Hydrogel Coatings for High-Speed Cell Sorting

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Statement of Purpose: Therapies based on the use of differentiated stem cell populations are emerging as the next frontier of medicine, offering a plausible path forward in the treatment of many devastating diseases. Critically, the unintended inclusion of undifferentiated cells offers an undesired possibility for the unregulated growth of tumors from these highly proliferative populations. The future of these and other therapies requires the purification of the differentiated cells at purities higher than is typical for magnetic sorting. Further, for differentiated stem cell treatment to broadly impact public health, cells must be purified at rates which greatly exceed the fluorescent sorting infrastructure.

We report a lysis-based method of sorting cells with purity rivaling that of fluorescent sorting, yet is fundamentally capable of sorting liters of blood per hour in a standard lab environment. Further, this process requires capital costs of ~$3,000 in off the shelf equipment. Briefly, polymerization initiators are bound to the targeted cell's surface through specific antibodies, polymer coatings are formed only on the outside of the desired cell, all untargeted cells are lysed, and the polymer coating is removed. Here, we investigate the potential for both exceptional cellular purity and viability for this completely new, high-throughput, biomaterials-based cell isolation process.

Methods: Cell mixtures are labeled with biotinylated antibodies and streptavidin-eosin (described here[1,2]) to localize eosin (photoinitiator) on antigen positive cells. The cells are suspended in a UV-cleavable PEG-diacrylate based monomer solution (monomer synthesis described here,[3] triethanol amine, vinyl pyrollidinone, in PBS), and irradiated under 530 nm light for 10 minutes. Targeted cells are now coated in a 100 nm polymer coating, and untargeted cells are unaltered. Cells are resuspended in 5% SDS in PBS, where uncoated cells are lysed for 5 minutes. Remaining cells are resuspended in PBS / EDTA and irradiated with 365 nm light for 10 minutes. Viability was measured by MTT and calcein, and both methods were in close agreement. Relative cell populations were determined by flow cytometry.

Results: A549 cells (EpCAM+) were isolated from a suspension with Jurkat cells (EpCAM-). A 90/10 suspension of Jurkat/A549 cells was prepared, and was resuspended in biotinylated antibodies against EpCAM. Cells were then resuspended in streptavidin-eosin, and rinsed. Upon resuspension in the monomer mixture and irradiation in 530 nm light, a polymer coating is clearly observed by both epifluorescent microscopy (aided by inclusion of fluorescent nanoparticles) and by SEM. Exposure of pure populations of uncoated cells to 5% SDS showed immediate (<<30 s) lysis of uncoated A549 and Jurkat cells. Contrastingly, polymer coated cells are intact, spherical, and viable in 5% SDS for up to several hours. In the 90/10 mixture, the uncoated Jurkat population was largely lysed, resulting in a population highly-enriched for the antigen positive cells.

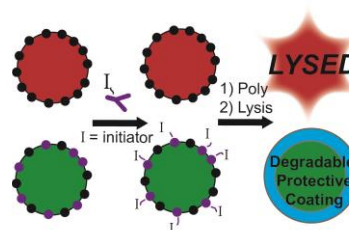

Figure 1. Overview of isolation process. Antigen-positive cells are coated by a temporary protective coating, and all uncoated cells are lysed.

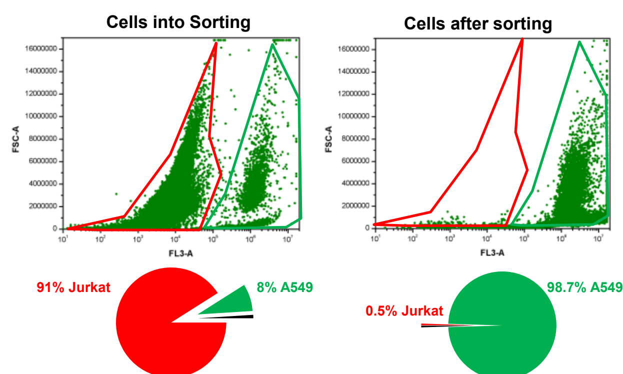

Figure 2. Isolation of A549 cells from mixture with Jurkat cells. First 10,000 events shown. Percent of total events within each bin represented in pie chart. Red = Jurkats, Green = A549, Black = outside of bins.

Control experiments, where Jurkats (CD45+) are isolated from a mixture with A549 cells (CD45-) using biotinylated antibodies against CD45 yielded a comparable enrichment for Jurkat cells.

The cells are then resuspended in PBS/EDTA and the polymer coating is removed by 10 minute 365 nm irradiation. Coating removal is verified by optical microscopy and the cell's capacity to once again proliferate in culture. Cell viability (~70%) is comparable to that of fluorescent sorting, yet the process costs a fraction of the capital costs and is presently accomplished in-lab at drastically superior timescales.

Conclusions: We introduce a completely new mode of cell isolation enabled by interfacial biomaterials. Viability and purity rival fluorescent sorting, while process throughput greatly exceed that of fluorescent sorting.

References:

1. Avens, HJ. J Hist Cyt Chem. 2011;59:76-87.
2. Avens, HJ. J NP Res. 2011;13:331-346.
3. Kloxin AM. Science. 2009; 324:59-63.

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