

The Effect of Growth and Differentiation Factor 5 in Tenogenic Differentiation

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INTRODUCTION: Growth and differentiation factor 5 (GDF-5 or bone morphogenetic proteins 14) is a multifunctional protein molecule that plays an important role in tissue differentiation¹. Although its role in tendon repair has been described, its use *in vitro* tenogenic differentiation from MSC has not been determined. Owing to the limitation in getting allograft for tendon repair, mesenchymal stem cell provides an alternative cell based therapy for damaged tendons. We therefore conducted an *in vitro* trial to isolate, characterize, proliferate and differentiate rabbit bone marrow derived MSCs with GDF-5 in view of future *in vivo* applications.

METHODS: Rabbit MSCs isolated from rabbit bone marrow via gradient centrifugation methods and expanded using cell culture techniques were used in this study. Total RNA were extracted from MSCs and further analyzed using our custom designed primers based on sequence information attained from GenBank database. Concurrently, MSCs fixed and embedded in epon were viewed using transmission electron microscope (TEM). To study the possibility of tenogenic differentiation, MSCs seeded in six-well culture plates were maintained in culture medium supplemented with GDF-5 at different concentrations for 4 days. The collagen expression of the MSCs was determined using SircolTM collagen assay. Real-time PCR was conducted to assess the gene expression profile of the type-I collagen and Scleraxis in MSC in growth medium, differentiation medium with and without GDF-5, and tenocyte.

RESULTS: Plastic adherent mononuclear cells appear heterogeneous with the majority of cells possessing fibroblastic morphology. The mononuclear cells isolated expressed CD29 and CD166 proteins but were negative for CD 45, which is similar to that reported previously. TEM images demonstrated ultra structures which may be of interest for further biological understanding. Collagen expression in MSC appears to up-regulated with increasing GDF-5

concentrations (table 1). MSC grown in tenogenic medium showed an increase in their gene expression in type-I collagen and Scleraxis as compared to undifferentiated MSC and autologous cultured tenocytes.

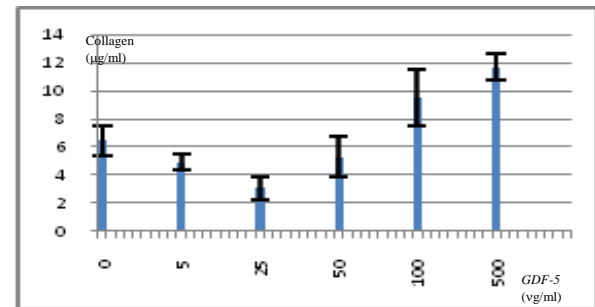


Table 1: Collagen expression of tenogenic MSC quantified by Sircol Collagen assay kit.

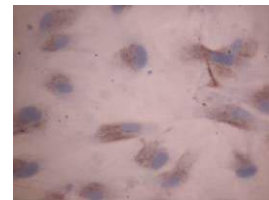


Fig. 2: Collagen type-I expression in rabbit MSC after induced by tenogenic medium (400X magnification).

DISCUSSION & CONCLUSIONS: Our technique of MSC isolation appears to produce replicable results validated using our characterization methods. Previous study reported that GDF-5 plays an important role in tendon repair^{1,2}. In our study, tenogenic differentiation appears achievable and is useful for future clinical application namely in tendon repair using differentiated MSCs.

REFERENCES: ¹ Chhabra A. et al. J Ortho Res, 2003, 21 826-35. ² Rodeo SA., Technique in Orthopaedics, 2007; 22(1): 10-13.

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