

# Alginate Microencapsulation Technology for Percutaneous Delivery of Human Adipose Derived Stem Cells (ADSCs)

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## Statement of Purpose:

Autologous fat is the ideal soft tissue filler; however, widespread application of fat grafting is limited due to variable clinical results and poor survival rates in well controlled experiments.<sup>1</sup> Engineered tissue fillers have the potential to maximize survival by adding conditioned cells to a scaffold designed to provide structure, shape, and the proper three-dimensional matrix for tissue and vascular in-growth. Adipose derived stem cells are an ideal transplant cell because of their growth and differentiation potential; however, they tend to migrate from the recipient site and further cell loss may result due to excessive shear during syringe injection. Alginate capsules of less than 200 microns facilitate mass transfer<sup>2</sup>, are biodegradable, and can protect against shearing. The purpose of this study was to determine if human ADSCs can be encapsulated in alginate microbeads and if cell viability is retained following injection of ADSC laden microbeads through a standard syringe.

## Methods:

Subcutaneous fat was excised from patients treated at Children's Healthcare of Atlanta. All patients gave written consent to the procedure and handling of fat. Tissue was processed and ADSCs were isolated via standard protocols.<sup>3</sup> Cells were passaged as necessary and grown to a specified number to guarantee a seeding density of  $1 \times 10^6$  cells per ml of alginate. Ultra pure, medium viscosity, guluronate predominant alginate powder (Pronova Biopharma, Norway) was dissolved in 0.9% saline to produce a 2% w/v solution. Second passage ADSCs were mixed with alginate at one million cells/ml and encapsulated using an electrostatic potential technique<sup>4</sup> under the following conditions: flow rate of 10ml/hr, needle inner diameter of 0.175mm, electric potential of 6kV, and temperature of 36°C. Alginate beads formed in a 50ml bath of 50 mM calcium chloride and 150 mM glucose. Electric potentials and flow rates were varied to assess effects on bead diameter.

ADSC microbeads were separated into two groups each containing three samples. The control group was decanted into tissue culture wells. The injection group was loaded into a 3ml syringe, mixed with 1ml of basal stem cell media, and injected through an 18 gauge needle into tissue culture wells. Microbeads were cultured in basal stem cell media at 37° Celsius and 5% CO<sub>2</sub> in humid air. Media was changed every 48 hours. Viability was measured using fluorescence confocal microscopy (Carl Zeiss MicroImaging Inc., NY) and a calcein/ethidium homodimer-1 stain.

## Results:

Increasing the electrical potential decreased the bead diameter for all flow rates tested. However, bead

morphology and regularity was negatively impacted as the voltage increased. Flow rate had a more predictable influence on bead diameter, with consistent results below 200 microns at flow rates below 5ml/hr (Figure 1).

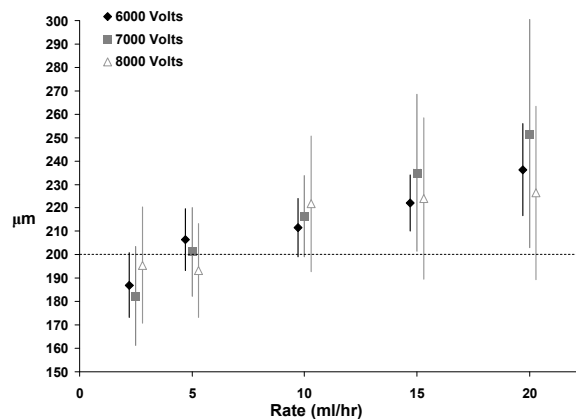


Figure 1: Bead diameter as a function of electrostatic potential and flow rate (n = 2 separate batches)

There were no differences in alginate morphology and ADSC viability between microcapsules placed into tissue culture wells versus those injected into tissue culture wells (Figure 2).

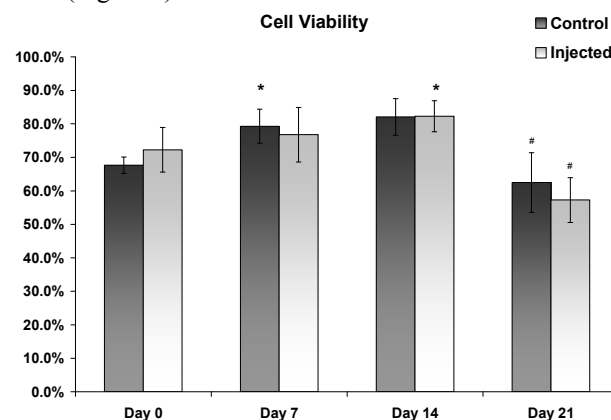


Figure 2: Cell viability in control and injected microbeads

## Conclusions:

ADSCs can be readily isolated, cultured, and encapsulated into alginate microspheres less than 200 microns in diameter. Cell viability in tissue culture is not adversely affected by injection pressures.

## References:

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3. Zuk et al. Human Adipose Tissue Is a Source of Multipotent Stem Cells. *Mol. Biol. Cell* 2002; 13(12):4279-4295.
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