In Vivo Chondrogenesis Using Chondrogenic Induced Human Bone Marrow Stromal Cells (BMSCs) Mixed With a Novel Ultra-purified Alginate Gel; a Report Of Preliminary Study

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Introduction: The articular cartilage potential for self-repair is limited which entails surgical interventions for possible lesions. Tissue engineering techniques as Autologous Chondrocytes Implantation (ACI) had been used for repair of such lesions which showed successful outcomes. However, prospective randomized controlled trials suggested that no higher potentials for ACI over other surgical procedures for the treatment of osteochondral defects1,2. Previous studies using novel ultra-purified alginate gel that we developed showed its high potential to stimulate the formation of cartilage like tissues3,4. This purified alginate has a quite low endotoxin level compared to other commercial-grade alginate gels. We hypothesized that Ultra-purified alginate is a potential novel scaffold material for cartilage tissue regeneration. We investigated the feasibility of the in vivo chondrogenesis of human bone marrow stromal cells (hBMSCs) using the ultra-purified alginate gel (UPAL) scaffold material.

Methods: This study was approved by our institutional review board. 3 groups of UPAL gel biomaterial with 3 different molecular weights 430kDa (AL20), 1000kDa (AL100) & 1700kDa (AL500) respectively were used as scaffold and mixed with hBMSCs obtained from acetabulum of one consented donor who underwent osteotomy surgery. As a control a group of gel only was used to exclude any host tissue invasion. In vitro chondrogenic induction of alginate beads for 2 weeks followed by 3 weeks of in vivo implantation in the back of nude mice (n=8). Extracted beads were evaluated for macroscopy; histology and immunohistochemistry staining (H&E, Safranin O and anti-Type II Collagen). mRNA expression had been also assessed by reverse transcription-polymerase chain reaction (RT-PCR).

Results: In vitro samples didn’t show significant differences among groups either macroscopically or histologically. While for in vivo results AL20, AL100 were better than AL500 as they maintained shape and size of beads throughout the experiment. Histologically AL20 and AL100 showed significantly higher collagen II intake than AL500 through 2-3 weeks, also exhibited signs of chondrogenic differentiation on cellular level (Fig.1).
Beads from control group didn’t show any changes throughout the experiment time course, moreover there was no evidence of host tissue invasion into the scaffold material (Fig.2).
RT-PCR results exhibited similar results as samples from AL100 in-vivo group showed higher mRNA expression for Type II collagen compared with the other two groups.

**Discussion:** This study showed that this novel ultra-purified alginate gel exerts potential chondrogenic capabilities. Our data suggest that UPAL gel effectively mediated the chondrogenic differentiation in vivo. Of the proposed molecular weights AL100 (1000kDa) showed best results as it showed better expression of type II Collagen as assessed by immunohistochemistry and mRNA expression. To our knowledge this is the first study to report the usage of this novel UPAL gel with human cells. This report showed data from our preliminary studies, further experiments utilizing statistically sufficient number of observations are needed to evaluate the entire chondrogenic differentiation process; for mRNA expression, DNA quantification and ELISA.

**Significance:** Articular cartilage injuries are typically common encounter in Orthopaedic surgery practice. Current procedures didn’t show superior outcomes. Novel materials are needed in order to regenerate hyaline cartilage like tissue and further improve the overall reparative process of an articular cartilage lesion.

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