Osteoarthritis and Cartilage 23 (2015) 319–327

Osteoarthritis and Cartilage

Passage-dependent relationship between mesenchymal stem cell mobilization and chondrogenic potential

A.R. Tan †, E. Alegre-Aguarón †, G.D. O'Connell ‡, C.D. VandenBerg §, R.K. Aaron ||, G. Vunjak-Novakovic †, J. Chloe Bulinski ¶, G.A. Ateshian † #, C.T. Hung † *

† Department of Biomedical Engineering, Columbia University, 1210 Amsterdam Ave, New York, NY 10027, USA
‡ Department of Mechanical Engineering, University of California, Berkeley, 5122 Etcheverry Hall, Berkeley, CA 94720, USA
§ Department of Orthopaedic Surgery, St. Luke's-Roosevelt Hospital Center, 1000 10th Ave, New York, NY 10019, USA
|| Department of Orthopaedic Surgery, Brown University, 100 Butler Drive, Providence, RI 02906, USA
¶ Department of Biological Sciences, Columbia University, 1212 Amsterdam Ave, New York, NY 10027, USA
# Department of Mechanical Engineering, Columbia University, 500 W. 120th St, New York, NY 10027, USA

ARTICLE INFO

Article history:
Received 1 April 2014
Accepted 6 October 2014

Keywords:
SDSCs
CD73
Galvanotaxis
Cartilage repair
Tissue engineering

SUMMARY

Objective: Galvanotaxis, the migratory response of cells in response to electrical stimulation, has been implicated in development and wound healing. The use of mesenchymal stem cells (MSCs) from the synovium (synovium-derived stem cells, SDSCs) has been investigated for repair strategies. Expansion of SDSCs is necessary to achieve clinically relevant cell numbers; however, the effects of culture passage on their subsequent cartilaginous extracellular matrix production are not well understood.
Methods: Over four passages of SDSCs, we measured the expression of cell surface markers (CD31, CD34, CD49c, CD73) and assessed their migratory potential in response to applied direct current (DC) electric field. Cells from each passage were also used to form micropellets to assess the degree of cartilage-like tissue formation.
Results: Expression of CD31, CD34, and CD49c remained constant throughout cell expansion; CD73 showed a transient increase through the first two passages. Correspondingly, we observed that early passage SDSCs exhibit anodal migration when subjected to applied DC electric field strength of 6 V/cm. By passage 3, CD73 expression significantly decreased; these cells exhibited cell migration toward the cathode, as previously observed for terminally differentiated chondrocytes. Only late passage cells (P4) were capable of developing cartilage-like tissue in micropellet culture.
Conclusions: Our results show cell priming protocols carried out for four passages selectively differentiate stem cells to behave like chondrocytes, both in their motility response to applied electric field and their production of cartilaginous tissue.

Introduction

Articular cartilage is the connective tissue that lines diarthrodial joints and serves to bear load and provide low friction and wear during motion[1]. When the tissue is damaged through physical injury or a disease such as osteoarthritis (OA), the healing response is inadequate due to the avascular nature and limited cellularity of adult cartilage[2]. We and others have investigated the use of mesenchymal stem cells (MSCs) found in the neighboring synovium, that are known as SDSCs[3,4], for the repair of articular cartilage. With the addition of appropriate lineage-specific culture medium, the population of SDSCs has been confirmed to be multipotent, capable of differentiating into several mesenchymal lineages, including chondrocytes, osteoblasts, adipocytes, and myocytes[5–7]. As such, harvested SDSCs expanded through multiple passages *in vitro* in the presence of a chondrogenesis-promoting growth factor cocktail[3,4] have been shown to produce extracellular matrix (ECM) components similar to chondrocytes (i.e., collagen II and aggrecan)[4,6,8] and to generate material and mechanical properties similar to native cartilage when encapsulated in agarose hydrogel[3]. SDSCs thus represent a promising new source for cell-based strategies for the repair of articular cartilage.

* Address correspondence and reprint requests to: C.T. Hung, Department of Biomedical Engineering, Columbia University, 1210 Amsterdam Ave, New York, NY 10027, USA. Tel.: 1-212-854-6542.
E-mail address: cth6@columbia.edu (C.T. Hung).

http://dx.doi.org/10.1016/j.joca.2014.10.001
1063-4584/

The delayed, ultimately poor healing ability of articular cartilage is thought to be due, in large part, to insufficient migration to the damage site by cells that have the potential to repair the lesion[9]. Thus, strategies that enhance and direct SDSC migration could amplify the intrinsic repair process. The use of direct current (DC) electric fields (EFs) of strengths ranging from 1 to 10 V/cm is known to induce directed movement (galvanotaxis) and shape change (galvanotropism) in a number of musculoskeletal cells including chondrocytes, fibroblasts, osteoblasts, osteoclasts, and meniscal fibrochondrocytes[10]. Endogenously generated EF gradients of this strength have also been shown to guide cell migration in developing embryos[11] and at the cut surface of wounds[12]. Hence, galvanotaxis may be a useful tool for encouraging and promoting the migration of a clinically relevant cell type to sites of articular cartilage defects.

Stem cells such as SDSCs exhibit phenotypic and behavioral changes as terminal differentiation is reached. Previously, we determined via flow cytometry, that changes in cell surface molecules are measurable throughout differentiation; some of these surface molecules may in fact serve as growth factor receptors or adhesion molecules that play a direct role in the differentiation process. Further, it was shown that manipulation of cell surface charges with chemical modifiers such as neuraminidase, an enzyme that removes sialic acids[13], or lectins[14], alters EF motility[15], pointing to a strong influence of surface molecules in directed migration. Taken together, these results suggest the hypothesis that differentiating SDSCs exhibit markedly different behavior depending on culture expansion age.

We are unaware of any previous studies that correlate a change in cell surface marker expression with a change in migration response to EF. Furthermore, it is not clear whether cells of a certain passage are preferentially more likely to adopt a phenotype favorable for cartilage matrix development. Thus, in this paper, we examine the surface markers and migration characteristics of SDSCs through four passages of culture; we hypothesize that as SDSCs reach a chondrocytic phenotype, their migration characteristics begin to resemble those reported for chondrocytes[16]. In parallel, through 3D pellet cultures using cells from each passage, we attempt to elucidate the passage at which SDSCs exhibit the greatest potential to synthesize cartilaginous matrix, in order to optimize their use for tissue-engineered constructs.

Methods and materials

Experimental design

Three controlled and concurrent cell culture studies are described herein, aimed at characterizing the behavior of juvenile bovine SDSCs. Specifically, study one examined the effect of cell passage number on the expression of four cell surface markers found on MSCs. Study two characterized the cellular response of these cells at each passage to an applied DC EF of comparable magnitude as those found at the cut surface of wounds in order to identify a cell population most suitable for wound repair. Finally, study three explored the development of tissue at each passage to investigate a potential correlation between cell migration behavior and matrix composition.

Cell isolation and expansion

The intimal layer of the synovium was harvested from bovine knee joints of four freshly slaughtered 2–4 week old calves, as described previously[3]. Medium supplemented with 1 ng/mL TGF-β1, 5 ng/mL bFGF, and 10 ng/mL PDGF-ββ[3] was changed every three days. At confluence, cells were trypsinized and then counted. One subset of the cells at each passage was replated for further expansion, another subset was used for flow cytometry analysis, a third subset was used for galvanotaxis experiments, and a final subset was used for 3D micropellet culture.

Cell surface marker assessment

The phenotype of bovine SDSCs at each passage (P1 to P4) was assessed by flow cytometry, as previously described[17]. Cells were stained with antibodies against CD31 (endothelial cell marker, Thermo Scientific), CD34 (hematopoietic cell marker, Abcam), and mesenchymal markers CD49c (Thermo Scientific) and CD73 (BioLegend)[18,19]; positive MSC classification requires the absence of CD31 and CD34 and presence of CD73[20], while CD49c is a constant marker of chondrogenic potential of MSCs, decreasing only in the presence of TGF-β3[21,22]. Cellular fluorescence was evaluated using a FACSCalibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ) and the resulting data analyzed with FlowJo software (version 9.3.2).

Galvanotaxis

After trypsinization, cells were allowed to equilibrate for 1 h before being plated at 2.65×10^4 cells/cm^2 onto sterile glass slides (Fisher Scientific, Pittsburgh, PA) with removable silicone wells. To investigate the influence of CD73 on P1 cells, one subset of cells was exposed to 100 μM 5′-(α, β-methylene)diphosphate (APCP)[23], a CD73 inhibitor, during the plating period. After cells were allowed to attach for 1 h in a 5% CO_2 incubator at 37°C, the slide was rinsed with medium to remove any nonadherent cells and placed into a custom galvanotaxis chamber under aseptic conditions [Fig. 1, described in Ref. 16].

A power supply (Kiethley Instruments) delivered a current of 3.3 mA (6 V/cm EF strength) through the chamber, and experiments were performed at room temperature for 3 h. Control slides were treated similarly without EF. Cell migration patterns were captured every 10 min using an Olympus IX-70 inverted microscope and digital camera ($n = 30$–40 cells observed in each field of view).

Cell migration parameters

The position of each cell was manually tracked via a custom MATLAB program[16,24]. Migration direction was quantified as sin φ, where φ is the angle between the x-coordinate axis and the migration vector, such that sin φ was defined as the value −1 when $\varphi = 4.71$ rad (270°), the direction of the cathode. The directional velocity, defined as the component of the speed directed toward the negative pole (e.g., Refs. 16,25), was obtained by multiplying a cell's speed by sin φ.

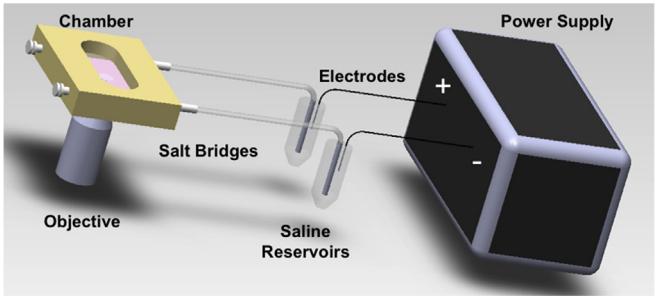

Fig. 1. Schematic of galvanotaxis setup. Cells seeded on a glass slide were inserted into a custom chamber and exposed to an electric field gradient for three hours.