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The effect of starch and starch-bioactive glass composite microparticles on the adhesion and expression of the osteoblastic phenotype of a bone cell line

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Abstract

There is a clear need for the development of microparticles that can be used simultaneously as carriers of stem/progenitor cells and as release systems for bioactive agents, such as growth factors or differentiation agents. In addition, when thinking on bone-tissue-engineering applications, it would be very useful if these microparticles are biodegradable and could be made to be bioactive. Microparticles with all those characteristics could be cultured together with adherent cells in appropriate bioreactors to form in vitro constructs that can then be used in tissue-engineering therapies.

In this work, we have characterized the response of MC3T3-E1 pre-osteoblast cells to starch-based microparticles. We evaluated the adhesion, proliferation, expression of osteoblastic markers and mineralization of cells cultured at their surface. The results clearly show that MC3T3-E1 pre-osteoblast cells adhere to the surface of both polymeric and composite starch-based microparticles and express the typical osteoblastic marker genes. Furthermore, the cells were found to mineralize the extracellular matrix (ECM) during the culture period.

The obtained results indicate that starch-based microparticles, known already to be biodegradable, bioactive and able to be used as carriers for controlled release applications, can simultaneously be used as carriers for cells. Consequently, they can be used as templates for forming hybrid constructs aiming to be applied in bone-tissue-engineering applications.

Keywords: Starch; Bioactive glass; Microspheres; Osteogenesis; Cell culture

1. Introduction

To try to regenerate bone has been a major goal of tissue-engineering research. A promising approach combines the use of scaffold materials together with autologous site-specific cells. In this way, it may be possible to construct a hybrid material that can repair an osseous defect. For this strategy to be successful, materials need to be generated that exhibit adequate physical and chemical properties, and at the same time enhance cell adhesion, proliferation and differentiation. An ideal substrate for the synthesis of bone should be able to promote the expression of the osteoblastic phenotype as well as provide a template for bone deposition [1]. Furthermore, it is desirable for excellent scaffold materials to release bioactive molecules in a controlled fashion such that cell adhesion, proliferation and other cellular functions are enhanced.

Starch-based materials were shown to possess a wide range of properties that support their potential for biomedical applications. Coupled with their biodegradable

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nature [2,3], the ability to be processed by diverse methods [4–6] and into diverse shapes (Three-dimensional (3D) porous scaffolds, microparticles, bone cements) [7–11], render these materials very attractive to be used as scaffolds. Blends of starch with different synthetic polymers have been studied for several biomedical applications, such as bone scaffolds [12–17] and drug release applications [7,18]. 3D porous scaffolds based on starch-based materials have been shown to be biocompatible and to possess excellent in vivo behavior [12,14,16,19].

In this work, we have focused on the production of starch-based microparticles, which are bioactive [20] and can release, in a sustained manner, molecules of biological interest [18]. However, to be used for biomedical purposes, their behavior regarding critical cellular functions such as adhesion, proliferation and maintenance of a defined phenotype needs to be well known. If osteoblast-like cells can adhere and grow at the surface of the starch-based microparticles these substrates could be used for: (i) non-load bearing applications or as part of a 3D-construct; (ii) cultivating anchorage-dependent cells in a dynamic bioreactor and (iii) encapsulating bioactive molecules in the microparticles and simultaneously growing cells at the surface of the microparticles that would release encapsulated growth factors to stimulate proliferation and differentiation of adherent cells. Cell proliferation and differentiation would occur, giving origin to a hybrid cell-material construct. After moving the construct to the in vivo location, the biodegradable nature of the microparticles would allow them to be replaced by newly formed tissue.

The aim of the present study was to evaluate the ability of starch-based biodegradable microparticles to support cell adhesion, viability and phenotypic expression of osteoblastic markers by MC3T3-E1 cells. In order to assess this, we have used cells of pre-osteogenic lineage and cultured them for periods up to 14 days at the surface of both polymer and composite starch-based microparticles.

2. Materials and methods

2.1. Materials

Starch-based polymer (SPLA, a blend comprised of 50 wt% corn starch and 50 wt% polylactic acid) and composite (SPLA/BG, comprised of SPLA and 30% Bioactive Glass 45S5 granules, with a composition of 46.1% SiO_2, 24.4% Na_2O, 26.9% CaO, 2.6% P_2O_5, mol%) microparticles were produced as described in a previous work [20]. Particle sizes between 210 and 350 μm were selected over smaller ones, due to the following reasons: compared with smaller sizes, they have larger surface area, which might allow the adhesion of higher number of cells per particle and yield bigger hybrid constructs.

The cell line used in this work—MC3T3-E1, subclone 4, derived from fetal mouse calvaria, was purchased from American Tissue Cell Collection (ATCC). The cells were cultured in DMEM medium supplemented with 10% FBS and 1% of antibiotics (penicillin—streptomycin) and cultivated in standard tissue culture conditions (37 °C, 5% CO_2).

2.2. Cell adhesion to the surface of starch-based microparticles

The ability of MC3T3-E1 cells to adhere to the surface of starch-based microparticles was evaluated for up to 6 h. To determine the adhesion efficiency, 1 μl volume of microparticles was added to a suspension of 2×10^5 cells. The cells were allowed to adhere for 30 min, 1, 2 and 6 h. After each time period, the microparticles were washed to make them free of non-adherent cells and transferred to a new vial. A volume of trypsin, enough to cover the microparticles, was added to detach the adherent cells, which was then neutralized by the addition of complete culture medium. Cells were then counted in a Neubauer chamber. Adhesion efficiency was determined as the percentage of adhered cells versus the total number of cells seeded. Data reports results from 3 independent experiments.

2.3. Cell seeding on the surface of starch-based microparticles

Before being used in any cell culture experiments, starch-based microparticles were sterilized with 70% ethanol, allowed to dry and then hydrated in DMEM culture medium prior to cell seeding. A volume of 10 μl of microparticles was then added to a suspension of 5×10^5 MC3T3-E1 cells. The microparticles and cells were mixed and centrifuged for 30 s at 100 rpm. After a maximum period of 12 h, the microparticles with seeded cells were transferred to 6-well plates containing 40 μm pore size cell strainers (BD Falcon, Bedford, MA, USA). The cell strainers prevented particle loss during culture medium changes.

The cells were cultured for 14 days and evaluated for cell proliferation, enzyme activity, gene expression and an end-point assay for mineralization by Alizarin Red staining.

2.4. MC3T3-E1 viability assessment using confocal laser microscopy

Viability of MC3T3-E1 cells adhered to starch-based microparticles was assessed by confocal laser microscopy (Inverted Confocal Microscope, Olympus FloView, Melville, NY, USA). For this purpose was used a viability fluorescent dye—CellTracker Green CMFDA (Molecular Probes, Eugene, OR, USA). This dye diffuses through the cell membranes and once inside the cell, the CellTracker, containing a chloromethyl group that reacts with thiols, is transformed into a cell-impermeant fluorescent dye-thioether adduct. Only living cells have the ability to allow this reaction to occur, and this principle was used to determine the distribution and viability of cells adhered to the surface of starch-based microparticles.

The medium from the samples (SPLA and SPLA/BG microparticles with adhered cells) was aspirated and replaced by a 1:1000 dilution of CellTracker in serum-free DMEM. After 30 min the working solution was removed and replaced with complete culture medium. After a second 30 min incubation period, the samples were analyzed in a laser confocal microscope, with an excitation laser of 517 nm. Images for SPLA and SPLA/BG samples were obtained by stacking of 20 μm planar slices.

2.5. MC3T3-E1 DNA quantification

DNA content, as a means of evaluating proliferation, was measured using the PicoGreen dsDNA kit (Molecular Probes, USA). PicoGreen dsDNA reagent is an ultra sensitive fluorescent nucleic acid stain for quantitating double-stranded DNA (dsDNA) in solution. At each time point in culture, cell strainers were removed and the contents (particles with cells) washed with isotonic saline solution and centrifuged. A minimal volume of 0.1 N NaOH was added to release DNA from the cells. An aliquot of the DNA suspension was added to 10 × TE (Tris-EDTA) buffer, to which was then added the PicoGreen reagent (previously prepared in 10 × TE buffer) in a 1:1 vol:vol, and fluorescence measured in a microplate reader at 485 and 535 nm excitation and emission wavelengths, respectively. Lambda DNA was used as standard. The data presents results of at least three independent experiments.