Self-assembling hMSC Sheets Incorporating Degradable Polymer Microspheres for Cartilage Tissue Engineering

Loran D. Solorio,[1] Chirag D. Dhami,[1] G. Adam Whitney,[1] Eben Alsberg[1,2]
[1]Department of Biomedical Engineering, [2]Department of Orthopaedic Surgery,
Case Western Reserve University, Cleveland, OH, USA

Introduction: Self-assembling cell sheets have tremendous potential for use in cartilage tissue engineering applications, as the densely cellular tissues provide an advantageous environment for *in vitro* chondrogenic induction of human mesenchymal stem cells (hMSCs). In the presence of chondrogenic factors, hMSCs can self-assemble into sheets on porous membranes and differentiate into chondrocytes depositing cartilaginous extracellular matrix (ECM) that may be harvested intact without disruption by enzyme treatment. By incorporating uniformly-dispersed gelatin microspheres within hMSC sheets, we could improve neocartilage tissue formation, as the microspheres could act as a bulking agent, increasing tissue thickness while helping to maintain construct shape. Additionally, the inclusion of microspheres could provide space for matrix accumulation within the tissues. Utilizing this system, it may be possible to tune the microsphere degradation rate to match the rate of ECM deposition within cartilage tissues. The gelatin microspheres may confer thickness and mechanical stability initially, and then degrade over time as cell-derived ECM accumulates and assume a greater percentage of the mechanical load. Here, we demonstrate a novel system that uniformly incorporates crosslinked gelatin microspheres into self-assembling hMSC sheets, increasing tissue thickness while allowing chondrogenic differentiation and glycosaminoglycan (GAG) deposition.

Methods: ***Gelatin microsphere synthesis.*** Microspheres were synthesized using gelatin and a standard single emulsion technique. Microspheres were crosslinked in a 1% genipin solution for 2 ("Low Gp") or 21 ("High Gp") hrs and UV sterilized. Light photomicrographs of crosslinked, hydrated microspheres were obtained and microsphere size was determined using Image J analysis software. The degree of crosslinking was measured with a ninhydrin assay (N=4). ***Microsphere-incorporated cell sheets.*** Crosslinked microspheres (1.5 mg) and $3x10^6$ P2 hMSCs were suspended in a chemically defined chondrogenic medium containing 10 ng/ml TGF-β1 and allowed to settle onto the membranes of 12 mm cell culture inserts (8 μm pore size). Experimental cell sheets and control sheets without microspheres were treated with chondrogenic medium every other day. Cell sheets were harvested after 2 weeks and measured with calipers, then assayed for DNA and GAG content (N=4). Data is reported as avg ± stdev and statistical analysis was done by two-tailed unpaired t-tests or ANOVA with $p<0.05$ considered significant.

Results and Discussion: Average diameters of the two microsphere formulations (32.5 ± 25.5 μm, "Low Gp", N=167; 27.7 ± 22.2 μm, "High Gp", N=208) were not significantly different. The "High Gp" microspheres were 67.6 ± 4.5% crosslinked, which is significantly higher than the 28.3 ± 7.2% crosslinked "Low Gp" microspheres. Examination via light microscopy revealed uniform microsphere distribution in the microsphere-incorporated cell sheets (**Fig. 1**). Sheets containing "Low Gp" microspheres and those containing "High Gp" microspheres were significantly thicker than control sheets without microspheres as shown in **Figure 2**. GAG and GAG/DNA contents were similar between sheets with and without microspheres at this early time point, but the DNA content in the "High Gp" group was lower than both the control and "Low Gp" groups (**Table 1**). Qualitatively, sheets containing microspheres were much easier to remove from the membrane and handle than control sheets, but mechanical testing will be required to quantitatively determine the mechanical properties.

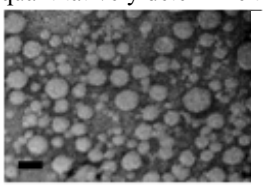

Figure 1: Light photomicrograph of uniformly distributed microspheres within a cell sheet (scale bar = 50 μm).

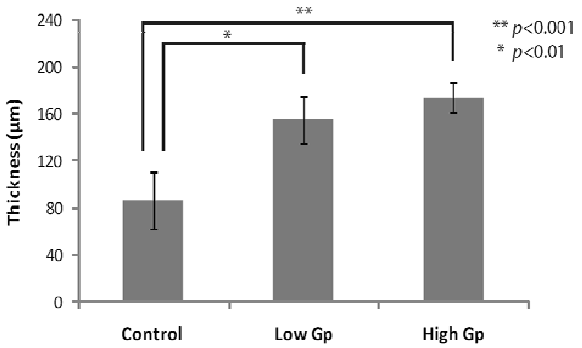

Figure 2: Thickness of cartilage sheets with and without microspheres after 2 weeks.

	Control	"Low Gp"	"High Gp"
DNA (μg)	**17.9 ± 0.2**	**18.2 ± 0.3**	**16.5 ± 0.7***
GAG (μg)	**25.2 ± 12.4**	**24.9 ± 10.8**	**52.2 ± 43.0**
GAG/DNA (μg/μg)	**1.4 ± 0.7**	**1.4 ± 0.6**	**3.2 ± 2.6**

Table 1: DNA and GAG content sheets after 2 weeks. (*indicates $p<0.01$ compared to other groups.)

Conclusions: We have demonstrated that crosslinked gelatin microspheres can be incorporated within self-assembled hMSC sheets, and that the microsphere-incorporated sheets are thicker than control sheets without microspheres. When treated with media containing TGF-β1, the microsphere-incorporated sheets are capable of producing GAG at levels similar to those of control sheets at 2 weeks. Longer culture time is being examined to determine how microsphere degradation rates affect neocartilage matrix deposition within the sheets and the mechanical properties of resultant tissues.