Acta Biomaterialia 23 (2015) 317–328

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Freeze gelated porous membranes for periodontal tissue regeneration

Saad B. Qasim [a], Robin M. Delaine-Smith [b], Tobias Fey [c], Andrew Rawlinson [d], Ihtesham Ur Rehman [a,*]

[a] Materials Science and Engineering Department, Kroto Research Institute, University of Sheffield, Sheffield S3 7HQ, United Kingdom
[b] Institute of Bioengineering, School of Engineering and Materials Science, Queen Mary University of London, Mile End Road, E1 4NS London, United Kingdom
[c] Department of Materials Science (Glass and Ceramics), University of Erlangen-Nuernberg, Martensstr. 5, 91058 Erlangen, Germany
[d] Academic Unit of Restorative Dentistry, School of Clinical Dentistry, University of Sheffield, Sheffield S10 2SZ, United Kingdom

ARTICLE INFO

Article history:
Received 11 January 2015
Received in revised form 2 April 2015
Accepted 4 May 2015
Available online 9 May 2015

Keywords:
Ascorbic acid
Guided tissue regeneration
Resorbable
Bioactivity
Osteoblasts

ABSTRACT

Guided tissue regeneration (GTR) membranes have been used for the management of destructive forms of periodontal disease as a means of aiding regeneration of lost supporting tissues, including the alveolar bone, cementum, gingiva and periodontal ligaments (PDL). Currently available GTR membranes are either non-biodegradable, requiring a second surgery for removal, or biodegradable. The mechanical and biofunctional limitations of currently available membranes result in a limited and unpredictable treatment outcome in terms of periodontal tissue regeneration. In this study, porous membranes of chitosan (CH) were fabricated with or without hydroxyapatite (HA) using the simple technique of freeze gelation (FG) via two different solvents systems, acetic acid (ACa) or ascorbic acid (ASa). The aim was to prepare porous membranes to be used for GTR to improve periodontal regeneration. FG membranes were characterized for ultra-structural morphology, physiochemical properties, water uptake, degradation, mechanical properties, and biocompatibility with mature and progenitor osteogenic cells. Fourier transform infrared (FTIR) spectroscopy confirmed the presence of hydroxyapatite and its interaction with chitosan. μCT analysis showed membranes had 85–77% porosity. Mechanical properties and degradation rate were affected by solvent type and the presence of hydroxyapatite. Culture of human osteosarcoma cells (MG63) and human embryonic stem cell-derived mesenchymal progenitors (hES-MPs) showed that all membranes supported cell proliferation and long term matrix deposition was supported by HA incorporated membranes. These CH and HA composite membranes show their potential use for GTR applications in periodontal lesions and in addition FG membranes could be further tuned to achieve characteristics desirable of a GTR membrane for periodontal regeneration.

1. Introduction

Destructive forms of periodontal disease such as chronic periodontitis affect the supporting tissues of teeth causing loss of gingival tissue, connective tissue, alveolar bone and periodontal ligaments. Initial treatment of these diseases includes the elimination of the primary causative factor (the dental plaque biofilm) by effective patient performed oral hygiene procedures and non-surgical treatment provided by a dentist or hygienist. While treatment usually halts disease progression, healing is characterized by repair of affected tissues with a long junctional epithelium, bone remodeling, and limited regeneration of the cementum and the lost periodontal ligaments that normally attach the tooth to the alveolar bone.

For these reasons, there has been much interest in developing methods for enhancing the regeneration of lost tissues in order to restore dental function and esthetics. This has been met with limited success using biologically active agents and guided tissue regenerative (GTR) or guided bone regeneration (GBR) membranes [1,2]. The ideal requirements for a GTR membrane include; a cell isolating occlusive biomaterial which meets minimum mechanical, physical, structural and biocompatibility requirements; ability to support organized and vascularized ingrowth and wound stabilization; protecting the underlying blood clot and thereby limiting the epithelial and unwanted connective tissue growth into the defect; promoting functional tissue regeneration from the relevant cells in the defect (avoiding healing by repair); and degrading in adequate time to provide space for newly formed periodontal tissue. The membrane surface facing the soft tissue should support cell attachment, growth and differentiation while the surface facing the defect acts as a biological seal [3].

A number of resorbable GTR/GBR membranes have now replaced the conventional non-resorbable membrane (expanded

* Corresponding author at: The Kroto Research Institute, North Campus, University of Sheffield, Broad Lane, Sheffield S3 7HQ, United Kingdom. Tel.: +44 (0) 114 222 5946; fax: +44 (0) 114 222 5943.
E-mail address: i.u.rehman@sheffield.ac.uk (I.U. Rehman).

http://dx.doi.org/10.1016/j.actbio.2015.05.001
1742-7061/

polytetraflouroethylene), which required a second stage surgical intervention to remove, resulting in damage to the newly formed tissue [4]. Many of the current resorbable membranes are based on the use of synthetic polyesters, such as, poly (lactic and glycolic acid) or polycaprolactone. Although these membranes have adequate mechanical and degradation profiles, they lack bioactivity [5,6]. The use of naturally occurring biopolymers, such as, collagen and chitosan (CH) has also been explored. Collagen based materials have shown favorable results due to excellent biocompatibility, however, there is batch variability and lack of control over the resorption rate which is a concern for clinicians. Moreover, the use of membranes derived from animal tissues is associated with the risk of disease transmission and has also raised certain ethical and cultural issues [7–9]. Using a functionally graded approach to fabricate a biomimetic GTR membrane has been proposed by Bottino et al. [2], which has the potential to fulfill all ideal GTR membrane criteria.

The use of CH as a potential biomaterial for tissue engineering and regenerative medicine has been investigated during the past 20 years. CH is produced by deacetylation of chitin, which is the second most abundant naturally occurring polysaccharide in nature. CH is a linear polysaccharide copolymer of β-(1-4) linked D-glucosamine and N-acetylated-D-glucosamine making up deacetylated and acetylated regions respectively [8,10–12]. The amide groups on the polysaccharide chain of CH can be positively charged and solubilized when the solution pH is below 6, hence becoming a polycationic polymer [13,14]. It has excellent biocompatibility, antimicrobial and wound healing potential as well as hemostatic properties and it has found popular use in the management of burns [15]. Hence, CH is an attractive biomaterial for future use in fabricating functionally graded GTR/GBR membranes [8].

The uses of bioceramic materials alone and as composites with biopolymers have revolutionalized the field of regenerative medicine. Synthetic HA has found wide use in tissue engineering applications due to its ability to mimic the natural inorganic bone component showing popular use in composites with other natural and synthetic polymers. The main advantages of HA are its good osteoinductive, osteoconductive properties and excellent biocompatibility [16]. However, its brittleness makes it difficult to shape or bind together. HA composites also impart the formation of a biologically active carbonated HA layer on their surface which is structurally and chemically similar in properties to the mineral phase of bone and enhances interfacial bonding in between tissues and biomaterials [17–19]. Composites of HA have shown to encourage bone cell attachment and proliferation as well as increased mechanical properties compared to the individual components [17,19]. CH can be molded into various patterns of fibers, thin films, and porous structures [20].

Numerous techniques have been used for the fabrication of porous cell-supporting membranes such as particulate leaching, phase inversion and freeze drying. However; drawbacks include difficulty in controlling the pore size, low interconnectivity, and residual salt and skin formation. Another method reported by Ho et al. [20] is freeze gelation (FG). This involves freezing a polymer solution to create frozen solvent and concentrated polymer phases, this phase separation mechanism being referred as solid–liquid demixing [20]. The solvent is extracted by a non solvent and the remaining space becomes porous, resulting in a polymer membrane. Various pore structures and morphologies can be achieved by varying the cooling rate, adjusting the polymer concentration, and changing the solvent system [20,21]. The technique of freeze gelation has also been reported to offer a more convenient, time and energy-efficient method to fabricating porous membranes compared with freeze-drying and offers an easy to scale up process [21]. The freezing process can be carried out in a more controlled manner to orient the growth of ice crystals in a particular direction [22,23]. More recently Park et al. have used directional freeze-casting with gelatine to mimic topographies with angular similarities of the alveolar crest and natural orientation of periodontal ligaments [24].

In this study, porous FG membranes of CH and CH–HA composites were prepared using two different solvents, ascorbic acid (ASa) and acetic acid (ACa). The preparation and biophysiochemical properties of these porous membranes are herein described with detailed characterization using scanning electron microscopy (SEM), micro computerized tomography (μCT), Fourier transform infrared spectroscopy (FTIR), swelling analysis, degradation studies and tensile testing. Membranes were also examined for their ability to support bone cell growth and matrix deposition by osteogeneic progenitor cells.

2. Materials and methods

2.1. Membrane fabrication

Chitosan (75–85% deacetylated, Sigma Aldrich, UK) having a molecular weight of 190–310 kDa was dissolved in 0.2 M L-ascorbic acid (ASa) (99.9%, Sigma Aldrich, UK) or 0.2 M acetic acid (ACa) (Fisher Scientific, UK) to create 3% w/v solutions with or without HA. This solution was kept stirring for 6 h before adding HA. HA was of medical grade, Captal S™ obtained from Plasma Biotal Limited, UK, (Batch No. P220), incorporated in a ratio of 50:50. After addition of HA, solutions were stirred for 12 h at room temperature and then cast into a petri dish before storing at 4 °C for 3 h and then at −20 °C for 12 h. A solution of Sodium hydroxide 3 M in 100% ethanol in a ratio of 1:1 (v/v) was pre-cooled to −20 °C and used to submerge frozen membranes for 12 h. Membranes were then washed with PBS (Oxoid PBS Tablets, used with distilled water to obtain a 100 mM solution, UK) to elude the remaining neutralizing solution and specimens were dried using a series of ethanol washes (70%, 80%, 90%, 95% and 100%) for 15 min each. After this the samples were immersed in a solution of glycerol (Fisher Scientific. UK) and distilled water in a ratio of 1:10 for 15 min and removed to air dry and stored at room temperature. In total four different membranes were fabricated denoted as ASa-CH, ASa-CH:HA, ACa-CH and ACa-CH:HA. Graphical illustration of a tri-layered GTR membrane is presented in Fig. 1.

2.2. Scanning electron microscopy (SEM)

SEM was employed to study the surface and cross sectional morphology of FG membranes (spot size: 3.0, voltage range 5–10 kV, Philips X-L 20 microscope). Samples were mounted on aluminum stubs with double-sided carbon adhesive dots and were sputter coated under vacuum with carbon using Speedivac carbon coating unit (Model 12E6/1598). Image J (National Institute of Health) software was used for measuring the pore sizes from the cross sectional images. The pore size/cell size and strut thickness were calculated using μCT (Skyscan 1172, Skyscan B.V., Koentich, Belgien). The X-ray tube was equipped with a tungsten target and operated at a voltage of 80 kV and a current of 100 μA. Exposure time per slice was 665 ms. Specimens were rotated through 360°, with one step per degree.

2.3. Fourier transform infrared (FTIR) spectroscopy

FTIR spectra of CH:HA membranes were obtained using a Thermo Nicolet iS50™ FTIR (Thermo Fisher Scientific Inc, USA) spectrophotometer in conjunction with a MTech Photo-Acoustic (PAS) sampling cell to allow analysis of neat samples without