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Development of a bioactive glass-polymer composite for wound healing applications

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ABSTRACT

This study reports the production and characterization of a composite material for wound healing applications. A bioactive glass obtained by sol-gel process and doped with two different metal ions was investigated. Silver (Ag) and cobalt (Co) were chosen due to their antibacterial and angiogenic properties, respectively, very beneficial in the wound healing process. Poly(ε-caprolactone) (PCL) fibers were produced by electrospinning (ES) from a polymeric solution using acetone as a solvent. After optimization of the ES parameters, two main suspensions were prepared, namely: PCL containing bioactive glass nanoparticles (BG-NP) and PCL with Ag_2O and CoO doped BG-NP (DP BG-NP), which were processed with different concentrations of BG-NP (0.25%, 0.5% and 0.75 wt%). The composite membranes were characterized in terms of morphology, fiber diameter, weight loss, mineralization potential and mechanical performance.

1. Introduction

Wound healing is a dynamic process consisting of four overlapping and programmed phases, namely: homoeostasis, inflammation, proliferation and tissue remodeling [1–3]. The microenvironment of the wound healing process is complex and involves the interaction of a large number of different types of cells and molecules [4]. To achieve and promote a better healing process, wound dressings play an important role and should include some essential features, such as: biocompatibility; ability to prevent bleeding and dehydration of the wound; protection of the wound against external contamination; permeability to gas and fluid exchanges; ability to absorb exudates from the wound area; thermal isolation; non-toxicity and non-allergenic profile [5–8]. Taking into consideration all these characteristics, a variety of biomaterials has been proposed for wound dressing applications, including chitosan, alginate, polyurethane or silicone foams, hydrofibers made from carboxymethyl cellulose fibers, collagen and poly(ε-caprolactone) (PCL) [3,9–12].

PCL is a biocompatible, low-cost, biodegradable, linear and aliphatic polyester with a melting point ranging from 55 °C to 60 °C [13–16]. Due to its chemical structure, it is possible to blend PCL with a large amount of polymers without losing most of its beneficial properties. Its semi-crystalline and hydrophobic nature leads to a very slow degradation rate of PCL (from 2 to 4 years, depending on the starting molecular weight) [14,15,17]. Although exhibiting these advantageous properties, PCL is not bioactive [18]. One of the main strategies proposed to overcome this limitation is the combination with bioactive inorganic materials used as fillers, and among them bioactive glasses (BGs) have been the focus of many studies [19–22]. BGs are biocompatible surface reactive inorganic materials with typical silicate composition based on SiO_2, CaO, Na_2O and P_2O_5 [23,24]. Such class of materials has shown the ability to bond not only to bone but also to soft tissue, avoiding the formation of an undesirable fibrous encapsulation [23,25–27]. Besides, the use of inorganic nanoparticles (NP) has been shown to reduce the hydrophobic behavior of PCL, speeding up its degradation process [28,29].

Analyzing the role of BGs in soft tissue, Day et al. have shown that BG particles have the ability to increase the release of vascular endothelial growth factor (VEGF) when cultured with rat fibroblasts [30]. Other reports indicate that BGs have the capacity to improve angiogenesis and

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neovascularization when in contact (direct or indirect) with cells, which is a beneficial feature for wound healing applications [31,32]. It has been also shown that BGs can be obtained as nanoparticles (BG-NPs) using sol-gel methodologies [27,33].

In order to modulate and achieve better biological response, research has also been carried out on developing different compositions of BGs, including different added elements, such as: magnesium, zinc, cobalt, strontium, iron, lithium, copper and silver among others [24,34–38]. The doping of BG-NPs with metallic ions is possible by including specific metallic precursors in the sol-gel reaction procedure [39].

One of the most important characteristics of silver nanoparticles is their strong inhibitory and bactericidal effect, as well as the wide spectrum of antimicrobial activity. These abilities are achieved by blocking the oxygen uptake of the microorganisms and also through the damage of bacterial RNA and DNA, inhibiting their replication [40–46]. Cobalt ions have been indicated as being promising for biomedical applications since they have the ability to interfere with the oxygen sensors in the hypoxia response pathway [47,48]. This phenomenon stimulates erythropoietin production leading to an increased oxygen-carrying capacity of the blood. Therefore the activation of hypoxia-inducible factor (HIF) leads to an encoding of angiogenic growth factors resulting in *in vivo* tissue growth [47,48].

Different methodologies have been proposed to combine NPs with polymers [24]. Electrospinning (ES) represents a suitable technique to produce fibers, with diameters in the nm-μm length scale, since it allows controlling fiber morphology, porosity and composition using relatively unsophisticated equipment. Typically, this electrohydrodynamic atomization process uses an electric field created between the polymer solution and the collector, which generates internal repulsive forces in the polymer solution and at a critical point causes the expulsion of the polymer solution in shape of fibers towards the collector [49–53]. The solid polymer fibers structure, a nonwoven web, resembles the skin structure making it possible to use it as transdermal patches for wound dressing [4].

In the light of the above findings, in this study bioresorbable composite membranes with wound healing capability were fabricated by electrospinning technique, incorporating silver-cobalt doped bioactive glass nanoparticles (DP BG-NPs) in the poly(ε-caprolactone) matrix. In a first stage, PCL fibermats were produced in order to optimize the electrospinning process parameters and then PCL/BG-NPs and PCL/DP BG-NPs fibermats were fabricated with different concentrations of nanoparticles, namely 0.25 wt%, 0.5 wt% and 0.75 wt%.

2. Materials and methods

2.1. Materials

For the development of the bioactive glass and Ag_2O and CoO DP-BG NPs, different precursors were used, including: tetraethyl orthosilicate (TEOS, 99.90% pure), diammonium hydrogen phosphate, calcium nitrate tetrahydrate (99%), absolute ethanol, citric acid mono-hydrate (99%), silver oxide (99%), cobalt chloride (98%) and ammonium water. All these chemicals were purchased from Sigma-Aldrich.

For the electrospinning process, polycaprolactone (PCL, M_W 70,000–90,000 Da), purchased from Sigma-Aldrich, was used as raw material for biopolymer matrix and acetone (Sigma-Aldrich) was used as solvent.

2.2. Ag_2O and CoO DP BG-NPs preparation

The preparation of BG-NPs was achieved following the protocol described by Luz et al. [27]. To obtain the Ag_2O and CoO DP BG-NPs with a composition of SiO_2:CaO:P_2O_5:CoO:Ag_2O (mol%) = 50:40:5:2.5:2.5, two main solutions were prepared. In the first one, calcium nitrate was used as a Ca precursor, TEOS as a Si precursor, diammonium hydrogen phosphate as P precursor and cobalt chloride was used as Co precursor. Ethanol (60 mL) was added as well as citric acid (30 mL) to promote the hydrolysis process. In the other solution, silver oxide was used as a Ag precursor and it was added to ammonium water. The first solution was added drop-by-drop to the second solution, which was kept stirring for 48 h. The DP BG-NPs were calcinated at 700 °C and resulted in a blue powder.

2.3. Suspensions preparation

Polycaprolactone suspensions with different concentrations of BG-NPs and DP BG-NPs (0.25%, 0.5% and 0.75 wt%) were prepared in acetone. First the nanoparticles were accurately weighed and added to 5 mL of ethanol. The suspension was ultrasonicated (Bandelin Sonorex RK 100, Germany) for 2 h for proper dispersion, and then was left to dry at room temperature for 48 h in order to allow the total evaporation of ethanol. After this step, 10 mL of acetone was added and the suspension was ultrasonicated for 2 h. A known quantity of PCL (1 g) was added to the previous suspension under stirring at 40 °C, so that the final suspension contained 10 wt% PCL. The suspension was magnetically stirred until PCL's total dissolution.

In order to be used in the electrospinning processing, the final suspension (10 mL) was poured in a glass syringe and electrospun on aluminum sheets. Finally, the obtained membranes were left to dry at room temperature overnight and were then peeled off the aluminum foil for further studies.

2.4. Electrospinning of PCL with bioactive glass and PCL with bioactive glass nanoparticles doped with Ag_2O and CoO

The PCL membranes filled with BG-NPs and DP BG-NPs were prepared by electrospinning 10 wt% PCL in acetone, after dispersing the nanoparticles. The electrospinning apparatus (Starter Kit 40KV Web) was assembled by Linari Engineering-Biomedical (Italy) and consisted of a syringe pump, a high voltage power supply and a 10 mL syringe attached to a needle (21G). The distance from tip to collector was selected as 15 cm and the DC voltage applied was maintained at 15 kV. The feed rate of the suspension was adjusted to 5 mL/h. A thin aluminum sheet was used as collector, with surface area of 290 × 190 mm^2. It was necessary to optimize the electrospinning process, since it is affected by several parameters, as summarized in Table S1 in the supplementary information.

2.5. Physical characterization

2.5.1. Scanning electron microsopy (SEM)

The morphology of the produced membranes, the size and distribution of the particles in the fiber mesh were assessed using SEM (Carl

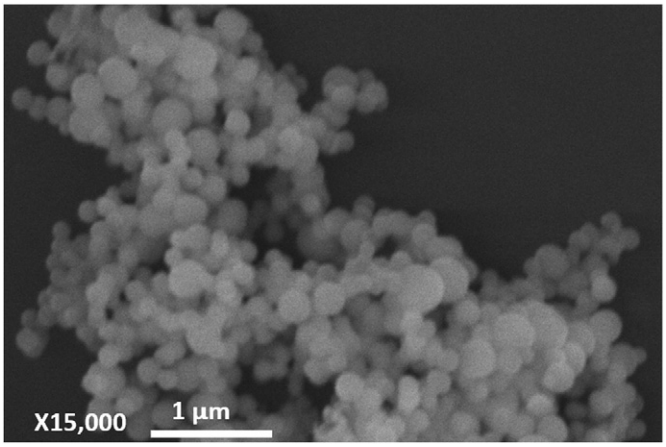

Fig. 1. SEM image of the Ag_2O and CoO DP BG-NPs.