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Triple-layered PLGA/nanoapatite/lauric acid graded composite membrane for periodontal guided bone regeneration

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ABSTRACT

This paper discusses the successful fabrication of a novel triple-layered poly(lactic-*co*-glycolic acid) (PLGA)-based composite membrane using only a single step that combines the techniques of solvent casting and thermally induced phase separation/solvent leaching. The resulting graded membrane consists of a small pore size layer-1 containing 10 wt% non-stoichiometric nanoapatite (NAp) + 1–3 wt% lauric acid (LA) for fibroblastic cell and bacterial inhibition, an intermediate layer-2 with 20–50 wt% NAp + 1 wt% LA, and a large pore size layer-3 containing 30–100 wt% NAp without LA to allow bone cell growth. The synergic effects of 10–30 wt% NAp and 1 wt% LA in the membrane demonstrated higher tensile strength (0.61 MPa) and a more elastic behavior (16.1% elongation at break) in 3 wt% LA added membrane compared with the pure PLGA (0.49 MPa, 9.1%). The addition of LA resulted in a remarkable plasticizing effect on PLGA at 3 wt% due to weak intermolecular interactions in PLGA. The pure and composite PLGA membranes had good cell viability toward human skin fibroblast, regardless of LA and NAp contents.

1. Introduction

Guided bone regeneration (GBR) has been established as a reliable therapeutic procedure for the treatment of bony defects in dental implantology as well as in other skeletal locations [1,2]. It is a surgical technique that aids the regeneration of lost bone tissues in periodontal defects [3]. In GBR, a barrier membrane is used to prevent fibroblastic cells from colonizing an intraosseous wound during healing, allowing slowly migrating bone cells to fill the defect, resulting in direct bone regeneration [2].

Periodontitis is a periodontal disease that affects the integrity of the periodontal system and leads to damaged periodontal tissues, such as connective tissues, bone support and eventually, tooth loss [4].

The conventional way of treating periodontitis by scaling and root planning is accompanied by the adjuvant administration of antibiotics [5]. The presence of oral pathogens such as *P. gingivalis* and *P. intermedia* may influence the success of periodontal regeneration in a negative manner. Therefore, there is a need for localized release of adjunctive antimicrobial agents in the GBR membrane to control and minimize the bacterial contamination of the periodontal defect in order to enhance periodontal regeneration [6,7]. It is advantageous to have a biodegradable sustained release antimicrobial agent delivery system that can be positioned into the periodontal pocket and maintain therapeutic concentrations for prolonged periods of time [8].

Maintaining barrier membrane integrity for at least 6 months in GBR procedures is important for new bone regeneration in membrane protected defects [3]. Furthermore, the ideal membrane should possess space-making properties, cell-occlusiveness, and clinical manageability [9]. Preferable surfaces are those that are compatible with osteoblast proliferation and migration for accelerated bone formation in the defect space. Finally, the membranes should be able to act as a localized controlled release system for antibiotic drugs [7]. For practical use, a GBR membrane design must utilize a compositionally graded structure with multiple compartments in order to meet the above requirements [10,11]. The high mechanical properties of GBR membranes are important for avoiding the deformation of the membrane and the collapse of bone regeneration space [12]. The GBR membrane is designed to have a smooth surface on one face to inhibit soft tissue penetration, which may prevent or delay bone formation, while the opposite porous face is capable of accommodating bone tissue ingrowth in vivo [11]. A membrane that is difficult to use because it is too malleable or stiff will often lead to complications in clinical reproducibility and it is not easily contoured, eventually leading to the exposure of the membrane [9].

Synthetic resorbable membranes have widespread uses in clinical medicine. However, inflammatory reactions due to the accumulation of acidic degradation products in poly(lactic acid) or poly(glycolic acid) membranes have been reported [3,13,14]. The abrupt release of these acidic degradation products triggers inflammatory and foreign body reactions *in vivo* [13,15]. Thus, the development of a membrane

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based on biocompatible synthetic materials for human use is highly anticipated [14].

Many researchers have attempted to develop a periodontal membrane with the required features [16–18]. Some studies have incorporated calcium phosphate (CaP) particles or therapeutic drugs [5,7,10,16,19,20]. CaP based materials are biocompatible, bioactive and osteoconductive, which enhances cell adhesion, proliferation, and metabolic activation [21,22]. These studies have demonstrated improved mechanical integrity, good control over the degradation rate of the membrane and the sustained release of drugs. In addition, the basic nature of apatites neutralized the acidic degradation products from the polymers using ionic interactions [10,16,23]. This emphasizes the need for polymer/ceramic composite materials that can combine the advantage of both materials. The incorporation of lauric acid (LA) is indispensable for imparting antimicrobial properties to the membrane, aimed to act against bacteria that can cause periodontal diseases in the oral cavity. LA is biodegradable and has the potential to inhibit bacteria at very low concentrations (ppm), which diminishes the toxic effects *in vitro* and is proven to be metabolized into omega hydroxylates by human liver microsomes [24–26]. It is likely that LA kills gram-positive bacterium by separating their inner and outer membranes, resulting in the cytoplasmic disorganization of the bacterium [27]. It is hypothesized that the incorporation of LA in GBR barrier membranes could possibly exhibit antibacterial activity.

GBR membranes are prepared through solvent casting [11,19], electrospinning [12,17,28,29], or dynamic filtration [18,30] using synthetic (e.g. poly(lactic-co-glycolic acid) (PLGA) and natural (e.g. chitosan) polymers. In this particular work, a new combination of modified solvent casting and thermally induced phase separation (TIPS)/solvent leaching techniques were employed to fabricate a triple layered composite PLGA membrane in a single step [31,32]. One of the most attractive characteristics of TIPS over other scaffold fabrication techniques is the formation of an interconnected porous space in one simple process that is scalable, fast, energy saving, and controllable [32,33]. Moreover, the ease of preparation and operation of solvent casting techniques without the need for specialized equipment is an added advantage in the preparation of GBR composite membranes [34].

In this study, a novel triple-layered PLGA/NAp/LA composite membrane was fabricated in a single step using new combination technique of solvent casting/thermally induced phase separation/solvent leaching. The gradient morphology of the layered membrane, cross-view in the presence of various nanoapatite (NAp) and LA contents in its composite phase were evaluated using scanning electron microscopy (SEM) and X-ray diffraction (XRD). The synergic effect of NAp and LA additions on mechanical strength and the elastic behavior of the PLGA membrane were determined by conducting tensile tests and revealed for the first time in this study. Finally, an *in vitro* cell viability test was conducted to confirm the favorable effect of leachable additives. Antimicrobial efficacy studies on LA added membranes were not included in this report.

2. Materials and methods

2.1. Materials

The starting materials included PLGA with a lactic to glycolic ratio of 85:15 and inherent viscosity of 0.55–0.75 dl/g in chloroform (Durect, LACTEL Absorbable Polymers, US), LA with 98% purity (Sigma, US), and NAp with composition of 40.08 wt% Ca, 18.58 wt% P, 1.84 wt% Na, 1.46 wt% Mg, 0.06 wt% K and 4.80 wt% CO_3^{2-}, and Ca/P ratio of 1.67 that was synthesized using the reaction method discussed in our previous study [35]. Dimethyl sulfoxide (DMSO, Fisher Scientific, US) was used as the solvent for both PLGA and LA when fabricating the membrane. The 85:15 ratio was intentionally used in this study due to its optimal degradation characteristics over 2 to 6 months, which made it a suitable candidate for GBR purposes [7].

2.2. Membrane fabrication

The graded triple-layered membranes were fabricated via solvent casting and thermally induced phase separation/solvent leaching techniques. It involved mixing PLGA and LA by dissolving in DMSO at final PLGA concentrations of 7, 9, 11, 13, 15, 17 and 20% (wt/wt) and 1–3% (wt/wt) of LA. The solutions were vigorously stirred until all components had completely dissolved and the solutions were visually clear. The NAp powder was mixed in the solution in the range of 10–100 wt% from the amount of PLGA used and sonicated for 30 seconds to 3 minutes. A total of three separate PLGA solutions were prepared by dissolving PLGA in DMSO for layer 1 (L1), layer 2 (L2) and layer 3 (L3) with graded composition of NAp and LA contents. LA and NAp were added to L1 and L2, whereas only NAp was added to L3 and ultrasonically mixed. The mixed solution of L1 was cast into a glass petri dish and frozen at −18 °C prior to the layering of L2. Similarly, L2 was quickly spread on the frozen L1 and frozen again at −18 °C. Finally, L3 was poured on L2 and subjected to prolonged freezing at −18 °C for 24 hours.

After the 24 hour period, the solidified triple-layered membranes in the petri dish were immediately immersed in pre-cooled water at 4 °C to leach out DMSO from the frozen polymer. Initially, the solvent leaching was carried out for 2 hours by replacing the pre-cooled (4 °C) water every hour. After the first hour, the top surface of the membrane was immediately precipitated by contact with water (non-solvent for PLGA), leaching out DMSO (solvent for DMSO) and then the sublayer was continuously precipitated by the diffusion of water into the phase separated PLGA-DMSO solids. In the second hour, the precipitated membrane was easily separated from the petri dish and transferred into fresh pre-cooled (4 °C) water for 1 hour and subsequently immersed in fresh pre-cooled (4 °C) water for 24 hours to ensure that the DMSO was completely removed. Finally, the triple-layered membrane was taken out of the water and dried at room temperature for 3 days in room air. A triple-layered pure PLGA membrane without NAp and LA additions was fabricated as a control without performing ultrasonication. The compositions and layering time of all the membranes are listed in Tables 1 and 2.

2.3. Characterization

2.3.1. Morphological and chemical characterization

The surface morphology of all the membranes was characterized by the means of variable pressure SEM (VPSEM; ZEISS, EVO LS10, UK). Prior to this examination, the membranes were coated with Pt using a sputter coater to prevent charging of the membrane surfaces in the SEM. A cross-sectional view of the triple-layered structure was obtained by sectioning the membrane along the vertical axis of the stacked layers followed by imaging a vertically tilted specimen. The phases of the membranes were determined and recorded using an XRD (Bruker D8 Advance, Germany) at ambient temperature using Ni-filtered Cu-Kα radiation ($\lambda = 0.15406$ nm). The data collected was in the range of 10°–80° (2θ), with a step of 0.02° and a scanning rate of 1.2° per minute. The membranes were mounted on an analytical cylindrical sample holder to obtain a flat upper surface. Then, both the L1 and L3 surfaces were characterized by analyzing the characteristic peaks of the present phases. Infra red (IR) spectra of the membranes were obtained by attenuated total reflectance (ATR) technique using a Fourier transform IR spectrometry (FTIR; Spectrum 2000, Perkin Elmer, USA). A piece of membrane (2 x 2 mm^2) was placed onto the ATR sample holder and pressed down to ensure contact. All spectra were collected between 600 to 4000 cm^{-1} wavenumber region with 4 cm^{-1} resolution and 16 scans.

2.3.2. Mechanical evaluation

The mechanical properties of the membranes were evaluated by uniaxial tensile testing using a universal testing machine (Tinius Olsen, H5KS,