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NANOPARTICLE-BASED PLATELET LYSATE RELEASE SYSTEMS FOR ENHANCED PROLIFERATION OF HUMAN ADIPOSE DERIVED STEM CELLS IN COMBINATORY TISSUE ENGINEERING STRATEGIES

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ABSTRACT

Recently, the delivery of growth factors (GFs) through controlled release systems is one of the top approaches for combined tissue engineering (TE) and regenerative medicine strategies. Polysaccharides have been motif of interest for their versatility and superior functional properties and can be used to design new protein delivery systems since they are capable of preserving the bioactive conformation and even enhancing the potency of the proteins. In this work, a polyelectrolyte complex (PEC) based on the electrostatic interaction of two oppositely charged polysaccharides, chitosan (CH) and chondroitin sulfate (CS) is proposed for the encapsulation of proteins, specifically platelet lysate (PL). PL is a promising source of GFs due to its autologous nature and rich composition, leading to synergistic GF actions. By a harmless and quick procedure for the entrapped proteins, spherical nanoparticles (NPs) slightly smaller than 200 nm were developed. The PL-loaded NPs were cultured with human adipose derived stem cells (hASCs) and it was observed a positive influence over cell viability and proliferation. The NPs were then included in a three-dimensional polyD,L lactic acid (PDLLA) scaffold to address the possibility of creating a multifunctional scaffold able to stimulate the proliferation and differentiation of seeded cells. Different protein delivery rates were achieved, which suggests this system as a promising approach for TE strategies.

INTRODUCTION

The emerging next generation of engineered tissues relies on the development of loaded scaffolds containing bioactive molecules in order to provide the necessary information or signalling for cell attachment, proliferation and differentiation to meet the requirement of dynamic reciprocity for Tissue Engineering (TE). This justifies the importance of delivery systems in TE applications (Causa et al., 2007).

Natural based chitosan/chondroitin sulfate nanoparticles (CH/CS NPs) were developed with the ultimate goal of encapsulating bioactive agents to promote and enhance bone and cartilage regeneration.

In the present study we investigate the potential of the CH/CS NPs to act as a controlled release system for PL, in order to stimulate the proliferation of human adipose-derived stem cells (hASCs) in 2D cultures.

The introduction of autologous platelet concentrate into clinical practice was previously suggested, underlining the several advantages of using this source of growth factors (GFs) for tissue healing. Besides their autologous nature, these concentrates do not present risks of disease transmission neither immunogenic reactions. The use of single GFs is not as effective as the application of combined GFs, which can act synergistically towards tissue healing, with a low cost (Hokugo et al, 2005). The main disadvantages come from the complexity of the cocktail of GFs present in the mixture, which are involved in different pathways and therefore might diverge the cell differentiation from the lineage we intend. There are some contradictory studies in the literature reporting the effects of platelet lysate (PL) on the osteogenic and chondrogenic differentiation of stem cells, resulting from different protocols for isolation of platelets and their further activation (Anitua et al., 2010).

We have also addressed the use of the developed NPs in a combinatory strategy with other 3D scaffolds based on PolyD,L lactic acid (PDLLA), to influence the viability, proliferation and differentiation of seeded hASCs. The combination of a NP releasing system with a supercritical fluid-produced 3D structure might result in an appropriate delivery system for TE strategies.

MATERIALS AND METHODS

Production of NPs: The CH/CS NPs were produced by polyelectrolyte complexation between CH and CS. Two different formulations were produced and further characterized: CH/CS 1/1 and CH/CS 2/1. After mixing both polymeric solutions at the indicated weight ratios, the suspension was stirred for 10min and further centrifuged at 13500 rpm for 30min. The supernatant was discarded and the NPs pellet was resuspended in distilled water.

Characterization of NPs: The NPs were morphologically characterized by Atomic Force Microscopy (AFM) and Dynamic Light Scattering (DLS). The zeta potential and the production yield were also assessed. The cytotoxicity of the NPs systems was evaluated with a cell line (L929), following the ISO/EN 109935 guidelines.

Protein incorporation: Bovine Serum Albumin (BSA) was used as a model protein for peptide encapsulation within the NPs. BSA was either mixed with CS before the complexation of CH with CS or adsorbed to previously prepared empty NPs. The encapsulation and adsorption efficiency were determined by quantification of the remaining protein in the supernatant, through the microBCA and BCA kits. The interaction of PL with the NPs was analysed following the same procedures as indicated for BSA.

PL release: After producing the PL-loaded NPs, 1mg of these were added to the culture of hASCs with DMEM, with and without fetal bovine serum (FBS), for 1, 3 and 7 days. During the week of culture, the ASCs viability, proliferation and morphology were assessed. The PL release profile was also quantified with the microBCA kit.

Scaffold production: The scaffolds were prepared by supercritical fluid foaming at 200 bar and 35 ºC. In each experiment c.a. 100 mg of PDLLA was loaded in a mould that was placed inside the high pressure vessel. Carbon dioxide was pumped into the vessel. The system was closed for 30 minutes to allow the plasticization of the polymer. Afterwards the system was slowly depressurized. The scaffolds impregnated with the NPs were prepared following the same procedure and characterized by scanning electron microscopy (SEM) and micro-Computed Tomography (μCT). In vitro release studies were performed by immersion of the structures in phosphate buffered saline (PBS) solution, pH 7.4, 37ºC and 60 rpm.

RESULTS

Spherical particles (< 200nm) were produced with a satisfactory production yield and have shown to be stable in a long-term basis. The NPs did not provoke a cytotoxic response as the viability of the L929 cultured cells was not affected. We observed that the encapsulation efficiency decreases with higher protein loadings. BSA and PL were successfully incorporated within the CH/CS NPs and the PL delivery was controlled and stable over the 7 days of test. The cultures of hASCs in presence of PL-loaded NPs showed higher viability and cell number.

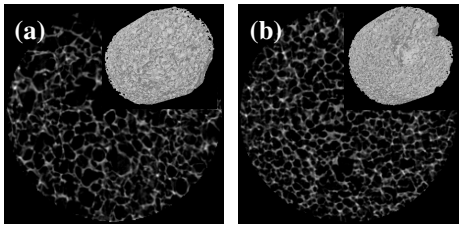

Figure 1. Representative Micro CT images of the 2D surface and 3D model (a) PDLLA matrices and (b) NPs-loaded PDLLA matrices.

The morphology (figure 1) and non-cytotoxic behaviour of the PDLLA scaffolds were not affected by the addition of the NPs. The inclusion of the NPs into the 3D structure seems to delay the protein release profile. Moreover, the entrapment of empty NPs within the PDLLA scaffold seems to decrease the burst effect observed in the release of proteins directly integrated into the scaffold.

CONCLUSIONS

The CH/CS NPs were able to successfully include both BSA and PL, and release the proteins in a controlled manner. The PL-releasing system improved hASCs viability and proliferation. The PDLLA-NPs hybrid structure shows the potential to be used as multifunctional scaffold for TE applications due to its porosity, interconnectivity and the possible different protein release rates, which can be achieved and designed with different ratios of NPs incorporation within the PDLLA scaffolds.

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