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Development of a PCL-silica nanoparticles composite membrane for Guided Bone Regeneration

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ABSTRACT

The pivotal step in Guided Bone Regeneration (GBR) therapy is the insertion of a membrane for support and barrier functions. Here, we studied the effect of the addition of silica nanoparticles (Si-NPs) in electrospun poly (ε-caprolactone) (PCL) membranes to improve the mechanical and osteoconductive properties of the membranes. To this end, Si-NPs were firstly synthesized and then suspended in PCL solutions containing a polar solvent (2,2,2-trifluroethanol) and water with the addition of an anionic surfactant. Nanocomposite membranes were fabricated from the solutions through an electrospinning technique. Morphology, structure and chemical composition, and tensile properties of the membranes were analyzed. Membrane stability was determined by visual examination of the membranes after immersion in phosphate buffered saline. The effect of the materials on osteoblastic differentiation was evaluated by in vitro culture of the membranes with MC3T3-E1 osteoblastic cells. The results indicated that Si-NPs were successfully incorporated in the interior of the PCL electrospun fibers during the electrospinning process. Tensile modulus was significantly increased for composition S50 and tensile strength significantly increased for compositions S25 and S50. Membranes containing Si-NPs have shown to be cytocompatible. The results obtained demonstrate that the Si-NPs were homogeneously incorporated in the electrospun fibers, resulting in an improvement of the tensile properties of the prepared materials.

1. Introduction

Guided Bone Regeneration (GBR) is a common therapy used for the treatment of lesions in the alveolar or mandible bone caused by infections or trauma. The principle underlying GBR is to create a secluded space in the wound site, which favors the proliferation of bone-forming cells and consequently results in new bone formation. An essential step in such a strategy is the use of a physical barrier, mainly in the form of a membrane, preventing the fast-growing fibroblasts from migrating into the wound site and keeping a space for the slow-growing bone tissue to regenerate [1,2].

Electrospinning is an ideal technique to produce polymeric GBR membranes. The application of an electric current to the polymeric solution leads to the creation of a jet and consequently to the production of polymeric fibers, due to the evaporation of the solvent [3,4]. Electrospun membranes share the advantages of possessing a high surface area and a microstructure which resembles the extracellular matrix structure (ECM) [5,6]. Electrospun GBR membranes are made of natural or synthetic polymers [7,8]. The use of natural polymers is justified by their great biocompatibility, possessing protein motifs that enhance cell adhesion and proliferation. However, these natural materials lack the ideal tensile properties for GBR applications and present a high degradation rate [9,10]. Synthetic membranes are often made of polyesters, e.g. polyglycolide (PGA) and polylactide (PLA), which possess easily controllable and tunable physicochemical properties. However, PGA or PLA release acidic degradation products, potentially leading to local inflammatory reactions [11]. Poly(ε-caprolactone) (PCL) is a known biocompatible polyester, having the advantage that its degradation products are not acidic. A major disadvantage of synthetic membranes is that they generally lack osteoconductive capacity to allow bone growth along their surface and promote bone healing or regeneration [12–14].

A known strategy for introducing functional properties in synthetic GBR membranes is the incorporation of compounds that lead to improved mechanical properties and/or trigger an osteogenic response. These additives can be organic, for example, proteins and peptides [15,16], or inorganic, such as calcium phosphate (e.g. hydroxyapatite) or silicate bioceramic particles [17–19]. The conjugation of inorganic particles and polymeric components: polymeric hydrogels [20], polymeric membranes [21] and polymeric molecular branches [22,23] are a

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current strategy used in the development of materials with new functional properties. Such new hybrid materials are used in the most diversified areas [24], including in the biomedical field: cancer therapy [25–27], gene therapy [28], development of new antibacterial therapies [29,30], optogenetics [31], drug-delivery systems [23] among others. Previously, we prepared GBR membranes based on PCL and nano-apatite rod-like particles [18]. The addition of nano-apatite particles led to improved mechanical properties and osteoblast-like cells differentiation. However, uncontrollable aggregation of the particles inside the fibers occurred, which negatively affected the mechanical properties of the membranes. Calcium biosilicates have shown in the past to induce osteogenic cellular differentiation and bone tissue formation [32,33]. More recently has been shown that silica nanoparticles (Si-NPs) can be an alternative type of bioceramic particles for osteoconductive purposes. Their application is based on their reported positive effect on the mechanical properties of polymeric membranes [34–36] and promotion of an osteogenic response from osteoblastic progenitor cells [37,38]. However, despite promising results, Si-NPs weight percentages above 30 wt% frequently result in adverse effects, e.g. particle aggregation [39,40], poor interaction between the polymeric components and the silica particles [34] or non-ideal mechanical properties [41].

The aim of this study was to develop a PCL-based GBR membrane, in which Si-NPs are more homogeneously dispersed inside the electrospun fibers at amounts above 30 wt%. To this end, Si-NPs were produced by a sol–gel Stöber's method to achieve mono-sized nanoparticles. Furthermore, membranes with a series of PCL:silica weight ratios (100:0, 100:25, 100:50 and 100:75) were fabricated by an electrospinning methodology. Morphology, structure and chemical composition were analyzed via scanning electron microscopy (SEM), transmission electron microscopy (TEM), attenuated reflectance-infrared spectroscopy (ATR-IR) and X-ray diffraction (XRD). Mechanical properties were evaluated using a tensile test. Stability of the membranes in wet conditions was evaluated by immersion in phosphate buffered saline (PBS) solution at 37 °C. Osteoblastic cell response was determined by in vitro culture of MC3T3-E1 cells on the membranes and by assessing cellular morphology, proliferation, and osteogenic differentiation.

2. Materials and methods

2.1. Preparation of silica nanoparticles

Si-NPs were prepared using a Stöber's method [42]. Briefly, a 6% v/v mixture of ammonia (ammonia solution 25%; Merck; Germany) in ethanol was prepared. Afterwards, tetraethyl orthosilicate (TEOS; reagent grade, 98%; Sigma-Aldrich; USA), was added to the mixture, under continuous stirring and left to react at room temperature for 30 min. The solution was kept at 4 °C overnight. Finally, the nanoparticles were washed first with ethanol, second a mixture of ethanol-water (50:50) and third milliQ water, with intermediate centrifugation and redispersion steps.

2.2. Preparation of the polymeric solutions and fabrication of the membranes

Four solutions were prepared by firstly dissolving PCL (average Mn 80,000; Sigma-Aldrich; USA) in a 80% v/v 2,2,2-trifluoroethanol (TFE; Sigma-Aldrich; USA)/milliQ water mixture with sodium dodecyl sulfate (SDS; Fluka; Germany) (Table 1). Afterwards, a specific amount of Si-NPs was added and the dispersion was left to mix overnight. Table 1 describes the Si-NPs:PCL weight ratio and silica weight percentages.

A commercially available electrospinning set-up was used for the fabrication of the membranes (Advanced Surface Technology, Bleiswijk, The Netherlands). The prepared solutions were fed into a glass syringe, controlled by a pump (KD Scientific Inc., Holliston, MA, USA), and connected by a Teflon tube to a blunt-end nozzle with an inner diameter of 0.5 mm. The electrospinning process was performed with a distance between the nozzle and a roll-drum collector of 20 cm, a voltage between 18–22 kV and a feeding rate of 2.0 mL h^{-1}.

Table 1
Si-NPs:PCL membranes composition.

Membrane	Si-NPs:PCL weight ratio	Si-NPs weight/%	SDS/%
S0	0:100	0	0.05
S25	25:100	20	0.05
S50	50:100	33	0.05
S75	75:100	43	0.05

2.3. Morphological and structural evaluation

The morphology of the membranes was observed using scanning electron microscopy (SEM; Zeiss, SIGMA 300) and transmission electron microscopy (TEM; JEOL, 1101). Images were taken at 1000 × magnification and 10,000 × magnification for SEM and 20,000 × for TEM.

The chemical profile of the membranes was determined by attenuated total reflectance-infrared spectroscopy (ATR-IR; UATR two, PerkinElmer, The Netherlands) with a resolution of 4.0 cm^{-1} and a scanning range from 400 cm^{-1} to 4000 cm^{-1}.

X-ray diffraction (XRD; Philips pw1830, The Netherlands) was performed to determine the crystallographic profile of the samples. Electrospun membranes as a thin planar layer were placed in a glass holder and scanned. XRD spectra were registered at 40 kV, 30 mA (Cu-K_α radiation with a wavelength of 1.54 Å) and a 2θ between 10 and 40°, at a step size of 0.005°.

2.4. Mechanical evaluation

To determine the effect of silica nanoparticles on the mechanical performance of the membranes, 6 samples of 10 mm in width and 50 mm in length were cut with a scalpel and tested for each group. The thickness of the membranes was measured with a caliper having a precision of 0.01 mm. The samples were attached to a tensile test machine (LS1; Lloyd instruments; Ametek) using 10 mm of the sample on both ends. The tensile properties of the membrane were tested with a 100 N load cell under a cross-head speed of 10 mm min^{-1}. The following mechanical properties were recorded or calculated from the stress–strain (σ–ε) curves: (1) the tensile modulus was calculated from the slope of the initial linear part of the curve, consisting of 10% of the initial strain values (2) the maximum strain at break was defined as the maximum strain that the samples could reach immediately before break and (3) the tensile strength was defined as the maximum stress at break [43].

2.5. Stability of the membranes in a wet environment

Circular membranes with a diameter of 15 mm were punched from the electrospun sheets. Each sample was immersed in 10 mL of phosphate buffered saline (PBS) solution in a test tube. All tubes (n = 3) were placed in a water bath at 37 °C under continuous shaking. After immersion periods of 14, 21 and 28 days the membranes were washed with milliQ water and freeze-dried. The membranes, before and after PBS immersion, were examined by SEM.

2.6. In vitro cell culture

MC3T3-E1 cells (ATCC, USA) were maintained in α-MEM medium (Gibco®, Life Technologies, Grand Island, USA) supplemented with 10% fetal bovine serum (FBS; Gibco®) and 1% mixture of penicillin/