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Modification of Bacterial Cellulose using Organosilanes to Enhance Cell Adhesion and Growth

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Modification of Bacterial Cellulose using Organosilanes to Enhance Cell Adhesion and Growth

Kristi Ferrato

Department of Chemical Engineering

**Honors Research Project**

Submitted to

*The Honors College*

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Dean, Honors College

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Reader (signed)
Executive Summary

Problem Statement
Wound healing applications that are made of low cost materials and can aid in the healing process, such as aid in forming new tissue, are highly desired. Tissue engineering also needs low cost scaffolding options that tissue can be grown on. Bacterial cellulose (BC) has the capability to serve in these applications as it is biocompatible, low cost, a renewable source, and does not require processing to remove harmful components. BC without modification cannot support cell growth seen on other surfaces due to its hydrophilic surface. Most of the modifications involves the incorporation of proteins, such as soaking the BC in gelatin prior to seeding with cells, to increase cell adhesion, increasing the cells per area. But, the use of animal products, such as proteins, could be quite expensive and has certain undesirable consequences, such as passing on diseases. Treating BC using inexpensive organosilanes such as 3-aminopropyltriethoxysilane (APTES) and (3-Mercaptopropyl)trimethoxysilane (MPTMS) that can help increased cell growth as these organosilanes introduce functional groups and/or create a hydrophobic surface could be a better alternative.

Results
The BC must be dried prior to treatment with the organosilanes so that the organosilanes react with the BC instead of the water in the natural wet state of BC. Depending on how the BC is dried, the BC may not be able to swell back if hydrogen bonds are formed between the layers of BC during drying. To prevent the BC structure from collapsing and thereby forming the hydrogen bonds that would prevent swelling, drying the BC with hexamethyldisilazane (HMDS) as well as freeze drying were assessed in this experiment. Neither the HMDS nor the freeze drying conducted in the experiment prevented the hydrogen bonds from being formed between the layers of BC as the BC did not swell
back. BC samples provided from our collaborator’s lab where the media consisted of coconut water, however, did swell back.

Treating the BC surfaces with APTES and MPTMS with a longer time (e.g., > 60 s) did create a hydrophobic surface, but only APTES significantly increased cells per area. The average cells per area for BC treated with APTES solution was found to be 373 cells/mm² compared to a control that only had 242 cells/mm², while the MPTMS had less cells per area than the control at 175 cells/mm². Neither the APTES nor the MPTMS treated BC showed significant results when the BC was treated for a short time (i.e., 60 s).

Soaking the BC in various concentrations of gelatin yielded different results. BC treated with 15% gelatin only had an average of 71 cells/mm², less than the control which had 187 cells/mm², which was almost a statistically relevant loss. BC treated with 1% gelatin, however, had significantly higher average with 374 cells/mm².

Conclusions

With the BC grown in our lab, freeze drying and treating the BC with HMDS to allow swelling of the dried BC when submerged in an aqueous medium did not work, but with BC grown in a different medium from our collaborator’s lab, these two drying approaches did allow the dried BC to swell back when placed in a liquid. Treating the BC with APTES for 30 s did significantly increase the cells per area, as did treating the BC with 1 wt.% gelatin. Treating the BC with APTES for 60 s did not significantly increase the cells per area, nor did treating the BC with MPTMS or treating the BC with 15 wt.% gelatin.

Implications

The implications from this study is that BC treated with APTES or low concentrations of gelatin has potential to be used in wound healing or tissue engineering.

Recommendations

As the drying methods in this study were heavily affected by the medium, it is suggested that an in-depth medium study be conducted, and on how the water in the BC should be frozen prior to being
placed in a freeze dryer. A more in-depth study should also be conducted to see what the limits are on the duration for placing the BC in the APTES solution, and for the optimal concentration of gelatin. It should also test to see whether the BC that promotes grow cells can be easily removed from the wound site once it is no longer needed, or if the cells would attach too strongly to the BC and patients wound, creating painful tearing when removed.
Introduction

Cellulose is utilized in a wide range of industries, from lumber to textiles. Cellulose is traditionally only thought of as being in plant cell walls, but it is also formed in a wide variety of organisms, including plants, bacteria and animals. Even cyanobacteria, the first prokaryote, was found to form cellulose. This abundance in sources of cellulose helps make cellulose the most prevalent macromolecule in the world, and its prevalence makes it a low-cost material [1]. Bacterial cellulose (BC) is a compound synthesized by a bacterial species: Acetobacter xylinum. BC varies from plant cellulose in that it can achieve a greater degree of polymerization, as seen in Figure 1, because no processing is required to remove lignin, pectin, and hemicellulose and other pollutants [2]. BC is commercially available and is widely used in food, particularly as dessert in the Philippines, and is now being used and studied in the medical field as a wound dressing and for tissue engineering.

![Figure 1: Compares the BC surface (a) to plant cellulose surface (b) using scanning electron microscope [3]. This is a picture from Handbook of Sustainable Polymers: Processing and Applications.](image_url)

Wound dressing materials must have good biocompatibility, and lately wound dressings that can aid in healing, such as form new tissue, have been studied [4]. Common wound dressings, such as gauze, require an additional topical treatment to prevent drying as moist environments aid in the healing
process more. Other common wound dressings, such as hydrogels, only keep the wound moist, and do not accelerate tissue growth [5]. BC, like most hydrogels, can maintain a moist environment but cannot support good cell attachment and growth. To address this moisture issue, materials composed of proteins, that are expensive and might bring in un-desired problems (e.g., the disease caused by animal proteins) needs to be incorporated into BC. Other modification methods that would avoid the contamination by proteins and using less expensive materials need to be sought after.

BC can be modified in several ways, including protein adsorption, gaseous plasma, and self-assembled monolayers (SAMs) of organosilanes [4]. Protein adsorption promotes cell attachment as proteins with a cellulose-binding module will attach to the BC while the adhesion molecules, found in peptides, promote cell growth [6]. Gaseous plasma with nitrogen can be used to increase cell adhesion on BC without changing bulk properties but must be done under vacuum [7]. SAMs also do not change bulk properties, including physical and mechanical. Organosilanes are commonly used as the head group attaches to the hydroxyl surface leaving the functional groups free to promote cell attachment. Organosilanes can create a hydrophobic surface, as opposed to the BC’s naturally occurring hydrophilic surface. As BC has a hydroxyl rich surface, SAMs is possible to use for modification [4].

One focus of this study is to quantitatively examine how chemically altered BC modified using organosilanes or gelatin affects cell growth. This altering of the BC was done by studying various ways of drying the BC, different organosilanes used to modify the surface of the BC, using various concentrations of gelatin on the BC surface, and various staining methods for analyzing the cells per area. The effects of modifying BC with organosilanes 3-aminopropyltrithoxysilane (APTES), 3-Mercaptopropyltrimethoxysilane (MPTMS) and with gelatin on induced vascular progenitor cells (iVPC) and normal human dermal fibroblasts (NHDF) are qualitatively measured as number of cells per area and compared against non-treated BC for statistical relevance. Air dried BC stained red became the base method for analyzing the BC so that ImageJ could be used to auto count the BC. The effects of air
drying without HMDS versus HMDS, as well as the BC being treated with APTES and MPTMS and with
gelatin of varying concentrations were studied. The qualitative analysis was done by comparing the cells
per area of the altered BC versus the cells per area of the control, which was non-treated BC. BC
modified in 3-aminopropyltriethoxysilane (APTES) for 30 seconds was found to significantly increase the
cells per area, as was soaking the BC in 1 wt.% gelatin. Throughout this project many further
recommendations were created. In the future, it should be studied how chemically altering wet BC
varies from dried BC. It should also be studied how different mediums, such as a coconut-based
medium, affect the ability of the BC to swell after being dried.

**Background**

*Bacterial cellulose (BC) and its modification*

BC has a modulus similar to that of a cell, meaning that it is flexible and can conform to unique
shapes [2]. The Young’s modulus of elasticity for BC was found to be in excess of 15GPa, which is
considered very large [8]. However, this modulus is highly affected by drying, which affects physical
properties. The hydrogen bonding in BC is strong enough that when air dried the BC collapses and
cannot swell back. This flexibility, coupled with its ability to retain water and biocompatibility makes BC
a potential candidate for tissue engineering and improve its application in wound healing. In order for
BC to be successfully used in these medical applications, cells would be needed to be grown on the BC
beforehand as the BC would act as a scaffold for the cells to construct functioning tissue which can then
be grafted onto the body as needed [9]. However, as BC is a hydrogel, it is hydrophilic making it difficult
for cells to attach, in addition the force between cells, particularly fibroblasts is stronger than the force
between the cells and the BC, also making it difficult for the cells to attach to the BC [4].

*Drying of BC*
This chemical modification is permanent, but only if it is cured in the oven, otherwise it can be washed off with ethanol [10]. The BC was dried before treatment with the organosilanes, as the silanes would react with the water to polymerize instead of altering the surface of the BC. However, the BC would need to be in its original wet state to serve for wound healing, as the moist environment is required, therefore the BC needs to be able to swell up after drying. When air dried, however, the BC cannot swell back to its original thickness. This inability to swell back up is due to the hydrogen bonds that form between layers due to the sugar rings, as can be seen in Error! Reference source not found., and so the BC cannot swell back after air dried due to the numerous short hydrogen bonds. To allow swelling after drying, drying with hexamethyldisilazane (HMDS) as well as freeze drying were studied in this work. HMDS reduces the destruction of the BC structure by reducing the surface tension and also cross-links proteins [11]. Freeze drying also prevents the BC structure from collapsing as the water is frozen and then pulled, as vapor, by vacuum as opposed to turning into a liquid and followed by evaporating.

![Figure 2: Bacterial Cellulose chemical structure.](image)

**Modification agents for BC in this study:**

Three agents are used for modifying BCs in this study. The first two are organosilanes, 3-aminopropyltriethoxysilane (APTES) and mercaptopropyltrimethoxysilane (MPTMS), the chemical structures of which can be seen in Figure 3 and physical properties in Table 1 [12].
Figure 3: Chemical structures of the two organosilanes used in this study.

Table 1: Various physical properties of the organosilanes used in this study.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Molecular Formula</th>
<th>Molecular Weight (g/mol)</th>
<th>Boiling Point (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>APTES</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;N(CH&lt;sub&gt;2&lt;/sub&gt;)&lt;sub&gt;3&lt;/sub&gt;Si(OC&lt;sub&gt;2&lt;/sub&gt;H&lt;sub&gt;5&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;</td>
<td>221.37</td>
<td>217 at 760 mmHg</td>
</tr>
<tr>
<td>MPTMS</td>
<td>HS(CH&lt;sub&gt;2&lt;/sub&gt;)&lt;sub&gt;3&lt;/sub&gt;Si(OCH&lt;sub&gt;3&lt;/sub&gt;)&lt;sub&gt;3&lt;/sub&gt;</td>
<td>196.34</td>
<td>93 at 40 mmHg</td>
</tr>
</tbody>
</table>

Gelatin, a polypeptide made from the collagen of animals and the chemical structure of which can be seen in Figure 4, while brittle and has limited flexibility when dry, is a common substance used for drug delivery. It is an excellent material for bone, skin, and cartilage support as it is decomposable, low-cost, and biocompatible [13]. Gelatin is also known for increasing cell adhesion [3]. Too high of a concentration of gelatin, however, can be harmful as it can limit the amount of oxygen to the BC due to the high viscosity [13].

Figure 4: Chemical structure of gelatin.

By combining the BC with gelatin, the final product can have high mechanical strength and flexibility and be used in drug delivery. This experiment studied soaking the BC in various concentration of gelatin to see the effect on the cells per area on the BC/gelatin composite.
Experimental Methods

Growing the BC

The BC used in this study was synthesized the *Acetobacter xylinum* bacteria and grown in medium with the ratios of 1 Na2HPO4: 8.025 glucose: 1.25 peptone: 1.25 yeast: 0.625 citric acid. It was attempted to combine these dry parts in containers ahead of time, but it was found that the solution became too acidic when this was done, and the BC would not grow. Water was added to the BC and then autoclaved to ensure that it was sanitized. At one point, one of the containers of medium had mold growing in it, so it was sanitized again. The solution came out browner from having been sanitized again, but the BC continued to grow as normal.

Medium was poured into the desired sanitized dish and would be seeded with bacteria, then placed in the incubator at 30°C to grow for a week. Grown BC was kept in 1 wt.% solution of BC, to kill and remove the *Acetobacter xylinum*, and therefore purify the sheets. The sheets would switch to another container of 1 wt.% NaOH solution after a 24-hour period until the solution stayed clear. Purified sheets of BC were kept at 4°C until they were needed.

Drying the BC

Most of the BC for this study was left in the hood for approximately 48 hours until completely dried, or air dried. One of the difficulties with this study was taking good pictures of the BC with the microscope. The BC would not dry flat, so when pictures were taken only parts of the picture could be in focus. One method that helped to prevent this was drying the BC on saran wrap. The BC would stick to the saran wrap, but if the saran wrap was moved the BC would easily come off. Thus, if the saran wrap was laid out flat, the BC would dry flat. However, air dried BC would not work for wound applications as the BC cannot swell after air drying, because as water evaporates it tears down the structural integrity of BC.
Freeze drying helps to preserve the BC structure, as the water goes from a solid state to a gaseous state, preventing the layers from collapsing. While most BC was grown in Dr. Bi-min Newby’s lab, some BC was provided by Dr. Siriporn Taokaew, who grew BC in a coconut water-based media and sent the BC over freeze dried. When BC from our lab was freeze dried, by placing it in a freezer set at -20°C and then placed in a dryer with the condenser set at -80°C and under high vacuum. Dr. Siriporn Taokaew’s BC was freeze-dried in a similar fashion, except it was placed in liquid nitrogen for two hours before being placed in the freeze dryer.

Other BC was dried with HMDS, an organic solvent with a relatively low surface tension of 18.16 mN/m of 25°C [14]. To dry the BC with HMDS, the BC would be placed in ascending concentrations of ethanol/water solutions, from 70% ethanol to 90% ethanol, for ten minutes each. The BC would then be soaked in pure ethanol two times for fifteen minutes each. The BC was then put in ascending concentrations of HMDS and ethanol (25% HMDS to 100% HMDS) for ten minutes in each solution. The BC was then dried in a desiccator. The HMDS method was to help prevent the BC micro scaffolding from collapsing, which would allow the BC to swell up again after having been dried. It seemed that the BC grown from the medium as described in the previous section did not swell back up, but BC that was supplied from another lab that was grown in a medium that consisted of coconut water instead of water did.
Modifying the BC with Organosilanes

As can be seen in Figure 5, treating BC with APTES leaves amino groups on the surface. This is beneficial for cell growth as the proteins need nitrogen to grow, and nitrogen is even considered “life’s blueprint for all cells” [15]. Figure 5 also shows that MPTMS leaves mercaptan end groups on the BC surface. Sulfur is very important for cell growth as it is required for collagen growth. Approximately 0.2mL of the 1 wt.% organosilane in hexanes solution being used, either APTES or MPTMS, was placed in a glass dish. The BC was placed in the solution for either 30 seconds or 1 minute, of which the difference in results will be discussed. The BC would be taken from the solution and rinsed with pure ethanol and dried with air and placed in an oven at 100°C for 20 minutes, an important step as it ensures that the modification is permanent. The BC would then be soaked in pure ethanol for approximately two minutes to sanitize the samples. The modification to the surface is shown in Figure 5. It was checked that the organosilanes made the BC surface hydrophilic by checking the angle of a drop of water on the BC.

Cells on BC

NHDF or iVPC cells were seeded on the BC or modified BC to allow attachment and growth. The cells were either already in the incubator or taken out of long-term storage in a freezer. The cells would
be seeded onto the BC by and allowed to grow and spread for approximately 3 days to a week. After a
week the cells would be fixed and stained and then analyzed. For each set of BC modified, there was
always a control BC, or non-modified BC, to compare to the modified BC. This was done each time as
opposed to just once to make up for any changes allowed in the cells or growth time.

**Analysis of cells on BC**

![Image of cells on BC](image)

*Figure 6: From left to right is the original picture of the APTES modified BC with NHDF cells dyed using red staining, taken at 10x magnification. The picture made binary so that ImageJ could count the cells. The last picture is the cells ImageJ counted. This picture was counted to have 453 cells.*

In the beginning of this study, the BC would be stained using Acetic Orange after the cells had
grown and analyzed using fluorescent light. This method, however, would wear off quickly and didn’t
dye the nucleus of the cells, making it more difficult to analyze. Therefore, red staining was done with
Alizarin Red S to detect calcium mineralization. This dye did not require the fluorescent light and did not
fade. The pictures were taken by making sure the BC took up the whole picture (no blank spaces) and at
10x magnification to ensure the same amount of area was in each picture. ImageJ was then used to
count the cells and was checked that it was within ±10% by hand counting a picture. This can be seen in
Figure 6 as the original images were made as 8-bit, then binary and then analyzed. The size of the
particles analyzed was varied depending on the type of cell as iVPC cells were smaller than NHDF cells
and was accounted for by using ImageJ to find the average area of the cells and inputting that as the size
under Analyze Particles. On some pictures, the contrast had to be changed so that the cells could be translated into the binary picture.

**Data and Results**

*Drying of BC*

Hexamethyldisilazane (HMDS) can be used to maintain the structural integrity of the BC, and this study looked at how that treatment coupled with organosilane treatment affects the growth of cells on the BC. The first method for drying the BC used was to soak the BC for ten minutes in 70% ethanol, 80% ethanol, 90% ethanol, and then in 100% ethanol two times for fifteen minutes, and finally 100% HMDS for fifteen minutes then air dried in a desiccator. However, the BC did not exhibit swelling in alcohol after drying, indicating that the BC structure had collapsed. The next method used the same increasing concentrations of ethanol at the same intervals, then increasing concentrations of HMDS in ethanol for fifteen minutes, the concentrations being 25%, 50%, 75% and 100%, and then dried in the desiccator again. With this method some of the BC swelled when placed in alcohol, but not all. The BC that did swell was BC provided by Dr. Siriporn Taokaew’s, who used a coconut water media as opposed to the glucose-based media grown for this study.

The BC grown in our lab also did not freeze dry as expected. As can be seen in Figure 7, the BC that was freeze dried in Dr. Newby’s lab did not completely collapse, but also did maintain near original thickness as the BC in Dr. Siriporn Taokaew’s lab did. This would indicate that not all the water sublimed, and it is recommended to place the BC in liquid nitrogen rather than in a freezer to ensure this does not happen.
Modification of BC with APTES and MPTMS

Contact Angle

Table 2: Table showing the differences in angles between control, APTES, and MPTMS.

<table>
<thead>
<tr>
<th>Modification</th>
<th>Modification time (s)</th>
<th>Average Angle (°)</th>
<th>P (t)</th>
<th>t value</th>
<th>t critical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>20.70</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>APTES</td>
<td>30</td>
<td>65.35</td>
<td>9.66E-03</td>
<td>-1.01E+01</td>
<td>4.30E+00</td>
</tr>
<tr>
<td>APTES</td>
<td>60</td>
<td>57.53</td>
<td>1.04E-06</td>
<td>-1.57E+01</td>
<td>2.36E+00</td>
</tr>
<tr>
<td>MPTMS</td>
<td>30</td>
<td>43.38</td>
<td>2.24E-09</td>
<td>-1.57E+01</td>
<td>2.18E+00</td>
</tr>
<tr>
<td>MPTMS</td>
<td>60</td>
<td>42.85</td>
<td>1.57E-03</td>
<td>-7.65E+00</td>
<td>2.78E+00</td>
</tr>
</tbody>
</table>

Figure 7: Freeze dried BC grown in Dr. Newby's lab can be seen on the left, freeze dried BC grown in Dr. Siriporn Taokaew’s lab can be seen on the right.

Figure 8: ‘A’ shows a water droplet on non-treated BC, ‘B’ is a water droplet on APTES treated BC, and ‘C’ is a water droplet on MPTMS treated BC.

Table 2 shows the difference in average angles, as well as statistical information for modified BC compared to a control. As all P values are less than 0.05, and all t values are less than the respective
critical values, the null hypothesis can be rejected in all cases. Thus, the organosilanes were proven to make the BC surface hydrophobic, as shown in Figure 5. As the angle for APTES treated surfaces were larger than the angle on the MPTMS treated surfaces, it indicates that the APTES surfaces were more hydrophobic than the MPTMS treated surfaces, which can also be seen by the shape of the water droplet on the different surfaces in Figure 8, so it is expected that APTES is a better surface modifier than MPTMS. However, when the BC was treated with APTES for 30 seconds was compared to BC treated with APTES for 60 seconds, the P value was greater than 0.05 at a value of 0.2, so there should not be a significant difference between those BC in terms of cells per area.

Cells per Area

Table 3: Table comparing average cells/area as well as statistical information for BC sheets that were in organosilane solutions for 30 seconds versus 60 seconds.

<table>
<thead>
<tr>
<th>Modification time (s)</th>
<th>Modification</th>
<th>Cell Type</th>
<th>Cells/Area (cells/mm²)</th>
<th>P value</th>
<th>t value</th>
<th>t critical value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Control</td>
<td>NHDF</td>
<td>1192</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>60</td>
<td>APTES</td>
<td>NHDF</td>
<td>1221</td>
<td>0.966</td>
<td>-0.0433</td>
<td>2.23</td>
</tr>
<tr>
<td>60</td>
<td>MPTMS</td>
<td>NHDF</td>
<td>1649</td>
<td>0.569</td>
<td>0.602</td>
<td>2.45</td>
</tr>
<tr>
<td>60</td>
<td>Control</td>
<td>iVPC</td>
<td>224</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>60</td>
<td>APTES</td>
<td>iVPC</td>
<td>226</td>
<td>0.799</td>
<td>-0.272</td>
<td>2.78</td>
</tr>
<tr>
<td>60</td>
<td>MPTMS</td>
<td>iVPC</td>
<td>180</td>
<td>0.015</td>
<td>-5.05</td>
<td>3.18</td>
</tr>
<tr>
<td>0</td>
<td>Control</td>
<td>NHDF</td>
<td>242</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>30</td>
<td>APTES</td>
<td>NHDF</td>
<td>373</td>
<td>0.042</td>
<td>-2.72</td>
<td>2.57</td>
</tr>
<tr>
<td>30</td>
<td>MPTMS</td>
<td>NHDF</td>
<td>175</td>
<td>0.256</td>
<td>1.32</td>
<td>2.78</td>
</tr>
</tbody>
</table>

When the BC was placed in the APTES for 30 seconds, the average number of cells per area was determined to increase by about 54%, or 242E-04 cells/mm² for the control and 373 cells/mm² for the APTES treated for NHDF cells, as can be seen in Table 3. When a student T test was run on the results, a P(t) value of 0.042 was determined, which was less than the alpha value of 0.05 used, which meant the null hypothesis could be rejected. In addition, the t critical was determined to be 2.57 and the t value was -2.72. As the t value was less than the t critical, the null hypothesis was rejected. However, when
the BC was placed in the APTES for 60 seconds, and NHDF cells were again grown on the BC, the percent increase of cells on the modified BC was only 0.96%, and the P value was 0.97, greater than the alpha of 0.05 and the null hypothesis could not be rejected. The null hypothesis also could not be rejected when the BC was placed in the APTES solution for 60 seconds and iVPC cells were grown on the BC as even though there was a 2.4% increase of cells per area on the APTES modified BC as compared to the control BC, and the t value was less than the t critical value, the P value was 0.79, which was greater than the alpha value of 0.05.

When the BC was placed in MPTMS for 30 seconds, there were less cells per area on the MPTMS treated BC than the control BC by about 28%, and the P value was greater than 0.05, which can be seen in Table 3. When the BC was placed in the MPTMS for 60 seconds and NHDF cells were attached, the MPTMS treated BC compared to the control BC had more cells per area by about 19%, but the P value was greater than 0.05. However, when the BC was in the MPTMS solution for 60 seconds and iVPC cells were grown on the modified BC, there was a decrease of cells on the MPTMS cells as compared to the control by about 38%, and the P was 0.015 and the t value was less than the t critical value.

Modification of BC with Gelatin

Table 4 shows results from treatment of BC with various amounts of gelatin.

<table>
<thead>
<tr>
<th></th>
<th>average (cells/mm²)</th>
<th>stdev</th>
<th>P(t)</th>
<th>t_value</th>
<th>t_critical</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>1.87E+02</td>
<td>1.12E-04</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1% gelatin</td>
<td>3.74E+02</td>
<td>9.44E-05</td>
<td>3.15E-03</td>
<td>-3.86E+00</td>
<td>2.23E+00</td>
</tr>
<tr>
<td>15% gelatin</td>
<td>7.06E+01</td>
<td>3.22E-05</td>
<td>4.43E-03</td>
<td>4.43E+00</td>
<td>2.45E+00</td>
</tr>
</tbody>
</table>

As can be seen in Table 4, treating BC with 1% gelatin significantly increased the cells per area as the P value was less than 0.05, and the t_value was smaller than the t_critical. However, treating the BC with 15% gelatin decreased the number of cells, almost by a statistical amount as the P value was less than 0.05, but the t_value was larger than the t_critical.
It should be noted that the gelatin on two of the five samples with 15% gelatin slid off the sample as shown in Figure 9, as the melting point of gelatin is around room temperature. While this did not occur on any of the 1% gelatin samples, it is a problem that could occur on studies focusing on BC treated with gelatin.

Discussion/Analysis

As shown in Table 3, the only null hypotheses that could be rejected are for MPTMS treated for 60 seconds for iVPC cells and for APTES treated for 30 seconds for NHDF cells. This would indicate that the MPTMS required more time to react with surface of BC and APTES polymerized quicker, so it required a shorter time. APTES polymerizes with water and time and that is why a shorter treatment time encouraged more cells to grow than a longer treatment time. For MPTMS, it seems the number of cells/area merely plateaued. For example, in Table 3 the number of NHDF cells per area grown on the control and APTES for the 30 second trial versus the 60 second trial saw a significant increase, but the number of cells per area grown on the MPTMS BC between the trials was about the same. In general, APTES did increase the number of cells per area as compared to a control, but the method of modification easily affected whether the increase was by a statistically relevant amount, which was not
expected based on the angle measurements to determine if the BC was hydrophobic or not. As for MPTMS, the number of cells per area was only greater than the control in one instance, the 60 seconds of modification and with NHDF cells, but even then, it was not by a relevant amount, even though the MPTMS treated surfaces were proven to be hydrophobic. Therefore, APTES should further studied for tissue engineering, as it does increase the number of cells that could be grown on BC. It should be evaluated how long the modification needs to be, if a shorter period than 30 seconds can be used or not.

As can be seen by Table 4, treatment with 1 wt.% gelatin did significantly increase the number of cells per area. Soaking the BC in 15 wt.% gelatin, however, did not, as in one case the gelatin actually slid off the BC and in the cases where that did not happen, there were less cells on the 15 wt.% BC than on the control, which can be seen in Table 4. As such, it should be studied what the limit is on treating the BC with gelatin and to still see significant results.

**Conclusions**

This study found that MPTMS does not significantly increase the number of cells per area grown on BC. It did reinforce that APTES and gelatin can significantly increase the number of cells per area, but if APTES is going to be used to modify the BC, a more consistent method of drying that allows for swelling of the BC needs to be used. The method for altering the BC with APTES must have the BC come into contact with the APTES for approximately 30 s, and if gelatin is to be used it should be about 1 wt.% gelatin, and not 15 wt.% gelatin.
Literature Cited


