Influence of TGF β1concentration on chondrogenesis in mesenchymal stem cells

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Module 3 Report Figures:

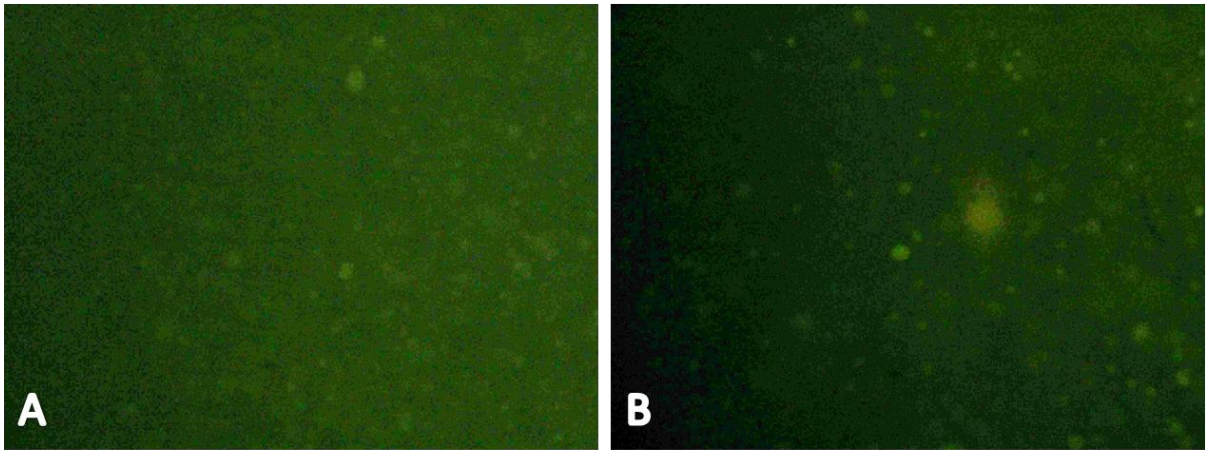

Figure 1: Live/dead fluorescence assay suggests cells for both samples were viable for further analysis. Images taken at 10x objective. For each sample, 3 alginate beads were recovered, tagged with dye, and subjected to a fluorescence assay. Cells were viewed using a FITC filter which returns green images for live cells and red images for dead cells. Both the standard concentration of TGF β1 (A) and the fivefold reduction (B) returned fields of view that had only green cells. These findings support conclusions that viable cells existed for each sample and the cells could be used for further analysis using RT-PCR and ELISA.

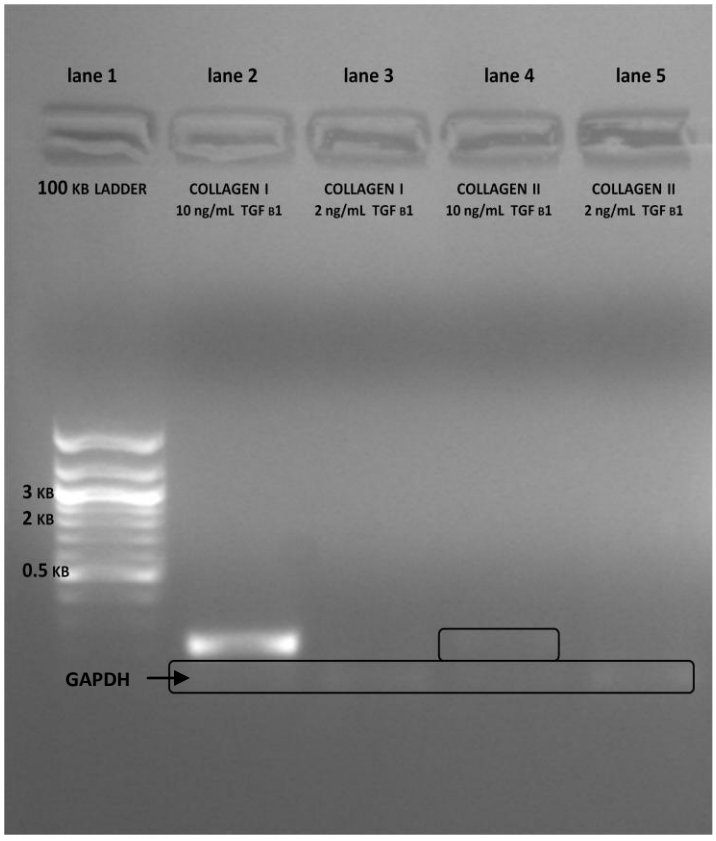

Figure 2: RT-PCR analysis suggests TGF β1 concentration marginally impacts chondrogenesis in MSC. Both the standard and fivefold reduction samples were subjected to RT-PCR analysis to examine whether TGF β1 concentration correlates with collagen II production. Samples were run on a 1.2% agarose gel (45 min, 125V) alongside a 100 bp ladder (lane 1). Despite faint bands, results indicate that collagen I production was greater for both samples than collagen II production. For the standard sample (10 ng/mL TGF β1) a bright band is visible for collagen I (lane 2) while a much fainter band is present for collagen II (lane 3). The same situation is visible for the fivefold reduction sample in which the collagen I band (lane 4) is more intense than the collagen II band (lane 5). These findings suggest TGF β1 does not directly induce collagen II production.

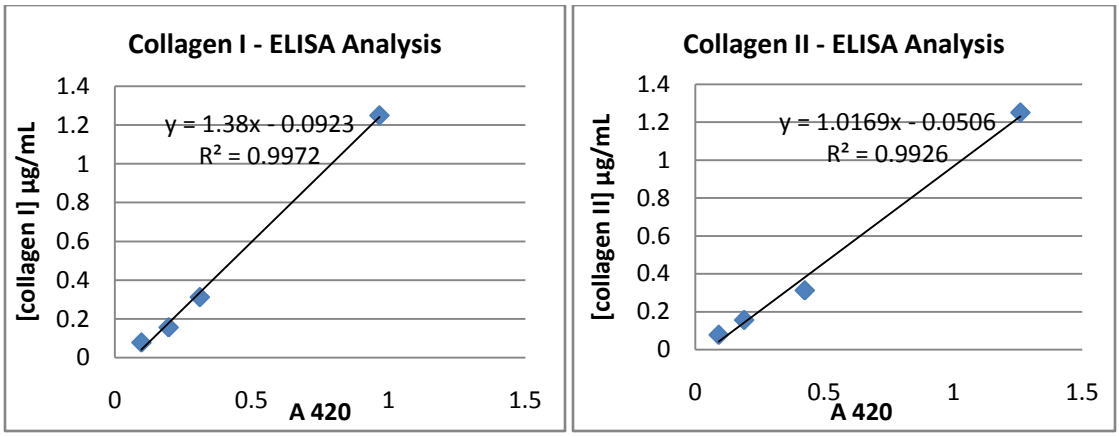

Figure 3: Known collagen concentrations used to analyze ELISA results. In order to analyze results obtained during the ELISA assay, a gradient of know collagen concentrations was assayed along with the samples. These concentrations and the absorbencies were used to calculated collagen I and collagen II levels for the 10 ng/mL and fivefold reduction samples.

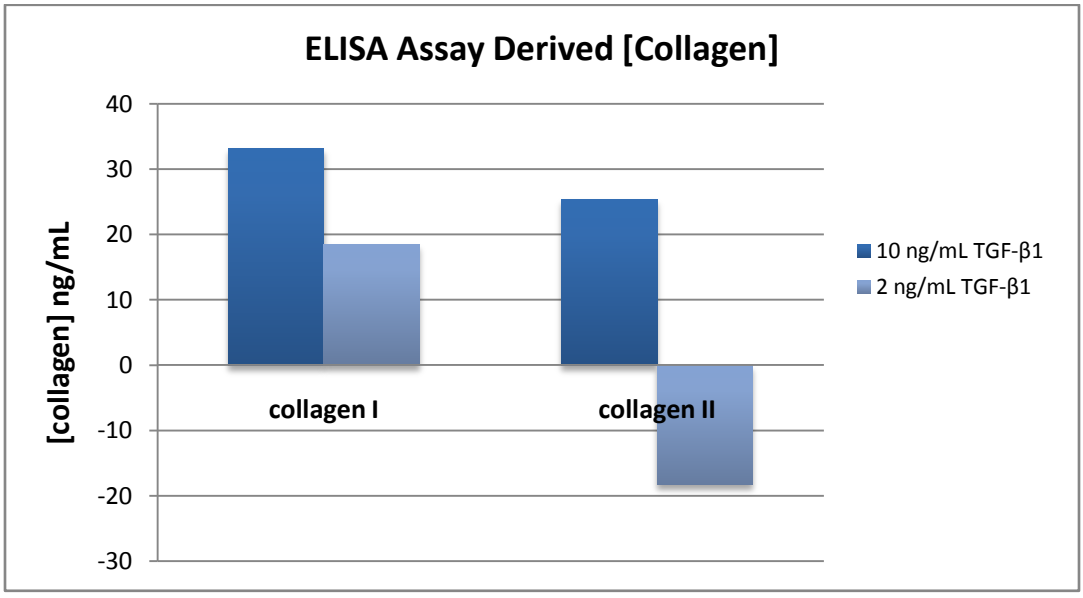

Figure 4: ELISA analysis suggests TGF β1 concentration marginally impacts MSC collagen production. An ELISA analysis was conducted for the standard and fivefold reduction samples in order to observe the impact of TGF β1 concentration on collagen production. Results for the standard (10 ng/mL) sample (darker columns) indicate that collagen I production was greater than that of collagen. This trend is also observed in the fivefold reduction (2 ng/mL) sample (lighter columns). Data also suggest that a greater amount of collagen production occurred in the standard sample in comparison to the fivefold reduction.