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Regular Article

Polymeric electrospun scaffolds for bone morphogenetic protein 2 delivery in bone tissue engineering

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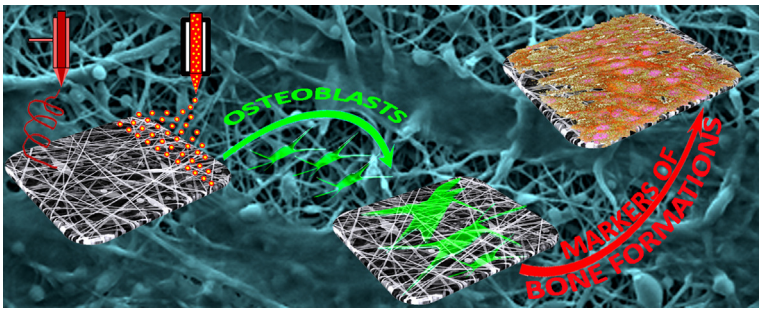
GRAPHICAL ABSTRACT



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ABSTRACT

Hypothesis: The development of novel scaffolds based on biocompatible polymers is of great interest in the field of bone repair for fabrication of biodegradable scaffolds that mimic the extracellular matrix and have osteoconductive and osteoinductive properties for enhanced bone regeneration.
Experiments: Polycaprolactone (PCL) and polycaprolactone/polyvinyl acetate (PCL/PVAc) core–shell fibers were synthesised and decorated with poly(lactic-*co*-glycolic acid) [PLGA] particles loaded with bone morphogenetic protein 2 (BMP2) by simultaneous electrospinning and electrospraying. Hydroxyapatite nanorods (HAn) were loaded into the core of fibers. The obtained scaffolds were characterised by scanning and transmission electron microscopy, Fourier-transform infrared spectroscopy, and thermogravimetric analysis. The *in vitro* potential of these materials for bone regeneration was assessed in biodegradation assays, osteoblast viability assays, and analyses of expression of specific bone markers, such as alkaline phosphatase (ALP), osteocalcin (OCN), and osteopontin (OPN).
Findings: PLGA particles were homogeneously distributed in the entire fibre mat. The growth factor load was 1.2–1.7 μg/g of the scaffold whereas the HAn load was in the 8.8–12.6 wt% range. These scaffolds were able to support and enhance cell growth and proliferation facilitating the expression of osteogenic and osteoconductive markers (OCN and OPN). These observations underline the great importance of the presence of BMP2 in scaffolds for bone remodelling as well as the good potential of the newly developed scaffolds for clinical use in tissue engineering.

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1. Introduction

The repair of bone defects is still a major challenge in orthopaedic and maxillofacial surgery [1]. Scaffolds play a crucial role in bone tissue engineering by acting as a template facilitating cell growth and differentiation within bone defects [2]. For these purposes, they should mimic the extracellular matrix (ECM); provide mechanical support; be biocompatible, osteoconductive, and osteoinductive; and possess high porosity provided by interconnected pores. Besides, they should be biodegradable to leave room for the new bone to grow. Fibers of mainly submicron sizes produced by electrospinning are a promising material to be used as scaffolds. These fibers resemble the ECM structure and are an excellent framework for cell adhesion, proliferation, and differentiation [3].

Among synthetic polymers, polycaprolactone (PCL) is widely used to obtain electrospun fibers because of its low cost, biocompatibility, and rheological and viscoelastic properties [4]. The electrospun scaffolds prepared with this polymer possess flexibility, good mechanical properties, and non-toxicity [3], though its hydrophobicity and low water adsorption may impair its biomedical applications. These limitations may be solved by its association with water-soluble compounds, such as tannins [5], proteins [6], or polysaccharides [7]. Furthermore, PCL osteoinduction and osteoconduction may be improved by addition of hydroxyapatite (HA) as we recently reported [4]. The addition of HA nanorods (HAn) to PCL/polyvinyl acetate (PVAc) core–shell fibers yields apatite formation on the nanofibre surface while the PVAc shell increases hydrophilicity and cell viability.

Even when HAn is an important osteoconductive biomaterial, incorporation of osteogenic growth factors, such as bone morphogenetic protein 2 (BMP2), is an interesting alternative way to increase the osteogenic activity [8]. Bone healing is a complex physiological process that is initiated and controlled by many growth factors such as bone morphogenetic proteins (BMPs). These proteins not only can enhance bone repair but also promote new blood vessel formation [9]. BMP2 is a necessary component of the signalling cascade that governs fracture repair because BMP2 is essential for initialisation of bone regeneration [10]. However, this protein may lose bioactivity after a short period owing to its short half-life under physiological conditions because of rapid degradation and deactivation by enzymes and other chemical and physical reactions that limit its local delivery [11]. To achieve therapeutic efficacy, a carrier is needed to deliver BMP2 locally at a stable concentration to avoid a burst release and uncontrolled ectopic bone formation in soft tissues [12].

Poly(lactic-*co*-glycolic acid) [PLGA] has been extensively used to encapsulate osteogenic growth factors into micro- and nanoparticles for a controlled drug release [13–15]. This polyester has interesting characteristics such as solubility in various solvents and approval by the US Food and Drug Administration (FDA). Several techniques have been developed for fabricating polymeric nanoparticles and for encapsulating drugs in a polymeric matrix, including emulsification. Even when this process is simple, it has multiple disadvantages, such as poor encapsulation and loading efficiency rates as well as possible denaturation of the encapsulated drug [16]. To overcome these drawbacks, electrospraying or electrohydrodynamic atomisation (EHDA) is a promising method for producing micro- and nanoparticles with high encapsulation efficiency of drugs (hydrophilic or hydrophobic molecules). It is a simple and inexpensive approach that enables researchers to preserve biofunctionalities of active ingredients [17]. A few examples of BMP2 encapsulation in polymer nanoparticles by electrospraying found in the literature show the sustained release of the protein for 35 days, thus allowing for mesenchymal-stem-cell proliferation and differentiation [18]. A stable release of BMP2 from PLGA electrosprayed spheres has been achieved, and new bone formation, accompanied by abundant in-growth of blood vessels, has been attained by *in vivo* implantation of these particles [1].

The combination of the unique properties of electrospun nanofibers with proven advantages of polymer particles for drug release can result in an innovative drug delivery system [19]. This approach allows for a homogeneous distribution of the BMP2-loaded particles along the entire fibre mats, thereby ensuring a continuous release of the growth factor, in contrast to the BMP2 immobilisation techniques that involve protein functionalisation only on the scaffold surface [20], limiting its efficiency.

In this work, for the first time, we developed novel composite electrospun scaffolds of PCL-HAn containing BMP2–loaded PLGA particles to provide the necessary biochemical cues for bone repair and regeneration. HAn-loaded PCL or PCL/PVAc core–shell fibers were decorated with BMP2–loaded PLGA particles via simultaneously electrospraying a solution of PLGA, bovine serum albumin (BSA), and BMP2 and coaxial electrospinning of PCL-HAn and PCL or PCL-HAn and PVAc solutions. Our aim was to evaluate the structural, physico-chemical, and biodegradation properties of the newly developed scaffolds and their ability to address the architectural, biochemical, and functional features of bone tissue. For this purpose, the scaffold bioactivity was tested by culturing human osteoblasts on the scaffolds and by monitoring cell viability for up to 4 weeks. The *in vitro* osteogenic activity of cells seeded onto the scaffolds was evaluated by assessing alkaline phosphatase (ALP) activity and the expression of osteogenic proteins osteocalcin (OCN) and osteopontin (OPN).

2. Experimental section

2.1. Materials

Poly(D,L-lactide-*co*-glycolide)lactide:glycolide 50:50 (PLGA) ester terminated at molecular weight 38,000–54,000 Da was purchased from Evonik Industries (Spain), and bone morphogenetic protein 2 (BMP2; ≥95%) from R&D Systems (US). The osteoblast growth medium (OGM) and human osteoblasts (HOBs) were acquired from PromoCell (Germany). PVAc and PCLc with an average molecular weight of 140,000 and 80,000 Da, respectively, calcium carbonate ($CaCO_3$; ≥95%), bovine serum albumin (BSA; ≥98%), albumin–fluorescein isothiocyanate conjugate (BSA-FITC), Tween® 80, 3-(3,4-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; ≥98%), human lysozyme (≥100,000 U/mg protein, lyophilised powder; ≥90%), lipase from *Aspergillus oryzae* (50 U/mg), N,N-dimethylformamide (DMF; ≥99.8%), dichloromethane (DCM; ≥99.8%), phosphoric acid (H_3PO_4; ≥85%), an ammonium hydroxide solution (NH_4OH; 28–30%) and CellCrown™ inserts (24-well plate inserts), were purchased from Sigma-Aldrich (Spain), whereas Trypsin-EDTA and Dulbecco's phosphate-buffered saline (DPBS) from Biowest (France).

2.2. Scaffold fabrication

Different types of fibers (PCL-HAn fibers for the core, and PCL fibers and PVAc fibers for the shell) and PLGA particles loaded with BSA and BMP2, were fabricated to obtain four types of electrospun scaffolds (Table 1).

PLGA particles were prepared using an Yflow 2.2.D-500 electrospinner (Electrospinning Machines/R&D Microencapsulation, Spain). BMP2 (10 μg) was dissolved in 100 μL of a BSA aqueous solution (2%), while PLGA (1.05 g) was dissolved in 10 mL of DMF. Then, the BSA aqueous solution was added to the PLGA solution, and the mixture was incubated with magnetic stirring for 12 h at 4 °C. The addition of a surfactant was not needed to obtain the