TARGETED DELIVERY OF STEM CELLS TO OSTEARTHritic CARTILAGE USING LACTOSE-MODIFIED CHITOSAN

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INTRODUCTION:
Osteoarthritis (OA) is a degenerative disease that results in loss of articular cartilage in the joints. Since mesenchymal stem cells (MSCs) have the capacity to differentiate into chondrocytes, the directed delivery of these cells to OA cartilage may lead to an enhanced repair response or to a reduction in cartilage loss. Chitlac, a lactose-modified chitosan, enables aggregation of chondrocytes and promotes collagen and glycosaminoglycan (GAG) production (1). There is evidence that cell surface galactin-1 (Gal-1) acts as the chitlac receptor in chondrocytes and that interaction with Gal-1 leads to the formation of chitlac aggregates. (2). Gal-1 is also expressed on the surface of human MSCs (hMSCs) and participates in extracellular matrix (ECM)-cell interactions (3). It seems possible, therefore, that chitlac may act as a cellular linker between transplanted MSCs and chondrocytes in OA cartilage and that this may represent an effective delivery strategy. The objective of this study was to assess the usefulness of Chitlac in this context and to determine if it could be used to achieve both targeted delivery and enhanced engraftment in OA. For this purpose a human OA cartilage explant culture system was used to assess tissue repair.

METHODS:
Isolation and Expansion of Human MSCs and Human Cartilage Explants: Bone marrow-derived human MSCs were obtained from the iliac crest of normal donors and cartilage explants were obtained from OA patients undergoing total knee arthroplasty. All procedures were performed with informed consent and approved by the Clinical Research Ethical Committee at University College Hospital, Galway. MSCs were isolated and expanded in culture as described previously by direct plating (4). Cartilage explants (1-2 mm thick and 2 mm diameter) were obtained from the tibial plateau of tissue obtained after total knee arthroplasty. Explants were placed in 10% fetal calf serum (FCS) containing media for 48 h at 37ºC and subsequently cultured in ICM for 24 h.

Chitlac Preparation: Chitlac, a gift from Prof. Sergio Paoletti. University of Trieste, Italy was prepared according to the procedure reported elsewhere (1). Chitlac (0.2%) was prepared by dissolving the freeze-dried material in incomplete chondrogenic media (ICM), followed by sterile filtration prior to use. hMSCs were resuspended in the chitlac solution (2mg/ml) and placed on a shaker for 2 h at room temperature prior to use.

Cell Growth: The effect of pre-treatment or continuous exposure by chitlac on the growth of hMSCs was assessed. hMSCs were either resuspended in chitlac solution (2mg/ml) for two hours on a shaker at room temperature or pre-treated with ICM alone. Additional cultures were established (using untreated hMSCs) to assess the effect of continuous exposure to 0.5, 1, 1.5 and 2 mg/ml chitlac. The culture media was changed every other day until day 6. Cell growth was determined at day 6 using the Viacount Assay on the GUAVA EasyCyte analyser.

Differentiation Assays: hMSCs were exposed to chitlac for 2 h at room temperature with agitation and placed in chondrogenic, osteogenic and adipogenic differentiation capacities as described previously (4). Untreated hMSCs were used as controls.

Chitlac-treated MSCs Targeted to OA Cartilage Explants: MSCs labeled with Cell Tracker Red and DAPI were exposed to chitlac as described previously and added to explants at a cell density of 1 x 10⁶ cells/ml for 20 min at 37ºC with agitation. Labelled MSCs were added to explants under the same conditions to serve as a control. Unattached cells were removed by washing and explants were either processed immediately or maintained in culture in complete chondrogenic medium (CCM) for 14 days. Cartilage disks incubated without cells were used as further controls. Formalin-fixed, paraffin-embedded sections were stained with haematoxylin and eosin (H & E), Toluidine blue, immunostained for collagen type I and II or processed for fluorescence microscopy.

RESULTS:
hMSCs cultured in the presence of chitlac showed no significant difference in proliferation over the 6 day period. Pre-exposure to the material for 2 h resulted in slightly reduced cell growth compared to control MSCs and MSCs cultured in the presence of varying concentrations of chitlac. Proliferation of cells appeared to gradually increase when cultured in the presence of increased concentrations of chitlac (Fig. 1). Cells pre-treated with chitlac for 2 h and cultured in the presence of chitlac appeared morphologically similar to control cultures. Differentiation of MSCs pre-treated with chitlac was not significantly different to that of untreated MSCs in each of the three lineages (Fig. 2 a-c). MSCs treated with chitlac and MSCs alone adhered to the OA cartilage at 20 min. Bound cells survived and started to differentiate to promote repair of the cartilage surface when exposed to CCM for 14 days.

DISCUSSION:
Results suggest that Chitlac had no significant impact on the ability of hMSCs to proliferate or differentiate along the chondrogenic, osteogenic and adipogenic pathways. MSCs treated with Chitlac and MSCs alone bound to the osteoarthritic cartilage at 20 min. Adherent cells underwent a change in morphology and elaborated a cartilaginous matrix over time. The ability of targeted cells to repair the cartilage surface can be assessed as explants can be maintained in culture for up to 14 days.

Strategies for stem cell-mediated repair of damaged cartilage as a result of OA will require targeting stem cells to damaged cartilage. Our results suggest that Chitlac does not impair the proliferation or differentiation capacities of MSCs thus making it an attractive biomaterial for stem cell delivery to damaged OA cartilage. We have established a functional explant culture system to evaluate in vitro MSC binding to OA cartilage to allow assessment of tissue-specific targeting strategies.

REFERENCES:

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