Stimuli-responsive Hydrogels for Reagent Storage and Delivery From Lab-on-a-Chip Cards

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Statement of Purpose: Sharply pH- and temperature-responsive hydrogels have been developed that stabilize antibody and enzyme reagents on plastic lab-on-a-chip cards in a collapsed, dried state. Upon hydration at the time of use, the gels swell rapidly to release the reagents into the flowstream as a well-controlled reagent plug. Copolymer hydrogels of poly(*N*-isopropylacrylamide) (pNIPAAm) with the hydrophobic acid monomer propylacrylic acid (PAA) have been prepared using reversible addition fragmentation chain transfer (RAFT) polymerization.[1] The NIPAAm-*co*-PAA copolymers exhibit tunable swelling properties that are poised to release at designed temperature and pH. This work examines their ability to preserve and deliver antibodies against blood- borne antigens for on-card immunoassays in global health oriented diagnostic devices.

Methods:

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO) and used as received unless otherwise noted.

Membrane modification

Biodyne C membranes (0.45 µm pore size; Pall Corporation, East Hills, NY), which have surfaces populated with reactive carboxyl groups, were exposed to a mixture of *N*-Ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide (EDC) and poly-L-lysine in DMF for 24 hours. Membranes were then rinsed with acetone and water and dried under vaccum. After drying, membranes were exposed to acrylic acid *N*-hydrosuccinimide in water to populate the surface with photopolymerizable vinyl groups. Membranes were rinsed again in water and dried under vacuum. Reaction efficiencies were assessed using the Ninhydrin assay, which quantifies amines on surfaces.

Synthesis of pNIPAAm-co-PAA hydrogels

Polymerization of pNIPAAm-*co*-PAA was carried out as as previously described[1], yielding a 20% PAA copolymer. Hydrogels were crosslinked to the surface of modified Biodyne C membranes by photopolymerization. NIPAAm-*co*-PAA, *N,N'*-methylenebisacrylamide (MBAm), and the photoinitiator Irgacure 2959 were dissolved in isopropanol. The solution was then and exposed to UV light for 1 hour to form a thin hydrogel layer covalently bound the membrane surface. Hydrogels were rinsed thoroughly in deionized water and dried under vacuum.

Protein loading and release

Gels were loaded with 20 µg of either Horseradish Peroxidase Conjugated Rabbit anti-Plasmodium Aldolase (ICL, Newburg, OR) or Peroxidase Conjugated Monoclonal IgG anti-Plasmodium falciparum (PfHRP2; ICL, Newburg, OR) in 10 µl deionized water, immediately frozen to -80ºC for 24 hours, and lyophilized. Protein release was assessed by incubation in water at either 25ºC or 45ºC. At specific intervals liquid was removed and protein content quantified by BCA assay.

Assessment of protein stability

Protein stability was assessed by colorimetric ELISA. Both dried protein alone and dried protein loaded into hydrogels were rehydrated and compared to equal amounts of protein stock stored at 4ºC.

Results/Discussion:

Static protein release

On average, 98.58 ± 0.57% of the total protein loaded into pNIPAAm-*co*-PAA hydrogels was released within 4 min of rehydration at 25ºC (Figure 1), and 98.45 ± 0.8% was released within 2 min at 45ºC, demonstrating rapid recovery of reagents from immobilized hydrogels.

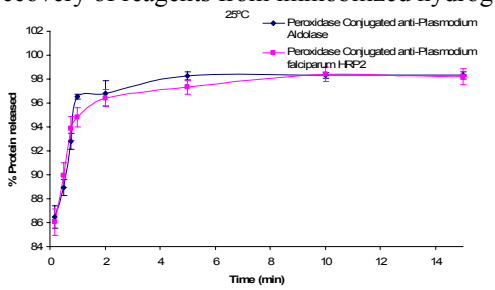

Figure 1. Protein release from membrane-bound pNIPAAm-*co*-PAA hydrogels occurred within 4 min at 25ºC.

The ability of stored hydrogels to release active protein was also observed. Protein stability, as assessed by colorimetric ELISA, was shown to be significantly greater with proteins freeze dried within NIPAAm-*co*-PAA hydrogels as compared to hydrogels freeze dried with no stabilizer (Figure 2).

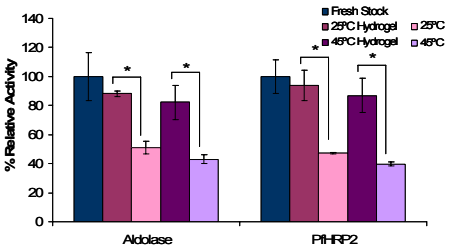

Figure 2. Relative activity of stored protein after 10 days compared to stock solutions stored at 4ºC. (*$p < 0.02$)

Conclusions: Our initial results show the potential of an immobilized hydrogel matrix as a stabilizer for protein reagent storage. The gels are poised to swell rapidly at instrumented temperatures upon hydration at neutral pH. These new materials may provide enhanced long-term reagent storage conditions for global health diagnostic applications.

References:

1. Yin X, Hoffman AS, Stayton PS. Biomacromolecules. 2006 May;7(5):1381-5.