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Effects of Crosslinking Conditions on Biofouling of Polymer Substrates with a Hydrogel Layer

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Honors Research Project

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Executive summary

Hydrogel polymers offer different advantages because they are soft and lubricious. However, these materials are usually not sturdy enough to be used for different applications. One of the advantages of some hydrogels is the prevention of fouling. Fouling is the accumulation of an unwanted, materials on a surface. This project consists in adding a hydrogel skin (thin layer) to the surface of other plastic materials. Specifically, it will be focused on the addition of a hydrogel layer to polystyrene. Polystyrene was chosen because of its great applications in the research field and also because it being a transparent surface it will allows for better imaging of bacteria or cell attachment.

A simple method previously reported (1) was used to crosslink the hydrogel, in this case polyacrylamide, into the polystyrene. The crosslinking process started with a hydrophobic initiator, which for this project benzophenone, was absorbed in the polystyrene. This initiator is what will allow the hydrogel and substrate to crosslink. Then the sample is introduced to the hydrogel monomer with hydrophilic initiator which will allow the monomer to polymerize. After curing it in an UV chamber, the substrate was rinsed to remove the bulk of the hydrogel. To measure and make sure that the surface was in fact hydrophilic, and the hydrogel crosslinking was successful, the contact angle was measured. Additionally, since the purpose of this project was to obtain an antifouling surface cell attachment was used as a way to prove that the antifouling properties were successful.

Some samples made with silicone rubber brand Ecoflex were made to have a better understanding of the process described in the reference article (1). After having a good sample of this material, the challenge was to achieve the same with polystyrene. .

The polystyrene was demonstrated to have some issues with the hydrogel crosslinking. The surface of the coated samples was not transparent. This brought some questions. It is known that polystyrene is not as porous as silicone rubber, therefore different conditions were tried to have a better understanding on whether the absorption of the hydrophobic initiator was causing this additionally to an inconsistency with the surface. There are two parameters that affect the absorption of a liquid into a solid, time of the sample soaked and temperature at which it is soaked. Therefore, these two variables were changed. The temperatures evaluated were 23, 50 and 85°C and the soaking times were 5 mins, 1hr, 24hr and 48 hr.

The best conditions were determined to be 50°C and 1hr. 50°C is higher than room temperature but much lower than the glass transition temperature of polystyrene (100°C) therefore this is why it was probably the best temperature. At longer time even though the samples were covered there is a possibility that the solutions could have evaporated therefore, that is probably why the 1 hr soak time works best. Overall, the 1 hr at 23°C sample was determined to be the best condition. This was determined by the lowest contact angle and transparency of the samples.

After the best condition was chosen which was the one soaked for one hour, the samples were tested for fouling by testing the cell attachment to the surface, Bovine Aorta Endothelial Cells were grown, and images were taken after a week under a microscope. The samples with the hydrogel coating showed significantly less cells in the imaging. This reduction in cell attachment proves that the coating was successful in preventing fouling of this type. Further fouling tests can be done to observe success in other types of fouling.

Introduction

The introduction of a hydrogel coating to a surface has been looked at for different benefits. Certain hydrogels offer antifouling properties that are beneficial to different applications like medical components. Previous attempts (2) (3)have been done to introduce a hydrogel surface to plastic substrates. One of them is to use grafting brushes which works properly but it is not sufficient since the layer is too thin and therefore can be damaged. The second method have a bulkier coating without crosslinking. Opposite to grafting brushes this method has too thick of a layer. Additionally, there is no crosslink therefore the coating is not very strong. A new method was developed and described in an article recently published in 2018 to add a layer of hydrogel coating to different polymers. It introduces different initiators to allow the crosslink of the hydrogel monomer and the polymer surface. They used different substrates and monomer combinations. However, one of the materials that were not used was polystyrene. Polystyrene has a good potential use of a hydrogel coating since it is used in a lot of applications such as in petri dishes, test tubes, and other medical devices. The goal was to obtain a good sample so that the lack of bacteria growth was able to be shown in the microscope. The transparency of this material would allow for observation.

The objective of this project is therefore to achieve a good hydrogel coating on a polystyrene surface. The idea is to provide a surface that has antifouling properties which will be proved using cell attachment tests.

Background

In the article "Multifunctional "Hydrogel Skins" on Diverse Polymers with Arbitrary Shapes" the authors develop a way to crosslink a thin, yet strong, monomer layer into a polymer substrate. (1) Other works (2) (3) in attempting to add hydrogel layers have done different methods and while they worked for their described purpose, it was not universal to different materials and applications. Additionally, they have their disadvantages of performance. Grafted hydrogels are too thin and not resistant to abrasion. Coatings are too thick and do not adapt to the shape of what you are trying to coat. The method described is very easy to apply with different materials and therefore it is appealing to try because of its simplicity.

Hydrogel polymers offer different advantages because they are soft and lubricious. Additionally, their main advantage is that of preventing fouling of different surfaces. Hydrophobic materials allow all bacteria to attach more strongly, especially when the bacteria are hydrophobic. Therefore, in certain cases it has been shown that a surface that is hydrophilic has antifouling properties (4). Hydrophobic cell fouling is the type of fouling that this paper is going to focus on. To know if the surfaces that were tested were hydrophilic, the contact angle of the surface was measured. The angle formed by the boundary where liquid, gas and solid intersect is called contact angle. This intersection is defined by the Young equation. When the contact angle is low it signifies that the liquid spreads in the solid. On the contrary, a high contact angle shows that the liquid does not spread. Zero contact angle indicates complete wetting and if the angle is greater than 90° the surface is not being wetted by the liquid. (5) In this case water is being used to measure the hydrophilicity of the surface since hydrogels, as its name says it, are very hydrophilic and therefore absorb water.

Polystyrene (PS) is a clear, amorphous, nonpolar commodity thermoplastic that is easy to process. It has excellent optical clarity due to the lack of crystallinity. It is not very porous, and it is very brittle. Polystyrene has a glass transition temperature of 100°C and a Melting temperature of 210-249 °C which is not very high compared to other polymers (6). Because polystyrene is not very porous and is easily dissolved in most organic solvents the absorption of the hydrophobic initiator was a hard task in the coating procedure.

Experimental Method

Materials:

Silicone Rubber substrate: Ecoflex 30 obtained by Reynolds Advanced Materials. Polyacrylamide monomer, Benzophenone and Irgacure 2959 obtained by Sigma Aldridge

Silicone Rubber Preparation

The first material that was tested to understand the method of adding a hydrogel skin was silicone rubber. The brand used was Ecoflex grade 30. The pack comes with two solutions that have to be mixed in a 1:1 ratio. 10 g of each liquid were measured in a scale on separate beakers and then A was poured into B to be mixed. The solution was degassed using a vacuum pump and a simple degassing chamber for 3 minutes to remove any bubbles formed when mixed. Then, the solution was poured in a Teflon mold that was laser cut to obtain small circular samples with a diameter of about 0.5 cm. The silicone rubber was allowed to cure for 4 hours at room temperature. Afterwards a post cure was done at 80°C in an oven for 2 hours.

Hydrogel crosslinking

The method involves two initiators, one hydrophobic and absorbed by the polymer substrate(i.e. silicone rubber, polystyrene) , and the other hydrophilic and added to the hydrogel monomer. First the solutions were made with 10% by weight of Benzophenone (BP) in organic solvent (acetone, isopropanol, or ethanol). The BP was measured in a scale and then the acetone was added using a pipette. Then the monomer solution was prepared with 10% acrylamide and 1% I-2959, then filled up with water using a pipette. After this the polymer substrate was cleaned using Isopropanol (IPA) and DI water and inserted in a UVO chamber to clean better and rinsed with IPA once again. The substrate was then inserted in the BP solution and was left there for the amount of time required depending on the condition seen in **Table 1** and if needed it was put in the oven. If left for a long period of time the solution needed to be covered very well otherwise the organic solvent would evaporate. After, the sample was removed and immersed in the monomer solution. It was then put in a UV chamber for 55 min. After this the sample was rinsed with water and the excess hydrogel was removed gently. The sample was finally allowed to dry. Three samples were obtained for each condition. The conditions are summarized in **Table 1.**

Table 1. Summary of conditions performed to different polymer substrates to add hydrogel layer. These describe the temperatures and times at which the polymer substrates were soaked in the hydrophobic initiator previous to crosslinking with the hydrogel monomer.

Substrate	**Solvent**	**Temprature**	**Time**
Silicon rubber	Acetone	23°C	5 mins
Polystyrene	Ethanol	50°C	5 mins
		85°C	
		23°C	1h
			24h
			48h
	Ispropanol	50°C	5 mins
		85°C	
		23°C	1h
			24h
			48h

Water absorption test

To test if the samples had in fact a coating, one way of knowing if they absorb water. This is because the plain samples are hydrophobic in nature. To know if the samples absorb water, a solution with 2% of food dye in water was made and the samples were soaked in this solution for 1 minute. The samples would then change color if they absorbed the water.

Contact angle measurement

To measure the contact angle a ramé-hart instrument co.)-model 100-00 goniometer was used. The sample was placed under a water syringe and a small droplet of water was placed on the surface. An amplified image was obtained, and the angle was measured later with Image J. Three measurements were made for each sample.

Cell attachment

The cell growth was performed with a similar method to that of a previously published article (7). Bovine Aorta Endothelial Cells were grown in a humidified incubator with 5% CO_2 at 37°C. The medium solution was made of DMEM with 10 % of fetal bovine serum, 1% sodium pyruvate, 1% nonessential amino acids and 2% penicillin streptomycin. The cell attachment was only performed in the samples that had the lowest contact angles and lowest transparency (i.e. Samples soaked for 1 hour in solvent with initiator before being cured to hydrogel monomer solution). The samples were transferred to individual wells and rinsed with PBS three times. Cells were collected by treating them with trypsin/ ethylenediaminetetraacetic acid (0.05%/0.53 mM) to detach the cells, then they were washed with PBS, and finally diluted in the culture medium to reach a final concentration of 10^5 cells/mL. The medium was changed every three days. Images were taken after a week of cell attachment using an EVOS xl core inverted microscope.

Results and discussion

The first part of this investigation involved proving that the method developed in the reference article was successful. The method involves the crosslinking in two steps. First, the hydrophobic initiator is absorbed by the polymer substrate. Then, the substrate is introduced to a hydrogel monomer solution and cured (with UV or heat). The polymer substrate crosslinks with the hydrogel thanks to the two initiators introduced to the solutions. (1). To prove the method worked with the materials provided, the conditions seen in **Table 1** were applied to the Ecoflex silicone rubber, these conditions were based on the reference paper. As seen in **Figure 1** the sample was soaked in a 2% food coloring solution in water. The sample absorbed the water because it was coated with the hydrogel and therefore it changed color. Additionally, it can be seen in **Table 2** that the contact angle was reduced from 92.48° to 31.35°. This reduction in contact angle further proved that the coating was successful.

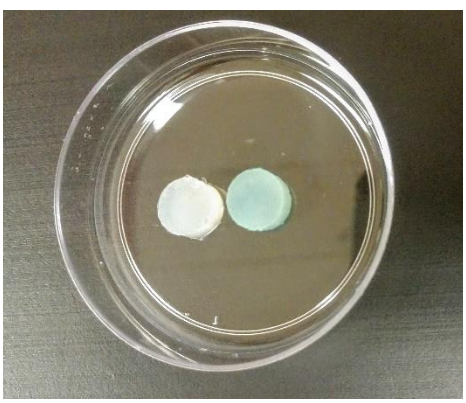

Figure 1. Pristine (left) and hydrogel coated (right) samples of Ecoflex Silicone Rubber(SR)

Table 2. Contact angle averages for Ecoflex silicone rubber.

Sample	Contact Angle Average	Contact Angle Standard dev.
SR coated in hydrogel	31.35°	0.51
SR control (pristine)	92.48°	0.56
SR soaked in hydrophobic initiator	106.34°	0.40

After this, began the study with a new substrate, polystyrene. Polystyrene is a much harder material and with a lower porosity. Therefore, it was a lot harder to achieve a good coating. One important change that had to be made with polystyrene was the solvent used to dissolve the hydrophobic initiator. Acetone could not be used since it dissolves the polystyrene. Therefore, two other solvents were tried: ethanol and isopropanol. Another issue that was encountered was that a transparent surface was preferred to allow the observation of cell attachment and with the first set of conditions (i.e. 5 mins at room temperature) this was not achieved. Therefore, a condition with the best contact angle and with the most transparency was the objective of this experiment. The hypothesis was that the polystyrene needed to absorb the hydrophobic initiator (BP) better. Consequently, by increasing the absorption time or the temperature at which the sample was soaked then it would have better absorption.

Three different temperatures were chosen to test if a higher temperature would allow the crosslinking to perform better. **Figure 2** shows the comparison of contact angle with changes in soaking temperature while keeping the time at 5 minutes. The best condition for changes in temperature for both Ethanol and IPA was the one at 50°C. This is probably because the glass transition temperature of polystyrene is around 100°C and therefore getting too close to that would not allow the polymer to absorb as well as at a medium temperature. Nevertheless, the change is not very significant, especially for ethanol that the contact angle has a big standard deviation. Three different samples were prepared for each temperature and three different sections of the sample were measured. Some samples or sections would have larger contact angles which suggests that they did not get coated evenly. One reason for this could be because when the samples were set to cure, they would float in the monomer solution or touch the bottom of the container where it was cured.

Four soaking times in hydrophobic initiator were chosen to compare how it would affect the crosslinking. **Figure 3** shows the comparison of contact angle with changes in soaking time at room temperature. The best condition was found to be 1 hour. **Figure 4** shows some examples of contact angle pictures taken for a control sample without any coating, and two with hydrogel coating prepared by soaking in hydrophobic solution with isopropanol and ethanol, respectively. The control sample had an average contact angle of 76.25° which compared to the other two (31.39° and 22.75°) it has a much higher value. This higher value means that the control sample is more hydrophobic compared to the other samples, thus confirming that they have a hydrogel coating. The increase in contact angle with time as seen in **Figure 4** after the 1 hr mark could be due to evaporation even though good care was taken for the solvent not to evaporate, there was still mild evaporation of the solvent. Even though the contact angles were not significantly different, the surfaces for the samples soaked for 1 hr were usually the most transparent samples. The transparency and contact angle were the determining factors to choose the 1-hour soaking condition to proceed with the cell attachment.

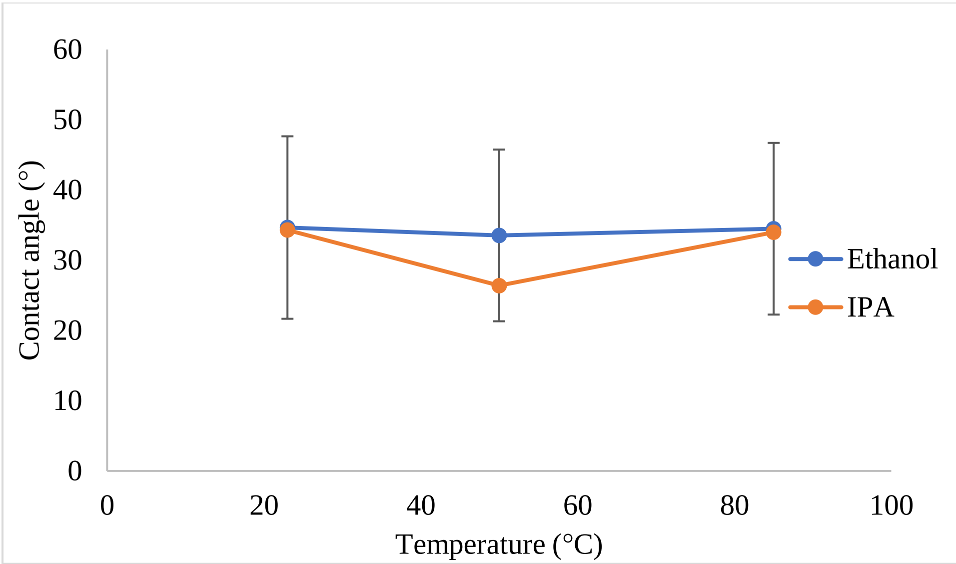

Figure 2. Contact angle average measurements when polystyrene samples were exposed at different temperatures while soaked in hydrophobic initiator (BP) solution in IPA and ethanol for 5 minutes before the hydrogel crosslink

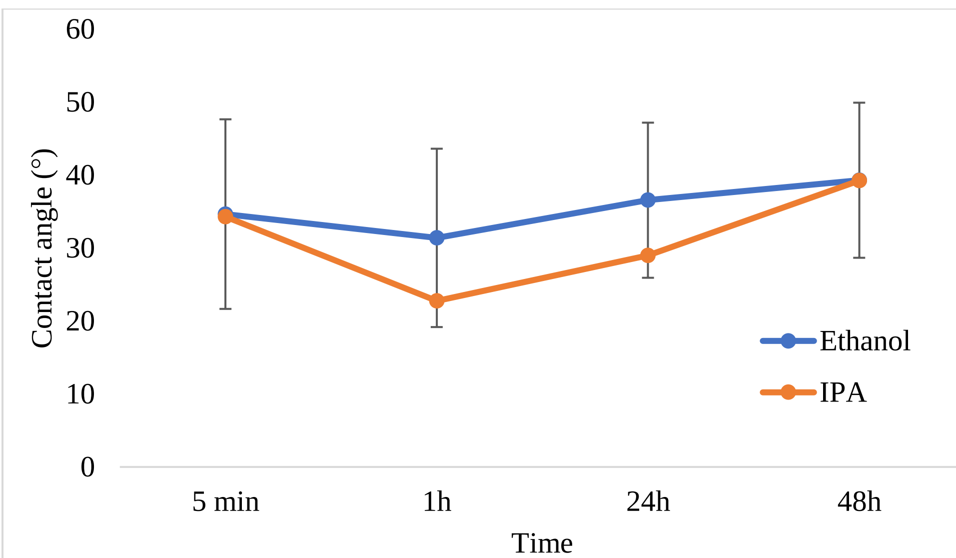

Figure 3. Average contact angle measurements for changes in soaking time of polystyrene in hydrophobic initiator(BP) in IPA and ethanol at room temperature to crosslink with the hydrophilic monomer

Figures 5 shows images of the samples that were used for the cell attachment portion of the research. These were prepared by soaking in hydrophobic initiator for 1 hour at room temperature which as mentioned above was the condition with best results. As it can be seen, the samples are not completely transparent, there are still some inconsistencies with the surface outcomes and more investigation would have to be made about the causing factors of the whitening. However, the cell preparation was still able to be performed. In **Figure 6** cell attachment to the polystyrene samples can be observed. The control sample (pristine) there were plenty of cell conglomerates observed. Some cells can be seen in the isopropanol sample, these however are very sparse and were only observed in one section of the sample. Very few cells can be seen in a small section of the ethanol sample. The images are not perfect, but the samples were imaged before proceeding with the cell growth, confirming that the cells shown in **Figure 6** are in fact cells and were not there before.

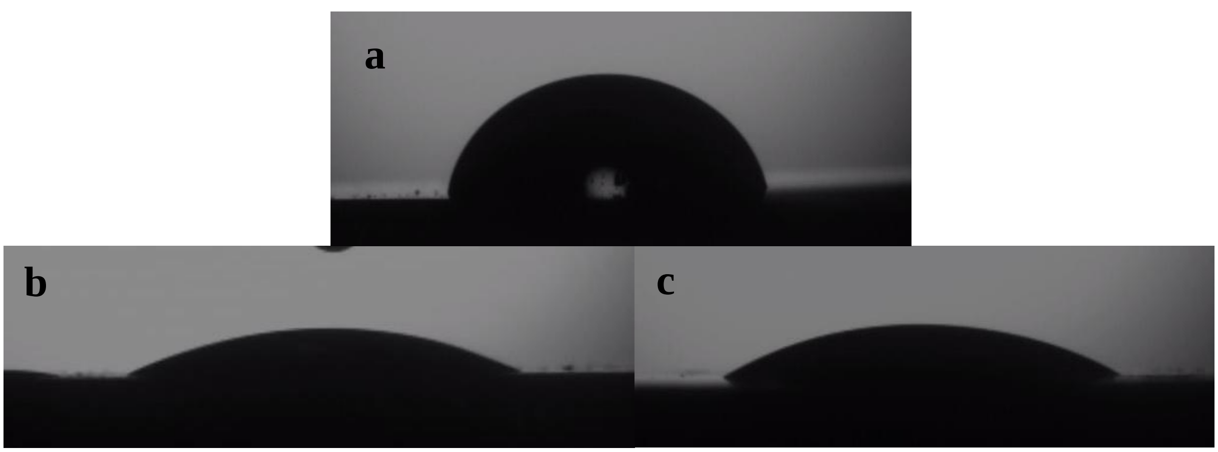

Figure 4. Contact angle sample images of a) pristine polystyrene, b)polystyrene coated with hydrogel using ethanol as solvent, c) using IPA as solvent for hydrophobic initiator

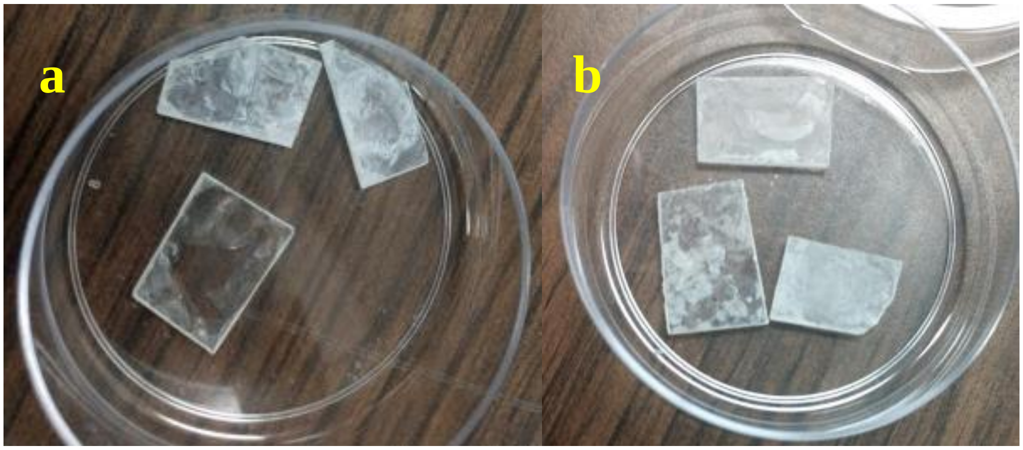

Figure 5. Samples of polystyrene coated with hydrogel by soaking in hydrophobic initiator dissolved in a)IPA and b)ethanol for 1hr

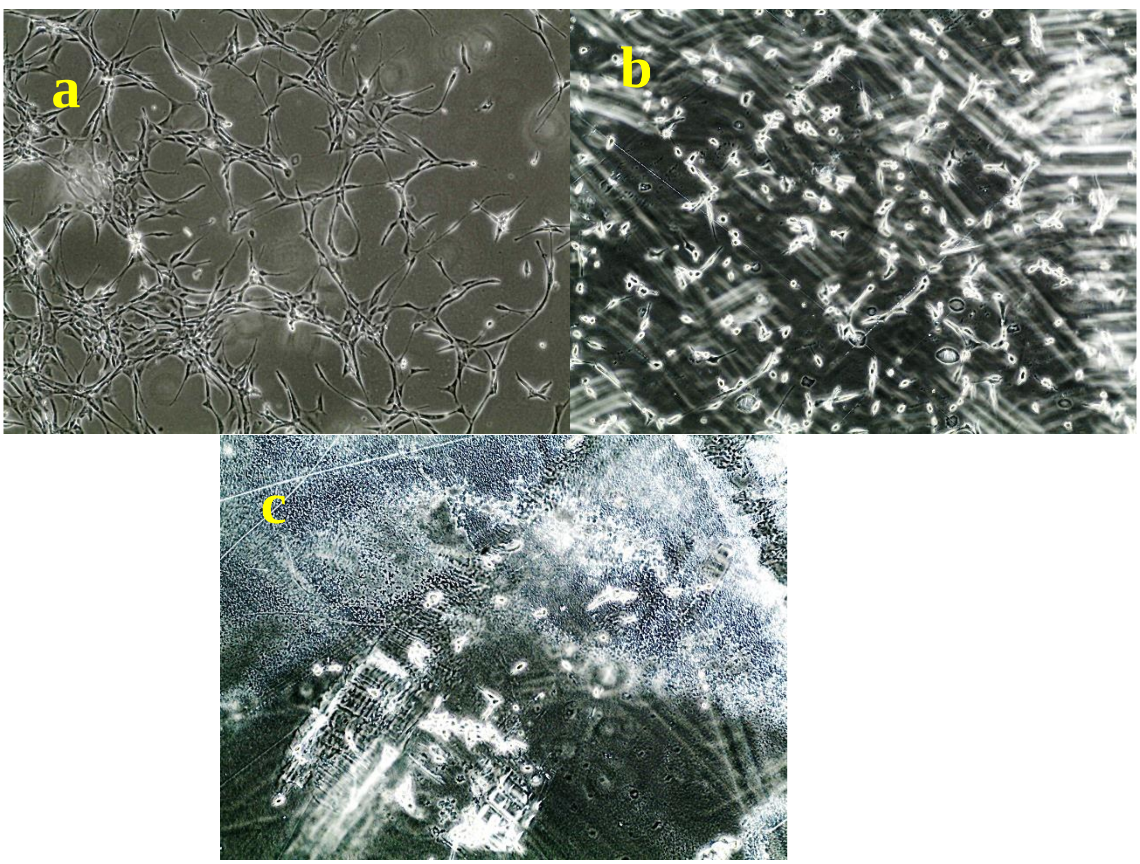

Figure 6. Cell attachment imaging for a) pristine polystyrene, b)polystyrene coated with hydrogel using IPA as solvent, c) using ethanol as solvent for hydrophobic initiator where it was soaked previous to crosslinking to the monomer.

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Appendix A

Table 3. All data for silicone rubber contact angles shown in Table 2

	Sample no.	Outside Angle	Inside Angle	average	stdev	% difference
SR + AAM	1	146.622	33.378			
		147.301	32.699			
		147.995	32.005	32.694	0.687	2.10%
	2	145.923	34.077			
		146.915	33.085			
		145.692	34.308	33.823	0.650	1.92%
	3	152.592	27.408			
		152.571	27.429			
		152.241	27.759	27.532	0.197	0.72%
SR- control	1	86.673	93.327			
		87.004	92.996			
		86.855	93.145	93.156	0.166	0.18%
	2	88.977	91.023			
		87.101	92.899			
		87.101	92.899	92.274	1.083	1.17%
	3	87.51	92.49			
		88.379	91.621			
		88.107	91.893	92.001	0.445	0.48%
SR- soaked in BP hydrophobic initiator	1	76.759	103.241			
		76.159	103.841			
		76.799	103.201	103.428	0.359	0.35%
	2	69.326	110.674			
		68.991	111.009			
		69.677	110.323	110.669	0.343	0.31%
	3	74.745	105.255			
		74.814	105.186			
		75.635	104.365	104.935	0.495	0.47%

Table 4. All data for samples soaked in ethanol as a solvent from figures 3 and 5

	Sample 1	Angle	average	stdev	% difference	Sample 2	Angle	average	stdev	% difference	Sample 3	Angle	average	stdev
Petri w/EtOH 5min RT		21.801					28.989					37.304		
		22.954					28.195					36.511		
	1	23.595	22.783	0.909	3.99%	1	30.964	29.383	1.426	4.85%	1	35.248	36.354	1.037
		26.928					33.864					51.34		
		28.072					33.254					51.033		
	2	26.147	27.049	0.968	3.58%	2	33.476	33.531	0.309	0.92%	2	50.29	50.888	0.540
		21.595					29.181					59.859		
		21.595					30.964					61.004		
	3	19.872	21.021	0.995	4.73%	3	31.827	30.657	1.349	4.40%	3	59.826	60.230	0.671
Petri w/EtOH 5min 85°C		30.964					40.236					41.522		
		31.675					39.588					39.719		
	1	30.854	31.164	0.446	1.43%	1	39.806	39.877	0.330	0.83%	1	40.946	40.729	0.921
		34.216					39.123					35.036		
		34.177					37.117					35.595		
	2	34.611	34.335	0.240	0.70%	2	38.118	38.119	1.003	2.63%	2	34.144	34.925	0.732
		29.116					27.022					35.655		
		28.511					27.597					34.472		
	3	29.055	28.894	0.333	1.15%	3	28.106	27.575	0.542	1.97%	3	34.095	34.741	0.814
Petri w/EtOH 5min 50°C		24.567					36.87					39.928		
		25.419					35.159					39.491		
	1	24.814	24.933	0.438	1.76%	1	36.656	36.228	0.932	2.57%	1	40.486	39.968	0.499
		47.07					34.114					31.931		
		46.975					33.986					31.908		
	2	45.644	46.563	0.797	1.71%	2	34.695	34.265	0.378	1.10%	2	32.905	32.248	0.569
		31.218					33.247					23.663		
		32.242					31.477					22.38		
	3	32.44	31.967	0.656	2.05%	3	32.057	32.260	0.902	2.80%	3	23.962	23.335	0.840
Petri w/EtOH 1h RT		23.518					30.606					28.686		
		23.199					30.005					29.01		
	1	23.259	23.325	0.170	0.73%	1	30.399	30.337	0.305	1.01%	1	29.795	29.164	0.570
		22.813					32.057					46.123		
		23.364					32.428					45.317		
	2	23.091	23.089	0.276	1.19%	2	32.067	32.184	0.211	0.66%	2	45.31	45.583	0.467
		31.185					29.197					37.659		
		30.211					30.677					39.657		
	3	30.124	30.507	0.589	1.93%	3	29.134	29.669	0.873	2.94%	3	38.752	38.689	1.000
Petri w/EtOH 24h RT		47.265					40.601					23.839		
		47.793					41.448					22.999		
	1	46.868	47.309	0.464	0.98%	1	41.186	41.078	0.434	1.06%	1	23.595	23.478	0.432
		50.054					36.254					16.46		
		48.972					37.255					16.975		
	2	50.932	49.986	0.982	1.96%	2	38.27	37.260	1.008	2.71%	2	15.762	16.399	0.609
		48.27					37.439					26.816		
		50.618					36.87					26.98		
	3	50.194	49.694	1.251	2.52%	3	36.995	37.101	0.299	0.81%	3	26.333	26.710	0.336
Petri w/EtOH 48h RT		47.643					49.456					24.362		
		47.684					48.18					24.747		
	1	49.467	48.265	1.041	2.16%	1	49.16	48.932	0.668	1.36%	1	23.839	24.316	0.456
		27.663					45.526					31.226		
		28.018					46.606					29.655		
	2	28.811	28.164	0.588	2.09%	2	45.498	45.877	0.632	1.38%	2	29.401	30.094	0.989
		53.344					46.614					28.951		
		53.276					45.796					29.852		
	3	52.595	53.072	0.414	0.78%	3	45.379	45.930	0.628	1.37%	3	28.244	29.016	0.806

Table 5. Data for samples soaked in IPA as a solvent from Figures 2 and 4

	Sample 1	Angle	average	stdev	% difference	Sample 2	Angle	average	stdev	% difference	Sample 3	Angle	average	stdev
Petri w/IPA 5min RT		32.928					36.682					27.451		
		33.154					37.875					25.133		
	1	33.69	33.257	0.391	1.18%	1	36.983	37.180	0.620	1.67%	1	26.565	26.383	1.170
		40.156					38.66					34.129		
		40.914					37.747					33.582		
	2	40.795	40.622	0.408	1.00%	2	38.367	38.258	0.466	1.22%	2	32.619	33.443	0.764
		32.307					33.261					33.536		
		33.14					34.579					33.024		
	3	32.106	32.518	0.548	1.69%	3	34.509	34.116	0.742	2.17%	3	32.142	32.901	0.705
Petri w/IPA 5min 85°c		38.577					39.289					26.822		
		38.66					39.806					28.652		
	1	39.382	38.873	0.443	1.14%	1	40.914	40.003	0.830	2.08%	1	27.734	27.736	0.915
		27.361					38.55					26.952		
		26.288					38.565					27.57		
	2	27.759	27.136	0.761	2.80%	2	39.417	38.844	0.496	1.28%	2	25.796	26.773	0.900
		30.774					42.357					33.389		
		31.185					41.496					34.461		
	3	30.141	30.700	0.526	1.71%	3	42.022	41.958	0.434	1.03%	3	33.977	33.942	0.537
Petri w/IPA 5min 50°c		23.518					30.114					22.62		
		23.121					32.735					22.655		
	1	24.944	23.861	0.959	4.02%	1	32.692	31.847	1.501	4.71%	1	23.136	22.804	0.288
		33.591					24.05					23.875		
		32.388					23.199					25.077		
	2	32.994	32.991	0.602	1.82%	2	24.114	23.788	0.511	2.15%	2	24.444	24.465	0.601
		27.284					24.305					24.158		
		28.811					25.115					24.47		
	3	28.465	28.187	0.801	2.84%	3	25.372	24.931	0.557	2.23%	3	25.364	24.664	0.626
Petri w/IPA 1h RT		12.943					27.216					23.749		
		12.901					26.565					22.306		
	1	13.519	13.121	0.345	2.63%	1	27.284	27.022	0.397	1.47%	1	24.261	23.439	1.014
		16.336					31.759					28.511		
		15.624					30.343					28.179		
	2	16.28	16.080	0.396	2.46%	2	31.065	31.056	0.708	2.28%	2	29.592	28.761	0.739
		17.939					23.015					24.319		
		18.06					23.691					24.011		
	3	17.684	17.894	0.192	1.07%	3	23.962	23.556	0.488	2.07%	3	23.369	23.900	0.485
Petri w/IPA 24RT		22.947					20.653					35.735		
		22.652					22.443					36.773		
	1	22.681	22.760	0.163	0.71%	1	20.442	21.179	1.099	5.19%	1	35.134	35.881	0.829
		37.432					22.874					35.036		
		38.941					23.334					35.47		
	2	37.569	37.981	0.834	2.20%	2	22.27	22.826	0.534	2.34%	2	35.981	35.496	0.473
		24.955					26.783					33.048		
		24.905					27.051					33.851		
	3	23.268	24.376	0.960	3.94%	3	26.075	26.636	0.504	1.89%	3	34.216	33.705	0.598
Petri w/IPA 48h RT		47.643					49.456					31.329		
		47.684					48.18					30.504		
	1	49.467	48.265	1.041	2.16%	1	49.16	48.932	0.668	1.36%	1	30.311	30.715	0.541
		27.663					45.526					22.91		
		28.018					46.606					23.66		
	2	28.811	28.164	0.588	2.09%	2	45.498	45.877	0.632	1.38%	2	23.939	23.503	0.532
		53.344					46.614					27.457		
		53.276					45.796					30.353		
	3	52.595	53.072	0.414	0.78%	3	45.379	45.930	0.628	1.37%	3	28.361	28.724	1.482