A potential route to hydrogel multifunctionalization
utilizing encapsulation of acrylate-conjugated streptavidin
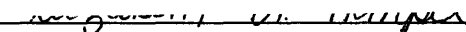
By

Elizabeth Hempel

SUBMITTED TO THE DEPARTMENT OF MECHANICAL ENGINEERING IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

BACHELOR OF SCIENCE
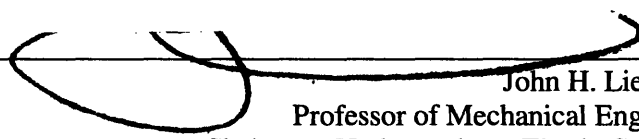AT THE
MASSACHUSETTS INSTITUTE OF TECHNOLOGY

JUNE 2006

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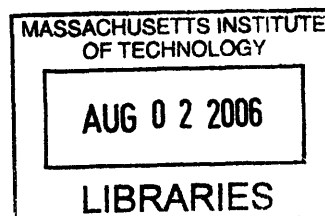
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A potential route to hydrogel multifunctionalization
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Submitted to the Department of Mechanical Engineering
On May 12, 2006 in Partial Fulfillment of the
Requirements for the Degree of Bachelor of Science in
Mechanical Engineering

ABSTRACT

Biologically active materials providing a range of applications from tissue engineering to microdevices have begun to revolutionize biomedical science. New chemistries, however, must be developed for functionalization of these materials with each different molecule. This paper explores a technique for developing multi-functional, biologically active hydrogels utilizing the high streptavidin-biotin binding affinity. Streptavidin was conjugated to acryl-PEG-N-hydroxysuccinimide, a commercially available molecule that allows chemical binding to poly(ethylene glycol) (PEG) diacrylate and dextran acrylate hydrogels. Such gels were made by photocrosslinking solutions of APN and streptavidin conjugated at various molar ratios, along with a gelling polymer under an ultraviolet (UV) lamp. Acryl group conjugation was confirmed through high performance liquid chromatography (HPLC) and mass spectrometry. Protein binding was assayed through the use of rhodamine-labeled streptavidin and fluorescent microscopy. Gels were incubated overnight in solution to determine diffusion. After 7 days, PEG showed no diffusion while dextran acrylate demonstrated 100% protein loss.

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1. Introduction

## 1.1 Background and Objectives

The development of materials with biologically active molecules has revolutionized biomedical research by providing new avenues of investigation into biomolecular and cellular interactions. Hydrogels have shown promise as bioactive materials which can be utilized in a variety of applications from scaffolds in tissue engineering[1] to microdevices.[2] The modification of synthetic materials with biological molecules such as peptides, proteins, and nucleic acids, have allowed for chemically specific adhesions.

Poly(ethylene glycol) (PEG) hydrogels have been of particular interest in development of synthetic biomaterials. They are non-toxic, non-immunogenic, and approved by the FDA for various clinical uses. Furthermore, PEG is chemically inert to most biological molecules, preventing protein adsorption onto materials which have had PEG previously adsorbed onto them. [3] PEG can also be modified through covalent bonding with acrylate, silane, and thiol groups, and through hydrogen or ionic bonding to enhance surface biocompatibility. [4] In the case of acrylate terminated PEG monomers, photocrosslinking under a UV light leads to the formation of a hydrogel. [5]

Currently, however, stoichiometric conditions must be individually considered for each molecule to be bound to PEG. As a result, the ability to synthesize a multifunctional hydrogel under simple, aqueous conditions would present a useful advance for bioengineering. The goal of this thesis was to develop and characterize multifunctional hydrogels encapsulating biological molecules which enable easy, robust conjugation to a variety of other biological molecules.

1.2 Theory

These multifunctional hydrogels aim to take advantage of unique properties of the protein streptavidin, a tetrameric molecule with four biotin binding sites. Additionally, streptavidin has several free amine groups which allow it to react with N-hydroxysuccinimide (NHS), a molecule that can be commercially obtained bound to acrylated PEG. Thus, as shown in Figure 1, streptavidin can be conjugated to PEG through the aminolysis reaction to form acryloyl-PEG-streptavidin (acryl-PEG-SA).

Acryl-PEG-N-hydroxysuccinimide   Streptavidin   Acryloyl-PEG-streptavidin
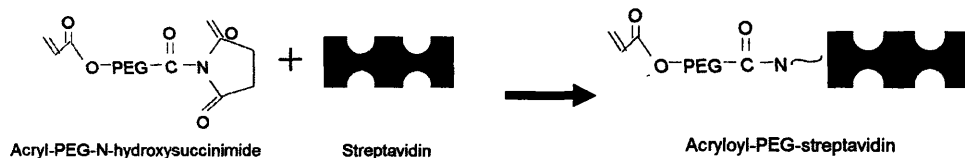
Figure 1: Procedure for obtaining a streptavidin modified photocrosslinkable PEG acrylate.

Additionally, streptavidin has an extremely high affinity for biotin, a small biomolecule that can be purchased readily attached to many other molecules of interest including

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proteins ($K_d \sim 10^{-15}$).[6] Thus, any biotinylated molecule can be bound into the PEG scaffold through a variety of procedures. As shown in Figure 2, a hydrogel can be photocrosslinked with acryl-PEG-SA with subsequent incorporation of biotinylated molecules. Previous studies have shown that the stoichiometric ratio of acryl-PEG-protein to PEG-diacrylate significantly affects both gel formation and functionality [7,8]; therefore, multiple molar ratios were to be examined.
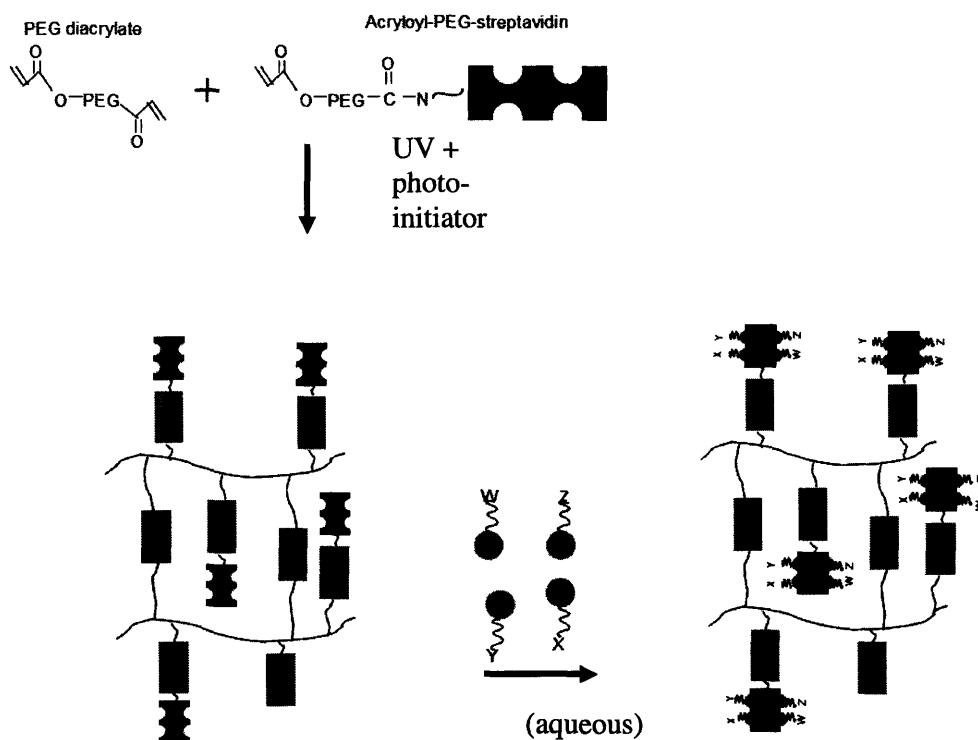
Figure 2: Procedure for formation of multi-functional hydrogel by addition of biotinylated molecules after copolymerization with PEG-diacrylate and acryl-PEG-SA.

2. Methods and Materials
2.1 Conjugation of Streptavidin to PEG-Acrylate Groups
Both rhodamine-conjugated and non-conjugated streptavidin, mw 55,000, were diluted to a 1 mg/ml concentration in 50mM sodium bicarbonate buffer, pH 8.2. Acryl-PEG-N-hydroxysuccinimide (APN), PEG mw 3400 Da, was also diluted in sodium bicarbonate buffer at various concentrations and then added to the streptavidin solution in a dropwise manner. Final solutions consisted of APN to streptavidin molar ratios of 1:2, 1:1, 2:1, 4:1, 8:1, and 16:1. The solutions were incubated for 2 hours on a rotor in the dark. Samples were then dialyzed using 10,000 MW dialysis cassettes (Pierce, Rockford, IL). The cassette membrane was wet briefly in distilled, deionized water (ddH$_2$O) before injecting the samples into them. Cassettes were suspended in ddH$_2$O for 48 hours in the cold room and kept in the dark. Water was changed every 12 hours. Samples were then

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drawn out of the cassettes. To ensure complete removal of sample, 1mL of ddH$_2$O was injected into cassette, swirled around, and added to the recovered sample. Samples were then lyophilized.

2.2 HPLC and Spectrophotometry

Lyophilized samples were diluted at a concentration of 1mg/mL in sodium bicarbonate; however, because mass measurements were unable to be obtained, 1mg of lyophilized streptavidin is an idealized value. A portion of this is likely to have been lost during the dialysis process.

To determine the concentration of the samples, various concentrations of unconjugated streptavidin were measured utilizing spectrophotometry. Spectrophotometry utilizes a spectrometer to generate light at a specific wavelength. That light is then passed through a sample where it is absorbed and/or transmitted. A photometer on the opposing side then measures then intensity of the light that passes through from the sample, thus giving information about the concentration of the sample if the concentration is color dependent. As all of the streptavidin was rhodamine labeled, the pinkish color enabled the use of this technique. Samples of .01% reaction product or unconjugated streptavidin at various concentrations were diluted in PBS and mixed by pipette. Care was taken to remove all bubbles. Samples were then placed in the spectrophotometer and automixed for 5 seconds. The analysis was run at 3 wavelengths, 285nm, 515nm, and 550nm.

High Performance Liquid Chromatography (HPLC) was utilized to determine the success of the streptavidin-APN reaction for the various molar ratios. In HPLC, pressurized sample is injected into a specialized column and then into a detector. The differing affinity of components within the sample for the column causes separation of the components upon exiting, those with the lowest affinity leaving the column first. As each band elutes from the column, an ultraviolet detector measures peaks in the sample. The time taken for each peak to elute determines the component; the area of the peak determines the relative quantity.

A mixture of 100μL of sample and 100μL of sodium bicarbonate was placed in HPLC tube. Streptavidin at a 0.5mg/mL dilution and sodium bicarbonate controls were also run. A C4 HPLC column was used, with 1% trifluoroacetic acid H$_2$O and acetonitrile as eluting buffers. Measurements were taken at 200nm wavelength, at 4mL/min.

2.3 Mass Spectrometry

Matrix assisted laser desorption ionization (MALDI) spectrometry was used with a time-of-flight (TOF) analysis to confirm the HPLC results. MALDI is a common form of mass spectrometry for proteins, peptides, and other non-volatile biochemicals of high molecular mass. One microliter samples of unconjugated streptavidin and conjugated streptavidin at 1:1, 2:1, and 4:1 APN to streptavidin molar ratios were mixed in a sinapinic acid matrix at a low concentration ratio. The sample was allowed to dry prior to insertion in the vacuum chamber. The acidic matrix prevents the direct energy of the laser from contacting the sample, a problem which often causes decomposition. Instead, the direct energy is converted into excitation energy in the sample, causing both matrix

and sample ions to be sputtered from the surface of the mixture. The ions then migrate due to an electrical field applied within the vacuum chamber towards the time-of-flight analyzer, as can be seen in Figure 3.

Analyzer

Flight

Ions released

Laser bombardment

Sample + Sinapinic Acid

High Voltage
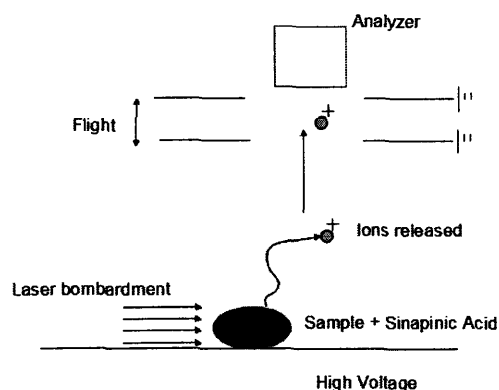
Figure 3: The interior of a MALDI-TOF mass spectrometer. The sample is absorbed onto the matrix where it is bombarded by a high energy laser. Ions are released and are guided by an electromagnetic field through a tube known as the flight to an analyzer.

Time-of-flight refers to the time which an ion takes to travel the length of the collection tube, known as the flight. Ions of heavier mass travel slower; lighter ions, conversely, travel much more quickly. The analyzer then collects information regarding the mass and charge of the ion bombardment. The MALDI analysis was then run in linear mode with a positive polarity on the ions.

2.4 Poly(ethylene glycol)
2.4.1 Gel Formation
Microscope slides were first methacrylated to ensure gel adhesion to the slides. One to two drops of trimethylsilyl methylacrylate (TMSMA) were dropped onto the face of a clean glass slides. A second slides was then placed on top, creating a TMSMA film between the two and evenly coating both surfaces. The same procedure was repeated for the second slide. Slides were let to sit for 10 minutes and then separated and placed in a slide rack in an oven at 110°C for another 10 minutes. Slides were allowed to cool and the rinsed in distilled water.

Polydimethylsiloxane (PDMS) molds were fabricated by casting PDMS (Sylgard 184 Silicon elastomer, Essex Chemical) against a complementary structure that was prepared by photolithographic method. Curing agent was mixed at a 1:10 ratio with the pre-polymer and incubated at 70°C for 1 h. The PDMS mold was then removed from the silicon wafer and cut prior to use. The molds had receding (negative) features.

Gel solutions consisted of 50μL of polymer, 50μL of lyophilized sample diluted to a concentration 0.6mg/ml in PBS, 1μL of 97% 2-hydroxy-2methyl-propiophenone were

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added to 899μL of PBS. Polymers utilized were 575MW PEG, 4000MW PEG (Sigma-Aldrich, Allentown, PA), and 70,000 MW dextran acrylate DS 20% made utilizing the protocol of Ferreira et al.[9] For controls, lyophilized sample was replaced with PBS or 1 mg/ml streptavidin in the gel solution. Fifty microliters of this mixture were placed on a methacrylated slide and firmly covered with a PDMS mold. Samples were then placed directly under a 365 nm, 300 mW cm_{-2} UV lamp (EFOS Ultracure 100ss Plus, UV spot lamp, Mississauga, Ontario) for 2.5 minutes. PDMS molds were then removed and gel was left in dark and kept moist until set.

1. Methacrylated slide

2. Gel solution

3. Cover with PDMS mold

4. Expose to UV light

5. Remove mold
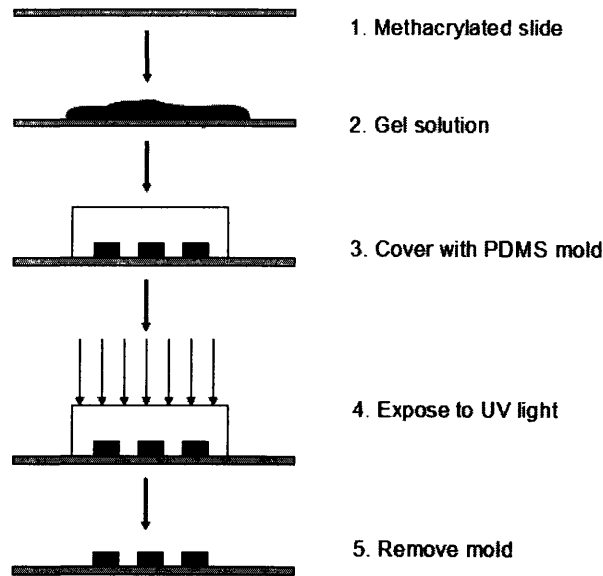
Figure 4: Process for forming the photocrosslinked hydrogels.

2.4.2 Biotin Incubation

Gels were also incubated in FITC labeled biotin (Pierce, Rockford, IL). Biotin was diluted in dimethyl sulfoxide (DMSO) at 5mg/100μL and then diluted to 50μg/mL in PBS. Gels were covered with biotin solution and incubated in the dark for 20mins. Gels were then rinsed twice for 8 minutes each in PBS. Gels were then viewed under fluorescent microscope for both FITC and rhodamine labels. Images were taken and analyzed using ScionImage Software by the National Institute of Health. The software takes a fluorescent image and converts it into a grayscale image before measuring the intensity. ScionImage measurements were then normalized against each other.

2.4.3 Overnight Incubation

Gels were incubated in PBS overnight on a rocker at 4°C. Samples were kept dark. Rhodamine images were taken every 24 hours and gels were returned to the rocker in fresh PBS. Dry controls were also maintained overnight without PBS incubation.

2.4.4 Salt Incubation

All APN-streptavidin reactions were performed at a pH of 8.2; after dialysis, the lyophilized product was diluted in PBS at a pH of 7.4. Both of these pHs are far above

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the pI of streptavidin, which occurs at pH of 5. Because the pI is the pH at which the molecule exists at an uncharged state, gels were incubated in solutions of various pHs to ascertain the effect of the charged state on non-specific binding within the gel. Gels were made as before using previous protocols. Sodium chloride solutions, 0.5M, were then pH adjusted to pH4, pH5, and pH7. Gels were incubated in their respective salt or PBS control solutions for 24 hours before being imaged under the fluorescent microscope. All images were analyzed using ScionImage software.

2.4.5 Surfaces

Methacrylated slides were cleaned in distilled water. Edges were lined with tape, leaving a small window in the center into which 150μL of 5% PEG and 0.1% photoinitiator in PBS solution was placed. The slides were covered with a thin, clean PDMS slide and clamped at the edges. The entire structure was then exposed to the UV lamp for 11 minutes. Surfaces were left to sit until dry and then the PDMS cover was removed. Slides were incubated in 0.1% BSA (non-fluorescent) in PBS for 30 minutes before being washed twice 15 minutes each in PBS; control slides were not incubated in the BSA solutions. Solutions of 0.05% BSA, 0.005% reaction product or unconjugated rhodamine-labeled streptavidin and 0.1% photoinitiator solutions were mixed and exposed on pegylated surfaces for 4 minutes under UV lamp while covered with PDMS slide. Control slides did not receive BSA in their mixtures. PDMS was then removed and surfaces were washed twice for 15 minutes each in fresh PBS. Fluorescence - microscopic images were taken under a rhodamine filter. Slides were then incubated in FITC labeled biotin for 30 minutes before being washed twice for 15 minutes in fresh PBS. Microscope pictures were taken with both rhodamine and FITC filters.
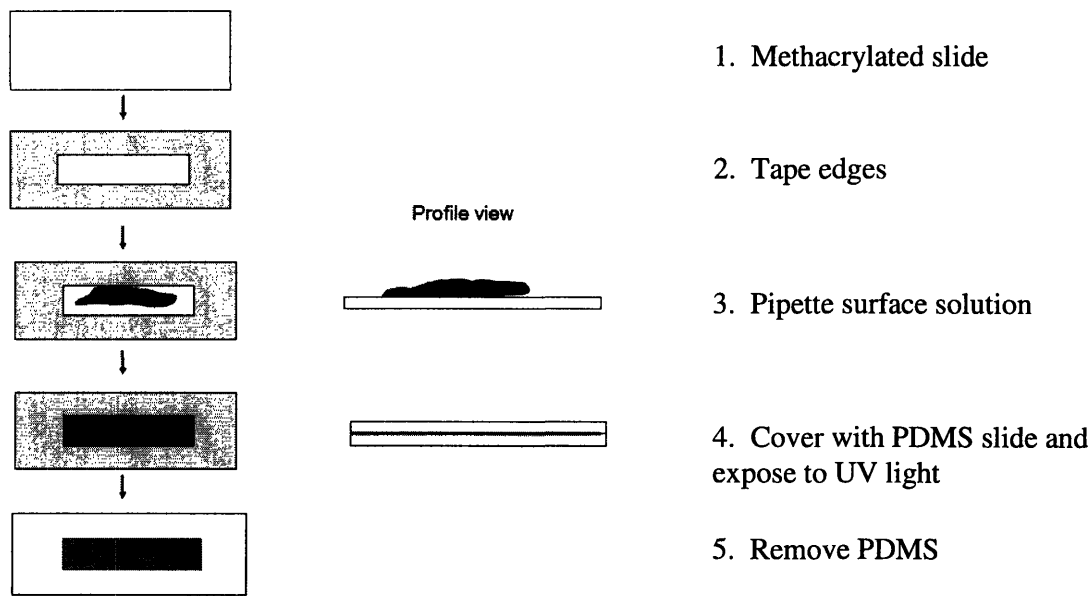
1. Methacrylated slide

2. Tape edges

Profile view

3. Pipette surface solution

4. Cover with PDMS slide and expose to UV light

5. Remove PDMS

Figure 5: Process for forming thin, photocrosslinkable surfaces with PEG diacrylate.

2.5 Dextran Acrylate Gels

Dextran acrylate gels were created using a similar procedure to the PEG gels. A solution of 10% dextran acrylate (20% acrylation), .005% reactant, and 0.1% photoinitiator was diluted in PBS, where the reactant was either streptavidin-APN, streptavidin, or plain PBS for various samples. The concentration of polymer was increased from 5% to 10% to account for the increased length of the dextran acrylate chain, which increased the difficulty in forming a solid gel. By increasing the concentration, the gel was more easily able to crosslink. The remainder of the procedure continued as before; however, crosslinking time under the UV lamp was reduced to 60 seconds based on optimal gelling times of trial runs. Dextran acrylate gels underwent an identical procedure for the overnight timecourse as the PEG gels.

3. Results and Discussion
3.1 Confirmation of acrylate conjugation to streptavidin
3.1.1 HPLC

HPLC results exhibited conjugation of the APN molecules to the streptavidin. Initial runs of streptavidin and sodium bicarbonate buffer solution indicated two control peaks. A peak occurring at 3.340 minutes appeared in all the sample runs, including the sodium bicarbonate control buffer and was thus concluded to be an error solution. A second peak at 2.065 minutes was found in all sample runs containing streptavidin and was thus determined to be the peak corresponding to the pure protein. All samples conjugated with APN exhibited a third peak at approximately 2.900 minutes, indicating the presence of a reaction product. The area under this third reaction peak increases with the molar ratio of APN to streptavidin in the conjugation reaction, indicating a larger presence of the reaction product. This trend continues clearly for APN to streptavidin molar ratios of 1:2, 1:1, 2:1, and 4:1. At higher molar ratios of 8:1, 16:1 and 32:1, peaks begin to merge

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together, indicating a change in the hydrophobicity of the reaction product. Streptavidin has multiple binding sites for APN; hence, the change in hydrophobicity indicates that multiple APN molecules are binding to the streptavidin at higher molar ratios of APN to streptavidin, thus changing the hydrophobicity of the reaction product and changing the HPLC results.

The graph shows Adjusted % React on the y-axis (0.00 to 60.00) versus APN:Strep on the x-axis (0 to 20), with the fitted equation:
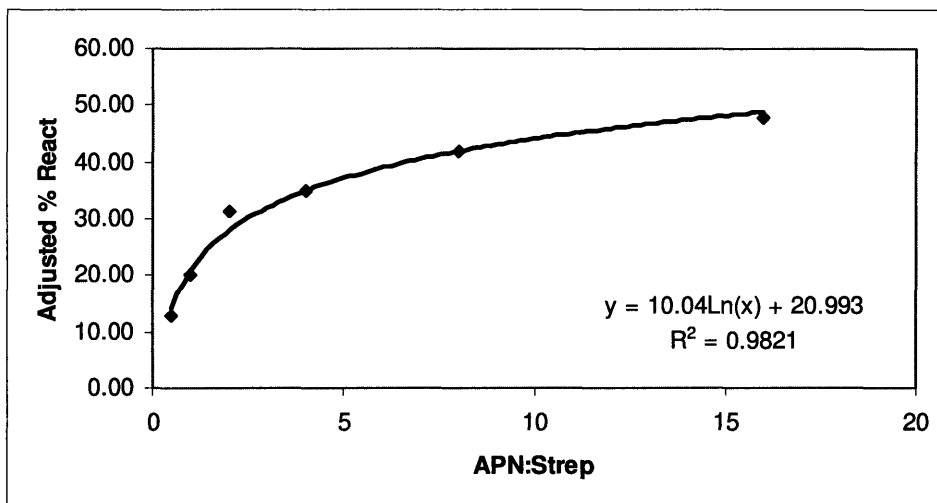
$$y = 10.04\text{Ln}(x) + 20.993$$
$$R^2 = 0.9821$$

Figure 6: Percent reaction product for various molar ratios of APN to streptavidin. Percentages have been adjusted to account for area underneath the buffer peaks.

3.1.2 Mass spectrometry

Mass spectrometry results supported HPLC results in showing a clear increase in mass corresponding to the molecular weight of APN. As can be seen in Figure 7, several primary peaks exist in the data, followed by secondary, tertiary, and sometimes even quaternary peaks. Each primary peak occurs at a point corresponding to the mass of the unconjugated streptavidin itself; the lower level peaks occur at regular intervals after this. The spacing, at roughly 3400 Da intervals, corresponds to the mass of the raw APN molecule itself. Thus, the results show an increase in mass which corresponds to the conjugation of APN to the streptavidin and even further indicate multiple bindings of

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APN molecules to single streptavidin molecules.

1:1

APN conjugation

APN conjugation

%Intensity

25877.99

20924.53 29245.84 28817.00 28499.84 33145.03 38890.28 39514.1 42610.79 45883.41 51915.45 55543.19 59042.74

Dimer

Trimer

Tetramer

%Intensity

25860.34

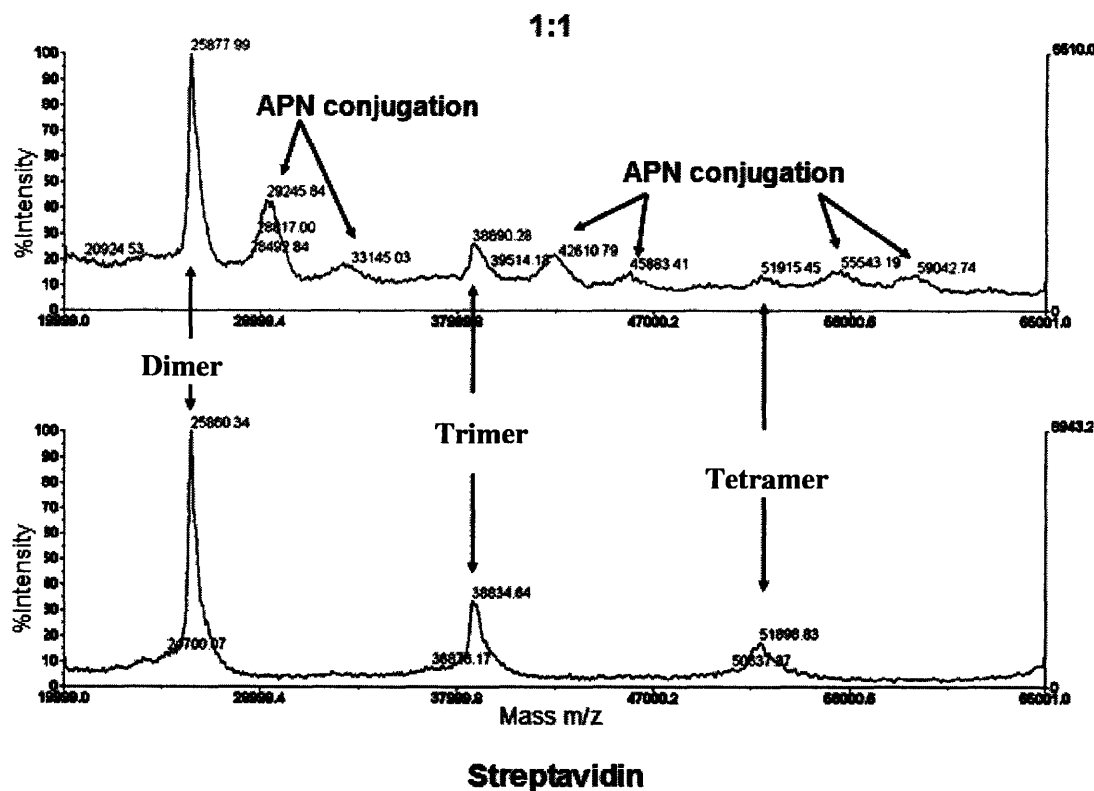26700.07 38834.64 36876.17 51896.83 50637.97

Mass m/z

Streptavidin

Figure 7: Results from MALDI-TOF mass spectrometry. Top chart illustrates 1 APN to 1 streptavidin molecular conjugation while bottom chart illustrates pure streptavidin. The positions of the dimer, trimer, and tetramer can be observed, as well as multiple APN conjugation sites. It should be noted that the full-range spectrum is not shown in order to better illustrate the data trends.

The mass spectrometry results also indicated a dissociation of the streptavidin subunits. Four large peaks are present on the streptavidin control plot; as streptavidin is a tetramer, these four peaks are hypothesized to represent each of the four possible mono- and multimeric conformations present in the protein. Because the mass spectrometry was run under acidic conditions, the dissociation could have been caused as a result of the assay itself; however, such occurrences are rare utilizing the MALDI mass spectrometry technique. Breakdown of the streptavidin protein is not clearly seen in the HPLC data. As a result, future work is being done to repeat these results under non-acidic conditions to exclude the possibility of protein break-down during the streptavidin-APN reaction itself. Also seen in the MALDO-TOF data are multiple bindings of APN. These can be seen to occur at all molar ratios, and can be seen to increase with higher quantities of APN, as shown in figure 8.
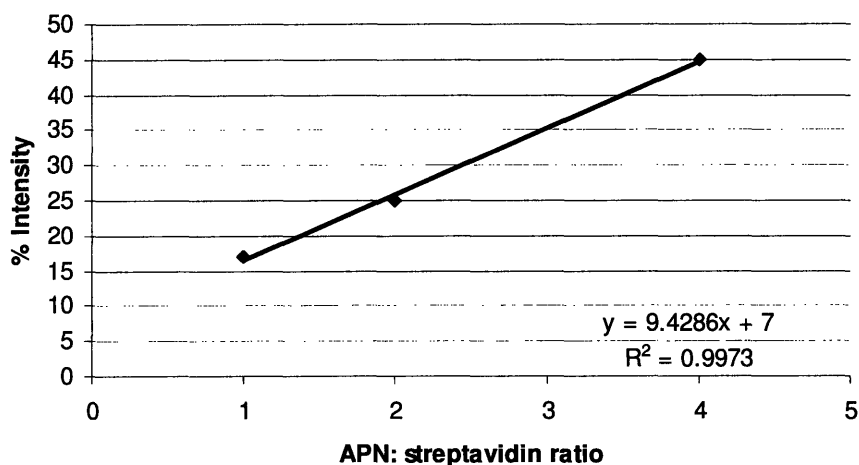
Figure 8: Correlation between APN:streptavidin molar ratio and % intensity of secondary APN binding peaks in mass spectrometry.

3.2 Spectophotometry

As previously noted, post-lyophilization mass of the APN-streptavidin conjugates was unknown due to difficulties in measurement. Samples were run through the spectrophotometer to determine a calibration curve for various concentrations of rhodamine-labeled streptavidin. The various samples of APN-streptavidin were then compared to the streptavidin calibration curve and values were utilized to calculate the concentration of product. Because the mass of APN is so small in comparison to the mass of the streptavidin, the mass of the sample was determined based solely on the streptavidin results. The average sample concentration was 0.6 mg/ml.

3.3 PEG

3.3.1 Initial data

Initial gels were made using the Irgacure 2959 (I2959) photoinitiator rather than the 2-hydroxy-2methyl-propiophenone noted in the methods and materials section because of the tolerance cells have exhibited to I2959 in previous studies.[10] Although other experiments had success with this photoinitiator[11], no gels were found to form after 15 minutes of exposure to the UV lamp; thus, the switch was made to a stronger initiator despite the potential harm to cells in future applications of the multifunctional hydrogels.

3.3.2 Overnight Timecourse

Figure 9 exhibits the data from the overnight timecourse of PEG hydrogels, one 575MW, the other 4000MW, with unconjugated, rhodamine-labeled streptavidin trapped inside. Because the streptavidin has no acrylate groups bound to it, the molecule should freely diffuse out of the gel when incubated overnight in solution. This diffusion out of the gel should appear in the chart as a drop in the level of fluorescence; however, instead of a decrease, an irregular pattern appears over the 7 days of the timecourse. The protein does not diffuse out of the gel. Two hypotheses were generated based on such data. First, the highest molecular weight of the PEG diacrylate tested was 4000 daltons, as opposed to

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the 55,000 of the streptavidin. Thus, the resultant mesh in the PEG diacrylate matrix could wrap so tightly around the streptavidin molecule that it cannot diffuse out of the gel. Secondly, the streptavidin molecule exhibits potential non-specific binding to the PEG diacrylate hydrogel itself, preventing free diffusion when incubated in solution.
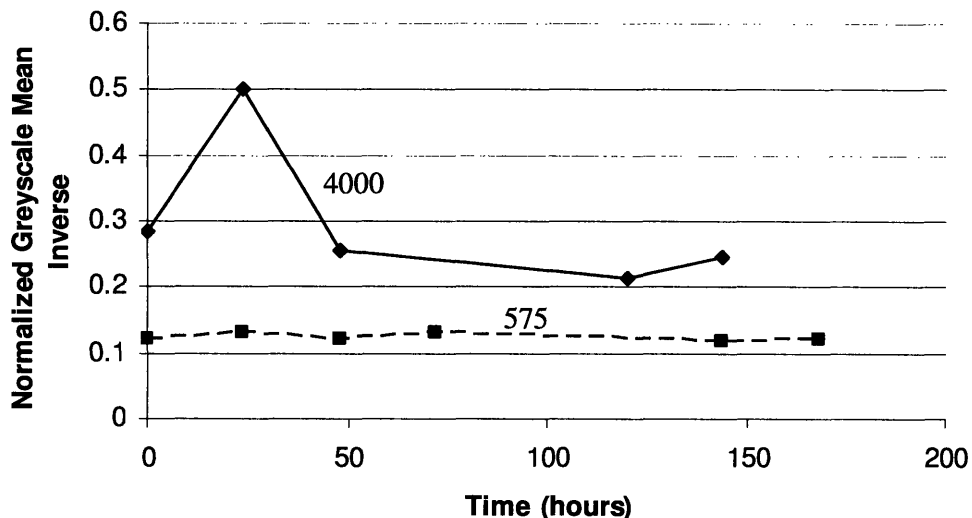

Figure 9: Overnight timecourse data for PEG-diacrylate gels. Dashed line represents data for 575 mw PEG diacrylate and solid line represents data for 4000 mw PEG diacrylate. Data has been normalized to 256,the value of the darkest colors in the ScionImage computer program.

3.3.3 Salt Incubations

Initial data showed the expected pattern, that the gels released streptavidin as they were incubated in solutions nearer to the pI of 5. The uncharged state of the streptavidin was less likely to non-specifically bind to the matrix of the gel. To confirm the results, a secondary trial was performed.

Shown in Figure 10 are the comparative results from the two experiments incubating gels in solutions at various pH. The key feature to note is the large differences in values between experiments one and two. Gels incubated in PBS showed a significant increase in fluorescence in the second experiment; other differences in patterns appear as well. The drastic changes between the two experiments demonstrate a lack of repeatability in the experimental procedure.
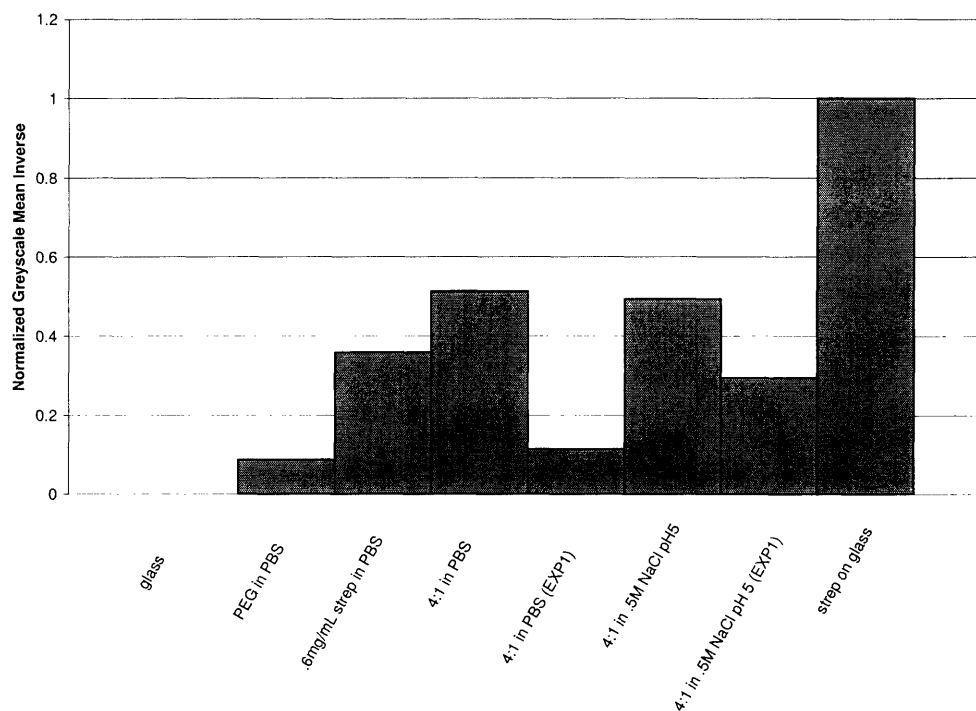
13

Figure 10: Comparative data for PEG diacrylate salt incubation experiments. Normalized fluorescence data for experiment 1 (EXP1) and experiment 2 are shown.

3.3.4 Surfaces

The results from the surface experiments proved to be non-repeatable. On certain occasions, the expected results were attained; however, when the identical experiment was repeated, the results followed different trends. Furthermore, the negative control, plain methacrylated glass, registered more fluorescence than the 4:1 APN-streptavidin gel. The lack of repeatability, combined with the relative brightness of the negative control, indicated an error in the experimental technique.

3.3.5 Photobleaching Analysis

The lack of consistent patterns in the data led to the hypothesis that the UV lamp was photobleaching the fluorescently labeled proteins encapsulated in the hydrogels, rendering the data inaccurate. As a result, gel solution was imaged on glass slides, underneath PDMS; the solution was not photocrosslinked. These images were compared to hydrogels formed using the standard crosslinking procedure under the UV lamp; PDMS molds were left on top of the hydrogels to eliminate any effects these might have in the fluorescence. The results can be seen in Figure 11 A clear pattern is exhibited in that samples exposed to the UV lamp exhibit far lower fluorescence than non-exposed samples. Furthermore, fluorescence values for non-exposed samples can be seen to steadily increase with the percentage of protein, whereas no correlation can be made between protein percentage and fluorescence in the exposed samples. This data indicates a clear connection between exposure to the UV lamp and bleaching effects in the rhodamine-labeled streptavidin, thus accounting for the lack of repeatability in previous
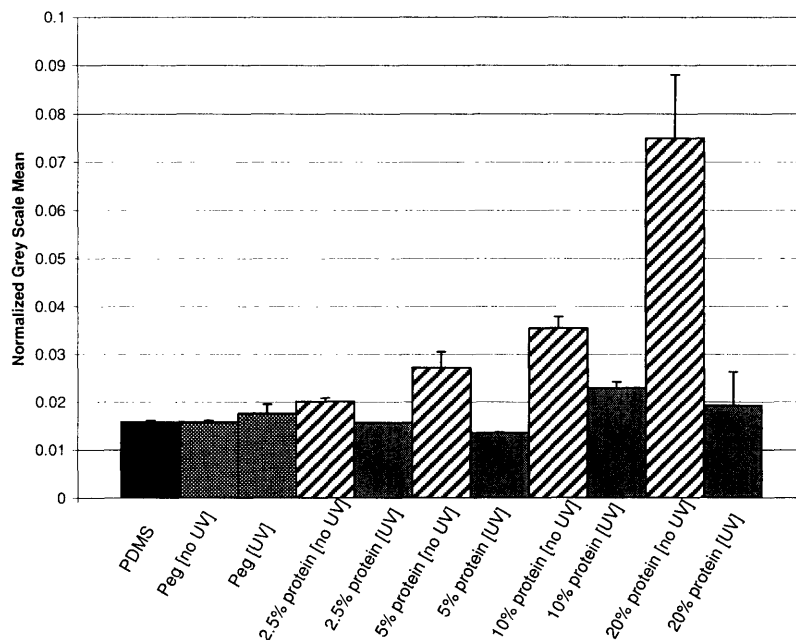
14

experiments.

Figure 11 Normalized fluorescence data for photobleaching experiment. Striped bars indicate samples not exposed to UV light while gray bars indicate UV exposure.

3.4 Dextran acrylate

The results from the PEG trials indicated that neither conjugated nor unconjugated streptavidin diffused out of the gel mesh after a period of 7 days. Given these results, a literature search was performed to determine the likelihood of diffusion over an extensive period of time. Protein of a similar size, bovine serum albumin (BSA), was found to remain entrapped within the gel for longer than 280 days. [12] The protein thus was not considered to diffuse sufficiently through the gel to remain viable. Another common biomaterial, dextran, was found to have adjustable diffusion properties[13] in addition to the ability to photocrosslink and be acrylated. Thus, the decision was made to investigate dextran acrylate gels as a potential alternative to poly(ethylene glycol). During the course of developing the gels, an additional advantage was discovered in the amount of time taken to form the dextran acrylate gels. Approximately 2.5 times lower than the gelling time for PEG, the reduced exposure to the UV lamp for dextran acrylate significantly lowered the effects of photobleaching on the fluorescence of the proteins, yielding far more consistent, reliable data.
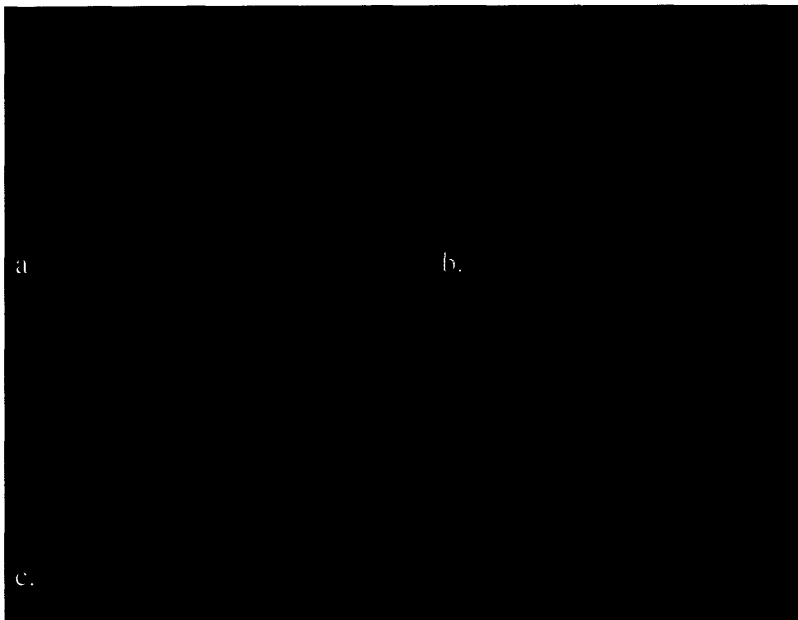
Figure 12: Dextran acrylate hydrogels with 4:1 APN to rhodamine-streptavidin modification after a 7 minute FITC labeled biotin incubation viewed under various filters. a) rhodamine b) FITC c) rhodamine-FITC overlay d) transmission

3.4.1 Overnight Timecourse

The overnight timecourse with the dextran acrylate exhibited protein diffusion out of the gels during an initial trial run. A second run was repeated under the same conditions and yielded similar results, which can be seen in Figure 13. The data in the figure can be separated into three distinct groups: protein encapsulated gels which were incubated overnight in PBS, protein encapsulated gels which were not incubated overnight in any solution, and gels which contained no protein. The gels which contained polymer alone and no protein can be seen to run along the bottom of the plot, as would be expected for negative controls. The gels which encapsulated protein and were not incubated in solution overnight can be seen to run across the top portion of the graph, indicating that no protein leached out of these gels. Furthermore, the maintenance of the fluorescence indicates that the rhodamine labeled streptavidin did not photobleach during the course of the experiment. This had been a concern due to the repeated removal of gels for imaging under the microscope. The third group of gels, those with protein that were incubated overnight, appear in the middle section of the graph and have a clear decreasing trend in their fluorescence pattern. The data indicates that the fluorescently labeled streptavidin is thus diffusing out of the gel. Of note, however, is the close similarity between the pure streptavidin sample and the APN-streptavidin sample. No statistical difference can be determined between the two samples, indicating that the molecules diffuse out of the same rate regardless of conjugation to an acrylate group. However, based on the results from the HPLC analysis only 35% of the sample contains APN, the remainder being unconjugated streptavidin. This suggests the need for purification of the reacted samples in order to eliminate the potential effects of the unconjugated protein and thus differentiate the two samples. Additionally, the viability of the acrylate groups has been

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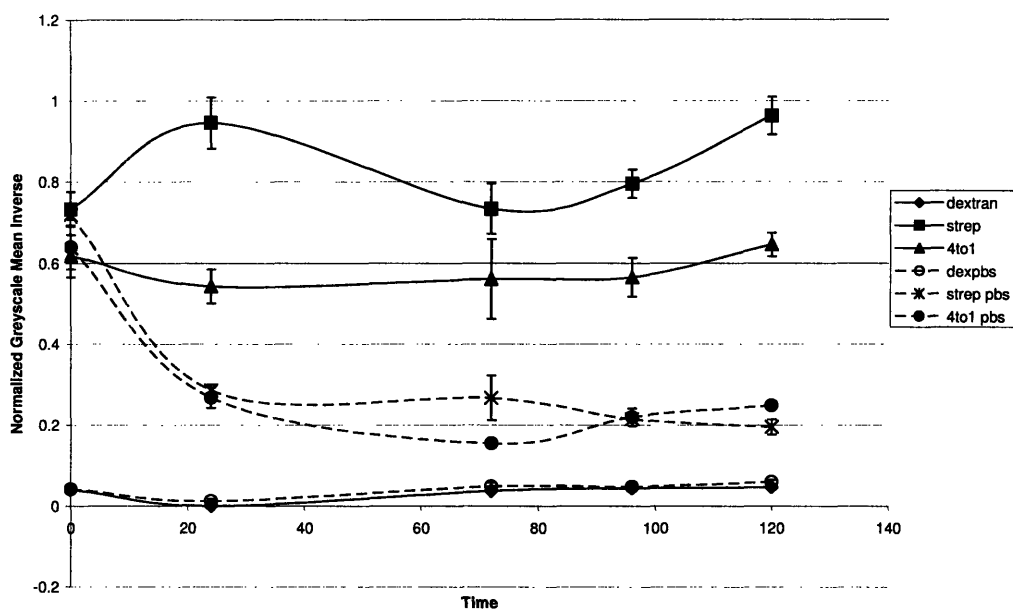called into question for further investigation.

Figure 13: Overnight timecourse data for dextran acrylate gels. Solid lines indicate gels which were incubated overnight in PBS while dashed lines indicate dry controls.

4. Conclusions

Streptavidin was successfully conjugated to APN at various molar ratios to form acryl-PEG-SA. Conjugation was confirmed using HPLC and mass spectrometry, both of which techniques indicated multiple bindings and dissociation of the tetrameric structure of the protein. Protein was subsequently encapsulated into poly(ethylene glycol) diacrylate and dextran acrylate gels and photocrosslinked to create specific binding between acryl groups on the acryl-PEG-SA and the polymers. Gels were characterized using fluorescent microscopy. Overnight incubations indicated little to no diffusion in PEG gels over the course of 7 days and complete diffusion in dextran acrylate gels. Photobleaching was found to occur significantly in PEG gels, most likely due to the long crosslinking times.

Additional work will include further studies into dextran based materials, including other percentages of acrylation, to determine the effects on diffusion through the gels. Additionally, dextran derivatives such as hydroxyethyl methacrylated dextran also provide alternatives to regulate the diffusion of proteins through the gel as they have also proved amenable to adjustability.[14]

Furthermore, the primary crux of the work thus far has been in verifying the viability of streptavidin conjugated hydrogels. The effect of the experiments on the biotin binding affinity has yet to be determined. Previous studies with avidin, the vertebrate analog of streptavidin, have shown that pegylation yields little or no change in protein conformation unless multiply conjugated.]15,16] Future experiments are planned to

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confirm that the binding of acryloyl-PEG, when kept to a minimum, has little effect on the biotin binding affinity of streptavidin.

5. References

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