® 3i	Colbar R&D, Ltd, Ramat Husharon, Israel	[68]
	Collagen type I, bovine	BioMend®	Zimmer Dental, Carlsbad, CA, USA	[69]
	Alginate	Non Comercial		[51]
	Chitosan	Non Comercial		[46-48, 55]
	Chitosan/tri-calcium phosphate	Non Comercial		[45]
	Platelet lysate	Non Comercial		[57]
	Platelet-rich fibrin	Non Comercial		[56]
Synthetic-Resorbable	Poly(lactic-co-glycolic acid)/collagen/hydroxyapatite	Non Comercial		[70]
	Poly(l-lactic acid)/chitosan	Non Comercial		[71]
	Polycaprolactone/ starch / calcium / silicon	Non Comercial		[43]
	Polycaprolactone/ calcium carbonate	Non Comercial		[50]
	Poly(e-caprolactone)/silica	Non Comercial		[42]
	Poly(trimethylene carbonate)	Non Comercial		[44]
	Poly(lactic-*co*-glycolic acid)	BioMesh	Samyang's, Daejeon, Korea	[72]
	Poly(DL-lactic acid)/ Bioglass®	Non Comercial		[49]
	Poly(DL-lactic acid)	EpiGuide®	THM Biochemical, Inc., Duluth,MN,USA	[73]
	Poly(DL-lactide-co-glycolide)	Resolut®	Gore-Tex Regenerative Material; W. L. Gore & Associates, Flagstaff, AZ, USA	[27]
	Polylactic acid	Guidor Matrix Barrier®	Guidor AB. Huddinge, Sweden	[65]

Other synthetic polymers, such as polycaprolactone or poly (trimethylene carbonate) were studied for GTR. Fujihara, et al. [50] developed two different membranes made of spun polycaprolactone incorporating calcium carbonate nano-fibers, one with PCL dominant and other with $CaCO_3$ dominant (75:25 wt%). These membranes are susceptible to fracture when subjected to strain, so they are not suitable for jaw bone regeneration due to the perpendicular forces of mastication which cause high stress. Given that, these membranes were suggested to be more appropriate for periodontal regeneration since the mechanical supporting PCL layer provides adequate tensile properties. Human osteoblasts cells attachment was verified in both the two types of membranes *in vitro*. Still, PCL dominant membranes provide increased cell proliferation with respect to CaCO3 dominant membranes. Nonetheless, the authors suggest that both membranes showed potential to be used in for periodontal tissues regeneration [50]. Other study on PCL membranes was developed by Eun-Jung Lee [42], who produced a nanostructured polycaprolactone membrane with silica xerogel. The *in vitro* results showed excellent cellular responses in terms of proliferation and differentiation of pre-osteoblast cells. The *in vivo* test was performed on 11 male Sprague–Dawley albino rats. Briefly, calvarial defects were generated on the bilateral sides of the midline in each animal. One defect was covered with the pure PCL membrane as a control and the other was covered with PCL–silica xerogel membrane. After 3 weeks the results showed that the hybrid membrane had a much higher bone formation rate.

More recently Requicha and co-workers [43,64] created bi-layered membranes based on a blend of starch and polycaprolactone (SPCL) composed by a flat SPCL layer and a SCPL fibre-mesh functionalized with Si groups. The influence of topography and the presence of Si groups stimulated the osteogenic differentiation of canine adipose derived stem cells (cASCs). The double-layered membrane was also assessed in a mandibular rat defect model and compared to a commercial collagen membrane (Parasorb Resodont, Resorba, Germany). The SPCL-Si scaffolds induced significantly higher new bone formation in 8 weeks showing better results than positive control [43].

2.3. The incorporation of nanotechnology in GTR

Since the introduction of the concept of nanotechnology by Feynman in his famous talk “There's Plenty of Room at the Bottom” in 1959, this approach as emerged as one promising tool for many engineering fields. The nano-materials have usually a larger surface to volume ratio, increased wettability, and protein adsorption when compared to conventional biomaterials. In addition, many nanobiomaterials have superior mechanical characteristics [74,75]. Moreover, the conjugation of nanobiomaterials with the traditional biomaterials used in tissue engineering, can provide better chemical and physical cues for cell adhesion and differentiation [76].

The surface topography is especially critical to guide cellular behaviour. Different nano-topography patterns have been shown to modulate the adhesion, migration, and differentiation of cells.

Aligned nano-patterns can be extremely useful in cell alignment and migration. Behring et al., reviewing the morphology, attachment, proliferation, and migration of cells cultured on collagen barrier membranes, found that membranes with organized arrays of collagen promote fast and directional migration of the fibroblasts [77]. Moreover, Lamers and co-workes showed that the speed and direction of osteoblasts migration is determined by the width of the parallel nanogrooves, being higher for the 300nm width grooves than for grooves with 150nm, for instance [78].

More than the dimensions, the organization of nano-topography and nanostructures can be tailored for different purposes. While parallel nanogrooves or nanofibers are associated with alignment and cell migration, patterns of nanopits have being reported to direct cell morphology and differentiation. Dalby et al. demonstrated that nanospits can be used to stimulate human mesenchymal stem cells (MSCs) to produce bone mineral in vitro, in the absence of osteogenic supplements [79] Lamers and co-workers controlled the pre-osteoblasts morphology and behaviour using patterns of anisotropic nanopits [80].

The coating or incorporation of nano-particles has been shown to improve other functional characteristics of the membranes such as stiffness, bioactivity, drug and antimicrobial delivery and protein or molecules carriers.

The incorporation of nano-particles on the GTR membranes allows not only the reinforcement of the stiffness [42,46] but also, depending on the material, can play osteoconductive and osteointegrative functions [41,42,46,81], as previously described. These advanced features of membranes have been studied incorporating nanoparticles such as calcium salts [41,50,70,82] and silica xerogel [41] or bioglass [46,81].

Moreover, the high specific surface of nano-structured materials, makes them potential carriers for the controlled delivery of proteins and other molecules [83] such as drugs or antimicrobial compounds [84].

In sum, the development of asymmetric membranes with well-defined nanotopography holds enormous potential to drive the regeneration of functional periodontal tissues, by one hand favouring the oriented migration of progenitor cells from the preserved PDL and alveolar bone, while avoiding the migration of epithelium and ginvival cells for empty space of the periodontal wound. Furthermore the incorporation of nanoparticles already

showed to have potential to improve the stiffness and bioactivity of the materials commonly used for the development of GTR membranes.

2.4. GTR as a stem cell therapy vehicle

The presence of periodontal origin cells was largely proved to be essential for the regeneration of the lost periodontal tissues [4]. In professor Okano's group, the pioneer technique of cell sheet has been applied to the regeneration of PDL, with encouraging outcomes [85, 86]. The autologous application of cells sheets of PDL origin cells associated to a PGA membrane in three-wall infrabony defect in dog fully restored an anatomically organized periodontium [87]. These results suggest the potential of the PDL origin fibroblasts and stem cells to fully regenerate the periodontal tissues. However, given the limited amount on PDL stem cells available in a patient suffering of periodontal tissues loss, other autologous sources of stem cells have been investigated (revised in ref [88]). Moreover, in recent studies [57,64], the development of barrier membranes envisioning its use as cell carriers has been proposed as an hybrid tissue engineering/GTR technique, which is expected to predicatively regenerate functional periodontium, by supplying the defects with the adequate support and environment for new tissues growth, as well as the adequate progenitor stem cells.

3. Conclusions

During the last decades, the research in GTR barriers has provided a large number of alternatives to the clinical treatment of periodontal defects. We are 30 years distant from the first Millipore membranes used to test the hypothesis in the basis of GTR: that the inclusion of a mechanical barrier to avoid the population of the periodontal defect with the gingival tissues, while providing the adequate space for the regeneration of the periodontal tissues with the progenitor cells residing in the PDL and alveolar bone. In spite of the fact that the total regeneration of lost periodontal tissues anatomy and function is still illusive, big steps were achieved towards the development of GTR treatments that predicatively allow the recovery of tissue functionality of damaged periodontal tissues.

The development of new materials and processing techniques has evolved with the understanding of the complex dynamic of periodontal wound regeneration. The GTR evolved to potentiate the limited innate regeneration ability of the periodontal tissues.

The research so far provided us significant advances in the materials/techniques available and overall knowledge over the past three decades ago. New materials that are simultaneously biocompatible, stable and tissue integrative, with predictable degradability are available. In addition, they can be processed in different combinations to produce new composite with improved mechanical properties. These materials can also be coated and have different porosities in order to improve their integration, osteogenic properties, antibacterial properties, and preferential cell adhesion. Moreover, the association of stem cell therapy, either as cell sheets and/or using the GTR membranes as cell carriers may allow better outcomes and a more predictable regeneration of functional periodontium.

Acknowledgements

Pedro S. Babo acknowledges *Fundação para a Ciência e Tecnologia* (FCT) for PhD Grant SFRH/BD/73403/2010.

References

[1] F.M. Chen, Y. Jin, *Tissue Eng Part B Rev*. **16**, 2 (2010).
[2] H.L. Wang, M. Modarressi, J.H. Fu, *Periodontol 2000*. **59**, 1 (2012).
[3] C. Susin, U.M.E. Wikesjö, *Periodontol 2000*. **62**, 1 (2013).
[4] G. Polimeni, A.V. Xiropaidis, U.M. Wikesjo, *Periodontol 2000*. **41**, (2006).
[5] A.H. Melcher, C.A. McCulloch, T. Cheong, E. Nemeth, A. Shiga, *J Periodontal Res*. **22**, 3 (1987).
[6] T. Karring, S. Nyman, J. Gottlow, L. Laurell, *Periodontol 2000*. **1**, (1993).
[7] D. Buser, K. Warrer, T. Karring, *J Periodontol*. **61**, 9 (1990).
[8] J. Gottlow, S. Nyman, T. Karring, J. Lindhe, *J Clin Periodontol*. **11**, 8 (1984).
[9] J. Gottlow, S. Nyman, J. Lindhe, T. Karring, J. Wennstrom, *J Clin Periodontol*. **13**, 6 (1986).
[10] R.G. Caffesse, W. Becker, *Dent Clin North Am*. **35**, 3 (1991).
[11] R. Hardwick, B.K. Hayes, C. Flynn, *J Periodontol*. **66**, 6 (1995).
[12] T.V. Scantlebury, *J Periodontol.* **64**, 11s (1993).
[13] H. Tal, O. Moses, A. Kozlovsky, C. Nemcovsky, in: H. Tal (Eds.), Bone Regeneration, InTech, 2012, pp. 112-137
[14] U.M. Wikesjo, W.H. Lim, R.C. Thomson, W.R. Hardwick, *J Clin Periodontol*. **30**, 7 (2003).
[15] W.H. Hiatt, R.E. Stallard, E.D. Butler, B. Badgett, *J Periodontol*. **39**, 1 (1968).
[16] Y. Zhang, X. Zhang, B. Shi, R.J. Miron, *Ann Maxillofac Surg*. **1**, 1 (2013).
[17] J.M. Carbonell, I.S. Martin, A. Santos, A. Pujol, J.D. Sanz-Moliner, J. Nart, *Int J Oral Maxillofac Surg*. **43**, 1 (2014).
[18] H.U. Toygar, E. Guzeldemir, U. Cilasun, D. Akkor, N. Arpak, *J Biomed Mater Res B Appl Biomater*. **91**, 2 (2009).
[19] D.K. Christensen, I.K. Karoussis, A. Joss, C.H. Hammerle, N.P. Lang, *Clin Oral Implants Res*. **14**, 6 (2003).

[20] N.U. Zitzmann, R. Naef, P. Scharer, *Int J Oral Maxillofac Implants*. **12**, 6 (1997).
[21] V. Lekovic, E.B. Kenney, M. Weinlaender, T. Han, P. Klokkevold, M. Nedic, M. Orsini, *J Periodontol*. **68**, 6 (1997).
[22] H.A. Marouf, H.M. El-Guindi, *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. **89**, 2 (2000).
[23] B.K. Bartee, *Implant Dent*. **4**, 1 (1995).
[24] B.K. Bartee, J.A. Carr, *J Oral Implantol*. **21**, 2 (1995).
[25] M.N. Sela, D. Steinberg, A. Klinger, A.A. Krausz, D. Kohavi, *Clin Oral Implants Res*. **10**, 6 (1999).
[26] A.S. Monteiro, L.G. Macedo, N.L. Macedo, I. Balducci, *Med Oral Patol Oral Cir Bucal*. **15**, 2 (2010).
[27] N. Donos, L. Kostopoulos, T. Karring, *Clin Oral Implants Res*. **13**, 2 (2002).
[28] P.J. Louis, R. Gutta, N. Said-Al-Naief, A.A. Bartolucci, *J Oral Maxillofac Surg*. **66**, 2 (2008).
[29] A. Aurer, K. Jorgic-Srdjak, *Acta Stomatol Croat*. **39**, 1 (2005).
[30] D. Rothamel, F. Schwarz, M. Sager, M. Herten, A. Sculean, J. Becker, *Clin Oral Implants Res*. **16**, 3 (2005).
[31] T. Hockers, D. Abensur, P. Valentini, R. Legrand, C.H.F. Hämmerle, *Clin Oral Implants Res*. **10**, 6 (1999).
[32] G. Zellin, A. Gritli-linde, A. Linde, *Biomaterials*. **16**, 8 (1995).
[33] S. Pitaru, H. Tal, M. Soldinger, A. Grosskopf, M. Noff, *J Periodontol*. **59**, 6 (1988).
[34] M. Minabe, T. Kodama, T. Kogou, T. Tamura, T. Hori, Y. Watanabe, T. Miyata, *J Periodontol*. **60**, 1 (1989).
[35] T. Kodama, M. Minabe, T. Hori, Y. Watanabe, *J Periodontol*. **60**, 4 (1989).
[36] D.M. Verissimo, R.F. Leitao, R.A. Ribeiro, S.D. Figueiro, A.S. Sombra, J.C. Goes, G.A. Brito, *Acta Biomater*. **6**, 10 (2010).
[37] D. Rothamel, F. Schwarz, A. Sculean, M. Herten, W. Scherbaum, J. Becker, *Clin Oral Implants Res*. **15**, 4 (2004).
[38] H. Petite, V. Frei, A. Huc, D. Herbage, *J Biomed Mater Res*. **28**, 2 (1994).
[39] T. Takata, H.l. Wang, M. Miyauchi, *J Periodontal Res*. **36**, 5 (2001).
[40] T. Takata, H.L. Wang, M. Miyauchi, *Clin Oral Implants Res*. **12**, 4 (2001).
[41] E.J. Lee, S.H. Jun, H.E. Kim, Y.H. Koh, *J Biomed Mater Res A*. **100**, 4 (2012).
[42] E.J. Lee, S.H. Teng, T.S. Jang, P. Wang, S.W. Yook, H.E. Kim, Y.H. Koh, *Acta Biomater*. **6**, 9 (2010).
[43] J.F. Requicha, T. Moura, I.B. Leonor, T. Martins, F. Munoz, R.L. Reis, M.E. Gomes, C.A. Viegas, *J Orthop Res*. **32**, 7 (2014).
[44] A.C. van Leeuwen, J.J. Huddleston Slater, P.F. Gielkens, J.R. de Jong, D.W. Grijpma, R.R. Bos, *Acta Biomater*. **8**, 4 (2012).
[45] S.M. Kuo, S.J. Chang, G. Cheng-Chie Niu, C.-W. Lan, W.T. Cheng, C.Z. Yang, *J Appl Polym Sci*. **112**, 5 (2009).
[46] J. Mota, N. Yu, S.G. Caridade, G.M. Luz, M.E. Gomes, R.L. Reis, J.A. Jansen, X.F. Walboomers, J.F. Mano, *Acta Biomater*. **8**, 11 (2012).
[47] H. Hong, J. Wei, C. Liu, *Composites Part B* **38**, 3 (2007).
[48] A.F. Fraga, E.d.A. Filho, E.C.d.S. Rigo, A.O. Boschi, *Appl Surf Sci*. **257**, 9 (2011).
[49] A.I. Leal, S.G. Caridade, J. Ma, N. Yu, M.E. Gomes, R.L. Reis, J.A. Jansen, X.F. Walboomers, J.F. Mano, *Dent Mater*. **29**, 4 (2013).
[50] K. Fujihara, M. Kotaki, S. Ramakrishna, *Biomaterials*. **26**, 19 (2005).
[51] K. Ishikawa, Y. Ueyama, T. Mano, T. Koyama, K. Suzuki, T. Matsumura, *J Biomed Mater Res A*. **47**, 2 (1999).
[52] S.V. Madihally, H.W. Matthew, *Biomaterials*. **20**, 12 (1999).
[53] P.R. Klokkevold, L. Vandemark, E.B. Kenney, G.W. Bernard, *J Periodontol*. **67**, 11 (1996).
[54] S. Ming Kuo, S. Jen Chang, Y. Ting Hsu, T. Wei Chen, *Conf Proc IEEE Eng Med Biol Soc*. **5**, (2005).
[55] T.W. Chen, S.J. Chang, G.C.-C. Niu, Y.T. Hsu, S.M. Kuo, *J Appl Polym Sci*. **102**, 5 (2006).
[56] V. Gassling, T. Douglas, P.H. Warnke, Y. Acil, J. Wiltfang, S.T. Becker, *Clin Oral Implants Res*. **21**, 5 (2010).
[57] P. Babo, V.E. Santo, A.R.C. Duarte, C. Correia, M.H.G. Costa, J.F. Mano, R.L. Reis, M.E. Gomes, *Inflamm Regen*. **34**, 1 (2014).
[58] J. Choukroun, A. Diss, A. Simonpieri, M.O. Girard, C. Schoeffler, S.L. Dohan, A.J. Dohan, J. Mouhyi, D.M. Dohan, *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. **101**, 3 (2006).
[59] M. Matsui, Y. Tabata, *Acta Biomater*. **8**, 5 (2012).
[60] G. Mabilleau, E. Aguado, I.C. Stancu, C. Cincu, M.F. Basle, D. Chappard, *Biomaterials*. **29**, 11 (2008).
[61] K.A. Athanasiou, G.G. Niederauer, C.M. Agrawal, *Biomaterials*. **17**, 2 (1996).
[62] A. Stavropoulos, N. Mardas, F. Herrero, T. Karring, *J Clin Periodontol*. **31**, 11 (2004).
[63] Y. Kang, Y. Yao, G. Yin, Z. Huang, X. Liao, X. Xu, G. Zhao, *Med Eng Phys*. **31**, 5 (2009).
[64] J.F. Requicha, C.A. Viegas, F. Munoz, J.M. Azevedo, I.B. Leonor, R.L. Reis, M.E. Gomes, *Tissue Eng Part A*. **20**, 17-18 (2014).
[65] A. Piattelli, A. Scarano, P. Russo, S. Matarasso, *Biomaterials*. **17**, 8 (1996).
[66] H.D. Barber, J. Lignelli, B.M. Smith, B.K. Bartee, *J Oral Maxillofac Surg*. **65**, 4 (2007).
[67] T. Takata, M. Miyauchi, H.l. Wang, *Clinical Oral Implants Research*. **12**, 4 (2001).
[68] Y.K. Kim, S.G. Kim, S.C. Lim, H.J. Lee, P.Y. Yun, *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. **109**, 6 (2010).
[69] M.N. Sela, D. Kohavi, E. Krausz, D. Steinberg, G. Rosen, *Clin Oral Implants Res*. **14**, 3 (2003).
[70] S. Liao, W. Wang, M. Uo, S. Ohkawa, T. Akasaka, K. Tamura, F. Cui, F. Watari, *Biomaterials*. **26**, 36 (2005).
[71] S. Chen, Y. Hao, W. Cui, J. Chang, Y. Zhou, *J Mater Sci*. **48**, 19 (2013).
[72] S.-C. Lim, M.-J. Lee, H.-H. Yeo, *Pathol Int*. **50**, 8 (2000).
[73] H.l. Wang, M. Miyauchi, T. Takata, *J Periodontal Res*. **37**, 5 (2002).
[74] Q. Cheng, K. Rutledge, E. Jabbarzadeh, *Ann Biomed Eng*. **41**, 5 (2013)

[75] Z.-G. Zhang, Z.-H. Li, X.-Z. Mao, W.-C. Wang, *Cytotechnology*. **63**, 5 (2011).
[76] C.J. Frandsen, K. Noh, K.S. Brammer, G. Johnston, S. Jin, *Mater Sci Eng C Mater Biol Appl*. **33**, 5 (2013).
[77] J. Behring, R. Junker, X.F. Walboomers, B. Chessnut, J.A. Jansen, *Odontology*. **96**, 1 (2008).
[78] E. Lamers, J. te Riet, M. Domanski, R. Luttge, C.G. Figdor, J.G. Gardeniers, X.F. Walboomers, J.A. Jansen, *Eur Cell Mater*. **23**, (2012).
[79] M.J. Dalby, N. Gadegaard, R. Tare, A. Andar, M.O. Riehle, P. Herzyk, C.D.W. Wilkinson, R.O.C. Oreffo, *Nat Mater*. **6**, 12 (2007).
[80] E. Lamers, R. van Horssen, J. te Riet, F.C. van Delft, R. Luttge, X.F. Walboomers, J.A. Jansen, *Eur Cell Mater*. **20**, (2010).
[81] S. Srinivasan, R. Jayasree, K.P. Chennazhi, S.V. Nair, R. Jayakumar, *Carbohydrate Polymers*. **87**, 1 (2012).
[82] F.Z. Mei, J.; Yang, X.; Ouyang, X.; Zhang, S.;Hu, X.; Ma, Q.; Lu, J.; Ryu, S.; Deng, X. , *Biomacromolecules*. 8 (2007).
[83] V.E. Santo, M.E. Gomes, J.F. Mano, R.L. Reis, *J Tissue Eng Regen Med*. **6 Suppl 3**, (2012).
[84] P.A. Norowski, *J Biomater Nanobiotechnol*. **03**, 04 (2012).
[85] I. Ishikawa, T. Iwata, K. Washio, T. Okano, T. Nagasawa, K. Iwasaki, T. Ando, *Periodontol 2000*. **51**, 1 (2009).
[86] T. Iwata, K. Washio, T. Yoshida, I. Ishikawa, T. Ando, M. Yamato, T. Okano, *J Tissue Eng Regen Med*. (2013).
[87] T. Iwata, M. Yamato, H. Tsuchioka, R. Takagi, S. Mukobata, K. Washio, T. Okano, I. Ishikawa, *Biomaterials*. **30**, 14 (2009).
[88] J.F. Requicha, C.A. Viegas, F. Munoz, R.L. Reis, M.E. Gomes, *Anat Rec (Hoboken)*. **297**, 1 (2014).