Tenogenic Expression of Differentiated Mesenchymal Stem Cells is Dependant on Growth and Differentiation Factor-5

INTRODUCTION:
Although the use of growth-differentiation factor-5 (GDF-5) and mesenchymal stem cells to repair damaged tendons has been previously described, the optimal amount of GDF-5 concentration required to elicit a favourable tenogenic response from mesenchymal stem cells (MSCs) has not been clearly established. To determine this relationship, a study was conducted to ascertain the amount of tenogenic expression of mesenchymal stem cells at different GDF-5 concentrations in vitro and to compare these changes to native tenocyte.

MATERIALS & METHODS:
Ethics approval for this study was granted by the Animal Care and Use Committee in University of Malaya (Reference number: PM/24/06/2008/TKE). Rabbit tenocytes and bone marrow derived MSCs from New Zealand White rabbits were harvested, processed and expanded following methods previously established. Characterization of the MSCs and tenocytes was performed to confirm the homogeneity and validity of the appropriate cell lineage utilized in the current study. To determine the dose response between GDF-5 and the phenotypic expression as the result of MSC transformation, MSCs seeded in six-well culture plates were maintained in serum free culture medium supplemented with GDF-5 at different concentrations for 4 days. MSCs cultured in basal cell culture medium (supplemented with 10% FCS) and tenocytes cultured in serum free medium were used as controls. The total collagen expression of the MSCs was determined by using Sircol™ collagen assay of rabbit MSC in culture medium and tenocytes cultured in serum free medium were used as controls. The total collagen expression of the MSCs was conducted to ascertain the amount of tenogenic expression of MSCs transformation, MSCs seeded in six-well culture plates were maintained in serum free culture medium supplemented with 10% FCS and tenocytes cultured in serum free medium were used as controls. The total collagen expression of the MSCs was determined by using Sircol™ collagen assay. Four (n=4) samples were used in this experiment, each conducted in triplicates. Real-time PCR was also conducted to assess the gene expression profile of the tendon specific marker, Scleraxis (Scx) and type-I collagen (Col-I) in: (1) tenocytes, (2) MSCs cultured in growth medium supplemented with 10% FCS, and (3) MSCs treated with GDF-5. Data attained from the study was analyzed using SPSS statistical software package (ver. 13.0).

RESULTS:
Cells isolated and cultured from rabbit bone marrow exhibited mesenchymal stem cell characteristics. Primary tenocytes attained from rabbits demonstrated increased expression of type I/III collagen and none to collagen type II. Increasing GDF-5 concentrations in cell cultures increased collagen expression proportionately (Figure 1). Although there was a reduction in the total collagen expressed in cultures supplemented with low concentrations of GDF-5 (i.e. 5 and 25 ng/ml), these differences were not significant when compared to untreated MSCs. (Mann Whitney-U test: p>0.05). Total collagen expression was significantly higher in cell cultures treated at higher concentrations of GDF-5 (i.e. 100 and 500 ng/ml) than that at lower concentrations (i.e. 0, 5, 25 ng/ml). At high concentrations, total collagen was also comparable to that of tenocyte cultures.

DISCUSSIONS:
This study demonstrated a correlation between total collagen expression and the increasing dose of GDF-5 in MSCs. Based on the dose response curve attained from this study, it appears that GDF-5 concentration between 100-500ng/ml produced comparable collagen expression to that of native tenocytes. However, tenogenic specific gene expressions appear to be highest when treated with 50ng/ml of GDF-5. While previous studies have demonstrated the importance of GDF-5 in MSCs transformation, our study contributes to further observation that a minimum amount of 100ng/ml of GDF-5 concentration is required to elicit optimal tenogenic differentiation in T-MSCs.

CONCLUSION:
The use of GDF-5 in the differentiation of MSCs to T-MSCs is dose dependent with an observed optimal GDF-5 concentration of 100-500ng/ml required to produce an appropriate tenogenic response. However, at the concentration of 50ng/ml of GDF-5, T-MSCs expressed tendon specific genes (SCX and Col-I) compatible or better than the native tenocytes.

REFERENCES:
2. Tan et al., International Society for Stem Cell Research (ISSCR) 7th Annual Meeting 2009; Pg226.