

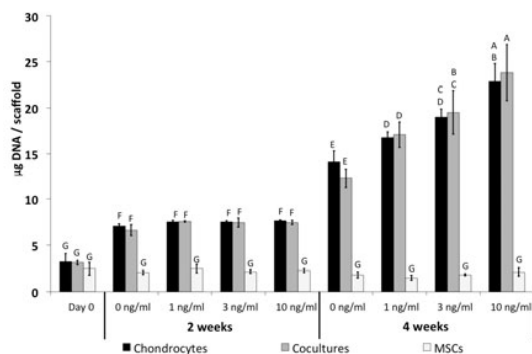
## Dose Response to TGF- $\beta$ 3 of Co-Cultured Chondrocytes and Mesenchymal Stem Cells on Porous Polymer Scaffolds

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**Statement of Purpose:** Articular chondrocytes (ACs) and mesenchymal stem cells (MSCs) are two common cell sources for articular cartilage engineering; however inherent disadvantages are associated with the use of each cell population. For this reason, co-cultures of ACs and MSCs have been investigated and shown to be capable of achieving equal or greater levels of chondrogenesis compared to ACs or MSCs alone,<sup>1</sup> yet further investigations should be conducted to enhance culture conditions for this cell population. In this work, it was hypothesized that such co-cultures would be more sensitive to chondrogenic growth factors, such as transforming growth factor- $\beta$ 3 (TGF- $\beta$ 3), and thus compared to ACs and MSCs, would require a reduced dosage of TGF- $\beta$ 3 in order to achieve particular level of chondrogenesis.

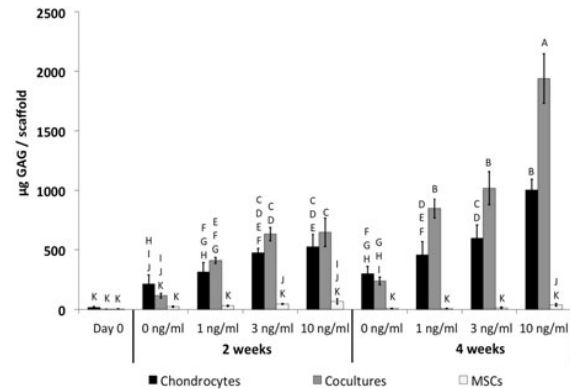
**Methods:** Poly( $\epsilon$ -caprolactone) was electrospun with an average fiber diameter of  $\sim$ 10  $\mu$ m, and scaffolds 8 mm in diameter were cut using a dermal biopsy punch. Bovine ACs were isolated from the femoral condyles of 7-10 day old calves, and expanded for one passage. Rabbit bone marrow-derived MSCs were isolated from the femora and tibias of 5 week old rabbits, and expanded for 3 passages. Three cell populations (ACs, MSCs, and a 1:3 ratio of ACs to MSCs) were seeded at a density of 225,000 cells per scaffold (n=5). Sensitivity to TGF- $\beta$ 3 was evaluated by supplementing the serum-free culture medium with four different dosages (0, 1, 3 and 10 ng/mL). After 2 weeks, TGF- $\beta$ 3 induction was removed and samples were cultured for an additional 2 weeks. At both times, samples were analyzed for DNA and glycosaminoglycan (GAG) contents as well as histological appearance. Statistical analysis was performed using ANOVA and Tukey's post hoc test (p<0.05). Data are presented as mean  $\pm$  standard deviation. Groups not connected by the same letters are significantly different.



**Fig. 1** DNA content after 2 and 4 weeks

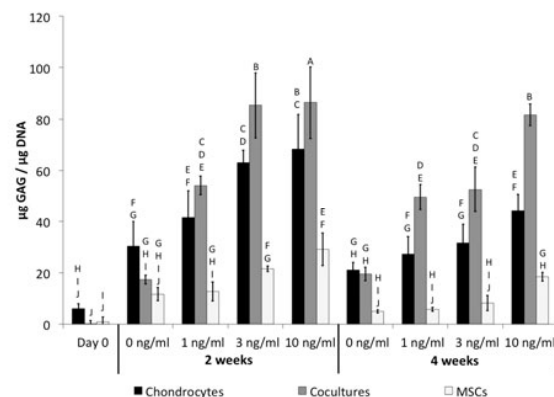
**Results:** Both the AC and co-culture populations displayed an increase in DNA content (Fig. 1) over the course of the study with no difference between the two cell populations. After 2 weeks of continuous exposure to TGF- $\beta$ 3 no difference in DNA content was observed between any of the dosages. Two weeks later, after TGF-

$\beta$ 3 removal, both cell populations displayed a dose-dependent increase in DNA content with the highest DNA content in the 10 ng/mL dose. In contrast, cellularity of the MSC group remained unchanged and significantly less than other cell populations in all cases.



**Fig. 2** Glycosaminoglycan content after 2 and 4 weeks

Similarly, a larger dose-dependent effect on GAG content (Fig. 2) was seen at 4 weeks compared to 2 weeks of culture. Looking at both the GAG and GAG/DNA (Fig. 3), it can be seen that even with the lowest dosage (1 ng/mL), the co-culture population displayed a significantly increased GAG content compared to the AC population at 4 weeks, and even achieved equal or greater GAG and GAG/DNA levels than the AC population with highest amount of growth factor at both 2 and 4 weeks. Additionally, while all groups saw a decrease in GAG/DNA with the removal of TGF- $\beta$ 3, the effect appeared to be reduced in the co-culture groups. These results were supported by the histological analysis (not shown); however, further evaluation of the chondrogenic



**Fig. 3** GAG/DNA after 2 and 4 weeks

gene expression should be performed.

**Conclusions:** This study indicates that co-cultures of ACs and MSCs are more sensitive to TGF- $\beta$ 3 compared to ACs or MSCs alone, require a reduced dosage for chondrogenesis, and may retain their phenotype better upon the removal of the growth factor.

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**Ref.:** 1. Meretoja VV. Biomaterials. 2012;33:6362-6369.