Agarose Microgels for Protein Delivery within Embryoid Body Microenvironments

Andrés Bratt-Leal,[1] Richard Carpenedo[1], Todd McDevitt[1,2]
[1]The Wallace H. Coulter Department of Biomedical Engineering,
[2]The Parker H. Petit Institute for Bioengineering and Bioscience,
Georgia Institute of Technology and Emory University, Atlanta, GA, USA

Introduction. Embryonic stem cells (ESCs) are pluripotent cells derived from the inner cell mass of the blastocyst stage embryo. The potential exists to use ESCs to generate a variety of different cells for regenerative therapies; however, controlled differentiation strategies remain a challenge. Suspension culture of ESCs induces the formation of embryoid bodies (EBs) which spontaneously differentiate into a heterogeneous population of primitive cell types. Typical methods of controlling EB differentiation rely on the addition of soluble factors to the culture media; however, steric barriers to diffusion within EBs may create concentration gradients that lead to the heterogeneous differentiation observed. Our group has previously demonstrated that incorporation of PLGA microparticles releasing small, hydrophobic morphogens within EBs can induce more homogeneous differentiation of ESCs. The purpose of this work is to develop hydrogel microparticles to deliver larger, hydrophilic molecules, such as growth factors and extracellular matrix proteins, within EBs for further controlled differentiation of the cells.

Materials and Methods. Agarose was dissolved in PBS at 60°C at 3% w/w. Microgels were formed using water-in-oil emulsion followed by gelation for 20 minutes at 4°C. Alexa-Fluor 488-conjugated BSA was mixed with liquid agarose prior to emulsion for EB incorporation studies, and gelatin types A and B were mixed with liquid agarose prior to microgel formation for release kinetic studies. Microgels were critically point dried and imaged using a Hitachi S800 scanning electron microscope. A Coulter Multisizer 3 was used to measure the size distribution of the microgel particles. A profile of release kinetics was generated with microgels loaded either with gelatin A or gelatin B incubated with PBS at 37°C under rotary conditions (40 rpm). The PBS was removed and sampled for protein content at various time points using a bicinchoninic acid (BCA) assay. Microgels were pre-coated with either gelatin type A (1mg/mL), gelatin type B (1mg/mL) or poly-L-lysine (0.1mg/mL) and mixed with $2x10^6$ mouse ESCs in a 100mm petri dish at a ratio of 7.5:1 on a rotary orbital shaker (40 rpm) for the formation of EBs. EBs were imaged using a Zeiss LSM 510 confocal microscope after one day of culture.

Results. Agarose microgels were formulated with a average diameter of 5.3±2.5µm. SEM analysis indicated that the microgels were largely spherical with rough edges (Figure 1A). While each pre-coating condition resulted in microparticles within EBs, poly-L-lysine coating (Figure 1D) was the most effective at promoting incorporation. All EBs displayed microgel incorporation and 35% of the EBs had large clumps of incorporation. Patterns of BSA fluorescence, imaged through confocal microscopy, were that of point source diffusion (data not shown). Protein release kinetics depended on both the charge of the protein and the percentage of agarose (Figure 1B). Gelatin type B, negatively charged at a physiological pH, was released faster than gelatin type A, which is neutrally charged at a physiological pH. Also, protein release from 6% agarose particles was slower than 3% agarose microgels. Protein incorporation was more efficient at the lower percentage agarose (13.8-24.5%) compared to the higher percentage agarose (8.9-10.1%). It is of note that the rate of diffusion of fluorescent BSA appeared to slow once the microgel was incorporated into EBs as demonstrated by the additional length of time the microgels remained fluorescent under culture conditions.

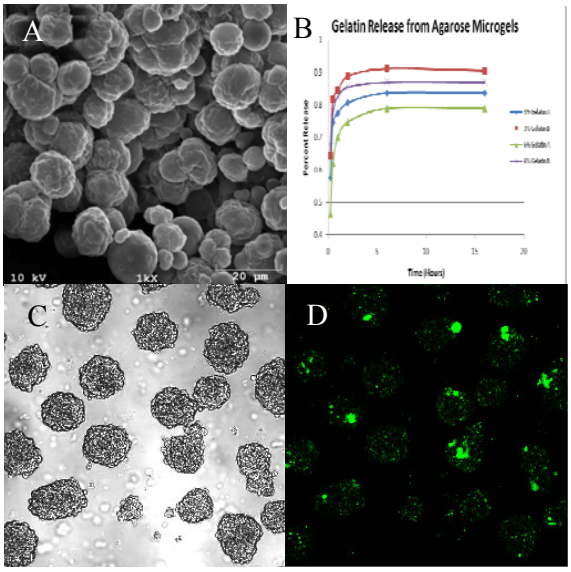

Figure 1. A) SEM image of Agarose microgels (1kX) B) Profile for the release of gelatin C) Phase image of EBs with microgels. 10x magnification. D) FITC image of C showing fluorescent microgels incorporated within the interior of EBs.

Conclusion. The hydrophilic properties of agarose microgels allow for incorporation and delivery of proteins that largely retain their bioactivity. Protein delivery from microgels can be modulated through agarose percentage and charge of protein incorporated. Fluorescent imaging of EBs with incorporated agarose microgels and protein release profiles suggest that agarose microgels may be further investigated as a controlled system to deliver proteins within EBs for directed stem cell differentiation. Future studies to deliver growth factors known to influence ESC differentiation will be performed to compare microparticle protein-mediated presentation to soluble delivery methods.