

ECM X: Stem Cells for Musculoskeletal Regeneration. Production of a cartilage-like tissue by coculturing human articular chondrocytes with human bone marrow MSC in a 3D system

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INTRODUCTION: Therapeutic approaches of hyaline articular cartilage defects rely on expanded chondrocytes, which progressively lose their chondrogenic potential during *in vitro* culture. Mesenchymal stem cells (MSC) represent an attractive alternative cell source providing that their default pathway towards osteogenesis can be prevented. In this study, human articular chondrocytes (HAC) and human bone marrow MSC were cocultured in a 3D system to examine possible cellular crosstalk leading towards stable chondrogenesis.

METHODS: Hyaline cartilage samples were collected postmortem from human knee joints. After isolation, HAC were expanded for 8 days in monolayer. Human bone marrow MSC were isolated by density gradient centrifugation and culture in monolayer until passage 3. HAC and MSC were labeled with PKH 67 and PKH 26 respectively. Cells were mixed at different ratios and HAC and MSC alone were used as control. Chondrogenesis was analyzed in pellet cultures for 3 and 6 weeks in high glucose DMEM serum free medium with and without TGF- β 1 (10ng/ml) and dexamethasone (10^{-7} M) (\pm T \pm D). Collagen II, collagen X and S100 protein expression were assessed via immunohistochemistry. GAG and DNA content of the pellets were quantified using DMMB assay and CyQuant kit.

RESULTS: PKH-fluorescence of labeled cells indicated an equal distribution of both cell types throughout the cocultured pellets. Macroscopically, 100% HAC generated a translucent disc-like pellets while 100% MSC formed small round pellets. The increasing number of MSC relative to HAC resulted in more round shape of cocultured pellets. 100% HAC pellets produced cartilage specific matrix proteins regardless of the presence or absence of chondrogenic factors. In contrast, 100% MSC pellets underwent chondrogenesis and expressed the hypertrophic marker collagen X only when induced with chondrogenic factors

(+T+D). The presence of 25% to 50% HAC increased proteoglycans, collagen II, and S100 expression compared to 100% MSC in both cultured condition (\pm T \pm D), and suppressed collagen X synthesis in (+T+D) condition (Figure 1), even after 6 weeks of coculture.

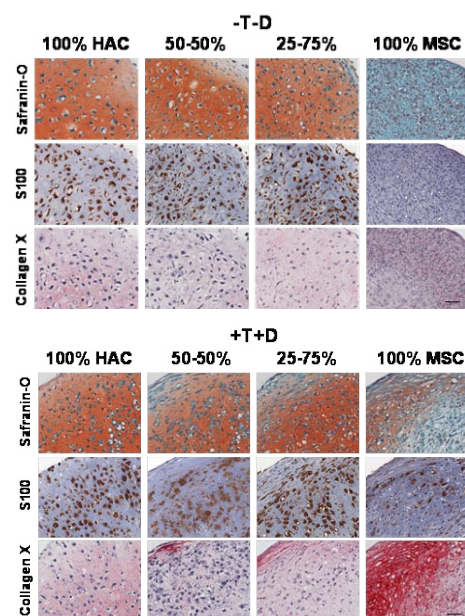


Figure 1: HAC induce chondrogenesis and prevent hypertrophy of MSC in 3 weeks cocultured pellets.

Expression of chondrocytic marker S100, detected in most cells in 3 weeks (\pm T \pm D) cocultured pellets, decreased after 6 weeks mainly in (+T+D) pellets. Biochemical analysis of pellets confirmed the histology results.

DISCUSSION & CONCLUSIONS: Detection of cartilage-specific matrix proteins in cocultured HAC and MSC pellets incubated in the absence of external chondrogenic factors suggests crosstalk from chondrocytes to MSC inducing chondrogenesis and preventing further hypertrophic differentiation of MSC towards osteogenesis.