POLYELECTROLYTE COMPLEX BARRIER MESHES FOR THE PREVENTION OF ABDOMINAL ADHESIONS

By

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ABSTRACT OF THE THESIS

Polyelectrolyte Complex Barrier Meshes for the Prevention of Abdominal Adhesions

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Adhesions are a painful and expensive result of abdominal surgeries, specifically in the peritoneal cavity. This complication is surprisingly common and requires a second surgery, adhesiolysis, to remove it. Current solutions to adhesions either lack efficacy or produce an inflammatory response in the peritoneum. This project focuses on developing a post-surgical adhesion prevention polyelectrolyte complex (PEC) to both combat the problem of adhesions and promote an anti-inflammatory response. Initially, material properties of the PEC were investigated. Fibroblasts and macrophage cell viabilities were also studied on the PEC since both cell types play a central role in adhesion formation and inflammation. Finally, preliminary in-vivo studies were conducted to determine the true capability of the material as a post-surgical adhesion prevention method.

Acknowledgements

I would like to thank Dr. Noshir Langrana for giving me the opportunity to initially enter the field of research at Rutgers University and for guiding me throughout both my research and academic career. In addition, I would like to thank Dr. Rene Schloss for her support and guidance in this research project. I would also like to acknowledge my fellow members of Langrana Lab, Karishma Desai and Jordan Tutnauer, for their help in my studies. And finally, Aresty Research Center for initially helping me get involved in research as well as funding my conference trips to BMES in 2013 and 2014.

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Table of Contents

ABSTRACT OF THE THESIS ii

Acknowledgements iii

List of Figures vi

List of Tables vii

Section 1: Introduction 1

Section 2: Methods and Materials 4

2.1 Manufacturing PEC 4

2.2 Absorption Testing 4

2.3 Degradation Testing 4

2.4 Mechanical Rheology Testing 5

2.5 Fibroblast Cell Viability 5

2.6 Cellular Toxicity Study 5

2.7 Macrophage Cell Viability 6

2.8 TNF-α Quantification 6

2.9 In-vivo Study 6

Section 3: Results 9

3.1 Absorption Testing 9

3.2 Degradation Testing 12

3.3 Mechanical Rheology Testing 13

3.4 Fibroblast Cell Viability 14

3.5 Cellular Toxicity 18

3.6 Macrophage Cell Viability 19

3.7 TNF-α Quantification 23

3.8 In-vivo Study 24

Section 4: Discussion 28

Section 5: Conclusions 32

Section 6: References 33

List of Figures

Figure 1: Manufacturing of Buttons 7

Figure 2: Animal - Time 0 with Buttons 7

Figure 3: Animal – Time 0 with Material Covering Buttons 8

Figure 4: Percent change in mass of PEC after being soaked in PBS 10

Figure 5: Normalized Absorption Data 11

Figure 6: Percent change in mass of PEC after being soaked in PBS solution with 0.5 mg/ml of lysozymes. 13

Figure 7: G' Values of PEC Films 14

Figure 8: Fibroblast Cell Attachment after 2 and 4 days 15

Figure 9: 4 day Fibroblast Images at 4x magnification. 17

Figure 10: Fibroblast Viability after 2 and 4 days 18

Figure 11: 4 day 40c Images at 4x Magnification 19

Figure 12: Macrophage Cell Attachment after 8 hours 20

Figure 13: 8 Hour Macrophage Images at 4x magnification 21

Figure 14: Macrophage Viability after 8 hours 22

Figure 15: TNF-α Quantification 24

Figure 16: 195 μm 40c PEC at 1 week (left) and 2 week (right) 25

Figure 17: 260 μm 40c PEC at 1 week (left) and 2 week (right) 26

List of Tables

Table 1: Summary of Buttons and Adhesions for In-Vivo Study..... 27

Section 1: Introduction

Adhesions are the formation of an abnormal fibrous bridge between two tissues that are not normally connected. This results in numerous medical issues including restriction of movement, pain, obstructive bowel movements, and infertility in women. Adhesions are caused by trauma to a region of the body and include disease, infection, foreign body placement, or one of the biggest causes, surgery. Post-operative adhesions are especially common in the peritoneal cavity, the area between the abdominal organs and the abdominal wall. Furthermore, postoperative adhesions call for an additional surgery, adhesiolysis, to remedy the original adhesion [1].

Peritoneal adhesions are generated through a deviance in the normal wound healing process. The first layer of the peritoneal cavity is comprised of mesothelial cells. The second layer is composed of collagen, extracellular matrix, fibroblasts, lymphocytes, and macrophages [2]. After injury to the normal mesothelial cells overlaying the peritoneal surface, the healing process begins. Subsequently, release of vasoactive substances such as histamines and kinins increase vascular permeability and cause the deposition of a fibrin-rich exudate that covers the injured area. The fibrin polymers in this exudate interact with fibronectin and thrombin to form the fibrin gel matrix which consequently produces fibrin bands between the injured areas. At the same time, fibrinolysis starts. Fibrinolysis is the destruction of the fibrin bands formed between the injured areas and a key factor in determining the amount of adhesion formation [3]. Fibrinolysis within 5 days of injury encourages proper healing of the peritoneum without adhesion formation. However, disruption of the epithelial layer and inflammatory reactions impair fibrinolysis. This imbalance results in the persistence of the fibrinous

mass [4–6]. Inflammatory mediators such as tumor necrosis factor-α (TNF-α) and interleukins, reduce the fibrinolytic ability of the peritoneum causing increased adhesion formation [2,7–10]. Subsequently, fibroblasts from the sub-mesothelial region invade the fibrin matrix and deposit extracellular matrix and collagen, which contributes to the strengthening of the initial fibrous mass further perpetuating the adhesions [11].

Several different methods have been generated to prevent adhesions from occurring but none are completely effective. Pharmaceutical methods prevent adhesions, but none of them directly disrupt the connective tissue from forming. There are many indirect methods, but these only target a small part of the adhesion pathogenesis. Fluids and gels are another method to prevent adhesions, but fluids and gels cannot perpetually reside in the wound inflicted area resulting in their inefficiency. Mechanical barriers are physical barriers that prevent the two tissues from attaching and are currently the most effective mechanisms for adhesion prevention [12].

Seprafilm® (Genzyme) and Interceed® (Johnson and Johnson) are both solid barrier methods and are the two most widely used products in the United States. Seprafilm, a solid sheet of biodegradable carboxymethylcellulose and hyaluronic acid, has shown the ability to diminish the occurrence of adhesions[13,14]. However, Seprafilm is brittle and tends to break when in a dry state. Interceed is a biodegradable sheet of oxidized regenerated cellulose that can perform similarly to Seprafilm. However, Interceed is completely ineffective in the presence of blood [11,13,15,16]. Furthermore, one of the biggest issues with both Seprafilm and Interceed is that both materials are only a physical barrier to prevent adhesion formation and have no biological basis for adhesion prevention [12].

The major problems of the current adhesion prevention products propagated the need for a better material to prevent adhesions. Therefore, studies in our lab investigated a Chitosan-Polygalaturonic Acid (Chi-PgA) polyelectrolyte complex (PEC) biomaterial to act as a post-operative adhesion prevention method. Chitosan is positively charged, used in many biomaterial applications, and anti-inflammatory while PgA is negatively charged, a property correlated to adhesion prevention [1,17–19] Furthermore, using a concentration of the PEC that has a higher amount of the negatively charged PgA should yield a PEC of a net negative charge that is conducive to anti-cellular adhesions. Hence the Chi-PgA PEC should not only act as a barrier but will also be anti-adhesive, anti-inflammatory, fibrinolysis-promoting, and malleable, qualifying it an ideal adhesion prevention material.

Section 2: Methods and Materials

2.1 Manufacturing PEC

Chi solution is prepared by dissolving 300 mg of Chi in 30 mL of deionized water with 1 mL of 1 M hydrochloric acid (HCl). PgA solution is prepared by dissolving 300 mg of PgA in 30 mL of deionized water with 1 mL of 1 M Sodium Hydroxide (NaOH). Then appropriate amounts of Chi are added dropwise to PgA to create a certain concentration PEC (PECs are named in terms of Chi concentration, 40% Chi is denoted as 40c). The mixed solution is then sonicated using the Branson Digital Sonifier 450 and air-dried overnight. To make thicker samples the sonicated solutions are put on top of previously air dried samples.

2.2 Absorption Testing

Circular pieces approximately 6 mm in diameter of each PEC concentration were prepared and initial masses were recorded. PECs were incubated with 1 mL of 1X Phosphate Buffer Saline (PBS) at 37ºC and 5% CO_2. Masses were then checked periodically from 1 hour to 8 days of incubation.

2.3 Degradation Testing

Circular pieces approximately 6 mm in diameter of each PEC concentration were prepared and initial masses were recorded. PECs were incubated with 0.5 mg/mL lysozymes per 1 mL of 1X PBS at 37ºC. Masses were then checked periodically from 3 days to 17 days of incubation. PECs were also visually inspected at these time points for signs of degradation.

2.4 Mechanical Rheology Testing

Triple thickness PECs were formulated and then piled on top of one another to form a stack approximately 7 mm high. Each stack was subjected to a load. The corresponding load and displacements were then measured (using the Mark-10 Force Gauge Series-Model EG5) and analyzed for mechanical properties.

2.5 Fibroblast Cell Viability

24 well plates coated with 300 μL of different concentrations of the PEC, pure PgA, and pure Chitosan were sterilized with UV light using the XL 1500 UV Crosslinker for 15 minutes, plated with 25,000 3T3 fibroblast cells per well, and incubated at 37ºC and 5% CO_2. Cells were then imaged using calcein am and ethdium homodimer-II (live/dead) staining after 2 and 4 days.

2.6 Cellular Toxicity Study

24 well plates with 1 μL dots of different concentrations of the PEC, pure PgA, and pure Chitosan were sterilized with UV light using the XL 1500 UV Crosslinker for 15 minutes, plated with 25,000 3T3 fibroblast cells per well, and incubated at 37ºC and 5% CO_2. Cells were then imaged using calcein am and ethdium homodimer-II (live/dead) staining after 2 and 4 days.

2.7 Macrophage Cell Viability

24 well plates coated with 300 μL of different concentrations of the PEC, pure PgA, and pure Chitosan were sterilized with UV light using the XL 1500 UV Crosslinker for 15 minutes. Primary peritoneal macrophages were extracted from female, adult, Sprague dawley rats. Half of the cells were treated with 1 μg/mL of Lipopolysaccharides (LPS) for 24 hours. Both LPS-treated macrophages and non-LPS treated macrophages were plated on the material at 25,000 cells per well and incubated at 37ºC and 5% CO_2. Cells were then imaged using calcein am and ethdium homodimer-II (live/dead) staining after 8 hours.

2.8 TNF-α Quantification

Supernatants from 8 hour macrophage cell viability studies were collected and stored at -20ºC. An enzyme linked immunosorbent assay (ELISA) was conducted on these samples to detect TNF-α secretions by following the vendor's given protocol (BioLegend TNF-α ELISA MAX Deluxe Catalog # 438204).

2.9 In-vivo Study

Initial in-vivo evaluation of the PEC was conducted using a rat ischemic button adhesion model [20]. Even though both 40c and 60c exhibited anti-inflammatory effects and inhibition of fibroblast adhesion in the in-vitro studies, 40c was selected for this study because it would theoretically be more anti-adhesive due to its negative charge. Sprague-Dawley rats (200–250 g) had aseptic midline laparotomies conducted while anesthetized. Each animal had a ventral midline incision, with 3 intra-abdominal peritoneal "buttons"

created on each side. A portion of the lining of the abdominal wall was grasped, then encircled with ligature, and tightened to ~ 5mm diameter to create buttons as seen in figure 1 [21].

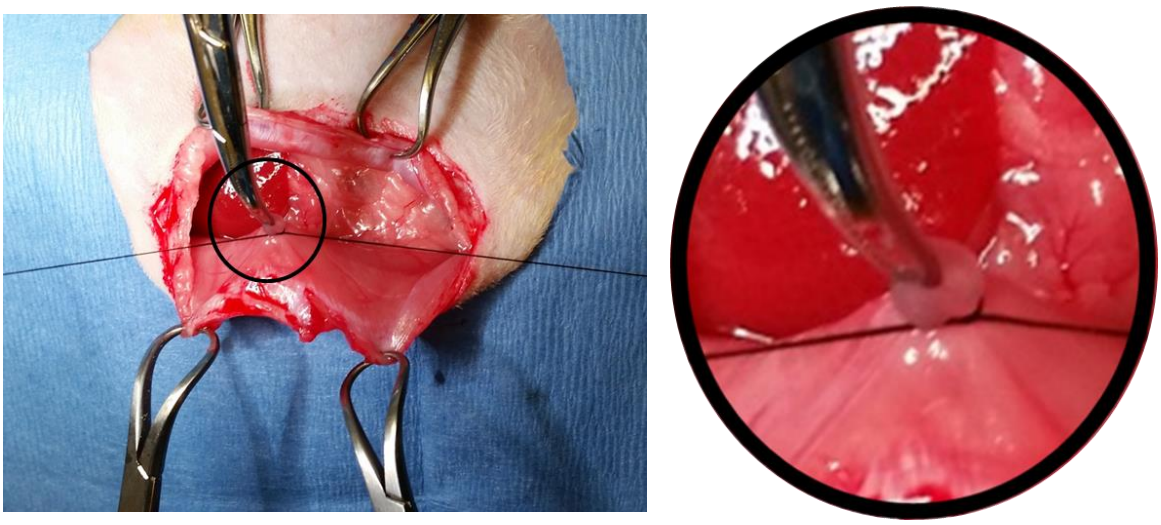


Figure 1: Manufacturing of Buttons

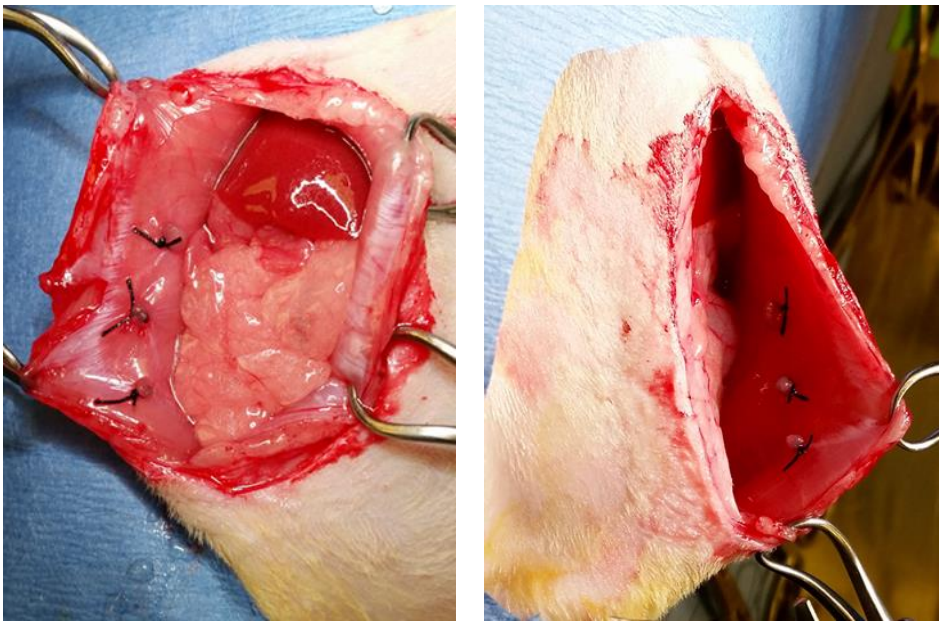
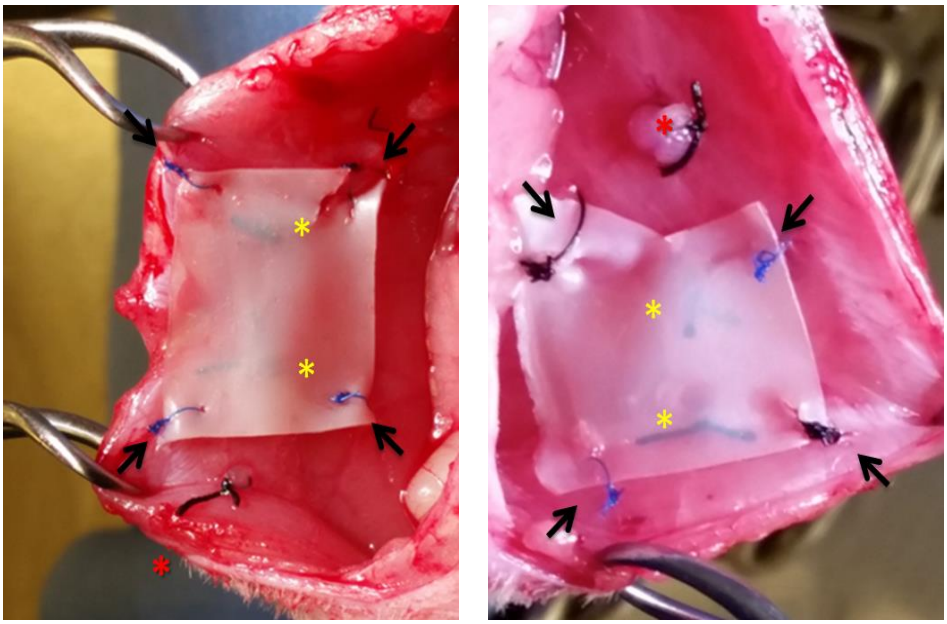


Figure 2: Animal - Time 0 with Buttons

This process initiates necrosis and then the inflammation which leads to adhesions. 40c PEC films, approximately 35 mm by 45 mm in size, were inserted in the peritoneum

cavity in such a way that it covered two buttons. One button was left without the film to act as a control. On the right side, 1 button acted as a control while the other two were covered with 195 µm 40c PEC. On the left side, 1 button acted as a control while the other two were covered with 260 µm 40c PEC. The film was held in place by 4 corner sutures.



Red Asterisk (*) - Control Button
Yellow Asterisk (*) - Experimental Buttons
Black Arrow (→) - Corner Sutures

Figure 3: Animal – Time 0 with Material Covering Buttons

After the material was implanted the abdomen was closed. 1 week and 2 week implantation time points were evaluated as well as 195 µm and 260 µm PEC thicknesses. One rat was used per time point. The experimental rats were sacrificed after 1 and 2 weeks to examine the in-vivo adhesion formation through visual inspection.

Section 3: Results

3.1 Absorption Testing

Both mass stability and low absolute swelling are desired for an implantable biomaterial to prevent any potential side effects. Therefore, to explore the swelling behavior of the PEC, absorption testing was conducted. Figure 4 depicts the overall percent mass change of each concentration of the PEC with respect to its initial weight. There was a large increase in mass during the first hour for all conditions. After the first hour, the mass change was relatively consistent with the initial mass increase at the one hour time point resulting in a relatively flat line for all conditions. The smallest absolute swelling was seen by the PEC concentrations with equivalent amounts of Chi and PgA (i.e. the 50c). As the difference between Chi and PgA increased, so did the absolute swelling. Hence, the 30c and 70c have the greatest mass percent increase, followed by the 40c and 60c, and the 50c has the lowest mass percent change. In addition, the swelling behavior of Seprafilm is approximately 225%, similar to the 40c and 60c [22]. All of PEC's mass percent changes were significantly different from each other (ANOVA of $p < 0.05$ followed by Tukey's test for multiple comparisons) except the 40c and 60c.

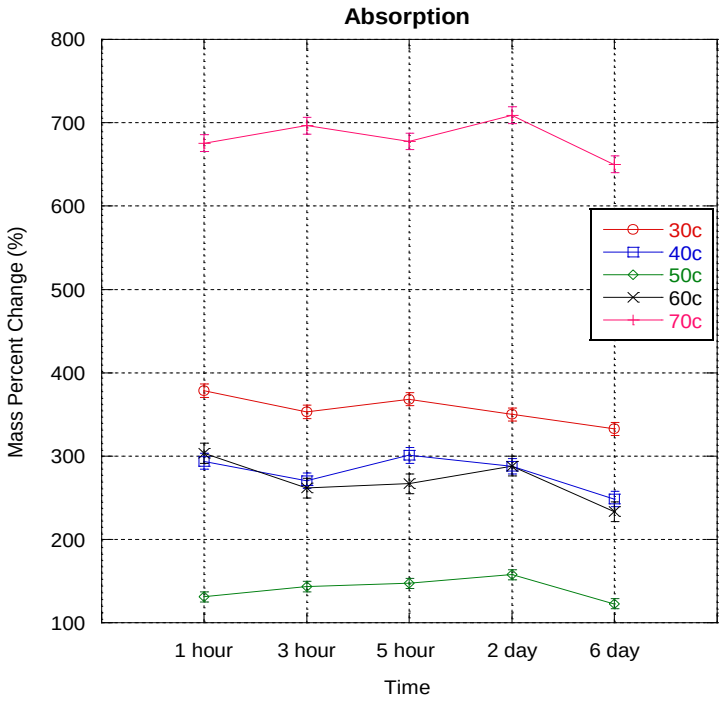


Figure 4: Percent change in mass of PEC after being soaked in PBS

Because most of the mass increase occurred during the first hour of incubation and fluctuated around that point for the rest of the study, the data was normalized to the mass change in the first hour. As seen in Figure 5, the change in mass after the first hour was small which indicated stability of the material after the initial mass increase. All PECs stayed in range of each other after the first hour of incubation with the highest percent mass change being 124% and the lowest being 71%. Furthermore, by 6 days all films decreased from the one hour mass, showing initial signs of degradation. Only the 50c and 60c films were statistically different from each other (ANOVA of $p < 0.05$ followed by Tukey's test for multiple comparisons). All concentration met the stability criteria, but the 30c and 70c had a high degree of absolute swelling which could cause potentially unwanted complications.

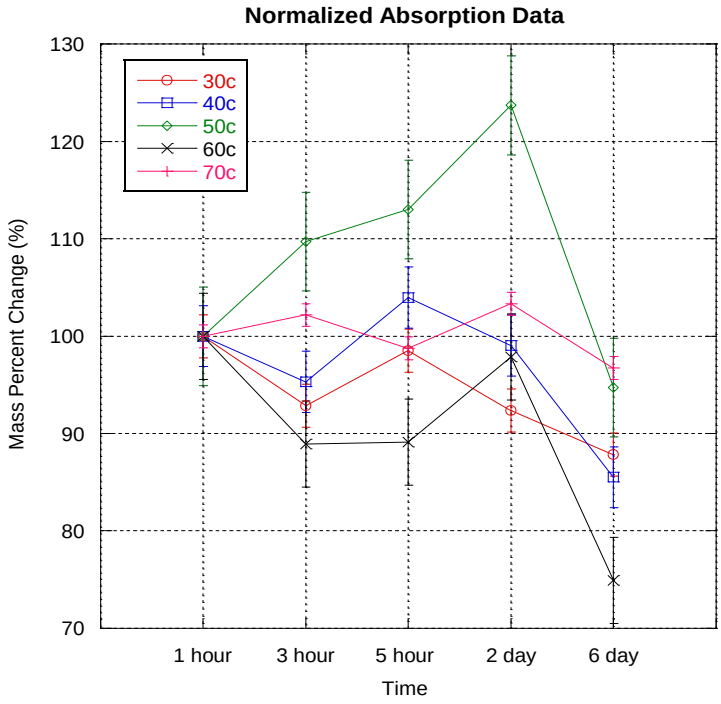


Figure 5: Normalized Absorption Data

Pairing the absorption data with the following equation:

$$\log\left(\frac{M_t}{M_\infty}\right) = \log(k) + nlog(t)$$

where M_t is the mass at time t, M_∞ is the steady state mass (6 day mass), k is a constant, and n is the unknown, allows researchers to determine the type of diffusion present in a biomaterial through plotting the data and solving for n. The n value of all films was determined to be less than 0.5 which is characteristic of less fickian diffusion. This particular type of diffusion is associated with a majority of the swelling occurring at the initial time points, which is observed in the PEC [23].

3.2 Degradation Testing

The material should naturally degrade inside the body slowly enough to prevent the adhesion but not too slowly that it interferes with the body long term. Lysozymes are secreted by macrophage cells as a response to remove foreign objects in the body. Hence, to mimic the in-vivo response, lysozymes were used in solution to observe the degradation profile of the PEC [24]. As seen in Figure 6, initially at the 3 day time point materials of all concentrations increased in mass except for the 50c material and by day 14 all concentrations are decreased mass. There was no statistical difference (ANOVA of $p > 0.05$) between all concentrations, showing that all materials behaved similarly. At the 14 day time points, materials of all concentrations became brittle when dry and difficult to handle when wet. Furthermore, particles of the PEC were seen in solution showing initial signs of degradation. Experts agree that adhesion pathogenesis begins 7 days after surgery occurs [12]. Hence, this is an acceptable degradation profile for PECs because the material needs to be intact for a minimum of 7 days to be present when formation begins. The material also needs to degrade in a timely manner to prevent additional complications which is seen at the 14 day time point.

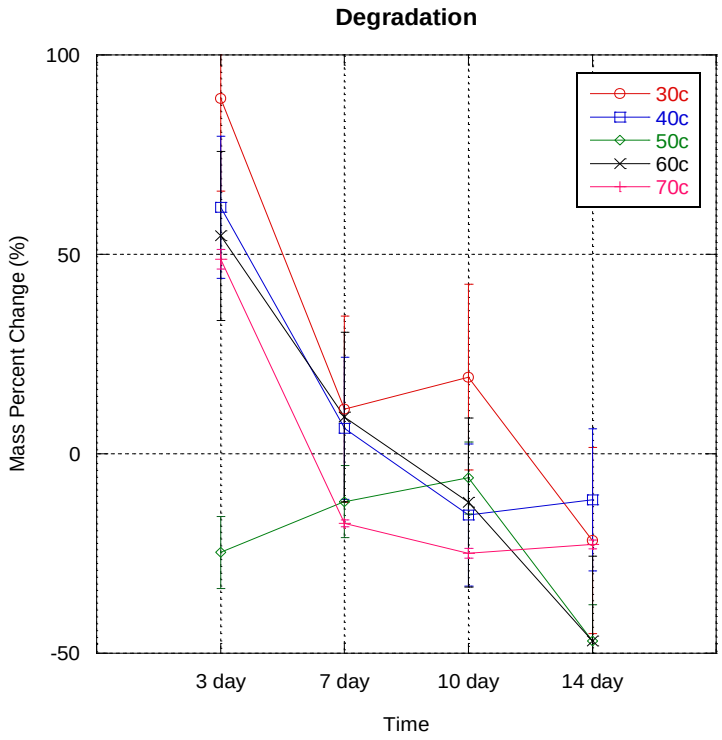


Figure 6: Percent change in mass of PEC after being soaked in PBS solution with 0.5 mg/ml of lysozymes.

3.3 Mechanical Rheology Testing

Previous adhesion prevention materials have the issue of tearing inside the body because they are too weak. On the other hand, if materials are too stiff they will cause irritation because they will not be able to bend with the natural movement of the body. An intermediate shear modulus (G') value is required to find a healthy medium between these two phenomena. As seen on Figure 7, the G' values of all PEC concentrations were within range of each other, the shear modulus of other known biomaterials (polyacrylamide gel ~ 230 kPa), and the shear modulus of soft tissue (~333 kPa) [25,26]. All PECs had significantly higher shear moduli than Seprafilm (shear modulus ~0.1 kPa), a material with a known problem of being weak and tearing [22]. The largest G' value was 2,293 kPa and the smallest G' value was 1,433 kPa. The G' values follow the

opposite trend that was seen in the absorption study. The extremes of 70c and 30c have the smallest G' values, 60c and 40c are in the middle, and 50c has the largest G' value. Films were not statistically different from one another (ANOVA of $p > 0.05$). The intermediate concentrations of 40c and 60c achieved an appropriate balance in mechanical properties.

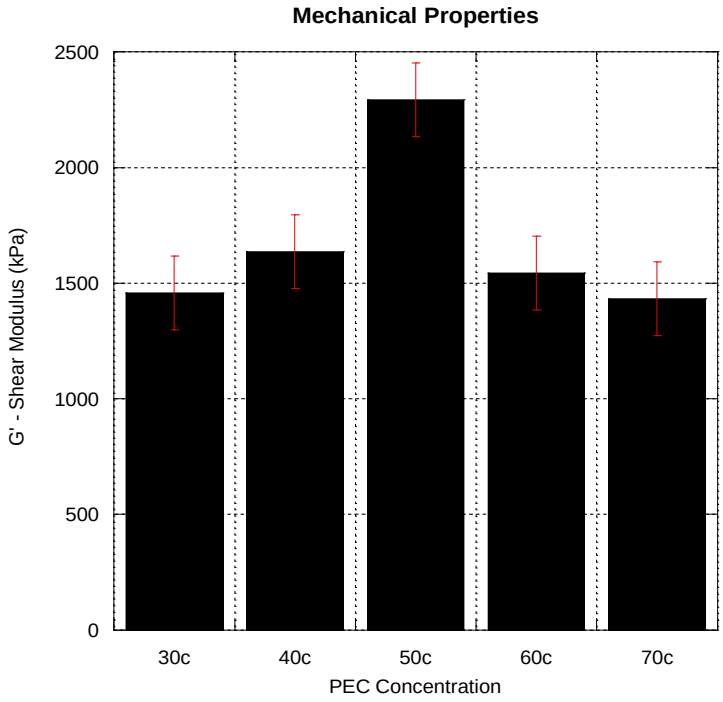


Figure 7: G' Values of PEC Films

3.4 Fibroblast Cell Viability

Fibroblast cell viability results are most indicative of the adhesion properties because fibroblasts are the cells that initially strengthen the fibrous mass causing the adhesion. Fibroblast cells attaching to the material would allow them the ability to secrete factors that would strengthen the initial fibrous mass, causing an adhesion. Hence, both a low attachment rate and a low viability of fibroblast cells would show anti-adhesive properties because the material would prevent the cells from secreting factors that would

promote adhesion. The presence of the material inhibited attachment of cells seen by the statistically significant difference (ANOVA $p < 0.05$ followed by Tukey's test for multiple comparisons) between the control and all material conditions.

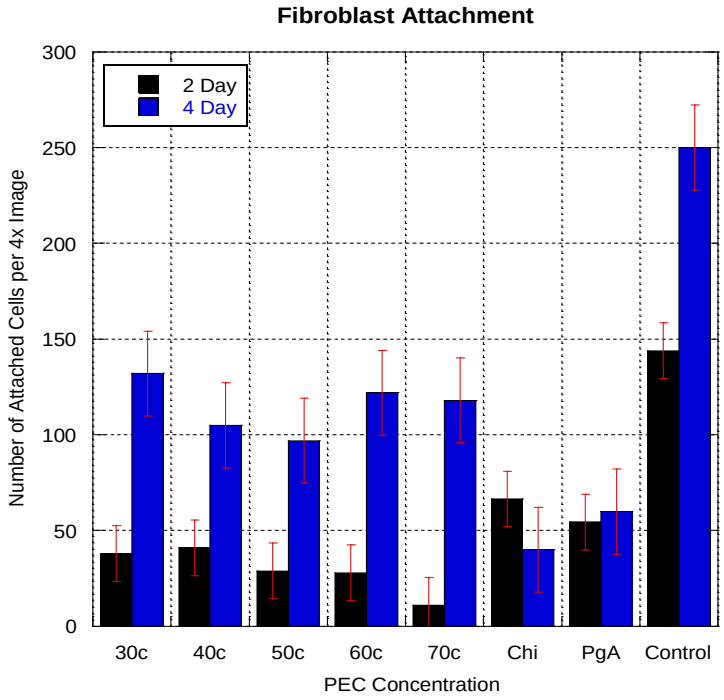


Figure 8: Fibroblast Cell Attachment after 2 and 4 days

All PEC concentrations exhibited similar viability results and all materials were statistically different (ANOVA of $p < 0.05$ followed by Tukey's test for multiple comparisons) from their respective controls except for the 2 day 50c. As seen in Figure 9, the fibroblast cells were mostly unviable on the material but the majority of viable cells were found in clumps. Live cells on the material were unhealthy due to their round appearance. This is the desired fibroblast viability profile for an anti-adhesive material. These fibroblast viability results paired with material data resulted in 30c, 50c, and 70c

being eliminated from future consideration as a PEC for adhesion prevention. All studies conducted from this point on would only use the 40c and 60c.

Theoretically, the Chi-PgA PEC will naturally degrade inside the body into its base components of Chi and PgA [27]. Hence, fibroblast viability was observed on both pure compounds in order to determine the behavior of the cells while the material is degrading. Once again, a low viability of these cells is desired to ensure anti-adhesive properties. The pure Chitosan had higher viability of fibroblast cells than that of pure PgA at both time points. Both Chi and PgA were also seen to have unhealthy cells because of their round appearance. Cell viability on PgA was statistically different from the Control while cell viability on Chi was not (ANOVA of $p > 0.05$). The pro-adhesive phenomenon of high fibroblast viability on Chi can be mitigated by the low fibroblast viability on PgA if a PEC of low Chi concentration is used.

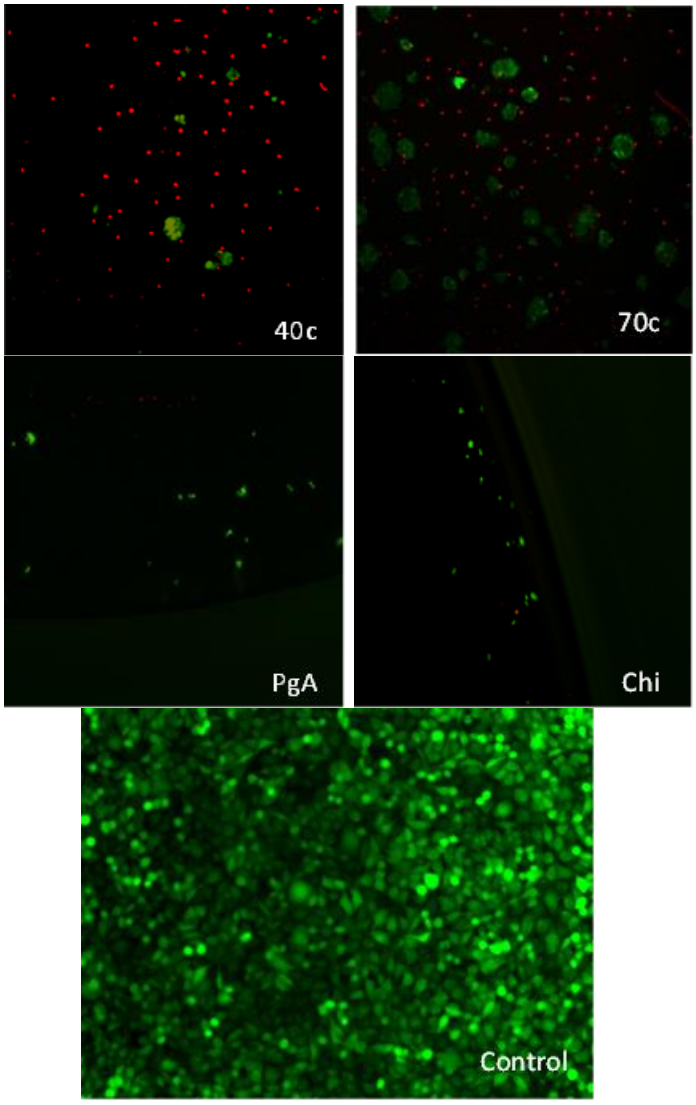


Figure 9: 4 day Fibroblast Images at 4x magnification.

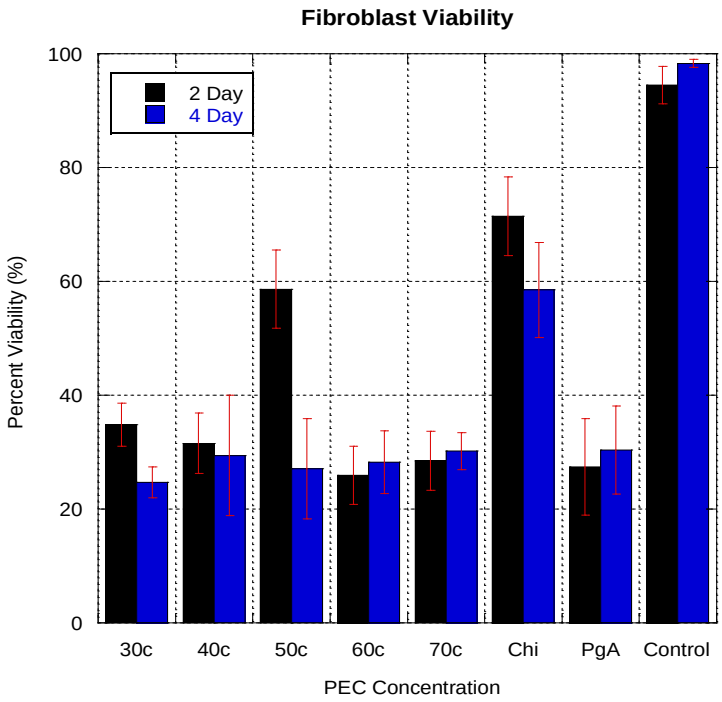


Figure 10: Fibroblast Viability after 2 and 4 days

3.5 Cellular Toxicity

The cellular toxicity experiment was aimed to determine whether the previous fibroblast viability study was due to the material creating a toxic environment or due to the material being a poor surface for cells to grow on. If the material created a toxic environment, it would cause unintended damage to other parts of the abdomen when implanted. The toxicity of the material was examined through comparison of the cells on the 1 µL dot of material versus cells not on the material. The live cells on the tissue culture plastic outside the PEC dot were all alive and healthy while the cells on the dot exhibited similar properties to that of the previous study, mostly dead but live cells seen in clumps. This shows that the material does not induce a toxic response in the body but only inhibits viability when cells are directly attached to it.

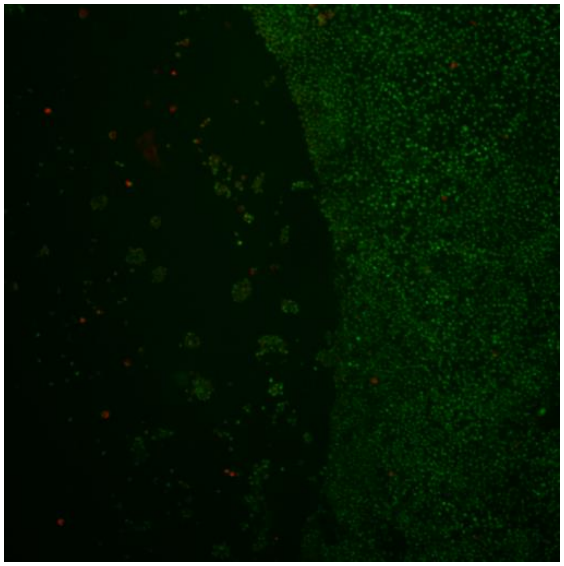


Figure 11: 4 day 40c Images at 4x Magnification

3.6 Macrophage Cell Viability

Macrophage viability studies were also conducted to test for the anti-inflammatory properties of the material. Macrophage cells release factors such as TNF-α which cause an inflammatory response; hence a low viability of these cells on the material would be consistent with an anti-inflammatory material property. There are two types of macrophage cells, M1 and M2. Generally speaking, M1 is indicative of an inflammatory response while M2 is telling of an anti-inflammatory response. In order to cover both types of macrophage cells, half the cells were treated with LPS to mimic the M1 variety while non-LPS treated cells represented non-activated macrophage cells [28]. On the PECs there were a greater abundance of LPS treated macrophages while on the pure substances there was an equal attachment of both cell types, as seen in Figure 12. Furthermore, there was large overall attachment on the Chi, low attachment on the PgA, and intermediate attachment on the PECs. Furthermore, the control had similar low

attachment to that of the PgA. Only the Chi LPS, Chi No LPS, and 60c LPS were significantly different (ANOVA of $p < 0.05$ followed by Tukey's test for multiple comparisons) from their respective controls. This provides evidence of inflammation being more likely on the PECs and Chi compared to that of the control and PgA. However, the secretions must be directly tested to be sure.

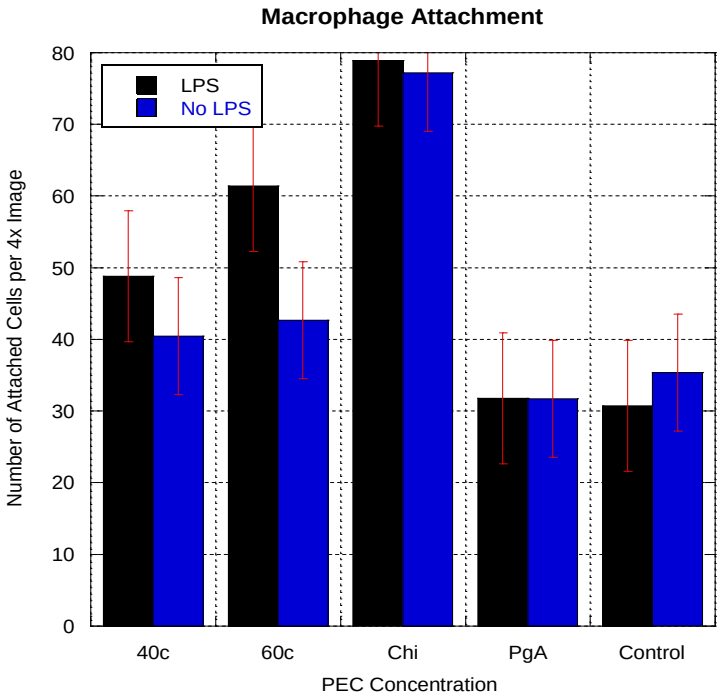


Figure 12: Macrophage Cell Attachment after 8 hours

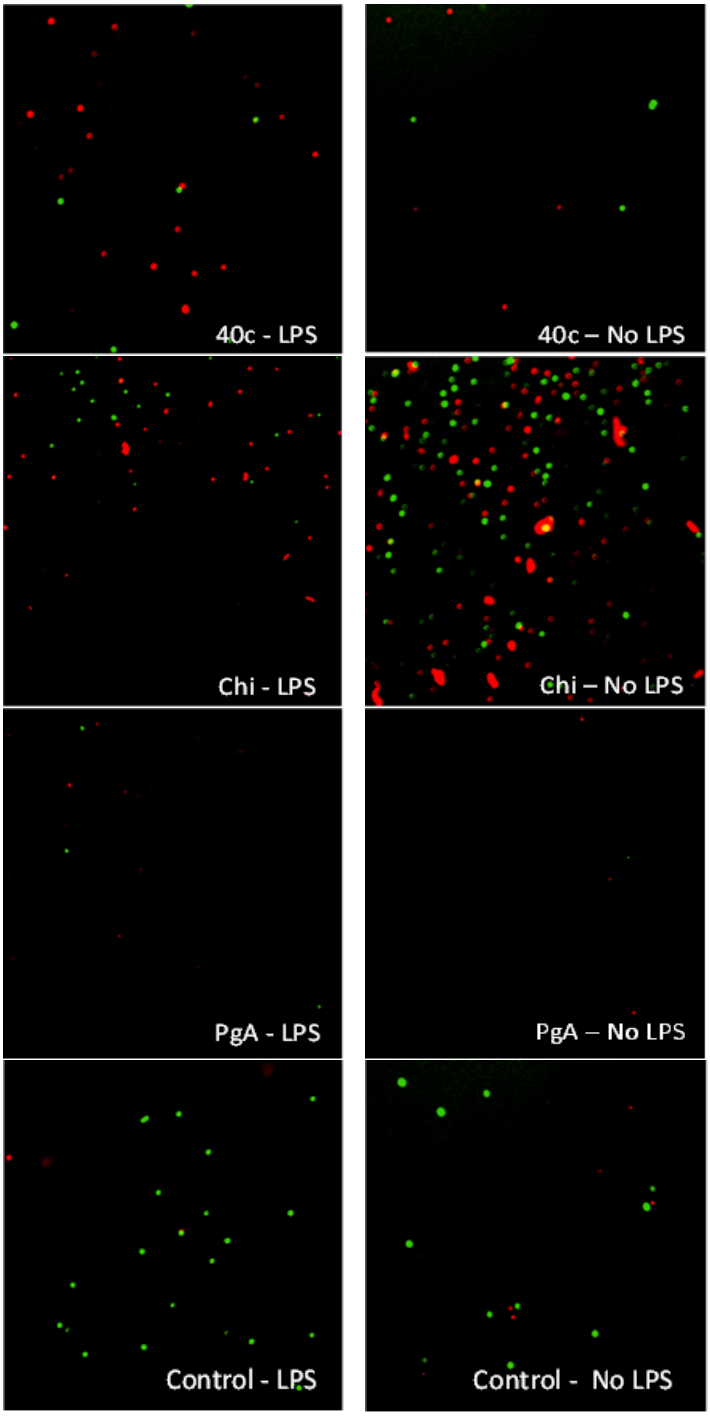


Figure 13: 8 Hour Macrophage Images at 4x magnification

Low macrophage viability, both LPS treated and non-LPS treated, was seen on all materials. All materials were statistically different from the control (ANOVA of $p < 0.05$

followed by Tukey's test for multiple comparisons) however, there was no statistically significant difference (ANOVA of $p > 0.05$) between LPS and non-LPS treated cells on the same material. Even though there was higher attachment of most materials compared to that of the control, the viabilities were low indicating that cells initially attached and then died. The low viability of all macrophage cells on both materials indicates that the cells will not be able to secrete inflammatory factors such as TNF-α. By this mechanism, both materials have decreased levels of inflammation. Furthermore, this anti-inflammatory response continues while the material is degrading because of the low viability on the pure substances of Chi and PgA.

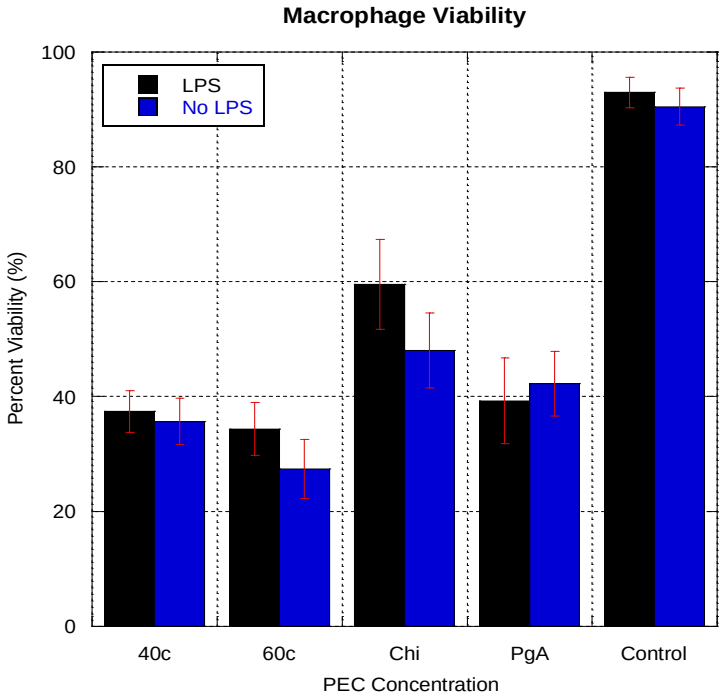


Figure 14: Macrophage Viability after 8 hours

3.7 TNF-α Quantification

To further understand the inflammatory properties of the material, the amount of TNF-α, an inflammatory cytokine secreted by macrophage cells, was quantified via ELISA [29]. A high level of secretion would indicate an inflammatory response while a low secretion, which is desired, would indicate a reduction in inflammation. All materials displayed lower secretion than the control, consistent with decreased inflammation. However, the differences between each condition and the control were not statistically significant (ANOVA of $p > 0.05$). Furthermore, LPS treated macrophage cells were expected to have a higher amount of secretion of TNF-α than non-LPS treated cells because LPS treated macrophages are representative of an inflammatory response while non-LPS treated macrophage are representative of an anti-inflammatory response [28]. This phenomenon, while observed in most cases, was also not statistically significant (ANOVA of $p > 0.05$).

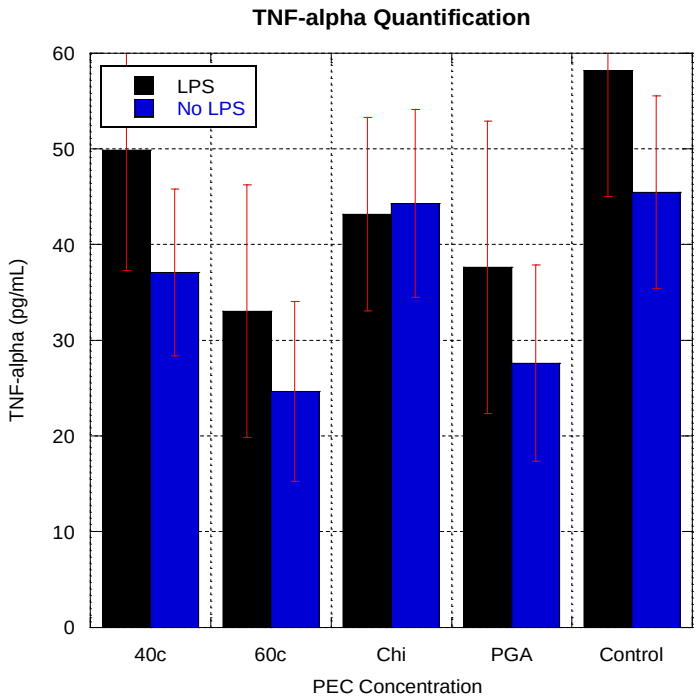
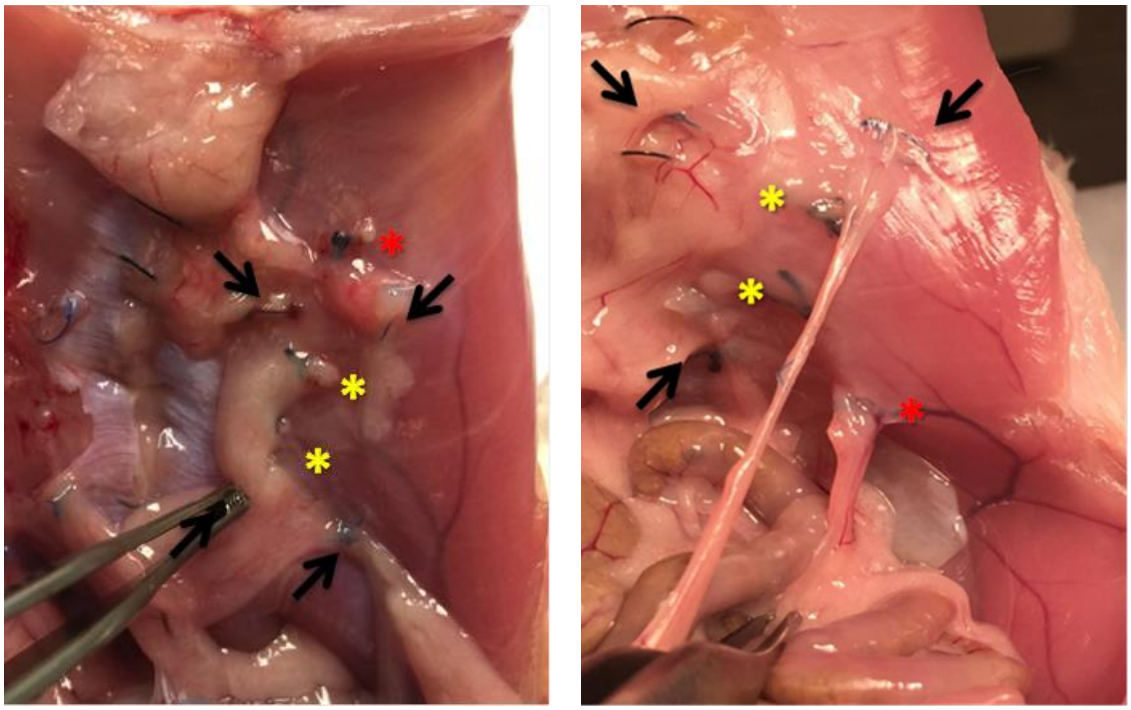


Figure 15: TNF-α Quantification

3.8 In-vivo Study

Preliminary in-vivo studies were conducted to explore the adhesion prevention potential of the PEC, the effect of different implantation times, and the effect of different implantation thicknesses. Implantation times of 1 week and 2 weeks were tested with thicknesses of 195 µm and 260 µm yielding four total experimental conditions. Buttons were analyzed for signs of adhesions after 1 and 2 weeks of material implantation. The buttons were used as controls to mimic adhesion formation inside the body. Hence, the control buttons not covered with material would ideally form adhesions while experimental buttons that were covered by the material would ideally not form adhesions. 3 out of the 4 control buttons formed adhesions, the lone exception being the 2 week 260 µm condition.

The 195 µm sample film at 1 week did not prevent adhesions in the experimental buttons but did prevent adhesions at 2 weeks. Furthermore, at both time points the material was dislodged from its initial location but the sutures were still intact in the abdominal wall. The material was found slightly degraded, seen by a reduction in size, and covered in proteinaceous material in the abdomen. Finally, all corner sutures were covered with adhesions.

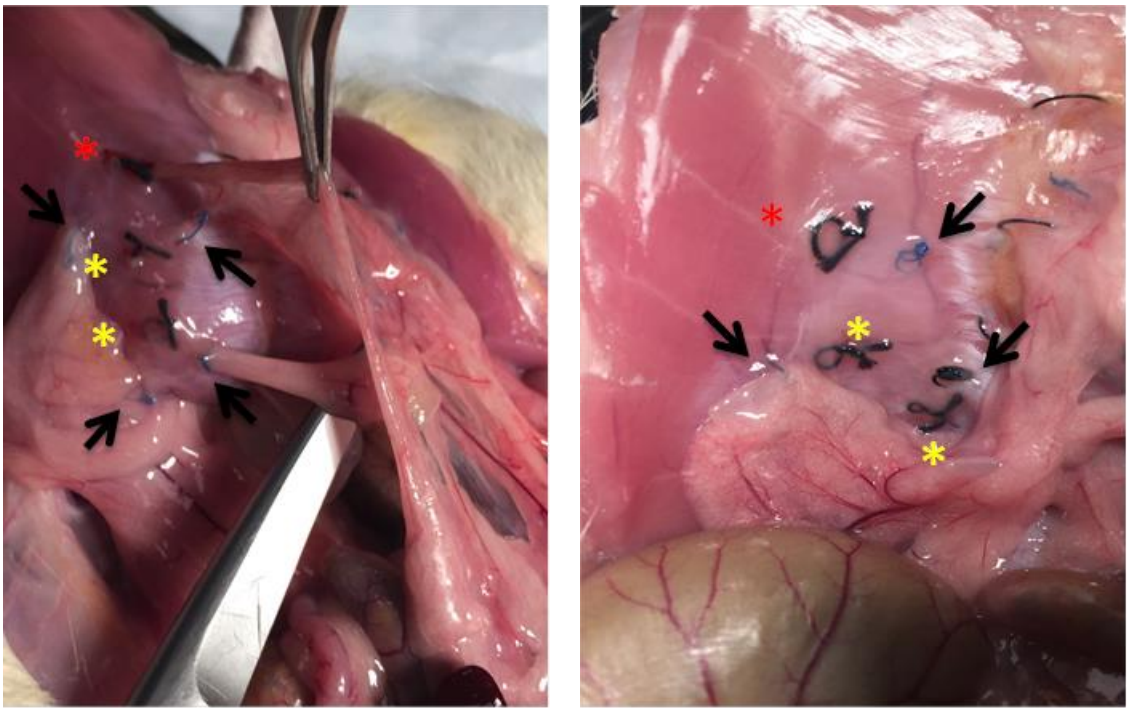


Red Asterisk (*) - Control Button
Yellow Asterisk (*) - Experimental Buttons
Black Arrow (→) - Corner Sutures

Figure 16: 195 µm 40c PEC at 1 week (left) and 2 week (right)

The 260 µm sample film at both time points prevented adhesions in the experimental buttons. The 1 week film was dislodged, degraded, and covered in proteinaceous material similar to the 195 µm film. However, the 2 week film was walled

off by the peritoneum. Finally, all but one of the corner sutures were covered with adhesions.



Red Asterisk (*) - Control Button
Yellow Asterisk (*) - Experimental Buttons
Black Arrow (→) - Corner Sutures

Figure 17: 260 µm 40c PEC at 1 week (left) and 2 week (right)

The PECs adhesion prevention efficacy depended on both material thickness and implantation time. The 195 µm material was only anti-adhesive at 2 weeks, demonstrating a time dependency. Furthermore, the 1 week implantation time only prevented adhesions for the 260 µm thickness material, indicating a thickness dependency. Hence, using a thicker material for a longer implantation time would be the most effective in preventing adhesions.

Summary of Buttons and Adhesions for In-Vivo Study					
		1 week		2 week	
	Control	195 µm	260 µm	195 µm	260 µm
# of Buttons	4	2	2	2	2
# of Adhesions	3	2	0	0	0

Table 1: Summary of Buttons and Adhesions for In-Vivo Study

Section 4: Discussion

The goal of these studies was to characterize a Chi based PEC for its material, anti-adhesive, and anti-inflammatory properties. Material properties were analyzed by conducting absorption, degradation, and mechanical testing. Anti-adhesive properties were analyzed via fibroblast viability testing. In addition, anti-inflammatory properties were characterized by quantifying both macrophage viability and TNF-α secretion. Finally, the true efficacy of the material was tested through preliminary in-vivo studies varying implantation time and material thickness.

As seen through the absorption testing results, all films exhibited similar characteristics. While the absolute changes in mass were not the same, mass increase in all films was evident within the first hour of incubation. In addition, the absolute changes in mass for the 40c, which was ultimately used in the in-vivo studies, were on par with that of Seprafilm[22]. The data was then normalized to the first hour to confirm that the PECs stay relatively constant in mass after the first hour. In addition, film diffusion properties were characterized as being less fickian. This means that the water penetration rate is significantly lower than the polymer chain relaxation rate, once again proving that most of the change in mass occurs within the initial time points [23]. Films with less overall swelling were desired in order to prevent large expansion of the film which could result in interference or moving of components in the abdomen.

Degradation studies showed that the film initially increased in mass after 3 days and began decreasing in mass and degrading after 14 days of incubation. It is important to note that while the studies reflected material changes, the in-vivo degradation profile will probably be faster due to the activity of a live organism. This profile was confirmed by

the in-vivo results showing that after 7 days and 14 days, the material post implantation was smaller than the original material, but still intact. Furthermore, adhesions begin to form approximately 7 days after the initial trauma (surgery), making the degradation profile of this material fit the disease because the material will be in tact long enough to prevent the adhesion and degrade in a timely fashion [12,30].

Mechanical properties of all films were relatively the same. Because low mechanical strength was a previously documented issue of current adhesion prevention products on the market, there was a need for a stronger material to avoid the same issue. The low mechanical properties of these other products resulted in ripping and tearing of the material in-vivo. All PECs were in range of both soft tissue and other known biomaterials [25,26]. Furthermore, the material was found to be stronger than other known adhesion prevention materials known for ripping, giving it ample mechanical strength to prevent ripping and tearing from occurring [12,22].

While most of the fibroblast cells on the PEC were dead and singular, live cells on the PEC were found mostly in clumps which is a documented property of fibroblast cells on biomaterials [31]. A majority of the PEC concentrations were shown to have low viabilities of fibroblast cells at both the 2 day and 4 day time points showing the anti-adhesive properties of the PEC. Pure Chitosan and pure PgA were also tested to see how fibroblast cells react to the material as it degrades since theoretically, the material should degrade into its base components. There was a high viability seen on the Chitosan which can be counteracted by a low viability seen on the PgA if a low Chi concentration is used, resulting in anti-adhesive properties as the material degrades. Chi and PgA conditioned media could also be used to understand cellular reactions to the degrading PECs.

Attachment of fibroblast cells was also inhibited on all materials at both 2 and 4 days. From the cellular toxicity study, it was determined that the material inhibits cell viability by acting as a poor surface for the cells to grow on and not by creating a toxic environment.

Macrophage cells were treated with LPS to mimic activated macrophage cells while cells that were not treated with LPS represented non-activated cells. Low viability was seen across both concentrations of the material which was representative of an anti-inflammatory response. In addition, low viability was seen on the pure substances as well which shows the anti-inflammatory properties of the material as it degrades. Furthermore, there was a large variation among the number of cells seen on each condition. Pure Chi had the most cells, the PECs in the middle, and pure PgA barely had any. Similar experiments at earlier time points must be conducted in order to truly understand this discrepancy.

The TNF-α secretion studies were designed to further understand the potential anti-inflammatory nature of the PEC. While the results showed that the materials reduced the macrophage cell's secretion of TNF-α, there was a large amount of variability across all conditions and hence while trends were apparent, no statistical significance was observed. Furthermore, combining the macrophage viability results with the TNF-α quantification, it showed the even though the PECs had a lower number of viable cells compared to that of the control, they released close to the same amount of TNF-α as the control. While each cell on the PEC secreted more of the cytokine, the overall inflammatory response experienced was still lower than the control and hence, was lessened because of the presence of the PEC. One of the possible causes of the large

amount of variability is the fact that the macrophage cells are primary. From each extraction of the primary cells, the cells could vary depending on the animal. Furthermore, a different cytokine could be tested to deduce anti-inflammatory PEC properties. For example, IL-10, an anti-inflammatory cytokine, could be measured to determine whether the material simply reduces inflammation or is actually anti-inflammatory [29].

The in-vivo data suggested positive results with respect to the ability of PEC films to prevent adhesions. Using both a thicker material and a longer implantation time, adhesions were better prevented from forming compared to a thinner material and shorter implantation time. However, all corner sutures that were meant to keep the material in place, also formed adhesions. The act of suturing in the material behaves as a trauma that also causes adhesions. There is a need for a way to better anchor the material inside the abdominal cavity. One possible solution would be to use an absorbable tack that would initially keep the material in place but eventually degrade in a predetermined amount of time. This would keep the material anchored long enough to prevent the adhesion but degrade before adhesions can form on the anchoring method. Another method would be to use a biocompatible and biodegradable glue [32]. This method would completely avoid causing a trauma resulting in no adhesion formation. Hence, while the PEC is seen to be efficacious in preventing adhesions, there are still other severe side-effects of the material implantation to mitigate.

Section 5: Conclusions

Through analysis of the material properties (absorption, degradation, and mechanical) of the Chitosan based PEC, it was seen that most of the PEC concentrations had sufficient material properties to be used as an implantable biomaterial. Furthermore, through analysis of the PEC for fibroblast viability, the PEC demonstrated anti-adhesive properties through a low viability. From the fibroblast studies paired with the material properties, all PEC concentrations were eliminated except for 40c and 60c because they displayed the most ideal material and anti-adhesive properties. The remaining concentrations demonstrated anti-inflammatory properties as a result of the low macrophage viability. The ELISA strengthened the anti-inflammatory characterization of the PECs by having all PEC concentrations have lower secretions of TNF-α than that of the control. Furthermore, fibroblast viability, macrophage viability, and ELISA experiments on the pure Chi and pure PgA showed anti-adhesive and anti-inflammatory properties as the material degrades.

The initial in-vivo studies showed that the material is efficacious in preventing adhesion formation inside the peritoneum. Adhesion prevention becomes more effective with a longer implantation time and a thicker material. While there may be an optimum time and thickness, further experimentation must be done in order to truly determine this. However, the current method of suturing in the material resulted in adhesions on a majority of the suture spots, inadvertently causing what the material was aimed to prevent. Other methods should be explored on how to anchor the material into the peritoneal cavity such as a fibrin glue or absorbable tacks.

Section 6: References

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