International Journal of Pharmaceutics 447 (2013) 139–149

Contents lists available at SciVerse ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Pharmaceutical nanotechnology

A cell-free nanofiber composite scaffold regenerated osteochondral defects in miniature pigs

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ARTICLE INFO

Article history:
Received 11 October 2012
Received in revised form 26 February 2013
Accepted 28 February 2013
Available online 7 March 2013

Keywords:
Cartilage
Fibrin
Nanofibers
Growth factors
Polyvinyl alcohol
Hyaluronic acid

ABSTRACT

The aim of the study was to evaluate the effect of a cell-free hyaluronate/type I collagen/fibrin composite scaffold containing polyvinyl alcohol (PVA) nanofibers enriched with liposomes, basic fibroblast growth factor (bFGF) and insulin on the regeneration of osteochondral defects.

A novel drug delivery system was developed on the basis of the intake effect of liposomes encapsulated in PVA nanofibers. Time-controlled release of insulin and bFGF improved MSC viability in vitro. Nanofibers functionalized with liposomes also improved the mechanical characteristics of the composite gel scaffold.

In addition, time-controlled release of insulin and bFGF stimulated MSC recruitment from bone marrow in vivo. Cell-free composite scaffolds containing PVA nanofibers enriched with liposomes, bFGF, and insulin were implanted into seven osteochondral defects of miniature pigs. Control defects were left untreated. After 12 weeks, the composite scaffold had enhanced osteochondral regeneration towards hyaline cartilage and/or fibrocartilage compared with untreated defects that were filled predominantly with fibrous tissue. The cell-free composite scaffold containing PVA nanofibers, liposomes and growth factors enhanced migration of the cells into the defect, and their differentiation into chondrocytes; the scaffold was able to enhance the regeneration of osteochondral defects in minipigs.

1. Introduction

Cartilage is an avascular tissue with a limited capacity for repair. Standard surgical techniques, such as debridement, penetration of subchondral bone, osteotomy, joint distraction, transplantation of autographs from a non-weight-bearing zone into a defect, and soft tissue grafts, have the potential to stimulate the formation of a new articular surface, and may decrease symptoms and improve joint function. However, they are not able to restore the articular cartilage (Buckwalter and Lohmander, 1994; Buckwalter and Mankin, 1998; Vaquero and Forriol, 2012).

The novel strategy of regeneration of chondral or osteochondral defects so-called matrix-associated involves autologous chondrocyte implantation (MACI) is based on autologous chondrocyte-seeded biomaterials (Trattnig et al., 2005). The matrices already in use in clinical practice include biopolymers, e.g. collagen I/III membrane (Cherubino et al., 2003; Marlovits et al., 2005), hyaluronan derivatives (Trattnig et al., 2005), fibrin (Visna et al., 2004), fibrin/hyaluronate scaffold (BioCart^{TM}II) (Eshed et al., 2012).

Recently, pluripotent mesenchymal stem cells from bone marrow, adipose tissue, or umbilical cord blood were found to differentiate into cartilage, bone or other tissues, depending on growth factors, chemical composition, and the physical and biomechanical properties of the matrices as well as different biomechanical loading of the material (Pittenger et al., 1999; Park et al., 2011a; Jelen et al., 2008). MSC-based therapy has already been used in clinical practice (Haleem et al., 2010). However, bone marrow harvesting is an invasive method that may be demanding for patients.

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http://dx.doi.org/10.1016/j.ijpharm.2013.02.056

In addition, the isolated cells require culture under special conditions, and further implantation. A promising new approach using biomimetic scaffolds guiding cell recruitment from a body niche, e.g. bone marrow, and their subsequent migration into functionalized scaffold has therefore been studied (Sun et al., 2012). This system has great potential in clinical practice, but the optimal parameters need to be found.

Nanofibers have a large specific surface and they can be functionalized with drugs, antibiotics, with bioactive peptides or proteins, RNA, and DNA. (Jannesari et al., 2011; Luong-Van et al., 2006). Sahoo et al. (2010a) reported faster bFGF release from blended nanofibers of poly(lactide-co-glycolide) (PLGA) than from coaxial nanofibers; however, both groups increased the proliferation of MSCs. In another study, a blended PLGA scaffold containing bFGF induced tendon/ligament-like fibroblastic differentiation, including synthesis of type I collagen and tenascin-C (Sahoo et al., 2010b). In our laboratory, we have recently developed systems of nanofibers or microfibers functionalized on the surface (Jakubova et al., 2011; Rampichová et al., 2012), in the core of nanofibers, or in blended nanofibers (Mickova et al., 2012; Buzgo et al., in press) in order to prepare a drug delivery system.

Fibrin gel alone or in composite scaffolds has already been used for repairing cartilage (van Susante et al., 1999; Hunziker, 2001; Eshed et al., 2012; Haleem et al., 2010), bone (Kang et al., 2011), meniscus (Longo et al., 2012), usually with growth factors. Fibrin serves as a drug delivery system where the rate of release is mediated by the interactions between fibrin and chemical, and may be modified by the composition of fibrinogen or thrombin (Spicer and Mikos, 2010).

Hyaluronic acid plays an important role in many physiological and pathological processes, e.g. cell recognition, cell migration, proliferation, cell differentiation, and inflammation. Most of these responses are mediated through the HA-CD 44 interaction (Maniwa et al., 2001; Laurent and Fraser, 1992). A matrix from hyaluronate benzylic esters (HYAFF) was reported to enhance regeneration of osteochondral defects either when seeded with MSCs or when the scaffolds are unseeded (Radice et al., 2000). Biodegradable polymers, such as hyaluronic acid, collagen and fibrin have been already used as scaffolds for drug delivery of growth factors (Holland and Mikos, 2003). Moreover, their three-dimensional structure provides cells with a suitable environment for adhesion, growth and differentiation that is influenced not only by chemical stimuli but also by biomechanical properties. They can also be combined with nano/microparticles, nano/microfibers, or liposomes, which can modify their structure or their biomechanical properties, or can serve for drug delivery.

PVA is a non-toxic, biocompatible material that has been used in medical practice, e.g. as a wound healing material, as an artificial lens, as a material for uterine artery embolization, for dry eye treatment, etc. (McCarron et al., 2011; Walker et al., 2007; Firouznia et al., 2008; Brodwall et al., 1997). The hydrophilic character of PVA means that it swells in water and is water soluble. Longer stability of the material should be achieved by chemical crosslinking (Alipour et al., 2009; Jiang et al., 2009), or by physical methods (Senna et al., 2010; Litvinchuk et al., 2009). As PVA can be successfully electrospun, it is a suitable material for external application, e.g. for wound dressing (Liu et al., 2010), and may be used as a drug delivery system.

Applying these advanced nanofiber scaffolds with a drug delivery system would undoubtedly be advantageous for tissue cartilage regeneration. However, no system of this type has yet been developed. In this study, we prepare nanofibers of a PVA/liposomes blend that are enriched with growth factors bFGF and insulin, and we test the release profile of the growth factors, and their effect on chondrocyte viability in vitro. Then the nanofibers are embedded in a fibrin/type I collagen/fibrin composite hydrogel, and we study their ability to enhance osteochondral regeneration in miniature pigs.

2. Materials and methods

2.1. Housing/animal care

For this study, five male and three female 7-month-old miniature pigs (29 ± 10.5 kg) were used. Animal care was in compliance with the Act of the Czech National Convention for the protection of vertebrate animals used for experimental and other scientific purposes, Collection of laws No. 246/1992, including amendments on the Protection of Animals against Cruelty, and Public Notice of the Ministry of Agriculture of the Czech Republic, and Collection of laws No. 207/2004, on Keeping and Exploitation of Experimental Animals.

2.2. Preparation and characterization of nanofiber scaffolds

50 mL of 12.8% (w/w) polyvinyl alcohol (PVA) solution (Sloviol®, CHZ, Nováky, Slovak Republic) containing 0.38% (w/w) glyoxal (40% (w/w) solution of glyoxal in water) and 0.51% (w/w) H_3PO_4 (85% (w/w) phosphoric acid in water) was prepared, and mixed with 3 mL of liposomes.

Multilamellar liposomes were prepared from 6% phospholipids (Asolectin, from soyabean, Sigma–Aldrich) by dry film method. Briefly, 25 mg of soybean phospholipids were dissolved in chloroform (1 mL) and subsequently evaporated under a flow of N_2 at 4 °C to form a thin lipid film. The dried lipid films were then resuspended in 3 mL of 100 mM phosphate buffer saline (PBS) at pH 7.3 for the preparation of empty liposomes. We used liposome mixture instead of pure phospholipids to facilitate the preparation of emulsion of phospholids in PVA solution for electrospinning. The emulsion was intensively stirred for 30 min. Electrospinning was carried out on a Nanospider™ device as described previously in detail (Lukáš et al., 2008). A high-voltage source generated voltages of up to 56 kV, and the polymer solutions were connected with the high-voltage electrode. The electrospun nanofibers were deposited on a grounded wire collector electrode. The distance between the top of the rotating drum and the collecting plate was 12 cm. All electrospinning processes were performed at room temperature (RT; ~22 °C) and a humidity of ~50%.

The nanofibers that were formed were crosslinked at 135 °C for 10 min and then dried in a desiccator. PVA nanofibers without liposomes were prepared as a control.

The surface morphology was studied using a Vega scanning electron microscope (Tescan, Czech Republic). The tested materials are not good conductive materials; a gold coating was therefore applied on the surface to increase the surface conductivity of the electrospun materials. From five SEM photomicrographs of both PVA + LIP and PVA nanofibers, the pore diameter and the pore size of the nanofibers were measured, and the porosities were calculated using LUCIA G Image Analysis, version 4.82 (Laboratory Imaging Ltd., Czech Republic). Nanofibers with the similar surface density were used.

2.3. Release of the growth factors from the nanofibers

PVA scaffold with liposomes was cut into round patches, with the area of 5 cm^2, weighing 11.3 ± 1.6 mg (mean and SD), and then four samples were incubated in PBS solution containing 200 μg/mL insulin (Actrapid inj. 100 IU/mL) and 200 ng/mL basic Fibroblast growth factor (bFGF, human recombinant, Roche Applied Science) at room temperature (RT) for 30 min (PVA + LIP). Four PVA nanofiber scaffolds without liposomes but with the same amount of growth factors were prepared as controls (PVA group). The scaffolds were