Mechanical Stimulation Increases Collagen Type I Gene Expression Of Murine Stem Cell-Collagen Sponge Constructs

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Introduction: A strategy that would permit investigators to monitor near real-time collagen gene expression could speed the tissue development process by avoiding the creation of inappropriate construct matrices. To that end, we created doubly transgenic mice with type I and II collagen promoters linked to Green Fluorescent Protein-Topaz (GFP-T) and Cyan Fluorescent Protein (CFP) respectively. As such, this technology allows us to track near real-time changes in col 1 (GFP-T) and col 2 (CFP) gene expression. Our objective in this study was to analyze the effects of tensile stimulation on GFP-T expression in mesenchymal stem cells (MSCs) cultured in 3-D collagen sponge scaffolds. We hypothesized that tensile stimulation would increase: 1) GFP-T fluorescence and col 1 gene expression compared to non-stimulated constructs and 2) GFP-T fluorescence and col 1 gene expression would be positively correlated.

Materials and Methods: MSCs were isolated from the bone marrow [1] of six 6-week old mice and cultured in monolayer until passage 2. Ten constructs for each animal (2 for day 0, 4 for day 7 and 4 for day 14) were created by seeding 0.5 x 10⁶ MSCs onto type I collagen sponge scaffolds in silicone dishes. Cell-sponge constructs were then acclimatized in the dish for two days before being stimulated for 5 hours/day in tension for either 7 or 14 days using a repeated profile (2.4%-peak strain for 20 seconds at 1 Hz followed by a rest period at 0% strain for 100 seconds). Control specimens were exposed to identical culture conditions without mechanical stimulation. Constructs were then prepared for evaluation of gene expression at all three time points (0, 7 and 14 days) using fluorescence analysis and real-time quantitative RT-PCR. To measure fluorescence, constructs were visualized in a Zeiss Axiovert fluorescent microscope with a filter set for GFP-T (XF104-2, Omega) and then digested in 4 ml of 150U/ml Type I collagenase (Sigma) for 40 min followed by 2 ml of trypsin (Invitrogen) for 20 min. GFP-T Relative Fluorescence Units (RFUs) in these digests were quantified using a spectrofluorimeter (SpectraMax M2, Molecular Devices) using excitation/emission/cut-off wavelengths of 491nm/531nm/530nm, respectively. Constructs subjected to gene expression analysis, were treated with RNAlater (QIAGEN Inc.,Valencia, CA) and frozen at -80°C. RNA was extracted using RNasy minikit (QIAGEN Inc., Valencia, CA). First-strand complementary deoxyribonucleic acid (cDNA) was generated using a reverse transcriptase (RT) reaction. Quantitative real time PCR was performed with a continuous fluorescence detector (MJ Research Inc., Waltham, MA). Mouse-specific primers were used for GAPDH, and col 1. Standard curves were created so that gene expression could be quantified and then normalized by calculating target gene to GAPDH ratios for each sample. RFUs and RT-PCR data were examined using a mixed-effect model (SAS proc mixed) with culture time and stimulation as fixed factors and animal as a random factor. Significance was set at α = 0.05.

Results: MSCs did not fluoresce at day 0. GFP-T fluorescence was observed in all constructs at days 7 and 14 but not in the presence of other filter sets. Fluorescing MSCs appeared elongated in both non-stimulated (NS) and stimulated (S) constructs (Fig. 1). Cells in the S constructs were not uniformly aligned in the tensile strain direction. Constructs loaded for 7 and 14 days showed higher col 1 gene expression compared to NS controls at the same time interval. S constructs showed a 2.3-fold increase in GFP-T RFU expression (p < 0.05) relative to NS controls at day 7 and a 1.5-fold increase at day 14 (p > 0.05) (Fig 2). S constructs showed a 12-fold increase in col 1 gene expression (p < 0.05) relative to NS controls at day 7 and a 5-fold increase at day 14 (p <0.05) (Fig 3). Col 1 gene expression measured by RT-PCR and fluorescence analysis were positively correlated (r = 0.89).

Discussion: Tracking real-time collagen gene expression using a fluorescent protein is very useful for studying effects of stimulation on tissue-engineered constructs, particularly at the cellular level. The fact that mechanical stimulation of these constructs increases collagen I gene expression of suggests that the tensile signal might trigger cell-surface receptor and adhesion sites, resulting in cascades that involve activation of genes responsible for the synthesis of extracellular matrix components [2]. The positive correlation found between collagen type I gene expression measured using real time quantitative RT-PCR and fluorescence analysis validates fluorescence as an indicator of gene expression not only at the whole construct level but at the cellular level where RT-PCR is less sensitive.


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