Development of a Catheter-Deployable Device for the Capture of Rare Analytes in Blood

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Statement of Purpose: The blood contains a wealth of information pertinent to monitoring treatment efficacy and disease progression in patients. Biomarkers are often at concentrations below standard sensitivity limits. Existing detection strategies focus on microfluidic devices that capture analytes from samples *ex vivo*. Biomarkers of interest may, however, be sufficiently scarce such that they are not present in clinical blood samples. We have extended the concept of integrating sensors **(**Ling Y, Nat Biotechnol 2011;29:273-7) to develop a device that can be deployed by catheter to reside in the bloodstream to capture and enrich biomarkers. Our device consists of a nitinol wire with a conformal silicone coating that has been functionalized with peptides, antibodies, DNA binding ligands, and poly(ethylene glycol) (PEG) to resist protein fouling. Allowing the device to reside in the bloodstream for 30 minutes has the potential to sample 40,000 times the volume of a traditional blood draw and capture cells, DNA, or proteins for analysis.

Methods: Nitinol wire was formed into spirals by heating the wire to 500˚C for 20 min around custom machined molds and quenched in water. Spirals were coated with poly(dimethyl siloxane) (PDMS, Sylgard 184, Dow Corning) by passing 1.5 A though the wire in a bath of prepolymer for 20 s. PDMS-coated wires were activated using a robotically controlled coronal discharge apparatus to oxidize the surface for subsequent chemical modification. Surface functionalization was monitored using a contact-angle goniometer (Attension). PEG-silane (1kDa, Gelest) was dissolved in toluene and reacted with activated PDMS surfaces. PDMS surfaces were also reacted with N-(p-maleimidophenyl) isocyanate (PMPI) or para-nitrochloroformate (pNP) dissolved in dimethyl formamide with equimolar triethyl amine. PMPI and pNP crosslinkers allowed further coupling with thiol or amine bearing biomolecules, respectively. The following molecules were conjugated to PDMS surfaces: biotinylated peptides (Cys-His6-Biotin); anti-dsDNA antibodies (Millipore) functionalized with 2-4 thiols per antibody using 2-iminothiolane; hoechst-amine (courtesy of N Murthy, UC Berkeley, synthesized according to (Dasari M, Org Lett 2010;12:3300-3)). Depletion assays were conducted to determine capture efficiency. Devices were incubated with streptavidin beads (Polysciences) or DNA. Concentrations of model analytes were determined though microscopy or Picogreen assay (Invitrogen).

Results: We have optimized a process to produce conformal PDMS coatings on nitinol wires by using the wire as a resistive heating element. We are able to produce coating thicknesses of 110 ± 10 µm by optimizing electrical current and heating time. These devices can be further functionalized with PEG-silane to generate hydrophilic surfaces that are stable for more than 4 weeks (contact angle <50˚; bare PDMS ~110˚).

We are able to create thiol-reactive surfaces to immobilize peptides and antibodies using PMPI crosslinking chemistry. Cys-His6-biotin peptides were conjugated to the PDMS surface and incubated with streptavidin-functionalized, fluorescent microparticles. PDMS functionalized with Cys-bearing peptides showed nearly three-fold more microparticles bound to the surface compared to control peptides (i.e. non-reactive Gly-His6-biotin peptides, Figure 1). These data represent up to 13% capture efficiency of the 1000 beads that were incubated with the device.

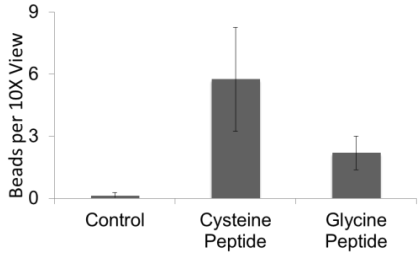

Figure 1. Streptavidin Microparticle Capture

PDMS surfaces were also functionalized with anti-dsDNA to demonstrate capture of DNA. PDMS surfaces captured 64 ng DNA/cm^2, which represents 60% capture efficiency based on area estimates (Figure 2). Non-specific DNA binding on bare PDMS and control surfaces showed a binding of 20-23 ng DNA/cm^2. We have also tethered the DNA binding dye, Hoechst, to surfaces and confirmed conjugation using FTIR.

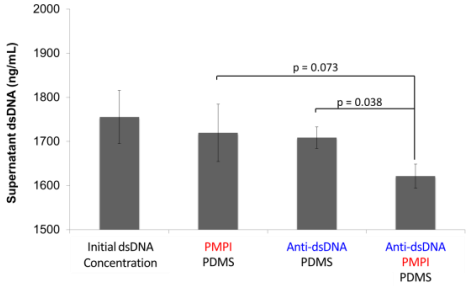

Figure 2. dsDNA supernatant depletion

Preliminary hemocompatibility was conducted using primary human platelets in adhesion tests. Adherent platelet counts were determined using a lactose dehydrogenase assay. Functionalized PDMS did not show an increase in platelet number compared to controls.

Conclusions: We present the design and fabrication of a device that can capture analytes from solution. The fabrication process is amenable to tethering a variety of capture ligands (antibodies, small molecules), providing a flexible platform for analyte enrichment. We demonstrate proof of concept of capture using streptavidin coated bead and DNA. Preliminary hemocompatibility studies suggest minimal platelet interaction and ongoing studies are focusing on flow cytometry-based characterization of platelet response to functionalized surfaces. We are currently identifying high-affinity ligands suitable for use *in vivo*. Future work will include capture of target cells and capturing and analyzing DNA from whole blood.