

## Enhancement of chondrogenesis of human bone marrow-derived mesenchymal stem cell by centrifugation or glucosamine sulfate

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**INTRODUCTION:** Bone marrow-derived mesenchymal stem cells (bMSCs) have generated significant interest in cartilage tissue engineering as an alternative for the autologous chondrocytes because of the potential ability to self-renewal and ability to differentiate into multi-lineage cell types, and the limitation of the chondrocyte availability. It has been shown that mechanical forces or glucosamine sulfate (GS) could stimulate proteoglycan synthesis. In this study, we investigated whether centrifugation or exogenous GS could enhance the chondrogenesis of bMSCs.

**METHODS:** Human bMSCs were isolated by plastic adherence and expanded in monolayer with  $\alpha$ -MEM supplemented with 10% FBS, 1% penicillin/streptomycin, 2 mM L-glutamine and 250 ng/ml fungizone before chondrogenesis. Human bMSCs at passage 2 (250,000 cell/pellet) were induced into chondrogenesis with chondrogenic medium, which included  $\alpha$ -MEM supplemented with 100 nM dexamethasone, 1% ITS+, 50  $\mu$ g/ml ascorbate-2-phosphate, 40  $\mu$ g/ml proline, 100  $\mu$ g/ml pyruvate, 500 ng/ml BMP-2 and 10 ng/ml TFG- $\beta_3$  for 21 days, or the chondrogenesis was performed with chondrogenic medium plus 100  $\mu$ M GS or centrifugation with 10xg for 10 mins at 37°C daily for 21 days. At the end of the culture, the samples were collected for histological staining of Toluidine blue, Safranin O and Hematoxylin & Eosin.

**RESULTS:** Histological staining of Toluidine blue (Fig.1) or Safranin O (Fig.2) showed that more proteoglycans found in the chondrogenic samples that were induced by the chondrogenic medium plus 100  $\mu$ M GS or centrifugation than that induced by chondrogenic medium. Macroscopic cell pellet induced by chondrogenic medium plus 100  $\mu$ M GS or centrifugation was bigger and stiffer than that from chondrogenic medium.

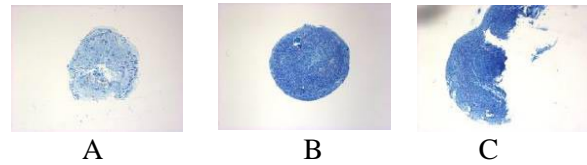


Fig. 1: Toluidine Blue (A: chondrogenic medium, B: chondrogenic medium + 100  $\mu$ M GS, C: chondrogenic medium + Centrifugation)

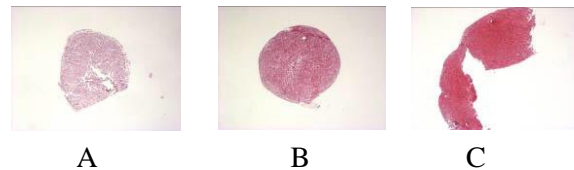


Fig. 2: Safranin O (A: chondrogenic medium, B: chondrogenic medium + 100  $\mu$ M GS, C: chondrogenic medium + Centrifugation)

### DISCUSSION & CONCLUSIONS:

Glucosamine (GlcN) is an amino monosaccharide found in cartilage, and has been used to treat patients with osteoarthritis as nutraceutical. Previously, 100  $\mu$ M or 1 mM GlcN enhanced human bMSC chondrogenesis and maintained cartilage matrix gene expression in chondrocytes (1). It has also been shown an increased expression of aggrecan and type II collagen in chondrocytes after the cells were exposed to 10xg gravity at 37°C (2). Centrifugation of chondrocytes in pelleted culture could also stimulate proteoglycan synthesis (3). We conclude from our preliminary results that exogenous GS or centrifugation may be simple procedures to enhance the chondrogenesis of bMSC, however, these results need to be further confirmed.

**REFERENCES:** <sup>1</sup>Derfoul A et al., Osteoarthritis and Cartilage, 2007, 15:646-655. <sup>2</sup>Basile V et al., Microgravity Sci. Technol. 2009 21:151-157. <sup>3</sup> Inoue H et al., J. Dent. Res., 1990, 69:1560-1563.

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