The hyaluronan pretreatment promotes chondrogenic differentiation of human adipose derived stem cells in a fibrin hydrogel

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Introduction:
Adipose derived stem cells (hADSCs) are an alternative cell source with several advantages including easy to harvest, high proliferation rate, and multilineage potentials. It also has been reported that hADSCs can be characterized by specific surface antigen like CD166, CD105, or CD44. Guiding stem cells differentiate into desired lineage may be important for stem cell based cartilage tissue engineering. Niche and cell surface antigen interaction plays an important role in influencing stem cells differentiation. Hyaluronan (HA) is one of the major extracellular matrix (ECM) of the embryonic mesenchyme, and our previous finding also showed that HA-microenvironment initiates chondrogenesis of ADSCs. The CD44 is the primary cell surface receptor for HA. We hypothesize that HA and CD44 interaction may contribute to the chondrogenic differentiation of ADSCs, and can be applied for more effective stem cell based articular tissue engineering. We investigated the effect of HA-pretreatment on promoting ADSC chondrogenesis in a fibrin hydrogel for future cell based articular cartilage tissue engineering.

Methods:
Human adipose derived stem cells (hADSCs) isolation: Adipose tissue was obtained from orthopaedic patients undergoing surgery. Cell specific surface antigen detection on hADSCs: CD105, CD166 and CD44 were detected by flow cytometry. HA-pretreatment and hADSCs encapsulation in fibrin hydrogel: hADSCs were expanded at 37°C under 5% CO2 in K-NAC medium containing Keratinocyte-SFM (Gibco BRL, Rockville, MD) supplemented with 5% Fetal bovine serum. hADSCs were incubated in HA in PBS or PBS only at 37°C under 5% CO2. After incubation, hADSCs were collected for further experimental analysis. To evaluate chondrogenesis, hADSCs were evaluated by examining mRNA expressions of chondrogenic (SOX-9, collagen type 2a1 and aggrecan) or osteogenic gene (cbfa1) using real time PCR. Sulfated glycosaminoglycan synthesis was determined by dimethylmethane blue (DMMB) assay. Sirius Red dye (Direct Red; Sigma) was used to stain total intracellular collagen. For histological procedures, the histological sections (5 μm thick) were stained for H&E staining. Statistical analyses were performed using Student’s t-test, with p values below 0.05 being considered significant.

Results:
The cell specific surface antigens (CD105, CD166 and CD44) were assessed using immunofluorescent staining and flow cytometry. CD105, CD166 and CD44 were detected on cell surface of hADSCs. Among those receptors, the CD44 is the main receptor for HA and have the potential for mediating HA signal into hADSCs. The flow cytometry data showed that 99.9% of the tested hADSCs possessed the CD44 receptor on cell surface (Figure 1). At 4 hours after encapsulation, our data showed that 99.9% of the tested ADSCs possessed the CD44 receptor on cell surface (Figure 1). At 4 hours after encapsulation, our result showed that nearly all of the hADSCs encapsulated in 3D fibrin hydrogels were remained viable as indicated by live (green)/dead (red) staining, and more cell aggregations were found in HA-pretreated group (Figure 2). The mRNA expressions of SOX-9, collagen type 2a1 and aggrecan of hADSCs cultured in HA-pretreatment group were significantly increased from day 1 to day 5 of culture comparing with the control cultures (without HA-pretreatment). The sulfated glycosaminoglycan and total collagen synthesis of hADSCs in fibrin hydrogel were also significantly increased at day 7 and day 14 of culture comparing with the control cultures. The mRNA expression of cbfa1, an osteogenic gene, was not changed in hADSCs cultured in HA-pretreated group. On the other hand, histological analysis show that the cells remained rounded in both groups, but better cell growth were observed in the central areas of HA-pretreated group comparing with non-treated group (Figure 3).

Discussion:
It is important for stem cell based cartilage tissue engineering to provide a 3-dimensional scaffold and appropriate cell-niche interaction for better chondrogenic differentiation. Our results indicate that HA-pretreatment promoted chondrogenic differentiation of hADSCs in a fibrin hydrogel. This finding suggests that HA-pretreatment may provide better cell-niche interaction that may be an alternative choice for applying to stem cell based cartilage tissue engineering.