# Nuclease-resistant Aptamers for the Affinity Capture of Endothelial Progenitor Cells

Mike D. Nichols, Casey McGinley and W. Monty Reichert
Duke University, Durham, NC

**Introduction:** The practicality of using of endothelial progenitor cells (EPCs) to endothelialize small-diameter vascular grafts is severely limited by the inability to quickly isolate a large quantity of cells from patients awaiting bypass surgery [1]. Current isolation strategies necessitate a 5-6 week post-harvest period to expand cells *ex vivo* before an endothelialized graft of typical size (4 mm ID, 10 cm long) can be prepared. This long wait period greatly increases the risk of adverse cardiac events in the interim and prevents clinical implementation of this otherwise promising technique. This expedited process would make EPC technology more clinically feasible and substantially reduce risks for waiting bypass patients.

The overarching goal of this project is to initially isolate a population of late-outgrowth EPCs from patients with coronary artery disease (CAD EPCs) that is 150-fold greater in number than achieved by current methods. This will enable a typical graft to be sodded within 2.5 weeks of isolation, which is more than twice as fast as current strategies. We hypothesize that this can be achieved by directly filtering whole patient blood via an ex vivo shunt through a column containing aptamers of exceptionally high specificity and affinity to circulating CAD EPCs. The *specific focus and first step of this work* is to develop and characterize 2'OMe-derivitized nuclease-resistant RNA (nrRNA) aptamers selective toward CAD EPCs but not the buffy coat cell population in which they reside.

**Methods**: Aptamers will be generated using a variation on the subtractive cell-SELEX technique detailed by Tan et al. [2] and summarized in Figure 1. Before beginning the

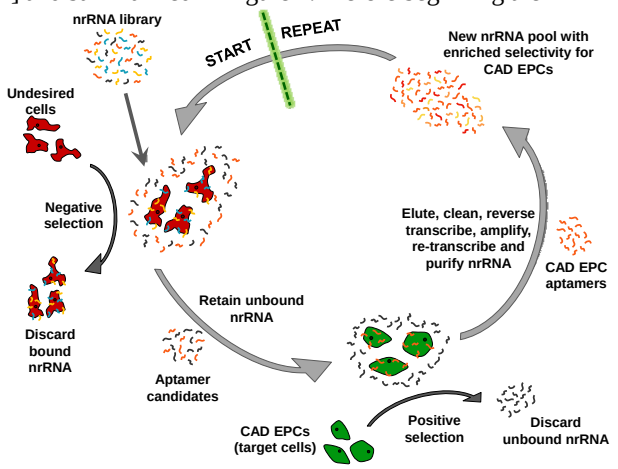


**Figure 1**: Overview of subtractive whole cell-SELEX

selection, a single-stranded nrRNA library (71 bp) consisting of a centralized 40 nucleotide random sequence flanked by constant regions will be used for selection and transcribed from a chemically synthesized random ssDNA library using a recombinant Y639F/H784A double mutant T7 RNA polymerase (YFHA) [3] produced in-house. Initially, a positive round of selection will be performed by incubating 10 nmol of library ~$10^7$ adherent CAD EPCs. The cells will then be washed and bound sequences will be liberated by mild proteolysis treatment using proteinase K. nrRNA aptamers will then be purified by phenol/chloroform extraction before being washed and concentrated in a spin filter. Retained sequences will be reverse transcribed to cDNA, amplified by PCR, transcribed back to nrRNA and then PAGE-purified for use in the next round of selection. Subsequent selection rounds will first employ a negative selection in which the nrRNA pool is incubated with freshly isolated buffy coat cells, and unbound sequences will be isolated by centrifugation and then directly used for positive selection as described above. Selection pressure applied during positive selection will be increased throughout the selection to retain only the strongest binders by (1) decreasing the cells:nrRNA ratio, (2) increasing wash stringency, (3) increasing binding reaction temperature, and (4) increasing binding buffer serum content. Enrichment in the selectivity of the library toward CAD EPCs will be monitored every other round by incubating fluorescently labeled aptamers derived from various selection rounds with cells and observing increased mean fluorescence intensity via flow cytometry. This procedure will be iterated until no further increase is detected with three subsequent rounds. After selection, aptamer binding affinities will be determined and their CAD EPC surface targets will be elucidated.

**Preliminary Results:** As proof of principle we have generated aptamers against human umbilical cord ECs (HUVECs). Five rounds of selection was sufficient to significantly enhance the binding capacity of the initially random ssDNA pool to HUVECs as indicated by an increase in the mean fluorescence intensity of fluorescent aptamer incubated cells (Figure 2). We have made YFHA enzyme, used it to generate nrRNA and started the selection against EPCs.

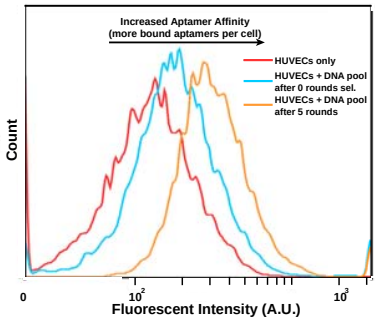


**Figure 2**: HUVEC selection data

**Conclusions:** The generated aptamers will enable the development of highly selective and efficient capture of this rare cell type, enabling rapid mass isolation of CAD EPCs and facilitating discovery of novel and unique biomarkers.

**References**:

1. Broxmeyer, H.E., et al., *Cord blood stem and progenitor cells*, in *Adult Stem Cells*. 2006. p. 439-473.
2. Sefah, K., et al., *Development of DNA aptamers using Cell-SELEX*. Nat. Protocols, 2010. **5**(6): p. 1169-1185.
3. Padilla, R. and R. Sousa, *A Y639F/H784A T7 RNA polymerase double mutant displays superior properties for synthesizing RNAs with non-canonical NTPs.* Nucleic acids research, 2002. **30**(24): p. e138-e138.