Polyethylene glycol applied to latex urinary catheters

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Abstract

Urinary catheterization occurs widespread in the United States with one quarter of all hospital patients experiencing it. Catheter problems include blockage, leakage, and infections and are predominately caused by proteins that adhere to the catheter surface and quickly build up on each other forming a protein layer. Current strategies to avoid these problems include coating a catheter with silver alloy to reduce bacteria on the catheter surface. An alternative solution is presented involving coating latex, a common urinary catheter material, with a microlayer (5-100 microns) of polyethylene glycol. This hydrogel is applied using an interfacial photopolymerizatoin process with ethyl eosin as the photoinitiator. A 25 PPM concentration of ethyl eosin provided the strongest gel to surface adhesion and significantly lowered protein adhesion when compared to an uncoated latex substrate.

Keywords: Hydrogel; Polyethylene glycol; Catheter; Urinary tract infection; Interfacial photopolymerization; Latex; Ethyl eosin

1. Introduction

A urinary catheter is a medical device inserted into the body and used to collect urine from the bladder. Typically a urinary catheter is a tube made of a soft, flexible material such as silicon, latex, or Teflon. This tube removes the urine from an individual and is used under a variety of conditions. Catheters are most often used to help with urinary incontinence and retention [1]. Urinary catheterization occurs widespread in the United States with one quarter of all hospital patients experiencing it [2].

Currently, patients using catheters for multiple days often encounter problems involving catheter blockage, leakage, and infection. These problems are a direct result of protein and bacteria adhering to the surface of the catheter. Once on the catheter surface, the bacteria can cause infection and the protein crystallizes to form obstructions. The obstructions cause discomfort, and usually result in an ineffective catheter which needs replacement [3].

The problems of long term catheter use are common and affect a large amount of users, cause extra time and effort for hospitals, and require the frequent replacing of catheters. Blockage is prevalent in half of all long term catheter users [3]. Patients using obstructed catheters can experience pain and trauma which require extra attention in a medical setting. Every time a catheter leaks, becomes blocked, or causes an infection it has to be removed, discarded, and replaced. By limiting these problems, fewer catheters need to be purchased and money can be saved by both home users and medical facilities. The healthcare industry spends an extra 1.8 billion dollars a year due to urinary tract infections that are directly related to catheter use [4]. As many as 62,000 people die from catheter related urinary tract infections each year, costing another 6.25 billion dollars [4]. The catheter that can solve these issues has the potential to help patients and reduce healthcare costs.

The aforementioned problems of leakage, blockage, and infection are caused by proteins that adhere to the catheter surface and quickly build up on each other forming a protein layer called a biofilm [5]. Bacteria from the urine and surrounding tissue can then easily colonize on

the biofilm along with other particles and microorganisms that exist in the urinary tract. As the layers build up they can crystallize, providing the major source of blockage and leakage. A non-magnified view of a catheter that has become encrusted by a crystallized biofilm can be seen in Figure 1 [3].

Fig. 1. A catheter that has undergone encrustation.

Once an initial layer of abundant adsorbed proteins forms on an implant the Vroman Effect follows. This phenomenon explains that subsequent layers of proteins replace other proteins while at the same time increasing the layers. This is an affinity and concentration dependent process in which the protein present in the largest amount has a greater affinity to adsorb than proteins with smaller concentrations [5]. The different proteins that adsorb onto implantable devices vary based on what proteins and phagocytes are present, along with the properties of the implantable device. Texture, charge, and material composition all contribute to which molecules adsorb and influences the interactions between the subsequent protein and bacteria that builds up on a surface [6]. Once the bacteria start to multiply, preventative measures, such as removing the catheter or washing out the area, are necessary to prevent infections. Figure 2 shows an example of a urinary catheter under a high powered microscope with common minerals struvite and calcium phosphate crystallized on its surface [3].

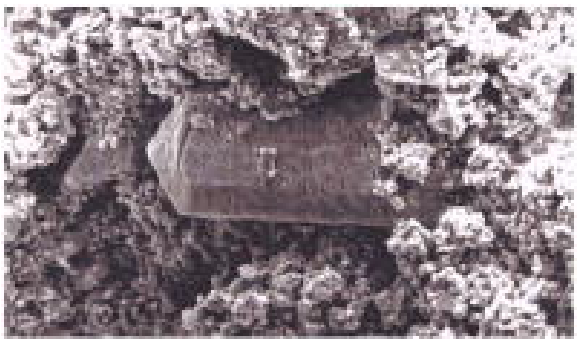

Fig. 2. Electron micrograph of calcium phosphate and struvite crystallized on the surface of a catheter.

Because of the widespread problems associated with catheter use, several procedures and products have been created in an attempt to limit the negative effects. Doctors try to identify patients who are prone to catheter problems and develop a strategy of avoidance [3]. Catheter

maintenance is often performed by cleaning the catheter with a wash out fluid [3]. This is done when frequent recatheterization is causing discomfort or getting too expensive, but the acidic fluid can irritate the lining of the bladder [3]. Physical modification of urinary catheters has also taken place in the form of a silver coating. Silver-coated urinary catheters showed a 57% percent decrease in urinary tract infections over non coated catheters [2]. However, silver alloy coatings can lead to increased silver resistance for bacteria. Because silver is already used as an antibacterial agent in many places in a hospital, it is even more possible that resistance can develop [2]. Coating catheters with a microlayer of PEG hydrogel could reduce catheter problems without irritating the patient, or requiring the high costs of silver coatings.

The problems of obstruction, leakage, discomfort, infection, and replacement can be reduced by limiting the protein that adsorbs to the surface of a catheter. Doing this will decrease the probability of a biofilm and protein crystallization. Removing this bacteria prone environment could decrease infections. Additionally, if an anti-bacterial agent can be introduced into the environment, urinary tract infections due to catheters may be decreased dramatically [7]. Polyethlyene glycol is an excellent medium to deliver antibacterial medication while also reducing protein adhesion to the catheter surface [8].

Herein, a unique process for creating and forming a microlayer of polyethylene glycol on the surface of latex is described. An interfacial polymerization process was utilized with a photoinitiator, ethyl eosin. The thickness, adhesion, and protein adsorption characteristics of this hydrogel are explored. Thickness was determined by optical microscopy and polystyrene beads. Adhesion to the latex surface was determined subjectively on a one to four scale. Finally, protein adsorption properties were measured using ultraviolet spectroscopy after samples had been exposed to bovine albumin solution and washed with a detergent to remove adhered proteins from the latex surface.

2. Materials and Methods

2.1 Hydrogel coating creation

Hydrogel coatings were created using a solution of PEG macromer. A 10x buffer solution was used to make the macromer solution. The 10x buffer solution was made by dissolving 5.35g of triethanolamine (TEOA) and 5.1g of potassium phosphate into 50ml of distilled water. This solution's pH was then altered using 2N hydrochloric acid to create a pH of 7.35.

The composition of the final PEG macromer including the following reagents to create a 5 mL solution of macromer: 3.35KL5A2 1.500 (g), distilled water 2.970 (g), 10X buffer 0.5 (mL), vinylcaprolactam 0.025 (g), fructose 0.005 (g), Fe-sulfate 0.00025 (g). This is summarized below in Table 1.

Table 1
Reagents and amounts used in PEG macromer solution.

Materials (5 mL Batch)	Weight (g)
3.35KL5A2	1.500
Distilled Water	2.970
10X Buffer	0.50 (mL)

Vinylcaprolactam	0.025
Fructose	0.005
Fe-Sulfate	0.00025

Commonly this formula was increased 4-fold; this made the Fe-Sulfate easier to weigh accurately and also increased the solution yield so that more experiments could be administered.

To test the effects of ethyl eosin on the experimental parameters, different concentrations of ethyl eosin solution were created. This was done by initially making a 200 ppm solution of ethyl eosin using acetone as the solvent. Dilutions of this solution were then made in the following concentrations: 100 ppm, 75 ppm, 50 ppm, 25 ppm, 10 ppm, and 5 ppm.

0.5 in. x 0.5 in. latex samples obtained from readily available latex gloves, were washed in acetone solution to remove any unwanted powders, moisturizers, or manufacturing agents. These samples were then soaked in the desired ethyl eosin solutions for 15 minutes. Upon removal from the ethyl eosin the samples were washed in acetone solution to remove any ethyl eosin that may not have directly adhered to the latex.

After the rinsing stage, each of the stained specimens were separately coated with a hydrogel. To do this each sample was placed into the macromer solution, where a xenon light source of 514 nm was applied for four 40 second cycles. After the light was administered, the samples were removed from the macromer solution and placed into a cell culture well filled with PBS to equilibrate before viewing and testing.

2.2 Thickness characterization

Thickness characterization was developed using a standard inverted optical microscope. PEG coated latex specimens were examined while in PBS solution kept at room temperature with a pH of 7.4. The coated sample can not be efficiently viewed under a standard optical microscope due to the width of the sample which results in substrate folding. Samples were cut with a razor blade into strips 2-4 mm thick which allowed for the sample to be viewed easily. Specimens were placed into PBS solution on side to allow for thickness to be measured. Six micron polystyrene beads were used to standardize the measurements. The widths of the beads were verified under a powerful and accurate microscope as shown in Figure 3.

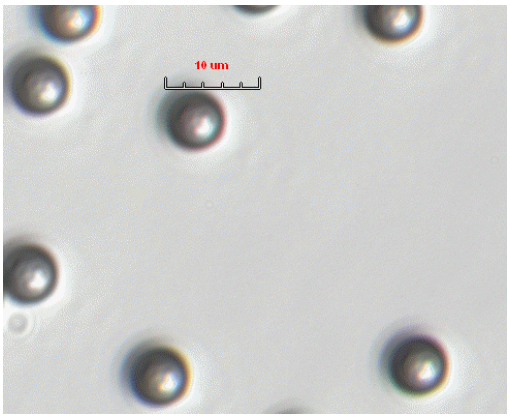

Fig. 3. Polystyrene beads with generated scale to verify diameter of six microns.

2.3 Adhesion characterization

Adhesion was subjectively characterized by scraping the gel off of the latex substrates. Varying ethyl eosin solutions were used to prepare the coatings. The samples were each given a score based on the following scale:

- 0 = Has fallen off
- 1 = Lifts off almost intact with mild force
- 2 = Lifts off in large chunks with some force
- 3 = Lifts off in small pieces with some force
- 4 = Does not delaminate even by destroying gel with pushing force

The same person tested the adhesion of the latex substrate and awarded the 0-4 score to ensure consistency throughout all trials.

2.4 Protein adherence characterization

Each of the 0.5 in. x 0.5 in. coated and uncoated latex substrates were submersed in 2 ml of 1% bovine albumin solution. The bovine albumin solution was used to imitate a physiological environment and to model for other substances that will be encountered by the end product when inserted into a patient. After incubating at room temperature for 24 hours, each substrate was placed in a 2 ml PBS bath. The substrates underwent 3 PBS baths, each lasting 24 hours. The PBS baths are used to allow all proteins that are not adhered to the substrate surface to diffuse into solution. Proteins may be absorbed into the hydrogels, but by utilizing multiple PBS baths only adhered proteins will be present on the substrates. Then, to remove the adhered proteins, the substrates were placed in microvials containing 1 ml of 1% sodium dodecyl sulfate (SDS) detergent solution and rotated for 24 hours. Along with the microvials containing proteins, blank SDS solutions were prepared and rotated for 24 hours in the same manner as the substrates of interest.

After the PBS baths and the substrates have rotated in SDS solution the absorbance assay was performed. The amount of protein adhered to each substrates surface was characterized using a UV absorbance assay. A Beckman DU 530 Life

Science UV/Vis Spectrophotometer was used to measure absorbance at 280 nm. Before measuring the absorbance of the protein present in the SDS solution, we blanked the spectrophotometer less than 30 seconds prior to measuring a sample of interest. The drift in the machine is such that it is necessary to blank immediately before taking each measurement. Also, when pipetting the SDS solution into a cuvette caution was taken not to extract any hydrogel. This can be a difficult task when dealing with a 200 ppm ethyl eosin hydrogel because the amount of hydrogel on the substrate is excessive.

3. Results and Discussion

3.1 Thickness characterization

Samples created with 10 ppm ethyl eosin solution or less showed no signs of hydrogel coating. This is due to the lack of photoinitiator adherence on the surface of the latex, causing no reaction when the light initiation sequence is applied.

Table 2 shows PEG coatings created on the latex substrate using 25-200 ppm ethyl eosin solution showing that thickness is dependent upon concentration.

Table 2
Thickness of PEG layer dependent on ethyl eosin concentration.

Ethyl Eosin Concentration (ppm)	**Thickness (um)**
5	0
10	0
25	5.33
50	12
75	22
200	31

As shown, increasing concentration increased thickness of PEG coating on latex. Although difficult to characterize analytically, a desirable coating is one which is homogenous in thickness across the sample. Samples created with 25-50 ppm ethyl eosin solution showed very uniform thicknesses across the entire sample. Coatings created above a concentration of 50 ppm ethyl eosin contained areas of heterogeneous thickness. When this occurred, a general measurement was taken in what was believed to be the average thickness across the entire sample and not the thickest portion of the coating. This heterogeneous coating will be further discussed after adhesion characterization (3.2).

3.2 Adhesion characterization

Due to the lack of PEG coating created with ethyl eosin solution concentrations 10 ppm and under, there is no adhesion data for these samples. Coatings created using 25-75 ppm ethyl eosin solutions created a PEG coating with high adherence for the latex substrate, averaging a 3 on the adherence scale used for our experiment. This adherence is the strongest achieved, though there is room for adherence improvement. Above 75

ppm ethyl eosin samples showed a deterioration in adhesion characteristics. We believe that the decrease in adhesion strength is due to sheer force created by the hydrogel's weight.

As mentioned in section 3.1, non-uniform coating thickness is undesirable. The pockets created by thin coatings adjacent to thick portions of the coating can easily be infected with bacteria. This could lead to the infection that the PEG coating is ultimately trying to avoid. For this reason, coatings with this undesirable characteristic are generally less useful in vivo. Additionally, samples with a heterogeneous thickness were generally less adhered to the latex substrate, making them even more undesirable for in vivo use. Coatings created using 75 ppm ethyl eosin and above showed heterogeneous thickness along with poor adherence. Satisfactory adherence was obtained with the coatings created using 25 and 50 ppm ethyl eosin solutions and results are displayed in Table 3.

Table 3
Adherence and characterization of PEG coatings.

Ethyl Eosin Concentration (ppm)	**Adherance**	**Uniform**
5	N/A*	N/A*
10	N/A*	N/A*
25	3	Yes
50	3	Yes
75	2.66	No
200	2	No

*N/A is used to denote conditions that did not produce coatings.

3.3 Protein adherence characterization

When preparing substrates with hydrogel coatings to test for protein adherence it is important to visually inspect each gel to ensure that the entire substrate is covered in a uniform coating. This can be done by comparing the coated substrate to an uncoated substrate. After the light treatments it is also important that the substrate be completely covered in PBS while equilibrating. This can be accomplished by using a tweezers to push the substrate to the bottom of the polystyrene well. These are two steps that can greatly improve the consistency of the hydrogel coatings. If this is not done, the latex substrates may float to the top of the PBS solution resulting in a portion of the coating not swelling.

Once the hydrogel coated substrates have swelled in the PBS solution they are then transferred to the albumin solution. At this time one should observe the uncoated control substrates. Diffusion of a substance from the uncoated latex control substrate was observed in over half of the uncoated samples. In the future an uncoated substrate should undergo a protein assay prior to placing it in a PBS bath. The researcher could then characterize the diffusing substance, which is believed to be loosely adhered protein, with greater certainty.

The 25 ppm ethyl eosin stain resulted in a 6 micron thick hydrogel with an adhesion score of 3. Using visual inspection, this hydrogel was the most uniform. The differences between amounts of protein per unit area for each set of ethyl eosin concentrations were compared using nonpaired t-tests at a $p < 0.05$. When compared to the uncoated control substrate, the 25 ppm ethyl eosin stain had significantly less protein adhered to its surface, 0.00087 mg/in^2 versus the uncoated substrate at 0.0014 mg/in^2.

In contrast to the 25 ppm ethyl eosin substrate, all other coated substrates did not have significantly less protein adhered to surfaces than the uncoated latex substrate. Also, the 200 ppm ethyl eosin substrate had significantly more protein than the uncoated latex. The absorbance reading of the 200 ppm substrate though may be inaccurate due to the large amounts of hydrogel present in the SDS detergent solution. Figure 4 shows the amount of albumin measured using the UV assay for five different ethyl eosin concentration. It also includes the average albumin amounts for uncoated samples and 25 ppm ethyl eosin coated samples that were not exposed to albumin.

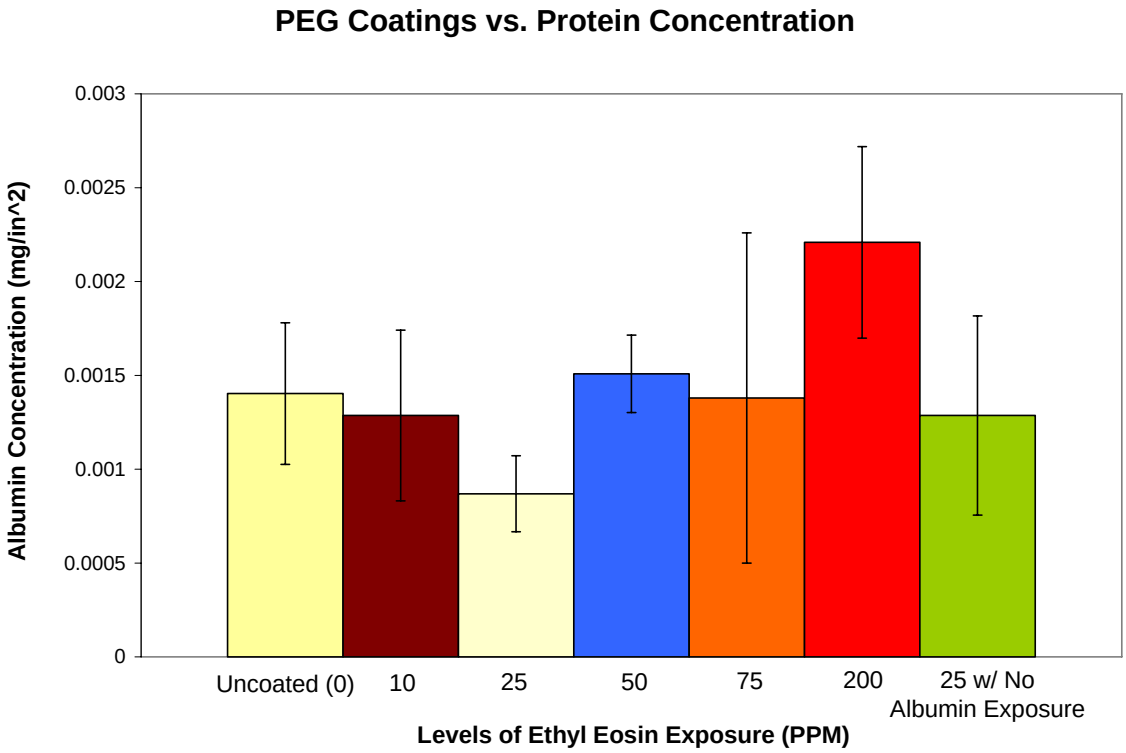

Fig. 4. Amount of albumin adhered to latex surface under varying ethyl eosin concentrations.

When comparing the 25 ppm ethyl eosin substrates to the 10, 50, 75, and 200 ppm substrates only two had a significant difference. Both the 50 and 200 ppm substrates had significantly more protein adhered to its surface than the 25 ppm substrate. The 10 ppm and 75 ppm substrates did not show a significant difference than the 25 ppm substrates. Furthermore, 25 ppm substrates that were never placed in bovine albumin solution did not show significantly less protein than the 25 ppm substrates that were submerged in the bovine albumin solution for 24 hours. From these results we hypothesize that there may be something other than protein absorbing at 280 nanometers. Something could be

leaching out of the latex or hydrogel which is corrupting our results. Thus, although the 25 ppm substrates showed significantly less protein absorption than the uncoated latex, a different protein assay should be pursued to better characterize the amount of protein adhered to the substrate surface.

4. Conclusion

In summary, we have optimized a unique process for applying a microlayer of PEG hydrogel to latex. The hydrogel coatings produced an optimal thickness and adhesion when using 25 ppm ethyl eosin as the photoinitiator. The 25 ppm ethyl eosin stain produced a uniform 6 micron hydrogel coating with the strongest adherence and significantly reduced protein adhesion compared to uncoated latex surfaces. Further testing and optimization of this process to coat urinary catheters could significantly reduce urinary tract infections. The application of PEG hydrogel to the surface of catheters could also be used to release antibiotics to further decrease infections.

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References

[1] Knowles, D. (2005, October 20). *Urinary catheters*. Retrieved April 8, 2007, from University of Maryland, Medical Center Web site: http://www.umm.edu/ency/article/003981.htm

[2] Rupp, M., Fitzgerald, T., Marion, N., Helget, V., Puumala, S., Anderson, J., & Fey, P. (2004, December). Effect of silver-coated urinary catheters: Efficacy, cost-effectiveness, and antimicrobial resistance [Electronic Version]. *American Journal of Infection Control*, 32(8), 445-450. Retrieved March 11, 2007, from ScienceDirect database.

[3] Getliffe, K. (2003, May). Managing recurrent urinary catheter blockage: Problems, promises, and practicalities [Electronic Version]. *The Journal of Wound, Ostomy, and Continence Nursing*, 30(3), 146-151. Retrieved March 11, 2007, from Ovid database.

[4] *Tech 25* (2004, November 14). Retrieved March 18, 2007, from http://www.techjournalsouth.com/news/article.html?item_id=747

[5] Kao, W. (2006). Biomedical engineering 430: Biological interactions with materials. Lecture presented at the University of Wisconsin-Madison, Madison, WI.

[6] Labarre, D. (2001). Improving blood-compatibility of polymeric surfaces [Electronic Version]. *Trends in Biomaterials and Artificial Organs*, 15(1), 1-3. Retrieved April 8, 2007, from http://medind.nic.in/taa/t01/i1/taat01i1p1.pdf

[7] Saint, S., Elmore, J., Sullivan, S., Emerson, S., & Koepsell, T. (1999, April). The efficacy of silver alloy-coated urinary catheters in preventing urinary tract infection: A meta-analysis [Electronic Version]. *The Journal of Urology*, 161(4), 1422. Retrieved April 8, 2007, from ScienceDirect database.

[8] DiTizio, V., Ferguson, G., Mittelman, M., Khoury, A., Bruce, A., & DiCosmo, F. (1998, March). A liposomal hydrogel for the prevention of bacterial adhesion to catheters [Electronic Version]. *Biomaterials*, 19, 1877-1884. Retrieved March 14, 2007, from ScienceDirect database.