Acta Biomaterialia 6 (2010) 436–444

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Microencapsulation and chondrogenic differentiation of human mesenchymal progenitor cells from subchondral bone marrow in Ca-alginate for cell injection

M. Endres [a,b,*], N. Wenda [c], H. Woehlecke [c], K. Neumann [a], J. Ringe [b,d], C. Erggelet [e], D. Lerche [c], C. Kaps [a,b]

[a] TransTissue Technologies GmbH, Berlin, Germany
[b] Charité University Medicine Berlin, Department of Rheumatology, Tissue Engineering Laboratory, Berlin, Germany
[c] Dr. Lerche KG, Rudower Chaussee 29, Berlin, Germany
[d] Berlin-Brandenburg Center for Regenerative Therapies, Charite University Medicine Berlin, Berlin, Germany
[e] Universitätsklinikum Freiburg, Department of Orthopaedics und Traumatology, Freiburg, Germany

ARTICLE INFO

Article history:
Received 2 February 2009
Received in revised form 29 June 2009
Accepted 7 July 2009
Available online 19 July 2009

Keywords:
Ca-alginate microcapsules
MSC
Chondrogenisis
Encapsulation

ABSTRACT

The application of stem cells is a promising therapeutic approach for cartilage regeneration. For cell therapies, a biocompatible injectable carrier, which improves retention and cell distribution and enables cell differentiation, is a prerequisite. In this study, Ca-alginate microcapsules containing human subchondral cortico-spongious progenitor cells were prepared and the chondrogenic differentiation potential was verified by real-time reverse transcription-polymerase chain reaction analysis of typical chondrogenic marker genes. The results confirmed that these cells were able to differentiate along the chondrogenic lineage when encapsulated in Ca-alginate microcapsules with a mean diameter of 600–700 μm and stimulated with TGF-beta3. Chondrogenic marker genes type II collagen, aggrecan and cartilage oligomeric matrix protein were induced together with type I collagen, whereas adipogenic and osteogenic marker genes showed no induction over 14 days. After 28 days, proteoglycans and type II collagen were evident histochemically and immunohistochemically. Mechanical stability as well as permeability of Ca-alginate capsules were analysed over the course of cultivation and found to be qualified for stable cell immobilization and sufficient exchange of solutes. Therefore, from the cell biology point of view, Ca-alginate, an established hydrogel scaffold material is suited for regenerative therapies of cartilage defects based on the injection of progenitor cells.

© 2009 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.

1. Introduction

In regenerative medicine, amongst other challenges, the method of application of cells into the human body in various repair approaches has not been sufficiently established. Clinically, the injection of pure stem- and progenitor cell suspensions into the defective site may be problematic because the cells do not adhere directly within the defect but are flushed into the surrounding milieu. Therefore, a biocompatible injectable carrier, which improves retention and cell distribution and enables cell differentiation, might be the answer. The combination of cells (especially mesenchymal stem cells, MSCs) with hydrogels like fibrin [1], sodium hyaluronan/gelatine [2] alginate [3], agarose [4], poly(JV-isopropylacrylamide-co-acrylic acid) (p(NiPAAm-co-AAc)) copolymer [5], collagen gel, matrigel and PuraMatrix peptide hydrogel [6] can decrease the flow properties by increasing the viscosity of the cell suspension and providing a three-dimensional environment for an even cell distribution. A comparative study on cell application methods showed that the use of hydrogels (e.g. fibrin) for cell injection into the intervertebral disc increase the cell transfer efficiency compared to the use of cell/medium suspensions. Using the example of augmenting lumbar intervertebral discs, leakage through the injection site in vitro and in situ resulted in the loss of more than 90% of the injected cells in a cell/medium suspension within the first 30 min [7].

Alginates are well known as excipients in drug products, e.g. release systems, and as a temporary matrix for cells in the field of tissue engineering. Their characteristics depend on the alginate concentration used, the concentration of the gelling solution (divalent cations, e.g. calcium ion) and the resulting crosslinking, which is responsible for the type and size of the pores [8]. An increased concentration and crosslinking in the alginate matrix results in a decrease in permeability, which is relevant for the diffusional exchange of solutes and nutrient supply for immobilized cells. Concentration and crosslinking of alginate also determine the mechanical strength of the capsules and hence the stability of the cell encapsulation.

It is still a challenge to fabricate alginate-based microcapsules where nutrients, oxygen and factors can be exchanged between

* Corresponding author. Address: TransTissue Technologies GmbH, 10117 Berlin, Germany. Tel.: +49 30 450 513 239; fax: +49 30 450 513 949.
E-mail address: michaela.endres@transtissue.com (M. Endres).

1742-7061/$ - see front matter © 2009 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.actbio.2009.07.022

the encapsulated cells and the surrounding environment without affecting the stability, mechanical strength, elasticity, swelling characteristics or size of the alginate beads [9].

The application of injectable autologous cell suspensions ranges from osteochondral defects in articular joints [2] and meniscus regeneration [10] to nasal augmentation [11]. Although mesenchymal cells from bone marrow are negative for immunologically relevant surface markers and inhibit proliferation of allogenic T cells in vitro [12], there are some concerns that MSCs from bone marrow may lose their immunosuppressive potential after expansion in vitro and after subsequent local implantation into allogenic recipients [13]. Therefore, graft vs. host reaction may be prevented by the use of encapsulated allogenic MSCs or by administration of autologous progenitors.

Progenitor cells suited for tissue repair, especially cartilage repair, reside in the subchondral bone marrow and are released by the microfracture technique. Small articular cartilage defects up to 8 cm^2 in size [14] can be treated by the microfracture technique. It is generally assumed that the microfractures create a connection between the subchondral cortico-spongious bone marrow and the cartilage defect, where mesenchymal stem and progenitor cells are flushed into the defect together with the bloodstream. Mesenchymal progenitors found in the trabecular bone, which display stem cell-like capabilities [15], as well as human mesenchymal progenitor cells from the subchondral bone marrow (CSP) [16] are of more the focus of interest. These CSP cells exhibit the typical cell surface antigen pattern known from human MSCs derived from bone marrow aspirates. Additionally, CSP cells have been shown to have an intrinsic osteogenic differentiation capacity and show low adipocytic development when stimulated with adipogenic medium containing insulin [16]. In contrast, stimulating CSP cells with TGF-beta3 strongly induced chondrogenic lineage development with induction of typical chondrogenic marker genes and deposition of cartilage matrix molecules like type II collagen and proteoglycan [16]. This suggests that CSP cells from the subchondral bone that are released by microfracture have a prominent chondrogenic differentiation potential and therefore are also promising candidates for cell injection into articular joints.

The objective of this work was to develop a suitable and simple technique to encapsulate CSP cells in alginate and to verify the maintenance of capacity of the incorporated cells to differentiate. In addition, we aimed to characterize the mechanical stability and permeability of the microcapsules in the course of cultivation and differentiation of the immobilized cells.

2. Materials and methods

2.1. Isolation and characterization of human mesenchymal progenitor cells from subchondral bone marrow

Human CSP cells were isolated from the lateral tibial head during high tibial closed wedge osteotomy (three donors: two male, one female, aged 40–62 years) as described previously [17]. The study was approved by the Ethical Committee of the Charite University Medicine, Berlin.

In brief, human cortico-spongious bone cylinders were cut into small pieces and treated enzymatically for 4 h in Dulbecco's modified Eagle's medium (DMEM; Biochrom), 10% human serum (German Red Cross), 100 U ml^{-1} penicillin, 100 mg ml^{-1} streptomycin (both Biochrom) and 256 U ml^{-1} collagenase XI (Sigma) in a spinner flask (Weaton) under gentle stirring at 37 °C. Subsequently, the bone fragments were placed into Primaria cell culture flasks (Becton–Dickinson) and cultured in DMEM supplemented with 10% human serum, 100 U ml^{-1} penicillin, 100 mg ml^{-1} streptomycin and 4 mM L-glutamine under standard cell culture conditions. After 5–7 days, the first cells were evident. After reaching 70% confluence, cells were detached with trypsin/ethylenediaminetetraacetic acid (EDTA) solution (Biochrom) and subcultured at a density of 8×10^3 cells cm^{-2}. After cell expansion over three passages, flowcytometric analysis (FACS) of the cells was performed. The CSP cells were characterized by FACS. Single-cell suspensions of 2.5×10^5 cells were washed once with phosphate-buffered saline (PBS) containing 0.5% bovine serum albumin (BSA; Sigma) and incubated with either fluorescein isothiocyanate (FITC)-labelled mouse anti-human CD105, FITC-labelled mouse anti-human CD45 (German Rheumatism Research Centre), FITC-labelled mouse anti-human CD44 or CD90, R-phycoerythrin (PE)-labelled mouse anti-human SH-3 (CD73), or PE-labelled CD166 or CD34 (Pharmingen) for 15 min on ice. Finally, cell samples were washed in PBS containing 0.5% BSA. Prior to the analysis with the FACSCalibur (Becton–Dickinson), cells were stained with propidium iodide (Sigma) to detect and exclude dead cells. Data were evaluated using the CellQuest software (Becton–Dickinson).

2.2. Encapsulation of cells and cultivation of cell-loaded Ca-alginate beads

Passage 3 cells were incubated with trypsin/EDTA (0.5 g l^{-1} trypsin, 0.2 g l^{-1} EDTA in PBS; Biochrom) for 5 min at 37 °C. Detached cells were centrifuged at 1000*g* for 5 min. The resulting pellet was resuspended in phosphate-free solution consisting of 8 g l^{-1} NaCl, 0.2 g l^{-1} KCl, 0.1 g l^{-1} $MgCl_2 \cdot 6\,H_2O$ and 0.1 g l^{-1} $CaCl_2$. After counting, cells were pooled in equal amounts, centrifuged again and resuspended in phosphate-free solution to a cell density of 2.4×10^7 ml^{-1}. The cell suspension was then mixed with an equal volume of 2.4% (w/v) sodium alginate solution (St. Louis, MO, USA) and dissolved in phosphate-free buffer to a final cell density of 1.2×10^7 ml^{-1} and a final alginate concentration of 1.2% (w/v). According to the manufacturer's data, its mean molar mass is between 75,000 and 100,000 g mol^{-1}, the viscosity is 0.25 Pa s (2% in water, 25 °C) and the M/G ratio is 1.56, corresponding to 61% mannuronate (M) and 39% guluronate (G). Sodium alginate solution was sterilized by filtration through a 0.22 μm filter (Stericup, Millipore GmbH, Schwalbach, Germany). For the in vitro studies described here, no additional purification of the Na-alginate was carried out.

Microspheres were produced using the laminar jet break-up technique with a vibrating nozzle, as described before [18]. The sodium alginate cell suspension was pressed through a 300 μm nozzle while loading with a frequency of 675 Hz to form equal-sized droplets under sterile conditions. The droplets fell into a stirred precipitation bath (0.1 mol l^{-1} $CaCl_2$) for solidification and forming of spherical Ca-alginate beads. The precipitation was stopped after 10 min. Cell-loaded Ca-alginate beads (approximately 2000 cells per bead) were rinsed twice with washing solution (0.01 mol l^{-1} $CaCl_2$, 0.15 mol l^{-1} NaCl) and transferred in equal amounts into differentiation medium or control medium. The microencapsulated cells were cultured in suspension cultures and either stimulated for up to 4 weeks with DMEM supplemented with insulin–transferrin–selenium (ITS + 1, Sigma), 0.1 μM dexamethasone, 1 mM sodium pyruvate, 0.17 mM ascorbic acid-2-phosphate, 0.35 mM proline (all purchased from Sigma) and 10 ng ml^{-1} TGF-beta3 (R&D Systems, Wiesbaden, Germany) for chondrogenic differentiation or in DMEM supplemented with ITS + 1, dexamethasone, sodium pyruvate, ascorbic acid and proline as control. The medium was replaced every other day. The cultures were stirred once a day. Ca-alginate spheres consisting of the same Ca-alginate but without cells were made in parallel and cultivated in control medium under identical culture conditions.

2.3. Determination of size and mechanical strength of the microspheres

The diameters of the cell-loaded and cell-free Ca-alginate spheres were measured microscopically using a calibrated micrometre eyepiece.

Download English Version:

https://daneshyari.com/en/article/2056

Download Persian Version:

https://daneshyari.com/article/2056

Daneshyari.com