Isolating rare cells through antigen specific hydrogel encapsulation

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Statement of Purpose: Cell based therapies offer a realistic solution to some seemingly incurable diseases. Many of the most promising therapeutic cells are rare constituents of native cell populations, such as hematopoietic stem cells and cardiac progenitor cells. Effective and affordable isolation of the rare cell population is necessary to be transitioned into therapeutic clinical use. Of the two widely used antigen positive isolation techniques - Magnetic Activated Cell Sorting (MACS) and Fluorescence Activated Cell Sorting (FACS), neither offers both high throughput and high purity sorting. However, a novel technique in development called Antigen Specific Lysis (ASL) is hypothesized to achieve these two criteria. This technique isolates cells by encapsulating the target population in a hydrogel protecting them from hypotonic solutions. In the current study, we isolate stem cell antigen-1 positive (Sca-1$^+$) cells from the mouse bone marrow using ASL technique. Sca-1$^+$ cells will be encapsulated in a hydrogel using a biotin labeled Sca-1 antibody. Hematopoietic Sca-1 cells are being used therapeutically to treat leukemia, lymphoma, and blood disorders. Sca-1$^+$ cells are shown to regenerate the cardiac tissue after myocardial infarction in a mouse model. Large scale isolation of these cells in short time and injecting to the heart, will help improve the cardiac function after myocardial infarction. The preliminary data on ASL technique also showed, the encapsulated Jurkat cells were resistant to hypotonic lysis.

Methods: Jurkats were first incubated in Biotin Mouse Anti Human CD45 (BD Biosciences, San Jose, CA) and then in streptavidin-eosin conjugate, where streptavidin binds to biotin and eosin acts as a photoinitiator. These photoinitiator tagged cells were resuspended in 25 wt% PEGDA 3500 (JenKem Technology USA, Plano, TX) having fluorescent nanoparticle. The cells were photopolymerized in a chip clip well (Whatman) under 530nm visible green light for 10 minutes at 30mW/cm^2 in the presence of nitrogen. Approximately 1.2 x 10^6 cells were taken for each polymerization. To test the coating affect, hydrogel coated and uncoated Jurkats were resuspended in water at a rate of 2 x 10^5 cells in 1mL of pure water for 10 minutes. To confirm the water lysis, approximately <10% of coated Jurkats are mixed with >90% of uncoated Jurkats and incubated in water for 10 minutes. In a cell isolation study, 10% Jurkats were premixed with 90% of A549, lung cancer cell line and polymerized Jurkats by specifically targeting antigen. Encapsulated Jurkat cells were isolated from uncoated A549 cells by water treatment.

Results: Jurkats were polymerized using PEGDA 3500 entrapping red fluorescent nanoparticles in the newly formed hydrogel. Microscope images showed a clear red color ring around the polymerized cell. Polymerization occurred only around the cell but not inside the cell. Flow cytometry data showed that the polymerized cells did not undergo lysis in water but whereas unpolymerized cells were immediately lysed in presence of water. In the Jurkats isolation study, after water treatment all the uncoated cells/A549 were lysed and only one population was seen in flow cytometry.

Conclusions: Jurkats were successfully encapsulated in the photopolymer by targeting antigen-antibody binding. The PEGDA coated cells were protected and escaped lysis whereas uncoated cells were immediately lysed in presence of water due to salt imbalance. 10% Jurkats were successfully isolated from binary cell mixture and these results show that ASL technique is a promising and reliable method for isolating rare cells at large scale in short time, with high throughput. Using a similar approach, we were also able isolate monocytes from mouse spleen, which are <10% in total. Preliminary data supports the scale up of this technique beyond 10^7 cell batches. Hydrogel coatings are degraded, by adding UV degradable groups to the monomer. In ongoing work, we are isolating Sca-1$^+$ cells from mouse bone marrow using a degradable monomer and also test the functionality of these cells after coating degradation.

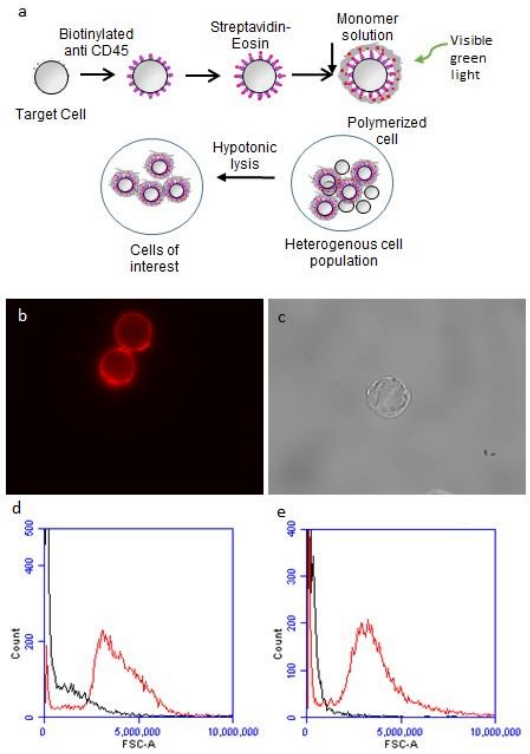

Figure 1. (a) An overview of the antigen specific lysis approach (b) Jurkat cells polymerized in PEGDA3500 having red fluorescent nanoparticle (c) brightfield image of a polymerized jurkat cell (Images at 100x) (d) flow cytometry data after water lysis of polymerized and unpolymerized jurkat cells (e) flow cytometry data after isolating jurkats from A549. Red line – intact jurkat cells and black line – cell debri after hypotonic lysis.