

Bioactive Polymer Microsphere-Driven Chondrogenic Differentiation of Human Mesenchymal Stem Cell Aggregate Cultures

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Introduction: Human mesenchymal stem cells (hMSCs) from adult bone marrow have enormous potential for use as a cell source for tissue engineering since they can undergo extensive self-renewal and, when provided with specific signals in their microenvironment, can be guided to differentiate into specialized connective tissue cells such as chondrocytes. A well-established technology, in which hMSCs are cultured in high cell density aggregates and treated repeatedly with exogenous transforming growth factor- β 1 (TGF- β 1) in defined media, has been used to consistently drive the differentiation of hMSCs down the chondrogenic lineage and induce cartilage formation. This culture system, however, requires repeated TGF- β 1 dosing in media changes and results in a non-uniform spatial delivery of the chondrogenic growth factor through the bulk of the aggregate due to diffusional limitations. In addition, *in vitro* culture of the aggregates for up to 3 weeks would be required prior to utilization in *in vivo* regeneration strategies. Here we present a novel technique to deliver growth factors to MSC aggregates via uniformly dispersed biodegradable polymer microspheres.

Methods: Poly(lactic-co-glycolic acid) (PLGA) microsphere synthesis. PLGA microspheres (50:50; 0.18 dL/g and 0.56 dL/g inherent viscosities) with TGF- β 1 (20 ng/mg polymer) were synthesized using a standard double emulsion technique. Scanning electron microscopy (SEM) images of the microspheres were obtained before and after 10 minute sterilization with UV. The size of the microspheres was determined using Image J analysis software. **Microsphere-incorporated hMSC aggregates.** UV sterilized PLGA microspheres and P1 hMSCs were suspended in a chemically defined medium and centrifuged in a 96-well microplate to form nonadherent cell aggregates. Experimental hMSC aggregates were cultured in defined medium which was changed every other day. Control hMSC aggregates without microspheres were treated with chondrogenic medium (medium supplemented with 10 ng/ml TGF- β 1) every other day. Aggregates were harvested after 6 days and processed for histological examination with Safranin O/Fast Green (N=2) or assayed for DNA and glycosaminoglycan (GAG) content (N=4).

Results: SEM images showed that UV sterilization did not change the surface morphology of the microspheres (Fig. 1). The average microsphere diameters of the two formulations ($6.9 \pm 3.9 \mu\text{m}$, 0.56 dL/g, N=575; $6.7 \pm 4.2 \mu\text{m}$, 0.18 dL/g, N=721) were not significantly different. When microspheres were incorporated into hMSC aggregates, histological examination showed relatively uniform microsphere distribution (Fig. 2). The size difference is due to the volume of incorporated microspheres. Quantification of DNA/pellet, GAG/pellet, and GAG normalized to DNA for aggregates cultured with incorporated microspheres for

6 days is presented in Table 1. No statistically significant differences in DNA or GAG content were found between experimental groups at this early time point. Safranin O/Fast Green staining confirmed regions of GAG synthesis after 6 days in aggregates containing TGF- β 1-loaded PLGA microspheres and control aggregates without microspheres that were cultured in TGF- β 1 supplemented media (data not shown).

Conclusion: We have demonstrated that growth factor-laden PLGA microspheres can be incorporated within hMSC aggregates and that the released TGF- β 1 can induce measurable chondrogenesis in terms of GAG production by as early as 6 days at a level approaching that of control aggregates cultured in chondrogenic media. Longer time in culture will be necessary to determine the progression of aggregate chondrogenesis. By supplying chondrogenic growth factors in this manner it may be possible to rapidly induce uniform chondrogenesis in hMSC aggregates *in vivo* without prior extended culture *in vitro*. Altering the polymer properties may provide control over the temporal presentation of these factors and allow for the use of lower concentrations compared to systems employing exogenously supplied TGF- β 1. Further optimization of TGF- β 1 loading concentration and PLGA formulation is currently being pursued.

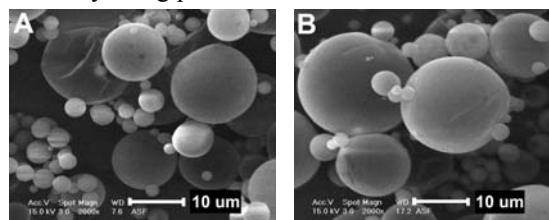


Figure 1: SEM images of PLGA (0.56 dL/g) microspheres encapsulating TGF- β 1 (A) before and (B) after 10 minutes of UV sterilization.

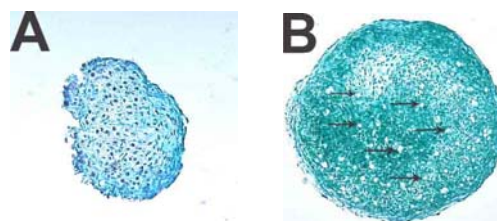


Figure 2: Safranin O/Fast Green histology of (A) control hMSC aggregate and (B) aggregate with uniformly distributed microspheres (0.56 dL/g) after 6 days of culture. (Black arrows indicate locations of several microspheres; Mag=100X)

	Control	50:50 (.56 dL/g)	50:50 (.18 dL/g)
DNA ($\mu\text{g}/\text{pellet}$)	$1.22 \pm .04$	$1.30 \pm .08$	$1.24 \pm .10$
GAG ($\mu\text{g}/\text{pellet}$)	6.1 ± 3.0	2.0 ± 2.5	3.9 ± 3.1
GAG/DNA ($\mu\text{g}/\mu\text{g per pellet}$)	5.0 ± 2.6	1.5 ± 2.0	3.1 ± 2.8

Table 1: DNA and GAG content in the hMSC aggregate formulations after 6 days (ave \pm stdev; N=4).