Functionalized Alginate Hydrogel Coatings for Endothelial Progenitor Cell Capture from Whole Blood

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Statement of Purpose: Over 400,000 coronary bypass surgeries are performed every year in the United States annually. Endothelial progenitor cells (EPCs), which are naturally present in circulating blood, are an especially interesting cell type because they have the ability to repair damaged blood vessels. EPCs have been utilized as precursors in the in vitro cultivation of vascular grafts. The conventional technique of isolating EPCs involves centrifugation followed by pre-plating. As tissue engineering and cell-based therapeutics begin the transition from the laboratory to clinical applications, the availability of robust and simple cell isolation techniques becomes significant. Microfluidic devices have recently been recognized as effective tools for separation. These devices can be fabricated using soft lithography techniques. This enables devices to be produced en masse in a cost effective and straightforward nature. The use of antibody coated channels for cell capture in microfluidic devices has recently been applied to several applications. The ability to release captured cells has been a challenge. Recently alginate has been shown to be an effective means of release (Plouffe B.D.et al. Lab on a Chip 2009;1507-1510). Selectivity can be improved by incorporation of 4-arm amine terminated poly(ethylene glycol) (PEG) molecules into the alginate hydrogel. Results show that these PEG molecules not only increase ligand density within the hydrogel but suppress binding of non target cell types. This novel hydrogel chemistry can be combined with a micro post array within a microfluidic chamber as shown schematically in figure 1a and a photo of the device if figure 1b. The resultant microfluidic device is suitable for highly specific capture and release of EPCs from untreated blood.

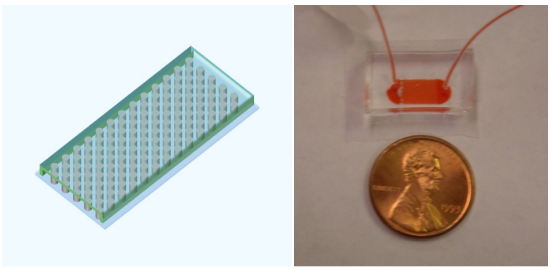

Figure 1. a) Schematic representation of the micropost array used with alginate coating. b) Photograph of microfluidic device with coating US Penny shown for scale.

Methods: The design and fabrication of the micro-post array devices followed previously described soft-lithography techniques. The device was fabricated from polydimethyl-siloxane (PDMS) and glass. (Green J.V. et al. Lab on a Chip 2009;677-685)
Alginate hydrogels were made by dissolving 22.5 mg alginic acid in 1 ml MES buffer. The alginate was then functionalized with anti CD-34 antibody using EDC-NHS chemistry (Hermanson G.T. Bioconjugate Techniques. 1996;) and 4-arm amine terminated PEG molecules under different mixing conditions and incubation times.
The hydrogels where injected into the microfluidic device using a syringe. A flow channel was created by injection of MES buffer at 10 µl/minute for 10 minutes and the gel was solidified by injection of 100 mM $CaCl_2$.at 10 µl/minute for 10 minutes. Blood was then injected into the device at 5 µl/minute for 60 minutes. The device was then rinsed and captured cells were released by injection of 50 mM solution of Ethylenediaminetetraacetic acid (EDTA) at 10 µl/minute for 10 minutes. Released cells were then incubated with florescent conjugated anti CD-133 and counted using flow cytometry.
Results: The EPC concentration in the released cell mixture for each type of gel is shown in figure 2. The use of PEG molecules increased the capture efficiency and selectivity of antibody conjugated hydrogels. By premixing the PEG and antibody the prior to addition of Alginic acid the selectivity can be further enhanced, it is believed that this is due to the proximity of the PEG and antibody molecules prior to the conjugation reaction being initiated. Additionally by varying the incubation time purity of the released cell stream was raised to over 70% CD-133 and FLK-1 positive cells as shown in Figure 1c. CD-133 and FLK-1 are known stem cell markers.

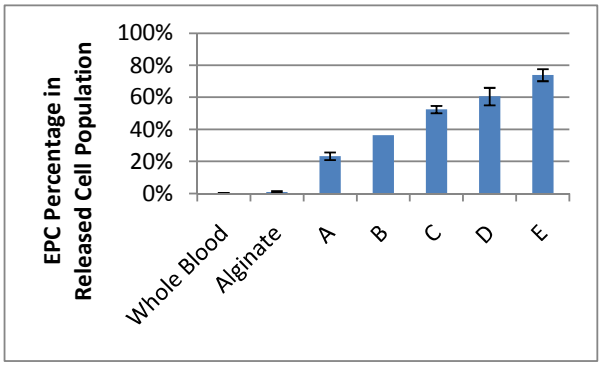

Figure 2. Bar graph shows the purity of the released cell population from microfluidic device as measured by flow cytometry. Gel A is alginate with capture antibody B-E are gels with PEG and antibody under different mixing conditions.

Conclusions: Using alginate hydrogels conjugated with PEG molecules and capture specific antibodies EPCs can be captured from whole blood within a microfluidic channel. Captured cells can then be gently released resulting in a suspension of high purity stem cells. Future work will place these released cells in culture to verify viability for use in tissue engineering.