In vitro and *In vivo* Characterization of Human Articular and Rat Growth Plate Chondrocytes Microencapsulated in Alginate using High Electrostatic Potential Technology

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Introduction

It has been difficult to expand articular chondrocytes *in vitro* for tissue engineering applications because they undergo a process known as dedifferentiation when expanded in monolayer culture. Three-dimensional alginate encapsulation has been shown to effectively reestablish a chondrocytic phenotype [1].

High electrostatic potentials can be used to reduce alginate bead size by disrupting the surface tension of the droplets. We have previously established a method for producing uniform spherical alginate beads approximately 180 μm in diameter with 70-80 chondrocytes per bead. The constructs can be maintained in culture for up to two weeks with greater than 98% viability [2]. The purpose of this study was to characterize the encapsulated chondrocytes *in vitro* and determine the potential of the microbeads as an *in vivo* cell delivery system.

Methods

Human articular or rat growth plate chondrocytes were microencapsulated as previously described [2]. After 0, 1, 3, 5, 7, or 14 days in culture the beads were washed with saline and the alginate dissolved using sodium citrate. The samples were centrifuged to pellet the cells and the alginate removed. RNA was extracted using the Qiagen miniprep kit and quantified by UV spectroscopy. Reverse transcription PCR (RT-PCR) was used to measure expression of collagen type 1, collagen type 2, aggrecan, and cartilage oligomeric matrix protein (COMP). RT-PCR was also performed on monolayer cultures as a control. Six different human donors between the ages of 16-39 were tested.

An athymic nude mouse intramuscular implant model was used to assess the *in vivo* response of the alginate microbeads. The implantation procedure involved a small skin incision over the calf region of each hind limb following disinfection, and a pouch was prepared in the gastrocnemius muscle by blunt dissection. One gelatin capsule filled with alginate microbeads containing rat growth plate chondrocytes was inserted into each pouch and the incision closed by clips. The animals were euthanized at 4 weeks and the legs harvested and processed for histological analysis.

Results

Monolayer culture of human articular chondrocytes showed expression of aggrecan, collagen type 1 mRNA, and COMP in all six donors, but no expression of collagen type 2 mRNA was detected. The monolayer rat growth plate chondrocytes were also positive for aggrecan and collagen type 1, but unlike the human cells continued to express collagen type 2. COMP mRNA was not detected in the rat growth plate chondrocytes.

Alginate microencapsulation for 14 days showed a decrease in aggrecan mRNA expression in the human articular chondrocytes, and did not reestablish collagen type 2 mRNA expression (Fig. 1). The rat growth plate chondrocytes continued to express constant levels of aggrecan, collagen type 1, and collagen type 2 during the 14 day culture period (Fig. 2).

The *in vivo* histology at 4 weeks showed intact alginate beads with living chondrocytes inside. There was a highly cellular tissue with a negatively charged matrix surrounding the implants that did not appear inflammatory. It was not clear if the cells were host or donor cells. There was not any noticeable production of cartilaginous tissue within the implant site.

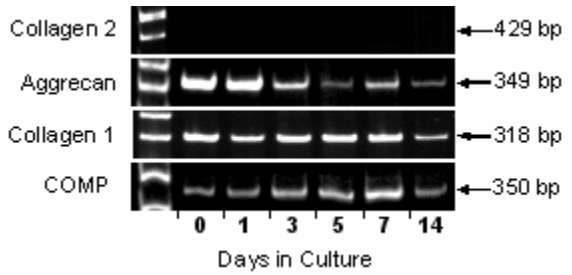

Figure 1: mRNA expression of human articular chondrocytes microencapsulated in alginate

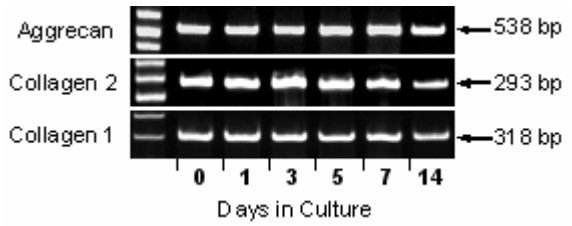

Figure 2: mRNA expression of rat growth plate chondrocytes microencapsulated in alginate

Conclusions

The microencapsulation method was not able to reestablish the phenotype of human articular chondrocytes after 2 weeks in culture. Longer incubation time may be required before collagen type 2 is expressed. The microbeads did not undergo significant degradation *in vivo* at 4 weeks, which most likely inhibited cartilage formation. Further experiments with lower molecular weight alginates and longer time points are required to further assess the potential as a cell delivery system.

References

1. Bonaventure J, *et al*. Exp Cell Res. 1994 May; 212(1):97-104.
2. Kinney RC, *et al.* 7th Annual TESI meeting. Oct 10-13, 2004; Lausanne, Switzerland.