DENTAL MATERIALS 29 (2013) 427–436

Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.intl.elsevierhealth.com/journals/dema

Asymmetric PDLLA membranes containing Bioglass® for guided tissue regeneration: Characterization and *in vitro* biological behavior

Ana I. Leal[a,b], *Sofia G. Caridade*[a,b], *Jinling Ma*[c], *Na Yu*[c], *Manuela E. Gomes*[a,b], *Rui L. Reis*[a,b], *John A. Jansen*[c], *X. Frank Walboomers*[c], *João F. Mano*[a,b,*]

[a] *3B's Research Group – Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Guimarães, Portugal*

[b] *ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal*

[c] *UMC St Radboud – Radboud University Medical Center, Department of Periodontology and Biomaterials, Nijmegen, The Netherlands*

ARTICLE INFO

Article history:
Received 22 May 2012
Received in revised form
10 January 2013
Accepted 22 January 2013

Keywords:
PDLLA
Bioglass®
Membrane
Periodontal ligament
Tissue engineering

ABSTRACT

Objective. In the treatment of periodontal defects, composite membranes might be applied to protect the injured area and simultaneously stimulate tissue regeneration. This work describes the development and characterization of poly(D,L-lactic acid)/Bioglass® (PDLLA/BG) composite membranes with asymmetric bioactivity. We hypothesized that the presence of BG microparticles could enhance structural and osteoconductivity performance of pure PDLLA membranes.

Methods. The membranes were prepared by an adjusted solvent casting method that promoted a non-uniform distribution of the inorganic component along the membrane thickness. *In vitro* bioactive behavior (precipitation of an apatite layer upon immersion in simulated body fluid, SBF), SEM observation, FTIR, swelling, weight loss and mechanical properties of the developed biomaterials were evaluated. Cell behavior on the membranes was assessed using both human bone marrow stromal cells and human periodontal ligament cells.

Results. Just the BG rich face of the composite membranes induced the precipitation of bone-like apatite in SBF, indicating that this biomaterial exhibit asymmetric osteoconductive properties. SEM images, DNA content and metabolic activity quantification revealed an improved cell adhesion and proliferation on the composite membranes. Composite membranes also stimulated cell differentiation, mineralization, and production of extracellular matrix and calcium nodules, suggesting the positive effect of adding the bioactive microparticles in the PDLLA matrix.

Significance. The results indicate that the proposed asymmetric PDLLA/BG membranes could have potential to be used in guided tissue regeneration therapies or in orthopedic applications, with improved outcomes.

* *Corresponding author at*: 3B's Research Group – Biomaterials, Biodegradables and Biomimetics, AvePark, Zona Industrial da Gandra, S. Cláudio do Barco, 4806-909 Caldas das Taipas, Guimarães, Portugal. Tel.: +351 253510900; fax: +351 253510909.
E-mail address: jmano@dep.uminho.pt (J.F. Mano).

0109-5641/$ – see front matter © 2013 Academy of Dental Materials. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.dental.2013.01.009

1. Introduction

Periodontitis is a disease that destroys the tooth-supporting tissues, including the alveolar bone, periodontal ligament (PDL) and cementum. This is the major cause of tooth loss in human adults [1]. The treatment of periodontal defects can be a complex process which may require surgery, but as a rule does not result in surgical intervention. However, periodontal defects, if left empty after open flap debridement, are filled with epithelial and fibroblasts, which are the first cells to reach the defect area, generating a core of fibro-epithelial tissues that does prevent the occurrence of an adequate regeneration process of the periodontal tissues [2]. In this context, Guided Tissue Regeneration (GTR) strategies consist in the application of a membrane that acts as a physical barrier to protect the defect site, preventing the epithelial cells, fibrous and gingival connective tissues to reach the injured area. The creation of segregated space for the invasion of blood vessels and osteoprogenitor cells protects against the growth of non-osteogenic tissues. This procedure favors the regeneration of lost and damaged tissue since it promotes cell repopulation of the periodontal ligament and adjacent alveolar bone. However, acting solely as physical barriers is a limitation on the clinical effect of these membranes: they provide no osteoconductive effects, enabling only minor contributions for new cementum and bone formation, which, by definition, is not true periodontal tissue regeneration [3]. Each side of an implanted membrane is in contact with a distinct biological environment, in which the osseointegration should be ideally promoted just in one of the faces. Nevertheless, this asymmetric bioactive behavior is almost inexistent in currently used GTR membranes and represents a possible challenge toward the development of innovative systems for the regeneration of periodontal tissues. GTR membranes can be obtained from natural or synthetic materials, either bioabsorbable or nonresorbable. Degradability is one of the most important requirements for GTR membranes and intends to avoid second surgical removing procedure. Natural resorbable collagen membranes have been widely used, not just because collagen is concretely one of the components of the alveolar bone and periodontal ligament but also because this material meets almost all the criteria required [4]. Collagen, however, presents some drawbacks such as its cytoxicity and xenogenic origin, poor mechanical strength and fast biodegradation by enzymatic activity [4,5]. In order to avoid these undesirable characteristics, maintaining the desirable ones, synthetic materials have been more frequently used, predominantly those from the poly(α-hydroxyesters) family [6]. The chemical properties of these polymers allow its hydrolytic degradation and the elimination of the resulting products by natural pathways [7]. Moreover their processing is easy compared to other polymers and the variety of existent molecular weights and copolymers permits a wide range of physical, mechanical and degradation rate related adjustments. Poly(D,L-lactic acid), PDLLA, is an amorphous polymer, with interesting mechanical properties and with degradation times in the order of 12–16 months [8]. It exhibits excellent biocompatibility *in vivo*, high mechanical stability and the possibility to be combined with drugs [9–11]. Nevertheless, PDLLA is not osteoconductive. Among different strategies that could be used to improve bioactivity in polymeric systems [12], the combination of osteoconductive inorganic particles has been widely used [7]. Bioglass® is a well known bioactive ceramic and has the ability to enhance the osteoblast activity and attachment between the biomaterial and the surrounding bone tissue, possibiliting the bone growth on the materials surface. Furthermore its dissolution products can control the gene expression in order to control the osteogenesis and consequently the production of growth factors [8], as well as counteracting the acidic degradation of the poly(α-hydroxyesters) providing a pH buffering effect [13,14].

In this work, Bioglass® microparticles were compounded with PDLLA to form a membrane using a solvent casting methodology. The conditions were optimized for the preparation of membranes exhibiting preferentially the BG in one of the sides of the membrane. It is envisioned that, upon implantation, the membrane side richer in BG could be faced to the defect side in which bone ingrowth should be stimulated while the more hydrophobic PDLLA rich side should act mainly as a barrier to avoid the invasion of soft tissue. Some relevant properties of the developed membranes were characterized and their biological performance was assessed, using two distinct cell types: human bone marrow stromal cells (hBMSC) and human periodontal ligament cells (hPDL).

2. Materials and methods

2.1. Materials

Poly(D,L-lactic acid) (PDLLA), (M_n = 31,750 and M_w = 100,000) with an inherent viscosity of 1.87 dL/g was purchased from Purasorb® (PURAC Biochem, The Netherlands) and was used as received. The 45S5 Bioglass®, with the composition: 45 SiO_2, 24.5 CaO, 24.5 Na_2O and 6.0 P_2O_5 in wt%, was supplied by US Biomaterials Corp. (Florida, USA). The particle size of the Bioglass® particles (BG), measured by laser scattering analysis (Coulter LS 100 particle size analyzer, Coulter, USA), was found to be lower than 20 μm. All the other reagents and solvents used were of reagent grade and were used without further purification.

2.2. Preparation of PDLLA and PDLLA/Bioglass® membranes

All PDLLA membranes were prepared based on a solvent casting technique. The PDLLA films were prepared by dissolving 0.50 g of PDLLA in 30 mL of chloroform. After total dissolution, the solution was transferred to a Petri dish with 9 cm of diameter and covered with an aluminum sheet. The Petri dish was settled in a horizontal position to facilitate the formation of a cast film with uniform thickness. The assembly was kept in a hood for 24 h, and chloroform was allowed to evaporate at a very slow rate. Then, the films were vacuum dried for 48 h at 40 °C.

The PDLLA/BG membranes were prepared in the exact same process as the pure PDLLA membranes. The PDLLA/BG dispersions were prepared by dissolving 0.40 g of PDLLA in 30 mL of chloroform. After total dissolution, 0.10 g of Bioglass®