Accumulation of Exogenous Activated TGF-β in the Superficial Zone of Articular Cartilage

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Supplemental Materials

Influence of cartilage matrix constituents on the measurement of TGF-β1 in guanidine hydrochloride extracts

A preliminary test was conducted to assess the potential influence of the cartilage matrix constituents present in guanidine tissue extracts on the TGF-β1 measurements performed in this study. Here, a solution of 6 ng/mL active human recombinant TGF-β1 was prepared in 4M guanidine-HCl (w/ protease inhibitors) to mimic the extracted levels of endogenous TGF-β1 from cartilage explants (at 10:1 tissue to solution volume ratio). Aliquots of this solution were supplemented with the following cartilage matrix consituents: Chondroitin sulphate (C6S shark cartilage, Sigma) at 0.2, 1.0, or 5.0 mg/mL, cartilage oligomeric matrix protein (COMP, R&D Systems) at 5 or 50 μg/mL, heparin sulphate (Sigma) at 0.01, 0.1, or 1.0 mg/mL, or maintained free of matrix constituents as a control. These constituent concentration ranges were selected to encompass their levels present in the guanidine after extraction of articular cartilage. As performed in the other experiments of this study, aliquots were diluted 20-fold in ITS-media. Diluted solutions were maintained at room temperature for 1 hour and subsequently subjected to ELISA for measure of their TGF-β1 content. Results confirm that matrix constituent supplementation had no effect on measured levels of TGF-β1 ($p>0.64$ relative to control). This result suggests that the ECM constituents extracted from cartilage in the experiments of this study had no adverse effect on TGF-β measurements.

Matrix constituent	Supplemented Concentration [mg/mL]	TGF-β1 concentration [ng/mL]
Control		5.4±0.1
Chondroitin sulphate	5.0	5.9±0.2
	1.0	5.5±0.4
	0.2	5.7±0.1
COMP	0.05	5.5±0.2
	0.005	6.0±0.4
Heparin sulphate	1.0	6.0±0.3
	0.1	5.4±0.2
	0.01	5.2±0.9

Table S1: Effect of matrix constituent presence on measured TGF-β1 concentration in 4M guanidine hydrochloride solution, as assessed by ELISA.