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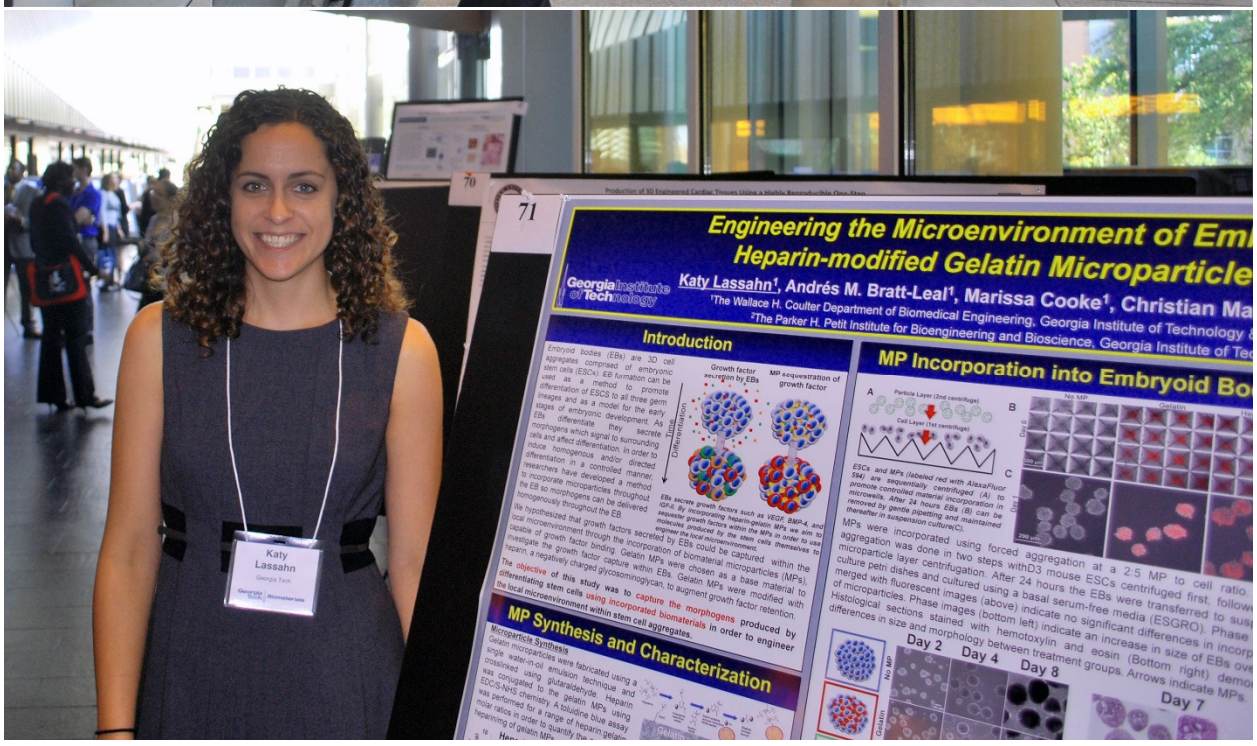
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Engineering the Microenvironment of Emb
Heparin-modified Gelatin Microparticle
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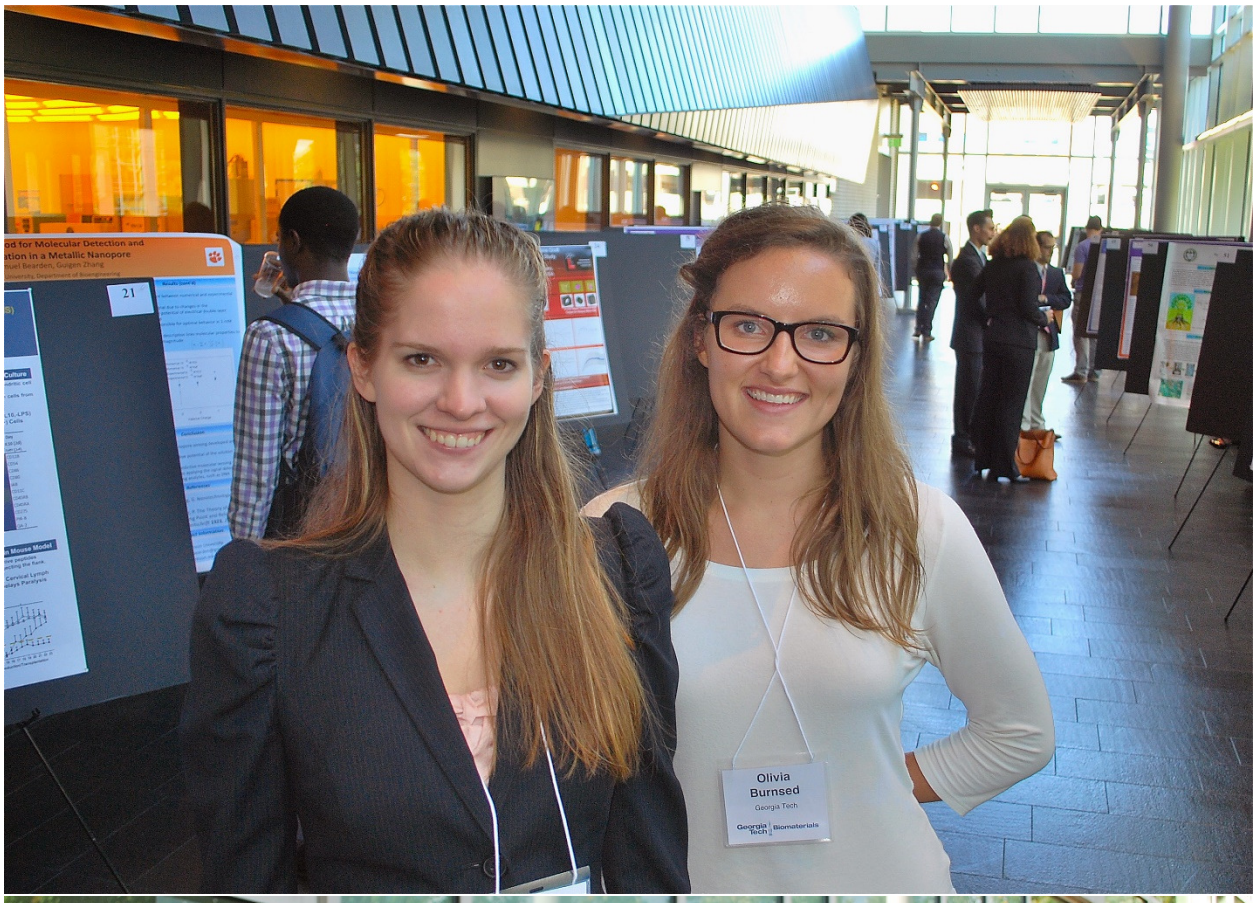
Introduction
Embryoid bodies (EBs) are 3D cell aggregates comprised of embryonic stem cells (ESCs). EB formation can be used as a method to promote differentiation of ESCS to all three germ lineages and as a model for the early stages of embryonic development. As EBs differentiate they secrete morphogens which signal to surrounding cells and affect differentiation. In order to induce homogenous and/or directed differentiation in a controlled manner, researchers have developed a method to incorporate microparticles throughout the EB so morphogens can be delivered homogenously throughout the EB.
We hypothesized that growth factors secreted by EBs could be captured within the local microenvironment through the incorporation of biomaterial microparticles (MPs) capable of growth factor binding. Gelatin MPs were chosen as a base material to investigate the growth factor capture within EBs. Gelatin MPs were modified with heparin, a negatively charged glycosominoglycan, to augment growth factor retention.
Growth factor secretion by EBs
MP sequestration of growth factor
Differentiation
EBs secrete growth factors such as VEGF, BMP-4, and IGF-II. By incorporating heparin-gelatin MPs we aim to sequester growth factors within the MPs in order to use molecules produced by the stem cells themselves to engineer the local microenvironment.
The objective of this study was to capture the morphogens produced by differentiating stem cells using incorporated biomaterials in order to engineer the local microenvironment within stem cell aggregates.
MP Synthesis and Characterization
Microparticle Synthesis
Gelatin microparticles were fabricated using a single water-in-oil emulsion technique and crosslinked using glutaraldehyde. Heparin was conjugated to the gelatin MPs using EDC-NHS chemistry. A toluidine blue assay was performed for a range of heparin:gelatin molar ratios in order to quantify the amount of heparinization of gelatin MPs.
MP Incorporation into Embryoid Bo
Particle Layer (2nd centrifuge)
Cell Layer (1st centrifuge)
ESCs and MPs (labeled red with AlexaFluor 594) are sequentially centrifuged (A) to promote controlled material incorporation in microwells. After 24 hours EBs (B) can be removed by gentle pipetting and maintained thereafter in suspension culture(C).
MPs were incorporated using forced aggregation at a 2:5 MP to cell ratio. aggregation was done in two steps withD3 mouse ESCs centrifuged first, follow microparticle layer centrifugation. After 24 hours the EBs were transferred to sus culture petri dishes and cultured using a basal serum-free media (ESGRO). Phase merged with fluorescent images (above) indicate no significant differences in incorp of microparticles. Phase images (bottom left) indicate an increase in size of EBs ov Histological sections stained with hemotoxylin and eosin (Bottom right) demo differences in size and morphology between treatment groups. Arrows indicate MPs.
No MP
Gelatin
Day 2
Day 4
Day 8
Day 7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