Developing Biologically Relevant Artificial Substrates for Bioadhesive Testing

By

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Thesis

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Abstract of Developing Biologically Relevant Artificial Substrates for Bioadhesive Testing

by Zakhar Lyakhovych, ScM., Brown University, May 2023

Bioadhesion, defined as the phenomena of adherence between natural tissue and synthetic materials, is an important property with relevance in a wide range of biomedical fields such as drug delivery and implantable devices. Understanding the bioadhesive properties of polymers and how to control them has significant implications for both oral and topical drug delivery. Current bioadhesive testing methods vary significantly across the field, utilizing a wide range of approaches to studying several different aspects of bioadhesion. With macroscopic drug delivery fracture theory is the dominant force and can be quantitatively measured. Current approaches to testing fracture theory often use cadaver tissue. This has limitations with viability, with tissue decomposition beginning almost immediately, and biological relevance, with moisture and tissue-to-tissue variation creating high variability in readings. By creating an artificial substrate that is meant to mimic a physiological environment, variability in bioadhesive measurements can be consistently reduced and provides a comparison across runs that cadaver tissue would not show as well as allowing extensive testing without the excessive use of ex-vivo models.

Chapter 1: Background

Bioadhesion generally refers to the adherence of a natural or synthetic material, usually a polymer, to a biological substrate[1,2]. This phenomenon is very similar to regular adhesion in terms of the effects between the two surfaces but the underlying mechanisms vary significantly between the two. Depending on the type of polymer and the type of tissue there are a range of mechanisms that dictate the strength and nature of the bioadhesive interactions between the two surfaces. One unique difference in bioadhesion is the potential for specific vs nonspecific interactions. While normal adhesion is nearly always nonspecific, in bioadhesion specific recognition of key markers can occur creating significant changes that have physiological significance[1].

Some of the parameters that govern the bioadhesive forces between two surfaces are polymer characteristics, substrate composition, adhesive Layer composition, and testing parameters. Bioadhesive bonds are usually present in the form of weak chemical bonds or in physical bonds[3]. Chemical bonds will primarily be in the form of secondary bonding such as electrostatic interactions, hydrogen bonding, or hydrophobic interactions between nonpolar groups. Given the high prevalence of charged groups in the mucosal layer such as hydroxyl and amino groups and the hydrophobic nature of the skin, secondary chemical bonds play a large role in the bioadhesive properties of a material. Physical bonds occur as a result of the physical entanglement of the substrate and the adhesive material[4].

Bioadhesive Theories

Wetting Theory

This is generally used to assess the performance of liquids or other low-viscosity materials that have a tendency to spread[5]. It is most closely related to the contact angle of a liquid on a surface. As a general rule liquid-surface interactions with a low contact angle will have a high measure of adhesion between each other[1,6].

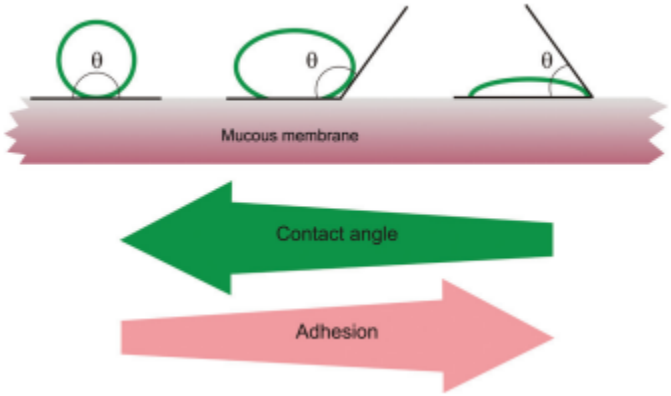

Figure 1: Diagram of the relationship between contact angle and adhesion in the wetting theory of bioadhesion[5].

Diffusion Theory

This theory relates heavily to the physical bonds that occur between a surface and a bioadhesive material. This creates a semi-permanent physical bond with the polymer chains and chains on the surface of the substrate. The physical bonding can be affected by an array of parameters such as the diffusion coefficient and contact time[7]. The diffusion coefficient is dependent on the polymer type, molecular weight, and cross-link density. Higher molecular weight polymers generally display stronger interpenetration of the adhesive layer but only up to an extent with large, bulky, highly cross-linked polymers having less interaction[8].

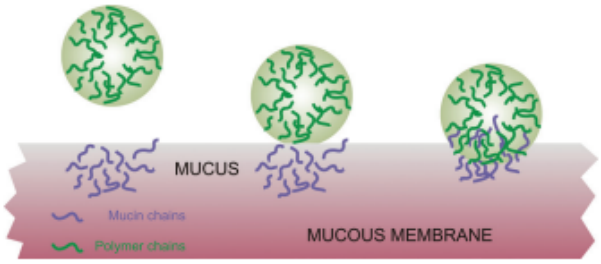

Figure 2: Schematic illustrating interactions between a polymer and the mucin chains of a mucous membrane[5]

Electrostatic Theory

When a material comes in contact with the network of molecules on the surface of the substrate a transfer of electrons takes place due to differences in electrical charge and structure. This can result in the formation of an electronic double layer at the boundary between the two surfaces. This leads to an electrostatic interaction between the two surfaces. This double layer resists separation and is discharged upon separation.

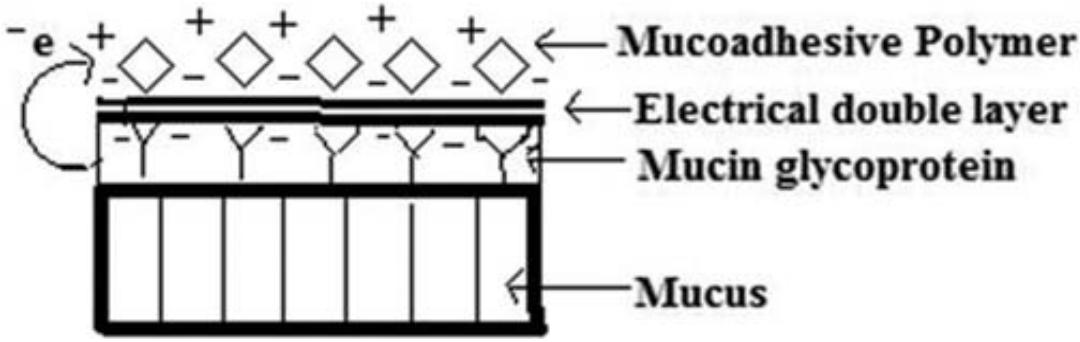

Figure 3: Schematic illustrating the electrical double layer that is created between a positively charged polymer and the negatively charged mucin substrate[9].

Fracture Theory

The fracture theory of adhesion considers the maximum tensile force that is developed during the separation of the material and substrate. By focusing on tensile force this theory

does not need the interpenetration of the two materials to occur. This gives an advantage since it allows the testing of polymers that don't necessarily have the flexible chains required for creating the physical entanglements required for diffusion theory. An additional advantage is that it can be used to test substrates that don't necessarily have complex networks and strands giving variability in testing substrates.

Bioadhesive Testing

Researchers have been working on improving the bioadhesion of various drugs and drug delivery systems for several decades. Shortly after bioadhesion was first investigated simple ex-vivo tensile testing followed, using excised organs from recently euthanized animals and volumes of water used to measure the detachment force of a pill on sections of the digestive tract[10]. Testing methods have improved significantly since that time and further research has shown strong correlations between the detachment force in vitro and the time it takes for passage through the gastrointestinal system[11].

These methods have the drawback of needing to have fresh animal tissue provided and provide a limited time for testing using a fresh sample, given that autolysis begins to occur in tissue minutes after death. There have also been concerns raised with the applicability of ex-vivo tissue testing compared to in-vivo results, which are difficult to measure directly[10]. They also limit the comparative value of different studies with different researchers using a range of variations and setups. Some researchers have made steps to create a more systematic method of testing bioadhesion, with the majority of the focus being on mucoadhesion[12].

The focus of these studies is often focused on fracture theory and the force of

detachment. However, while researchers may use similar methods to measure bioadhesion there are disagreements as to what are the most accurate predictors of bioadhesion, work of adhesion, or detachment force. These researchers did valuable work investigating separate parameters of bioadhesive measurements such as contact time, detachment speeds, and force applied to set a framework for creating a representative system.

Specific Aims

This project consisted of 3 consecutive investigations building off of previous bioadhesive research done at the Mathiowitz lab.

Aim I –Verify existing mucoadhesive substrate used to mimic intestinal tissue through model polymers

Investigate whether the artificial mucin substrate follows generally accepted trends of bioadhesion

Aim II – Investigate the bioadhesive effects of hydrophobic drug loading on chitosan and PCL using a mucoadhesive substrate

Verify if increasing drug loading or processing method changes can be detected by the substrate on two common biomedical polymers.

Aim III – Create a skin-mimicking substrate that could be used to test the bioadhesion of transdermal delivery systems, including water-soluble polymer

Expand the applicability of artificial substrates and have the ability to test water-soluble polymers.

Chapter 2: Methods

This film-casting method was developed and used to create consistent samples and have similar morphology across multiple samples. Chloroform ($CHCl_3$, Sigma), dichloromethane (CH_2Cl_2, Spectrum), and glacial acetic acid (CH_3COOH, Spectrum) were used as the solvents. High molecular weight chitosan (Sigma-Aldrich) and CAPA 640 PCL (Perstorp Holding AB, Molecular Weight: 37 kg/mol) were the primary polymers used. Curcumin (Sigma) was used for drug loading.

Film Casting

Polystyrene

For polystyrene samples 200 mg of polystyrene of various molecular weights (2,500 Da, 45,000 Da, and 125,00 Da) was measured on a high precision analytical lab scale (0.001g) and added to a 20 mL scintillation vial with 2 mL of dichloromethane. The solution was vortexed until fully dissolved. The solution was then poured into a 62mm glass petri dish making sure the solution was evenly spread over the dish. The dish was left under a fume hood for 24 hours. After 24 hours the dichloromethane evaporates leaving behind a solid film. The film was removed using tweezers.

Polycaprolactone

For pure PCL samples, 250 mg of PCL was measured and added to a 20 mL scintillation vial with 2 mL of chloroform. This was added to a 20mL scintillation vial. To solubilize the polymer the solution required 3 rounds of vortexing and sonication until complete dissolution occurs. The solution was then poured into a 62mm glass petri dish making sure the solution was evenly spread over the dish. The dish was left under a fume hood for 24 hours. After 24 hours

the chloroform evaporates leaving behind a solid film. The film was removed using tweezers. For curcumin-loaded samples, a similar procedure was used. To keep a constant amount of material, ensuring consistent thicknesses of the films the same total amount of material was added to each 20mL vial. Loaded films were prepared with different amounts of curcumin at 1%, 5%, 10%, 30%, and 50% of the total mass of 250mg. The two were combined with 2 mL of chloroform and were solubilized with the procedure above. The was then poured into a 62mm glass petri dish making sure the solution was evenly spread over the dish. The dish was left under a fume hood for 24 hours. After 24 hours the chloroform evaporates leaving behind a solid film. The film was removed using tweezers.

Chitosan

For pure chitosan samples, 200mg of chitosan was measured and added to a 20 mL scintillation vial along with 20 ml of 1% acetic acid solution. To solubilize the chitosan the solution was vortexed for 3 minutes and then placed into a sonicator for 10 minutes. The solution would be revortexed and placed in the sonicator until all solids had dissolved usually after 2 cycles. The 20mL of solution was then poured into a 62mm glass petri dish making sure the solution was evenly spread over the dish. The dish was left under a fume hood on a hot plate set at 60 °C for 24 hours. After 24 hours the acetic acid solution evaporates leaving behind a solid clear film. The film was cut around the circumference with a razor blade and removed using tweezers.

For curcumin-loaded samples, a similar procedure was used. To keep a constant amount of material, ensuring consistent thicknesses of the films the same total amount of material was added to each 20mL vial. Chitosan for 1%, 5%, 10%, 25%, and 50% was measured to be 198 mg,

190 mg, 180mg, 150mg, and 100 mg respectively. Curcumin for 1%, 5%, 10%, 25%, and 50% was measured to be 2mg, 10mg, 20mg, 50mg, and 100 mg respectively. The two were combined with 20mL of 1% acetic acid and were solubilized with the procedure above. The 20mL of solution was then poured into a 62mm glass petri dish making sure the solution was evenly spread over the dish. The dish was left under a fume hood on a hot plate set at 60 °C for 24 hours. After 24 hours the acetic acid solution evaporates leaving behind a solid clear film. The film was cut around the circumference with a razor blade and removed using tweezers.

Processing

For processing films removed from the Petri dishes were cut in even halves with identical semicircles of films being created. The first processing step is referred to as the first press procedure. Films are folded into a cylindrical dye and placed into a hand press (Specac Inc). The hand press was set to 10,000 lbs of pressure and the samples were kept under constant pressure for 15 minutes. This ensures each sample forms a consistent circular disk. After 15 minutes pressure is relieved and the disk is carefully extracted from the dye.
After the first press creates a disk designated samples were set aside to be heat pressed. A temperature-controlled hydraulic press was used (Carver, Wabash, Indiana) to apply pressure and heat simultaneously. The press was first set to the desired temperature and allowed to reach the target temperature. The disks from the first press were placed between two metal plates and placed on the bottom plate of the press. The press was then activated, moving the heated plates together with the sample between them. Samples were exposed to the target temperature without any pressure for 10 minutes. After 10 minutes the pressure component

was activated and the press applied 20,000 lbs of force to the samples for 5 minutes. The heating element was deactivated and the press was returned to normal temperature. The pressure was then removed, the plates were separated and the samples were retrieved.

Mucin Substrate

The mucin substrate consisted of high-gelling temperature agarose and mucin from the porcine stomach (Sigma-Aldrich). A standard 7% w/v mucin substrate was made by measuring 700 mg of mucin and adding it to 10 mL of DI water in a 20 mL scintillation vial. The solution was placed on a circular mixer and mixed for several hours until the mucin was dispersed. Once the mucin was fully dispersed, 400mg of agarose was added to the vial. The vial was replaced on the circular mixer for one hour until fully dispersed. The dispersed mixture was loosely uncapped and placed in a microwave for 5 seconds. The cap was tightened and the mixture was shaken vigorously. This step was repeated two additional times. The vial was loosely capped a final time and placed in the microwave for 3 seconds. The vial was vigorously shaken once again. The substrate was poured evenly into 5 mini glass Petri dishes with a diameter of 22mm and immediately covered with parafilm to prevent dehydration of the gel. These were placed into the heating block of the texture analyzer prior to testing to allow the temperature to reach 37℃. A 0.2% w/v artificial saliva mixture was created by weighing 20mg of mucin and adding it to 10 mL artificial saliva in a vial. This mixture was placed on a circular mixer until fully dispersed. Prior to running the test, 100ul of the 0.2% mucin in artificial saliva was pipetted onto each mini petri dish.

Skin Substrate

The skin substrate consisted of high-gelling temperature agarose and silicone oil. Initially, 400mg of high-gelling temperature agarose was added to a 20 mL vial. The vial was placed on the circular mixer for one hour until fully dispersed. 100 µL of silicon oil was added to the solution and vortexed.

This solution was immediately loosely uncapped and placed in a microwave for 5 seconds. The cap was tightened and the mixture was shaken vigorously. This step was repeated two additional times. The vial was loosely capped a final time and placed in the microwave for 3 seconds. The vial was vigorously shaken once again. The substrate was poured evenly into 5 mini glass Petri dishes with a diameter of 22mm and immediately covered with parafilm to prevent dehydration of the gel. These were placed into the heating block of the texture analyzer prior to testing to allow the temperature to reach 37℃. Prior to running the test, 100 µl of silicon oil was pipetted onto each mini petri dish.

Sample Preparation

Samples were placed on a metal plate that was cleaned with ethanol. A 5mm punch was then used to cut discs out of the samples. These were arranged to have the side that was in contact with the petri dish being facedown. A 4mm punch was used similarly on a double-sided adhesive with the cutting edge on the adhesive side. The disks of adhesive were fixed to the flat end of the nails with the covered side of the adhesive remaining. When all nails were prepared the adhesive coating was removed and the nail head with adhesive was pressed down onto the

5mm disks, with care taken to center the polymer disks on the adhesive center, and held for 10 seconds. The nail was then lifted and proper placement and attachment were confirmed.

Testing Procedure

A TA.XT*plus* Texture Analyzer (Texture Technologies Corp., Scarsdale, NY/Stable Micro Systems, Godalming, Surrey, UK) was used with a 1 kN load cell for measuring the tensile forces. A standard bioadhesion test present in Texture Exponent software was used to conduct the test and set the testing parameters. The following parameters were utilized for all testing:

Pre-test Speed = 5.00mm/sec
Test Speed = 0.20mm/sec
Post-test Speed = 5 mm/sec
Applied Force = 2.50 g
Retraction Distance = 45.00 mm
Contact Time = 10.0 sec
Trigger Force = 2.5 g

A prepared sample was attached to the load arm with the nail head facing down. A mini petri dish with the substrate was then uncovered and hydrated appropriately. The sides of the dish were then taped down to the heating block. The petri dish would be centered visually underneath the prepared sample. Upon running a test care was taken to make sure the probe made contact with the substrate, taking care to avoid the outer diameter of the dish due to cohesive interactions causing the mucin to creep up the sides of the dish. After contact is made the probe holds for 10 seconds and then returns to a distance of 45mm above the substrate.

Analysis

A previously created macro to analyze peak amplitude and area under the curve was used to analyze the data. The macro converts the graphed results into a peak force and work done by adhesion measurement based on the two factors above. After selecting the macro an anchor was dropped in the software before the probe made contact with the surface. The macro run results were transferred to a separate results file. The data from this file was uploaded for statistical analysis into GraphPad Prism (San Diego, California).

Chapter 3: Results

Polystyrene with various molecular weights was used to test if the system would be able to detect a difference in bioadhesion between the samples as would be predicted. Polystyrene was chosen as a stable, non-degradable polymer with high intestinal uptake. The results of testing different molecular weights of polystyrene on mucus substrate and the summary of peak force and work done by adhesion are shown in Figure 4.

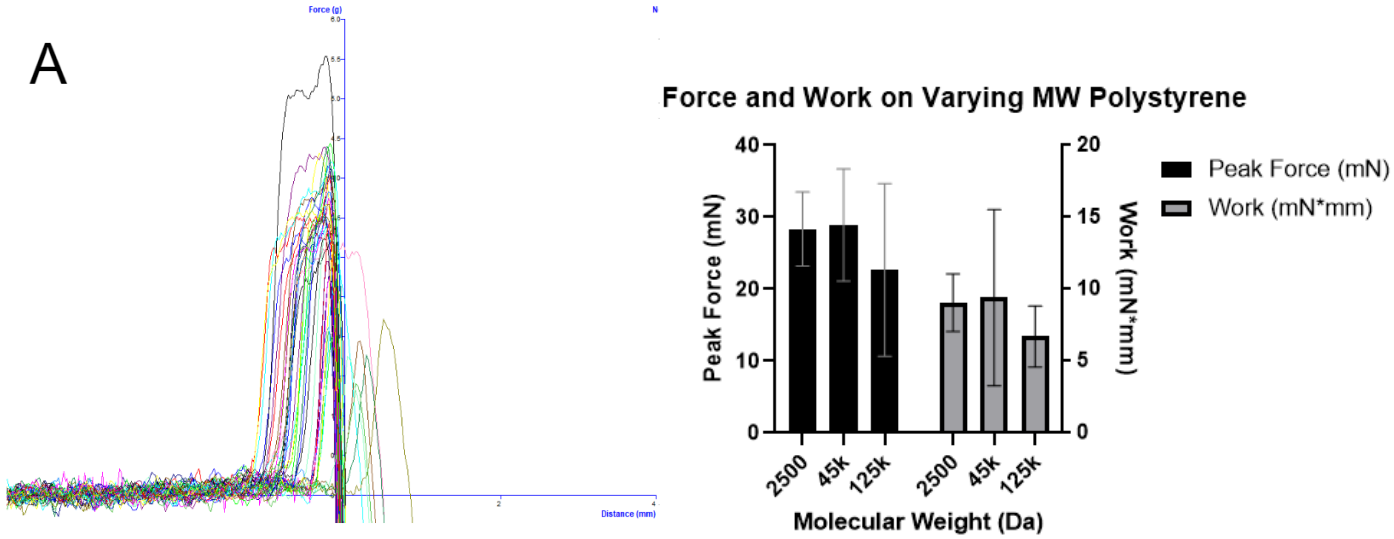

Figure 4: Trace of adhesive forces on a 5mm diameter probes with 2500, 45k, and 125k Da polystyrene (A), comparison between the peak force and adhesive work of three different molecular weight polymers (B)

The trace of the texture analyzer data can be seen in Figure 4A. The broad range of peak widths indicates there is high variation in the samples with no clear groupings being seen. The maximum peak heights can also be seen to have a high degree of variation with individual outliers being seen. The summary of the results in Figure 4B shows the overall results of the testing with peak force and work being measured. While it can be seen that there was a

moderate decrease in the peak force at 125k Da probe one-way ANOVA showed there was no statistically significant change between the groups for either peak force or work (p= 0.096 and p= 0.1170 respectively). This testing seems to indicate that bioadhesive changes can not be reliably recorded with this testing method and substrate.

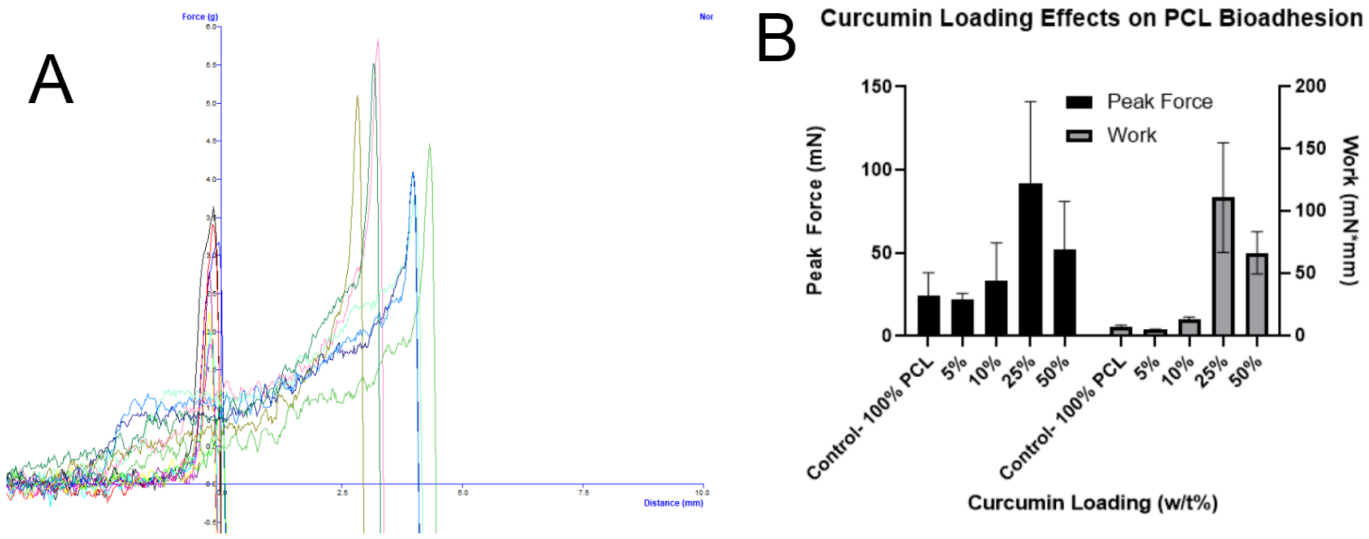

Figure 5: Trace of adhesive forces on a sample of 5mm diameter probes with film cast PCL at 0%, 5%, 10%, 25%, and 50% curcumin loading with the traces on the right side being from the 25% samples (A), comparison between the peak force and adhesive work of 0%, 5%, 10%, 25%, and 50% curcumin loading (B)

PCL was tested with increasing curcumin loading concentrations, up to 50% as well as 0% loading as a control. Looking at the trace seen in Figure 5A two groupings of graphs can be seen. The grouping on the left side consists of samples from the control, 5%, 10%, and 50% groups. These samples have sharp peaks and relatively low variation in the narrow width of the peaks. This indicates that there is rapid detachment of the probe from the polymer seen in the rapid drop-off of force. The grouping on the right shows prolonged muco-adhesion between the substrate and the probe which is shown in the prolonged force exerted on the probe even after

lifting off the substrate. In Figure 5B this prolonged mucoadhesion can be seen in the large increase in work, which corresponds to the increased area under the curve for the 25% samples which remains but to a lesser extent in the 50% sample but likely as a result of the higher peak force and not prolonged mucoadhesion. The samples also showed higher bioadhesion in both peak force and work for most samples when compared to polystyrene. ANOVA testing was conducted and showed that there were statistically significant differences between both peak force and adhesive work ($p<0.0001$ and $p<0.0001$ respectively) and indicating that bioadhesive changes as a result of drug loading can be measured and compared.

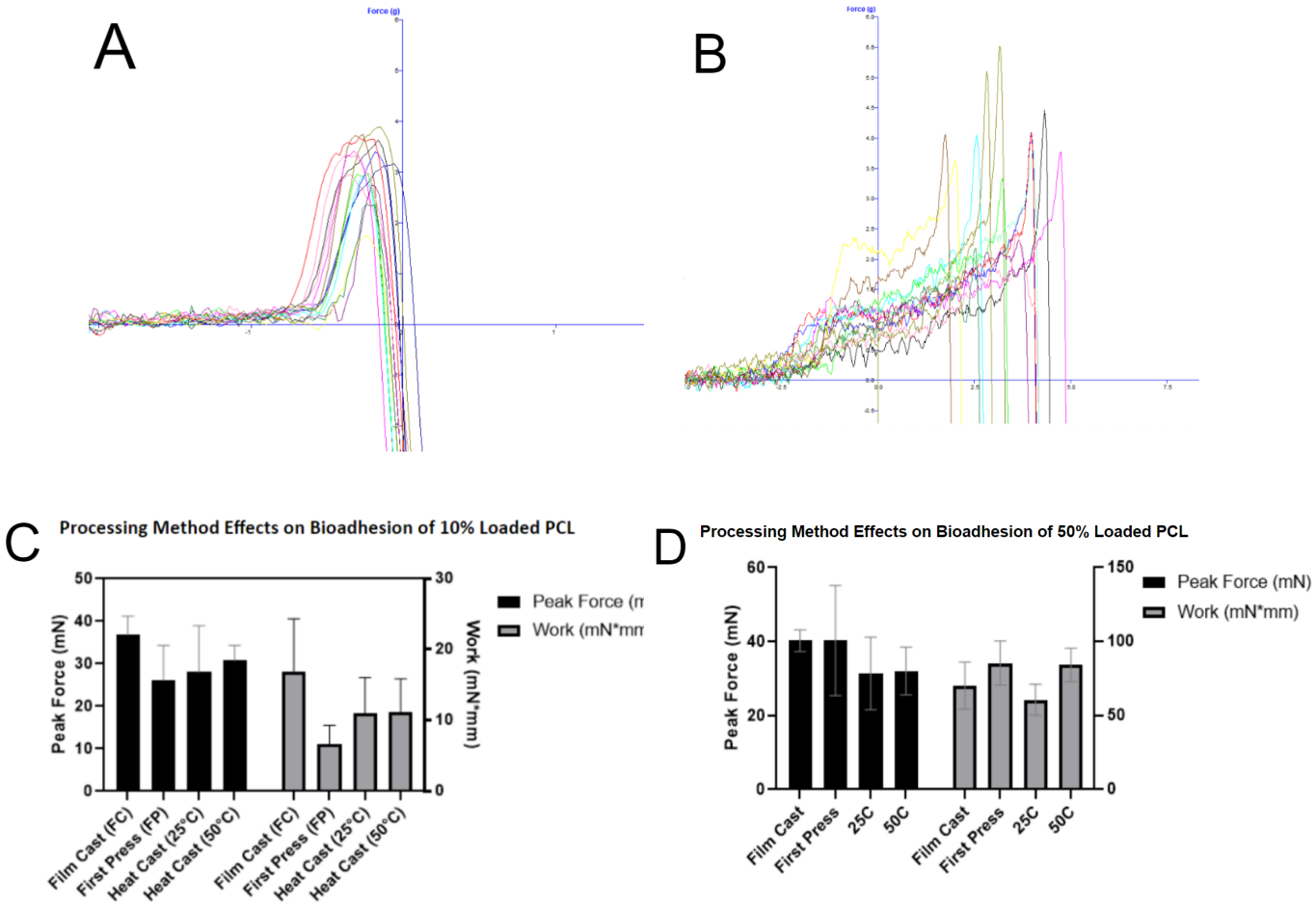

Figure 6: Trace of adhesive forces on a sample of 5 mm diameter probes with film cast, first pressed, 25℃, and 50℃ processed PCL loaded at 10% (A), trace of adhesive forces on a sample of 5 mm diameter probes with film cast, first pressed, 25℃, and 50℃ processed PCL loaded at 50% (B), comparison between the peak force and adhesive work of film cast, first pressed, 25℃, and 50℃ processed PCL loaded at 10% (C), comparison between the peak force and adhesive work of film cast, first pressed, 25℃, and 50℃ processed PCL at 50% (D)

Two different drug loadings for PCL were investigated to see the effects of processing on the bioadhesion of each loading. The trace seen in Figure 6A shows that all processed 10% samples showed rapid detachment while the trace seen in 6B shows prolonged mucoadhesion in the 50% samples as well as higher overall peaks for a number of samples. This is expected given the previous results that showed a trend that 50% would have higher adhesion than 10%.

The comparisons between the two different drug loadings showed different results with the film cast being the most adhesive at 10% (Figure 6C)and the first press having comparable bioadhesion in the 50% samples(Figure 6D). The work done by the 50% group shows a large increase in the scale of work done when compared to the 10% sample which is expected given the prolonged adhesion seen in the trace. ANOVA showed there were statistical differences between the peak force and work of adhesion in both the 10% ($p= 0.0407$ and $p= 0.0053$ respectively) and 50% sample ($p= 0.0085$ and $p<0.0001$ respectively) indicating that different processing effects have differing effects on the way they affect bioadhesion when loaded with hydrophobic materials potentially as a result of physical changes on the surface.

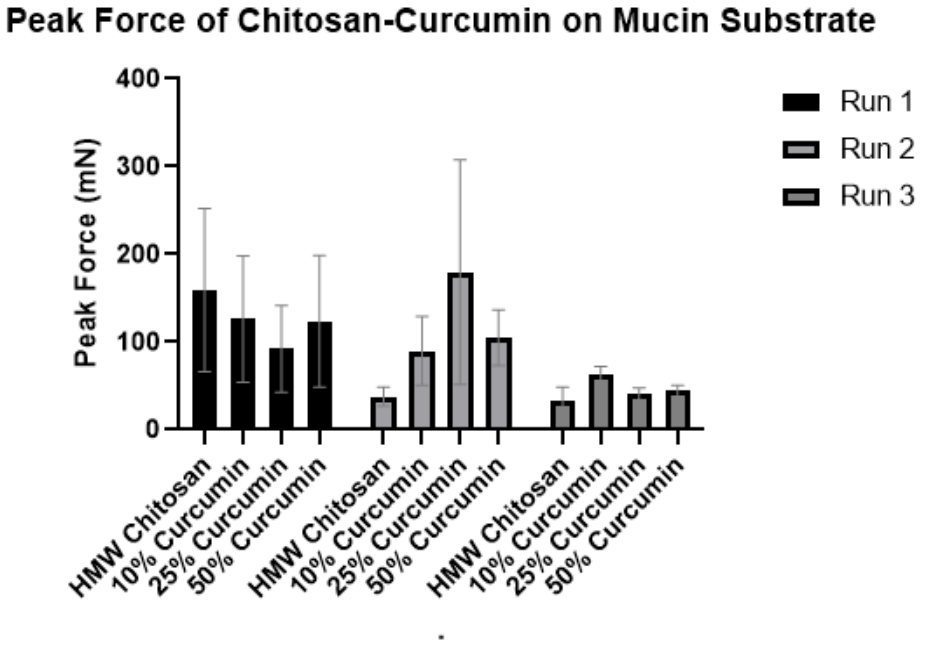

Figure 7: Three comparative runs of chitosan probes loaded with increasing curcumin concentrations (0%, 10%, 25%, 50%)

Chitosan was tested with the same mucoadhesive substrate as PCL with similar drug loading concentrations. When conducting tests with chitosan on substrate high variability was

seen when identical samples were tested. To confirm 3 separate runs (n=16 each) were conducted with 3 different concentrations of curcumin and control of high molecular weight chitosan. The averages of the peak forces for each run can be seen in Figure 7. The tests showed high variability both between different runs and within the runs themselves indicating that neither the substrates nor environmental conditions could have affected the results. ANOVA testing was done across the drug loading groups of different runs and showed statistically significant differences between all identically loaded probes apart from 10% (p=0.231). These results indicated that the mucoadhesive substrate was not compatible with polymers that have swelling characteristics. The probes, post-testing, were observed to have deformed disks that no longer had a flat consistent surface and demonstrated decreased structural integrity compared to untested samples, further indicating that swelling is compromising the results.

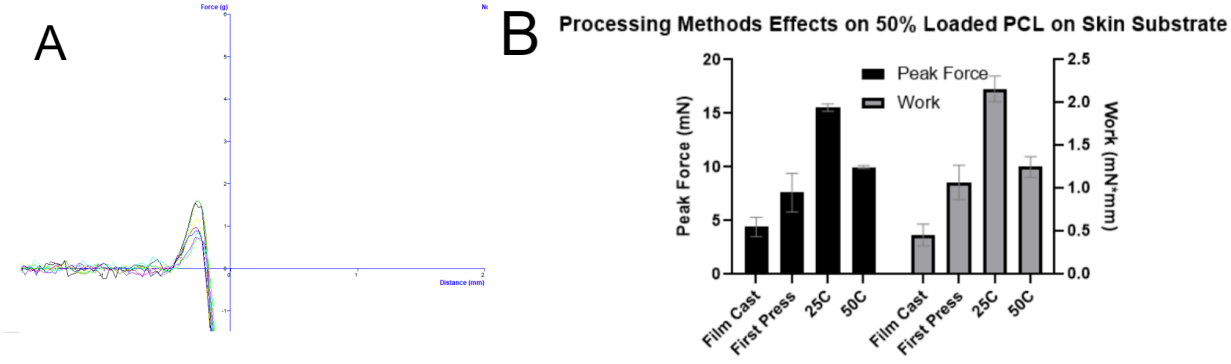

Figure 8: Trace of adhesive forces on a sample of 5 mm diameter probes with film cast, first pressed, 25℃, and 50℃ processed PCL loaded at 50% (A), comparison between the peak force and adhesive work of film cast, first pressed, 25℃, and 50℃ processed PCL at 50% (B)

A silicon-based skin was tested to attempt to address the inconsistencies seen when testing on a mucoadhesive substrate. PCL at 50% loading with different processing methods was chosen to assess the viability of using other substrates with this bioadhesive testing method

due to its increased hydrophobicity. The trace that can be seen in Figure 8A shows low variation in the samples as well as an overall smaller curve compared to previously tested samples. There appears to be some grouping of individual traces. The comparison in Figure 8B shows that the silicon-based skin displayed very good consistency in the samples tested with lower variations than seen in previous samples indicating it could viably assess the adhesion to it. The highest peak force and work done were seen in the 25℃ sample which differs from the mucoadhesive substrate where 25℃ processed samples had among the lowest adhesive forces. This indicates that different forces are acting on the samples compared to the mucoadhesive substrate and processing at 25℃ has an impact on the hydrophobic attraction between the substrate and polymer.

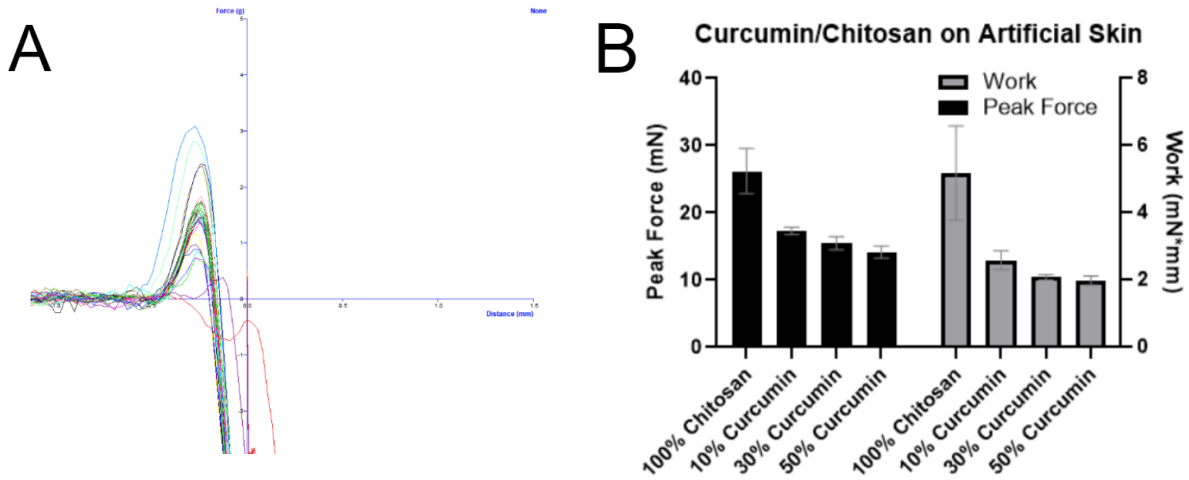

Figure 9: Trace of adhesive forces on a sample of 5 mm diameter probes with film cast chitosan at 0%, 10%, 25%, and 50% curcumin loading (A), comparison between the peak force and adhesive work of 0%, 10%, 25%, and 50% curcumin loading (B)

Chitosan formulations were also tested on the silicon-based artificial skin to assess if reliable results could be obtained. The trace seen in Figure 9A shows that samples had rapid detachment and didn't display prolonged adhesion. Groupings can be seen for the peak heights

and low variation can be seen in the width of most traces. Looking at the comparison in Figure 9B we can see that high molecular weight chitosan had the highest adhesion, which was a surprise given the hydrophilic nature of chitosan and the hydrophobic nature of the silicon skin. Initial drug loading shows a notable drop in both the peak force and adhesive work done but subsequent increases show only marginal changes. In comparison to the mucoadhesive substrates, the peak force and adhesive work measured on the silicon substrate both decreased by nearly a factor of 10. The low standard deviations seen indicated that drug loading has an effect on the adhesion of chitosan to hydrophobic substrates.

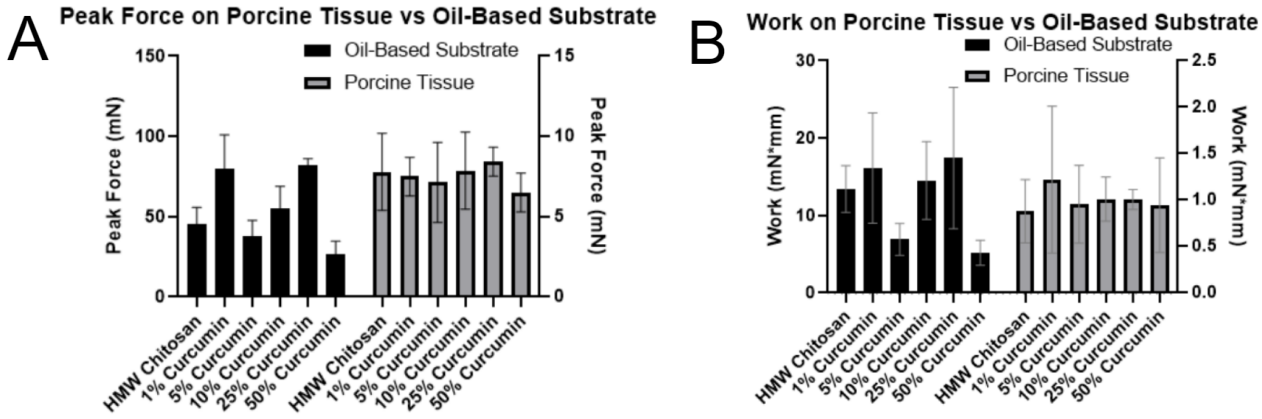

Figure 10: Comparison between the peak force of chitosan/curcumin formulations (0%, 1%, 5%, 10%, 25%, 50%) on Si-Oil based substrate and porcine skin tissue (A), comparison between the adhesive work of chitosan/curcumin formulations (0%, 1%, 5%, 10%, 25%, 50%) on Si-Oil based substrate and porcine skin tissue (B)

Having seen that hydrophobic surfaces are able to have detectable changes in bioadhesion a hydrophobic substrate was created to mimic the oil-based hydrophobic environment on the skin surface. To see if the results have physiological relevance porcine skin tissue was used as a substrate and tested with identical formulations as the substrate. A comparison of peak forces can be seen in Figure 10A. It can be seen that for the oil-based

substrate, 1% and 25% loading had the highest peak force while in the porcine skin, there were no discernible trends in peak force. The scale of the peak force was greater in the oil-based substrate by about 10 times. Overall this shows that changes in drug loading percentage can be detectable by a substrate but not on porcine tissue. When comparing the adhesive work in Figure 10B it can be seen that there was more variation in the measurements compared to the peak force but 1% and 25% loading still had the highest values on the oil-based substrate. Similar to the peak force the scale of the work done by the oil-based substrate was larger than in the porcine tissue by approximately a factor of 10.

While the testing did not display comparable trends in bioadhesion between the substrate and the porcine tissue it showed that fracture testing is effective on a range of artificial substrates. Additionally, these substrates can show more sensitivity that can point to changes in the polymer properties and structure.

Chapter 4: Discussion and Conclusions

Aim I –Verify existing mucoadhesive substrate used to mimic intestinal tissue through model polymers.

The first aim of this project was to see if the mucoadhesive substrate previously developed in the lab had bioadhesive relevance in various polymers as well as establish its ability to detect processing and drug loading changes. Polystyrene, a nonbiodegradable hydrophobic polymer, PCL, a degradable polyester, and chitosan, a degradable linear polysaccharide were used, the latter two having high biomedical relevance. Several different aspects were investigated, molecular weight, processing method, and drug loading.

Increasing molecular weights of polystyrene were used to investigate if the test methodology with the set parameters would be able to detect changes in molecular weight through changes in bioadhesion. Due to high variation between samples, it was difficult to detect any trends resulting from changes in molecular weight.

Processing methods, on the other hand, showed an ability to be measured and distinguished. At lower drug loading, film casting results in significantly more adhesion than other processing methods. When drug loading is higher however (50%) these differences shrink and the first press becomes comparatively adhesive to the film cast samples in terms of the peak force while adhesive work done by the film cast probes was marginally exceeded by the first press and 50-degree processing.

Aim II – Investigate the bioadhesive effects of hydrophobic drug loading on chitosan and PCL using a mucoadhesive substrate

Curcumin, the hydrophobic drug of choice, was incorporated into PCL and chitosan in concentrations up to 50%. PCL testing resulted in large changes related to drug loading in the film cast. There was a significant increase at 25% with changes that indicate higher bioadhesion seen in the trace of force.

Testing for chitosan with curcumin loading showed high variation when testing samples were used on the mucoadhesive substrate. The variation was present with high sample numbers and large changes existed when testing the same sample repeatedly. This made testing water-soluble polymers such as chitosan impractical due to the hydration of the sample even over the 10-second contact time.

Aim III – Create a skin-mimicking substrate that could be used to test the bioadhesion of transdermal delivery systems, including water-soluble polymers

Silicon, often used as an artificial skin for testing, was investigated as both a solid sheet to replicate skin and as part of a substrate formulation. Samples tested on silicon-based skin displayed low variation within samples and repeats. Testing indicated high molecular weight chitosan had the highest peak force as well as the highest work done by adhesion. Large drops in adhesion were seen with additions of curcumin in the artificial silicon skin. To judge physiological relevance, pig skin was used as a physiological representation. The trends seen in the silicon oil substrate were not seen in the porcine tissue. When using this substrate there were large changes between different drug-loading groups that could not be seen in the porcine tissue. These were present in both peak force and adhesive work. This demonstrates the increased sensitivity of the substrate when changes can't be seen in ex-vivo models.

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