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Development of an electrospun nano-apatite/PCL composite membrane for GTR/GBR application

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Abstract

In dental practice, membranes are used as a barrier to prevent soft tissue ingrowth and create space for slowly regenerating periodontal and bony tissues. The aim of this study was to develop a biodegradable membrane system which can be used for guided tissue or bone regeneration. Three types of composite fibrous membranes based on nano-apatite (nAp) and poly(ε-caprolactone) (PCL) were made by electrospinning, i.e. n0 (nAp:PCL = 0:100), n25 (nAp:PCL = 25:100) and n50 (nAp:PCL = 50:100) with average fiber diameters ranging from 320 to 430 nm. Their structural, mechanical, chemical and biological properties were evaluated. Tensile test revealed that n25 had the highest strength and toughness, indicating there is an optimal ratio of nAp to polymer for mechanical reinforcement. Subsequently, a simulated body fluid immersion test confirmed that the presence of nAp enhanced the bioactive behavior of the membranes. Finally, an in vitro osteoblast cell study showed that all membranes supported proliferation, but the presence of nAp facilitated an early cell differentiation. This study demonstrated that an electrospun membrane incorporating nAp is strong, enhances bioactivity and supports osteoblast-like cell proliferation and differentiation. The membrane system can be used as a prototype for the further development of an optimal membrane for clinical use.

Keywords: Nano-apatite; Poly(ε-caprolactone); Electrospinning; Rat bone marrow cells; Guided tissue regeneration

1. Introduction

In dentistry the concept of guided tissue regeneration (GTR) is frequently applied in reconstruction of periodontal defects, i.e. an occlusive membrane is used to prevent the faster-growing connective tissue from migrating into the defect and to allow time for periodontal ligament, cementum and bone to reconstruct [1]. Similar to GTR, the concept of guided bone regeneration (GBR) is applied at dental implant sites where a membrane is used to cover the bone defect to encourage new bone ingrowth while preventing the ingrowth of fibrous tissue into the grafted site [2,3].

In general, the commercially available GTR/GBR membranes are made of polymers, including non-degradable polytetrafluoroethylene (PTFE: Gore-Tex® (W.L. Gore & Associates, Inc., Elkton, MD, USA)) and TefGen® (Lifecore Biomedical, LLC, Chaska, MN, USA), biodegradable polylactide (PLA), polyglycolide (PGA), polycarbonate and collagen (Inion®, Inion Oy, Tampere, Finland), Guidor® (Guidor AB, Huddinge, Sweden) and BioGide® (Geistlich Pharma AG, Wolhusen, Switzerland)). Although present polymeric products show positive results in clinical studies, their weak mechanical properties and poor bone regeneration capacity are still major challenges. To overcome these problems, recent research efforts have included the incorporation of bone-like ceramics into the membranes, e.g. hydroxyapatite, tricalcium phosphate and calcium carbonate [4–6]. In these efforts, nano-sized ceramic

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particles are of particular interest as they mimic the mineral crystals as present in the natural tissue and have been shown to induce a significant increase in protein absorption and cell adhesion, compared to their micro-sized counterparts [7,8].

Although numerous membrane materials have been investigated, few studies have focused on the technique of membrane preparation. So far, most GTR/GBR membranes are made in the shape of porous foam, created by traditional methods such as particulate leaching, solvent casting or gas foaming [9,5]. Recently, a new technique has been introduced, which is called electrospinning, and allows the preparation of thin fibrous membranes [6,10]. Electrospinning makes use of a high electric voltage to draw polymer solutions/melts into a whipped jet, which becomes ultrafine fibers after drying in air [11]. Fibers obtained from electrospinning are in the range of 50 nm to a few microns in diameter and generally collected in the form of a non-woven structure. It has already been shown that electrospun membranes have the potential to promote osteoblastic cell function and bone regeneration [11,12]. More importantly, the pore size of the electrospun membranes in general is less than the average cell size, and previous studies have shown that such small pores do not allow cell penetration [13,14].

In view of the above mentioned, the aim of the current study was to develop a composite GTR/GBR membrane containing nano-apatite (nAp) particles and biodegradable poly(ε-caprolactone) (PCL) fibers by electrospinning, and to test the mechanical properties, bioactivity and osteoblast-like cellular behavior in vitro. In order to study the effect of nAp, three membranes with different nAp content were chosen. Our hypothesis was that electrospinning is a suitable method to fabricate GTR/GBR membranes; and that the addition of nAp improves the properties of polymeric membranes in the aforementioned terms, especially the mechanical properties and the bioactive behavior.

2. Materials and methods

2.1. Preparation of nano-apatite particles

Nano-apatite (nAp) was synthesized following a precipitation reaction. In brief, 0.3 M orthophosphoric acid (H_3PO_4) (Acros Organics, Geel, Belgium) was added drop-wise to a 0.5 M calcium hydroxide ($Ca(OH)_2$) (Mallinckrodt Baker, Phillipsburg, NJ, USA) suspension under continuous stirring overnight at room temperature, while the pH was kept above 10.5 by the addition of ammonia solution. Then, the obtained precipitate was aged for one week at room temperature and centrifugal washing with deionized water three times. After each washing step, an ULTRA-TURRAX® homogenizer (T25, IKA® Werke, Staufen, Germany) was used to re-suspend the particles. Finally, the resultant was freeze-dried to obtain very fine particles, thereafter named nAp.

To observe the morphology of the obtained particles, one drop of an aqueous dispersion of nAp was put on a Formvar-coated copper grid and air-dried at room temperature. The dried grid was examined by transmission electron microscopy (TEM; JEOL 1101, Tokyo, Japan). The crystallographic structure of the particles was verified by X-ray diffraction (XRD) using a θ–2θ X-ray diffractometer (PW3710, Philips, Eindhoven, The Netherlands) with Cu K_α radiation (45 kV, 40 mA). The scanning range was from 20 to 40° with a step size of 0.02°. The chemical structure of the particles was examined using Fourier transform infrared spectrometry (FTIR, Spectrum One, Perkin-Elmer, Waltham, MA, USA) with an attenuated total reflection (ATR) accessory.

2.2. Fabrication of membranes

Membranes were fabricated using the electrospinning technique. The electrospinning dope without nAp was prepared from poly(ε-caprolactone) (PCL, $M_n = 80{,}000$, Sigma). PCL was dissolved in 80% 2,2,2-trifluroethanol (TFE) (Acros Organics, Geel, Belgium) in deionized H_2O at a concentration of 12% w/v. For the electrospinning dope containing nAp, a defined amount of nAp was suspended in the solvent by ultrasonic and vigorous stirring before adding the polymers. Dioctyl sulfosuccinate sodium salt (0.05% w/v) was used as a surfactant and dissolved in the solvent to obtain stable particle suspension in the polymer solution. Three types of membranes were prepared and named n0, n25 and n50 based on the amount of nAp in the membranes. Their detailed composition is listed in Table 1. Note that the solvent for n0 was 80% TFE in phosphate buffer saline (PBS) as a preliminary study showed that one could not obtain uniformed fibrous structure when using 80% TFE in H_2O as solvent.

A commercially available electrospinning set-up (Advanced Surface Technology, Bleiswijk, The Netherlands) was used for the scaffold fabrication, as described previously [15]. Briefly, 10 ml of the prepared dope was fed into a syringe, which was controlled by a syringe pump (KD Scientific Inc., Holliston, MA, USA) at a feeding rate of 2 ml h^{-1}. A Teflon tube was used to connect the syringe and a blunt-end nozzle with an inner diameter of 0.5 mm, which was set up vertically. The distance between the nozzle and a grounded collector was adjusted to 12 cm. A high voltage of 18–22 kV was applied to generate a polymer jet. On the basis of a pilot study, the processing parameters were chosen such that uniform fibrous structures were formed. The resulting fibers were collected on a rotating mandrel, left in vacuum conditions overnight to eliminate solvent residues and then kept in a desiccator for further experimentation.

The morphology of the fabricated membranes was observed by scanning electron microscopy (SEM; JEOL 6310, Tokyo, Japan) and TEM. The TEM samples were prepared by directly depositing the as-spun fibers onto the Formvar-coated copper grids. The fiber diameters were measured from SEM micrographs obtained at random locations ($n = 50$). Pore size distribution of each membrane

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