3D Structured Biomaterials to Induce Vascularization after Ischemic Cardiovascular Events.

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Introduction Ischemic cardiovascular diseases are pathologies that can cause reduced blood supply to the heart. Because mature cardiomyocytes possess low regenerative potential [1,2], the self-healing process is limited in the heart after an acute ischemic event [3]. We propose to produce a collagen scaffold with a 3-dimensional internal geometry provided by alginate microspheres to guide host endothelial cell invasion and create a rudimentary vascular bed through the depth of the implant, which can be subsequently remodeled by the host for sustained perfusion. Drug or growth factor release from alginate microspheres is used to promote the neovascularization of the ischemic site.

Methods Alginate microspheres were produced using the VarJ30 bead generator (Nisco, Switzerland), extruding alginate solution (Sigma, 1% w/v in PBS) in 0.15M, 0.5M and 1M $CaCl_2$ (Sigma) at 18 ml/h and nitrogen pressure of 80 mbar. Release studies from alginate beads were performed using chymotrypsin as a model protein. Briefly, chymotrypsin (1 mg/mL) was mixed with alginate prior to the formation of microspheres. After preparation, microspheres were washed with mH_2O and incubated in PBS up to 14 days. Aliquots (25 µl) of the supernatant were taken at each time point and added with 200 µl BCA assay (Sigma). The absorbance was read in a SpectraMax M5 Microplate Reader at 562 nm to quantify the release of the protein. The 3D constructs were produced by embedding alginate beads in a 2.5 mg/mL rat tail collagen type 1 (Advanced Biomatrix) solution. For the production of cellular scaffolds, we tested mouse embryonic fibroblasts (MEFs), which were added to collagen solutions ($5x10^6$ cells/mL) before embedding alginate microspheres and allowing to gel at 37°C for 45 min prior to addition of MEF medium. Samples were embedded in fresh frozen blocks after 7 days and sections visualized by picrosirius red stain for collagen and hematoxylin & eosin staining.

Results and Discussion Alginate microspheres with an average diameter of 100 µm were successfully produced using the bead generator (Fig. 1).

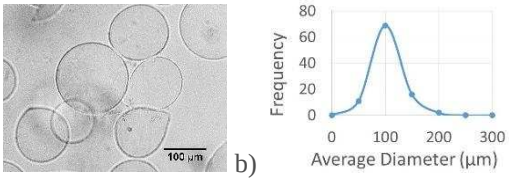

Figure 1. a) Optical microscope image (scale bar=100 µm) and size distribution of the produced microspheres.

Chymotrypsin release from alginate microspheres can be modulated by varying the concentration of $CaCl_2$: alginate microspheres gelled in 0.15M $CaCl_2$ solution exhibited a burst release of chymotrypsin (40% in 24h), and negligible release for up to 14 days. A greater burst release was observed for the beads gelled with 0.5M and 1M $CaCl_2$ (60% and 90% of the immobilized drug after 24h, respectively) with a low but constant release of chymotrypsin (3-5%) over the following 7 days.

Acellular and cellular tissue constructs were produced in custom rectangular PDMS molds and had dispersed microspheres after gelling and overnight culture (Fig. 2).

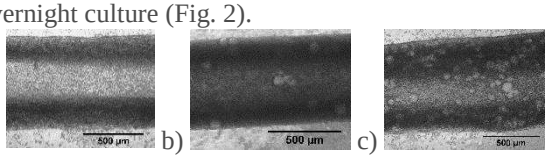

Figure 2. Cellular collagen tissue constructs with: a) 0, b) 0.5 and c) 2.4 mg alginate microspheres/100µl collagen.

In the cellular constructs, MEFs started to contract the scaffolds after 1 day of culture, and were able to grow in between the alginate microspheres (Fig 3).

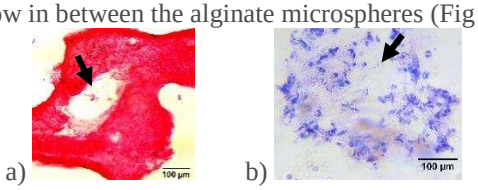

Figure 3. (a) Collagen (picrosirius red) and (b) nuclear (hematoxylin) staining of cellular constructs (2.4 mg alg microsph/100µl collagen). The arrows indicate the void space created by microspheres (scale bars=100µm).

Ongoing studies are now focused on VEGF release from alginate microspheres and the in vivo implantation of acellular collagen-alginate microsphere constructs in healthy and injured rat hearts. The angiogenic effects of unloaded and VEGF-loaded alginate microspheres will be evaluated with perfusion studies and traditional immunohistochemistry on the harvested hearts.

Conclusion. Our preliminary results support our hypothesis that collagen-alginate microsphere 3D constructs are an innovative approach to provide a 3D microenvironment with growth factor release for the formation of new vessels in engineered tissues for repair of ischemic myocardium.

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References

[1] Ahuja et al,Physiol Rev, 2007, 87:521-524.
[2] Pasumarthi et al, Circ Res, 2002, 90:1044-54.
[3] Hassink et al, Cardiovasc Res, 2008, 78:18-25.