DUAL DELIVERY OF ANTI-INFLAMMATORY AND ANTI-MICROBIAL DRUGS FROM AFFINITY POLYMER COATED SUTURES PARALLELS WOUND HEALING TIMELINE

by

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Dual Delivery of Anti-Inflammatory and Anti-Microbial Drugs from Affinity Polymer Coated Sutures Parallels Wound Healing Timeline

by REBECCA M HALEY

Abstract

Surgical sutures are vulnerable to bacterial infections and biofilm formation. At the suture site, pain and undesirable, excess inflammation are additionally detrimental to wound healing. The development of a polymerized cyclodextrin (pCD) coated surgical suture introduces the capability to locally deliver both anti-inflammatory and anti-microbial drugs throughout the phases of acute and chronic healing. Local delivery allows for the improvement of wound healing while reducing related systemic side effects and drug resistance. Through testing, it has been shown that the fabrication of this pCD coating minimally affects the suture's mechanical properties. *In-vitro* studies show measurable and consistent drug delivery for nearly five weeks. The therapeutic level of this delivery is sufficient to show inhibition of bacterial growth for four weeks, and free-radical scavenging (an *in-vitro* anti-inflammatory activity approximation) for two weeks. With this pCD coating technique, we maintain clinical performance standards while also introducing a long-term dual delivery system relevant to the wound healing timeframe.

Chapter 1 – Background

1.1 Surgical Sutures

Surgical sutures are used to hold tissue together after injury or surgery, and represent a market estimated to be around $1.3 billion annually.[1] Sutures come in a wide variety of sizes and materials, and their usage primarily depends on the location of the suture site. Absorbable sutures do not necessitate removal like their traditional non-absorbable counterparts, as they fully degrade after 4 to 8 weeks. This makes them effective for internal or inaccessible suture sites.[2,3] Recently, there have been successes in the creation of sutures which have the capability to improve wound healing. These include antimicrobial sutures[4-8] and bio-active sutures such as drug-eluting[9-11] and stem cell[12-14] or growth factor coated[15-18] sutures. These newly designed sutures represent a response to a critical market, as there has been extensive research done which indicates increased potential for bacterial growth and undesirable inflammatory response when traditional surgical sutures are used.[19-21]

The first FDA approved antibacterial suture was Vicryl Plus in 2002.[22,23] Made of a polyglactin suture coated with triclosan, it successfully reduced the risk of surgical site infections[24,25], and opened the door for the development of a variety of anti-microbial suture types. The growing resistance to triclosan[26], and a limit to the approved and effective usages of Vicryl Plus has caused suture alternatives with improved properties to be desired. With the inclusion of anti-microbial effects, sutures sites would be less likely to develop infections, and sutures themselves are less likely to build biofilms, a challenging complication of long-term bacterial colonization.[27] Addressing infections and

biofilm prevention will result in overall easier healing for the patient, and lower medical costs.

While a limited variety of antimicrobial sutures exist, infection prevention is still a major concern for any sutured wound. As mentioned previously, surgical sutures are vulnerable to bacterial biofilm formation. While biofilm incidence is low (with an estimated 5% occurrence rate)[28,29], cost of infection is high, as it requires high drug dosages and can even necessitate repeat surgery. Additionally, once biofilms have formed, the difficulty of eradicating infection is drastically increased, and typical antibiotic regimens can be ineffective. Preventative anti-microbial drugs would eradicate this issue, but as with the administration of NSAIDs, long-term systemic exposure is problematic.

Pain from the wound site is an inevitable outcome for patients requiring stitches, whether it be post-operative or simply due to injury. To treat this pain, drugs such as non-steroidal anti-inflammatories (NSAIDs) are often administered systemically. However, these drugs are not given throughout the entire healing process, to reduce systemic exposure to the drug. As a result, they do not exhibit effects throughout the entire healing window. There has been recent research into the production of anti-inflammatory sutures, which would allow for localized pain relief and better wound healing. Common designs are coatings on clinically used sutures[30,31], or entirely new suture fabrications.[32-35]

The rationale behind using sutures for drug delivery of both drug types is the simultaneous addressing of both issues with localized drug release, avoiding the need for excessive systemic levels. Surgical sutures are used in almost all surgical procedures. Therefore, the introduction of drug delivery to them allows additional therapy without the

need to introduce extra material or an implant, which can have detrimental effects on healing. By introducing anti-microbial and anti-inflammatory drugs locally via new drug delivery sutures, therapeutic concentrations can be achieved for sustained periods of time without risk of systemic toxicity or resistance development.

A commonly used antibiotic, rifampicin (Figure 1.1) is an ideal anti-microbial drug to use for this application due to its high level of efficacy and low MIC against common bacterial strains such as *S. Aureus*. Resveratrol (Figure 1.1) is a naturally occurring anti-inflammatory molecule, and is most commonly found in red wine. Its activity as a free-radical scavenger makes it useful for wound healing applications because it has decently low activity, working to reduce excessive inflammation without quenching levels below that of the natural wound healing process.

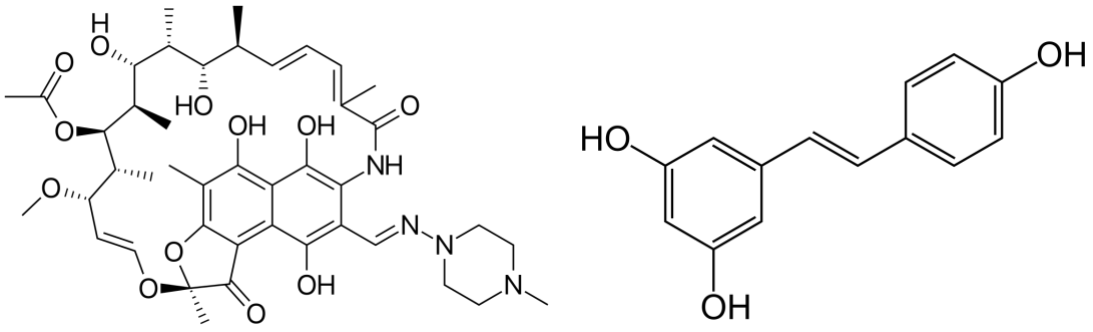


Figure 1.1 Molecular structure of the two drugs used in this system: the anti-microbial molecule rifampicin (left) and the anti-inflammatory molecule resveratrol (right).

While existing smart suture designs have been moderately successful, there are only limited suture systems which incorporate multiple therapies.[36] This project aims to produce a dual-delivery suture system, which shows both anti-microbial and anti-

inflammatory effects. In doing so, we hope to have the best possible wound healing outcome for patients.

Furthermore, existing smart sutures often only release drug for a short period of time, and the majority of research reports delivery time frames of 1-2 weeks.[37] Wound healing can be a slow process, with high cell infiltration and neutrophil activity giving way to macrophage/monocyte activity and tissue remodeling after 4 long weeks. In addition, the percutaneous nature of many sutures leaves them open to infection risk over the entire time they are in place, not just the first 1-2 weeks. To best impact wound healing and patient quality of life, therapeutic drugs should be used throughout this entire healing window. Even absorbable sutures can take up to 2 months to be fully broken down and removed from the body, further highlighting the chronic time scale of many suture applications. Based on these time scales, four weeks was chosen as a benchmark goal for drug delivery at therapeutic levels. While the majority of non-absorbable sutures are removed before this point, it is a good goal to meet if there is any interest in applying pCD to absorbable sutures as well, which can take 4 to 8 weeks to fully degrade.

Many existing methods for the fabrication of drug-eluting sutures significantly affect the mechanical properties of the suture itself, rendering it less useful clinically.[35] Currently used non-absorbable nylon sutures (4-0 monofilament) handle tensile loads up to 14N, while comparable polyester and polypropylene sutures can take about 11N of tensile force.[38] Tensile strength is the most important strength property for surgical sutures, therefore it is vital that drug-eluting sutures have strength equal to at least that of polyester and polypropylene sutures.

1.2 Polymerized Cyclodextrin

Cyclodextrin (CD) is a good material for generating a drug delivery coating due to its ability to be polymerized on a device, and its long-term drug release capabilities.[39,40] The capacity for CD to improve drug delivery rates is due to its molecular structure. The monomer form of CD is a cyclic oligosaccharide, and it is this ring-shaped structure which allows the molecule to act as a 'pocket' for holding small molecule drugs. The inner space of this pocket is hydrophobic, and drug molecules have an affinity to localize to this area. CD in the monomer form has been used pharmacologically to solubilize hydrophobic drugs for systemic delivery. Our lab creates an insoluble form of CD by forming a high molecular weight crosslinked polymer. In this form, we can take advantage of CD's molecular affinity to alter the rate of drug release beyond that achievable through diffusion alone.

Past work from our lab and others has shown that this affinity reduces the initial drug burst effect, retaining more drug to be delivered at later time points, and giving a release profile which is overall more consistent.[41] This has been done with a number of different drug types as well, from antimicrobial[39,42] and anti-fungal[43] therapies to corticosteroids.[44] Additionally, loading CD polymers is very straightforward, and can be done post-polymerization, allowing drug incorporation for dual drug loading and release.

By introducing a novel pCD coating technique to non-absorbable surgical sutures, as seen in Figure 1.2, it is our aim to maintain current clinical performance standards while also introducing a long-term dual delivery system relevant to the wound healing timeframe.

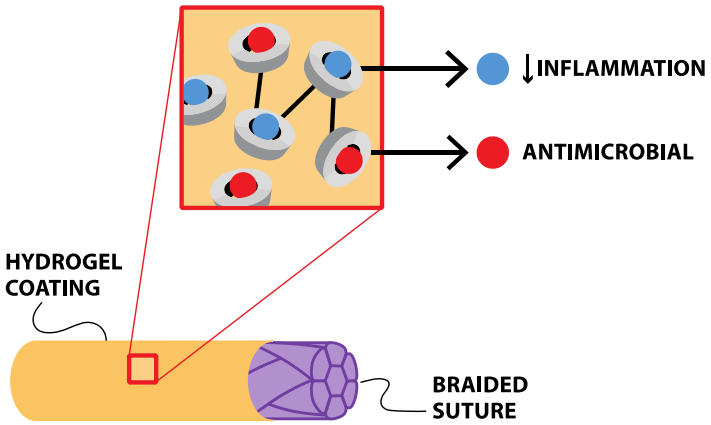


Figure 1.2 Schematic for proposed dual drug delivering suture system. Outer hydrogel (polymerized cyclodextrin) coating releases both antimicrobial and anti-inflammatory molecules to the local space.

Chapter 2 – Use of Affinity Allows Anti-Inflammatory and Anti-Microbial Dual Release that Matches Suture Wound Resolution[45]

2.1 Materials and Methods

2.1.1 Materials

A lightly epichlorohydrin-crosslinked β-cyclodextrin pre-polymer (βCD, 2-15 kDa, average 10 CDs per chain) was purchased from CycloLab Ltd, (Hungary). Braided Nylon sutures in size 0 (Surgilon) were purchased from eSutures. 1,6-diisocyanatohexane (HDI) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) from Sigma Aldrich (St. Louis, MO), N,N-dimethylformamide (DMF, extra dry) from Applied Biosystems (Foster City, CA), rifampicin (RMP) from Research Products International (Mt. Prospect, IL), and trans-resveratrol (RSV) from PureBulk (Roseburg, OR) were used as received. All other reagents, solvent, and chemicals were purchased from Fisher Scientific in the highest grade available. Green fluorescent protein (GFP)-labeled *Staphylococcus aureus* culture was kindly provided by Dr. Ed Greenfield (Case Western Reserve University).

2.1.2 Polymerization of CD onto existing non-absorbable nylon sutures

Cyclodextrin (CD) was polymerized according to previously published protocol.[46] Briefly, 250 mg of β-CD prepolymer is dissolved into 750 mL of dimethylformamide (DMF) and polymerized using a hexamethylene diisocyanate (HDI) crosslinker in a molar ratio of 1:0.32 (glucose residue:HDI) at room temperature. With two reactive groups on either end, HDI is able to react to two different CD hydroxyls, allowing CD to form an insoluble network polymer that is a hydrogel. The hydrogel structure remains well hydrated, resulting in good transport properties, and consistent drug delivery rates.

To produce polymerized cyclodextrin (pCD) coated sutures for testing, braided nylon (Surgilon) size 0 sutures were cut into 1 inch segments and hung. These segments were dipped into the CD mixture at hours 2 and 7 in the polymerization process, allowing the CD hydrogel to form onto the suture surface as the reaction progresses. In between these time points, the polymerizing CD mixture was kept at room temperature, and sealed to reduce solvent evaporation. After the 7 hour dip coating, sutures were left to dry overnight. This simple and safe reaction has no excessive heat or other toxic byproducts, which allows it to proceed and penetrate the microstructure of the suture material.

2.1.3 Characterization of surface coating – Scanning Electron Microscopy

Sutures both with and without pCD coating were characterized by Scanning Electron Microscopy (SEM)(JSM-6510 Series, JEOL) to determine surface morphology. To prepare samples for SEM, suture samples were coated with 5nm of palladium in vacuum by a sputter coater. Images were taken at an excitation voltage of 25 kV.

2.1.4 Evaluation of tensile properties – Pull Test

Suture samples of approximately 2.5” were used for tensile strength tests. Gauge length for each sample was 1”. Samples were kept in ambient environment before testing, and testing was carried out at room temperature as well. An Instron Model 1130 Universal Testing machine (CWRU, Advanced Manufacturing and Mechanical Reliability Center (AMMRC)) was used to obtain measurements. The samples were pulled at 10 mm/min until complete fracture occurred. The force was measured with a 100 N load cell, and data acquisition occurred at a rate of 100 Hz. Stiffness was taken as

the slope of the best-fit line acquired by performing linear regression on all data points in the elastic region of force-displacement curves between 45% to 55% of the maximum load (minimum 40 data points per specimen, all r^2 values exceeded 0.999). Replicas of 5 samples were used to ensure adequate statistical power as several properties were analyzed: max load, work to failure, stiffness, and strain to failure.

2.1.5 Loading of Rifampicin and Resveratrol into pCD suture coating

The usage of rifampicin (RMP) and resveratrol (RSV) is due to both their known interaction kinetics with CD[39,46], and their capability to be easily read in the same solution due to non-overlapping absorbance wavelengths and the fluorescent properties of RSV.

To load coated sutures equally with both rifampicin (RMP) and resveratrol (RSV), each 1 inch segment was immersed in 1 mL of highly concentrated, mixed loading solution of both drugs and left on a shaker at low speed at 23ºC for 72 hours. The optimal loading solution was determined through condition testing on pCD disks, which is illustrated in Supplementary Figure 1. Conditions tested include equal and non-equal drug amounts (1:1 and 2:1 v/v of RMP and RSV respectively), alternate drug concentrations (20 and 50 mg/mL w/v), and varying loading solvents (100 and 90% DMF v/v).

For the pCD-coated sutures, an equal mixture (1:1) of RMP and RSV was chosen as the drug-loading solution. Both drugs were dissolved in a 90% DMF/10% v/v water mixture at 5 wt% concentration. These conditions were found to show both high and comparable loading of RMP and RSV simultaneously.

Once the dual-loading solution was determined, drug loading of pCD was carried out as mentioned previously, and according to previously determined protocol.[47] After 72 hours of loading, sutures were blotted on KimWipes and briefly rinsed with water to remove surface DMF and any free drug which is not loaded. Rinsed sutures were left to dry at 23ºC overnight.

2.1.6 Evaluation of loading efficiency and total drug loading capacity of pCD suture coating – solvent extraction

To determine the total drug in the pCD coating which is available for release, an organic solvent extraction was carried out to measure the total weight percent of loaded/available drug. Dual-loaded pCD coated sutures were incubated in 1 mL of DMF. Every 12±4 hours, the DMF was removed and replaced with fresh to create infinite sink conditions. All DMF samples were added together in a single test tube, and the concentration of drug in this solution was determined spectrophotometrically, giving a quantitative measurement of the total released drug. For spectroscopy, extraction samples were diluted in PBS until DMF background was no longer significant.

Extraction samples were analyzed using UV/Vis at 321 nm with a standard curve of RMP in PBS to determine total RMP loading. The same samples were also analyzed using fluorescence, with an excitation wavelength of 318 nm and emission wavelength of 385 nm,[48] using a standard curve of RSV in PBS to determine total RSV loading.

2.1.7 Quantification of Rifampicin and Resveratrol release – Infinite sink model

Dual-loaded pCD coated sutures were incubated in 1 mL of phosphate buffer saline (PBS) pH 7.4 at 37ºC with mild shaking. Every 24±4 hours, the PBS release media was removed and replaced with fresh to create infinite sink conditions. The concentration of both RMP and RSV in each time point sample was determined spectrophotometrically using the same wavelengths and standards as in the leach experiment. The release was continued until concentration of drug was no longer accurately measurable (<1 ug/mL RMP and <0.5 ug/mL RSV). Measureable amounts are determined by the lowest point on the standard curve.

2.1.8 Evaluation of Anti-Bacterial Activity – in-vitro zone of inhibition study

A zone of inhibition assay was used to determine the efficacy of the anti-microbial properties of the loaded sutures *in-vitro*. Dual-loaded pCD coated sutures were evaluated against *Staphylococcus aureus (S. aureus)* according to previously published protocol.[49] *S. aureus* was cultured overnight and 70mL was then spread on a trypticase soy broth (BD BBL™) agar plate. A dual-loaded pCD coated suture was placed on this fresh *S. aureus* lawn and incubated at 37ºC overnight. After 24 hours, the zone of inhibition around each suture was measured with calipers at three points and recorded. Each suture was then transferred onto a new *S. aureus* plate and the process repeated daily until the zone is no longer visible (<0.5mm).

2.1.9 Evaluation of Anti-inflammatory activity – DPPH Scavenging

In order to determine the *in-vitro* efficacy of the anti-inflammatory properties of the loaded sutures, a 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay was used.

DPPH is a free radical which changes color when scavenged. RSV's anti-inflammatory activity comes from its antioxidant properties, scavenging free radicals involved in inflammation to help prevent chronic inflammation from occurring. A 100μM solution of DPPH in ethanol was created, according to published protocol.[50] This DPPH solution was incubated with dual-loaded pCD coated suture release samples in a 1:1 ratio for 30 minutes, after which scavenging can be determined by reading color change spectrophotometrically at 517 nm. A positive control of 1mg/mL RSV and a negative control of PBS were used to determine % DPPH scavenging.

2.1.10 Statistical Analysis

If not otherwise stated, experiments were carried out in triplicate for statistical analysis (n=3). Data displayed is the mean of each condition, and error bars represent the standard deviation of the triplicate set. Student's t test and ANOVA were done using Prism to determine reported statistical significance.

2.2 Results

2.2.1 Polymerization of CD onto existing non-absorbable nylon sutures

CD was successfully polymerized onto the surface of the braided nylon suture. This pCD coating can be seen by eye as a translucent surface. This surface can also be seen in digital camera photos. (Figure 2.1) The coating holds up to moderate handling and submersion in solutions such as water and PBS. However, extreme bending of pCD coated sutures seemed to cause microscale cracking of the coating at stress points. This would indicate that the dried coating is more brittle than the nylon suture core.

Nevertheless, once broken at a stress point, the coating remains adhered to the nylon suture core, indicating that cracking does not render the coated suture more susceptible to further breakdown or delamination.

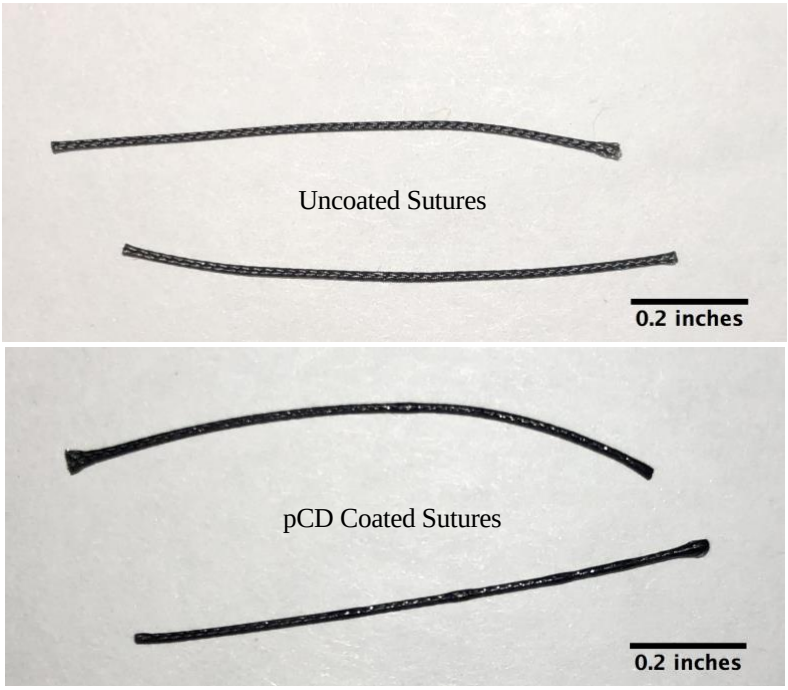


Figure 2.1. Digital camera images of non-absorbable nylon sutures with (bottom) and without (top) polymerized cyclodextrin (pCD) coating. Scale bar represents 0.2 in.

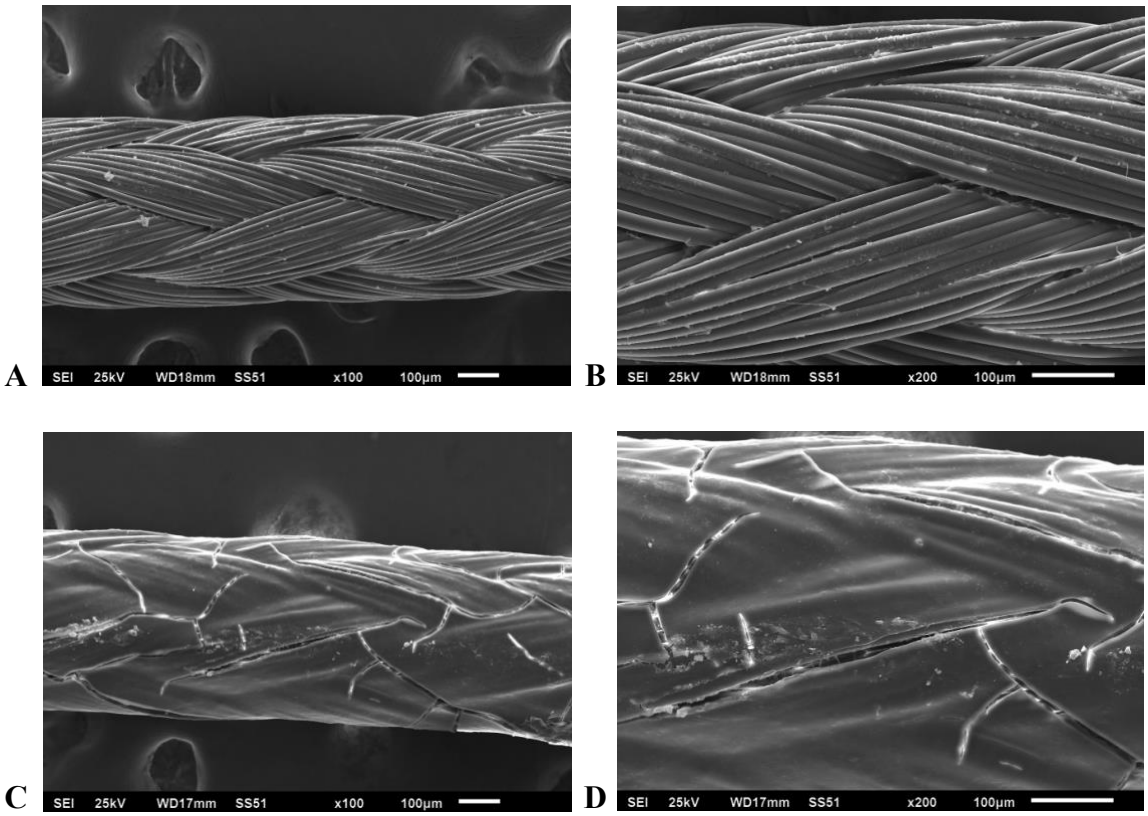


Figure 2.2. Scanning Electron Microscopy (SEM) images of non-absorbable nylon sutures before (A, B) and after (C, D) polymerized cyclodextrin (pCD) coating. Scale bar represents 100µm. pCD coated sutures show smoother surface in comparison to individual braided threads which can be seen in the uncoated image, but there is significant micro cracking throughout the coating itself.

2.2.2 Characterization of surface coating – Scanning Electron Microscopy

From the SEM images in Figure 2.2, it is clear that polymerized cyclodextrin has successfully coated the non-absorbable braided nylon sutures. In the non-coated images (Figure 2.2.a and 2.2.b), the individual nylon threads can be clearly seen. In the coated images (Figure 2.2.c and 2.2.d), individual threads are no longer readily apparent, due to

the pCD coating. The coating appears fairly uniform, and the braids can still be determined. However, the pCD coating shows noticeable cracking at this microscale.

The preparation process caused the pCD hydrogel to transition from an aqueous to dry state, due to the vacuum conditions required for SEM imaging. In the aqueous state, pCD is highly hydrated. Therefore, it is likely that the cracks shown in these images occurred are solely due to the stresses experienced during drying, and that hydrated coatings would not have these same cracks.

Interestingly, the majority of cracks appear to follow the lines of the braided nylon filament. The cracks do not appear to induce delamination while the suture is under tensile stress, but the cracks have the potential to propagate across the suture-coating interface if alternate stresses or strains are applied.

A few cross-sectional images of pCD coated sutures were also collected using SEM. (Figure 2.3) In these images, it can be seen that the outer pCD coating lies flush against the texture of the individual nylon fibers, creating a thin polymer with varying thickness. To further quantify the coating that was produced, sutures were weighed before and after polymerization. The results of these measurements can be seen in Table 2.1.

avg virgin suture weight (mg/in)	**avg pCD coated suture weight (mg/in)**	**avg pCD coating (mg/in)**	**Estimated CD monomer units (molecules/in)**
3.13 ± 0.11	4.05 ± 0.11	0.92 ± 0.15	4.88×10^{17}

Table 2.1. Nylon suture weights before and after pCD coating polymerization for further quantification of coating.

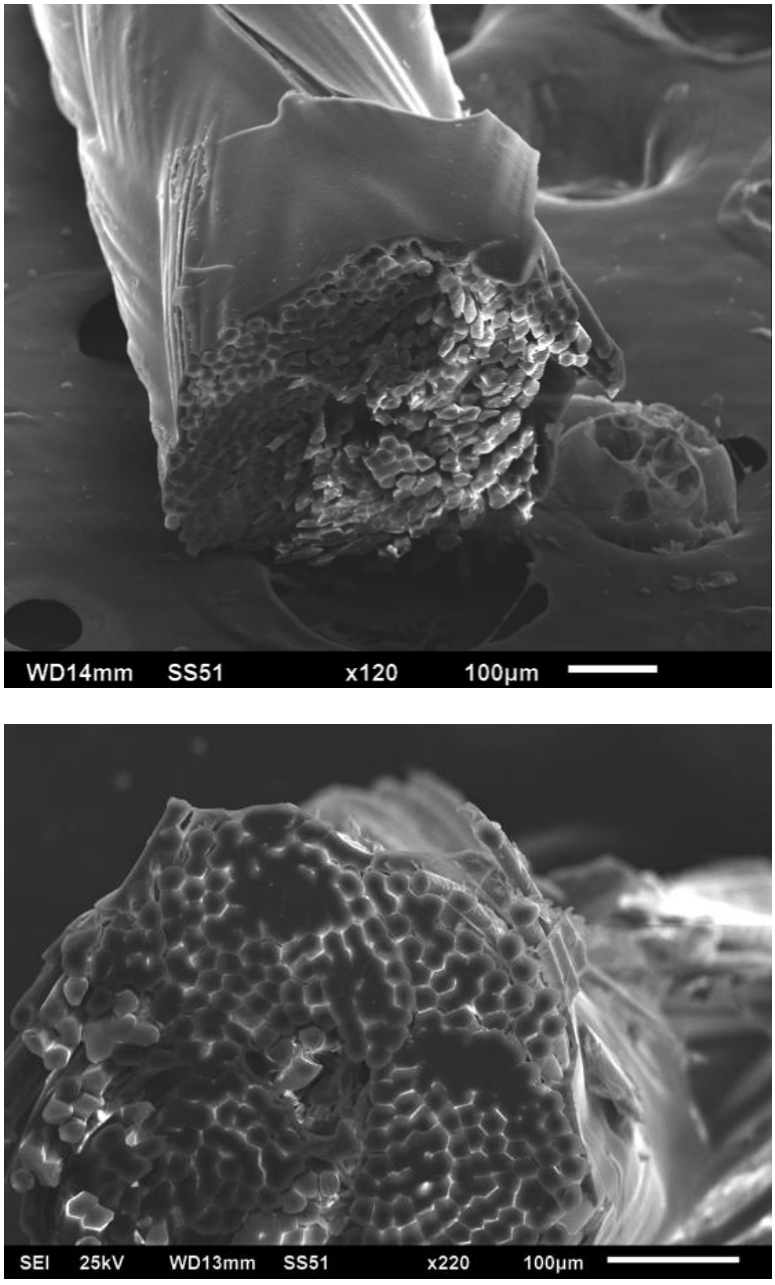


Figure 2.3. Scanning Electron Microscopy (SEM) cross-sectional images of non-absorbable nylon sutures with polymerized cyclodextrin (pCD) coating. Scale bar represents 100µm.

2.2.3 Evaluation of tensile properties – Pull Test

Force-displacement curves from suture tensile tests were analyzed to determine several properties, namely max load, work to failure, stiffness, and strain to failure. The overall shape of the force-displacements graphs for both pCD coated and uncoated sutures are very similar (Figure 2.4). However, the maximum displacement is reduced in the case of the coated suture samples, significantly affecting the values for max load ($p < 0.001$), work-to-failure ($p < 0.005$), and strain-to-failure ($p < 0.001$) for the coated sutures.

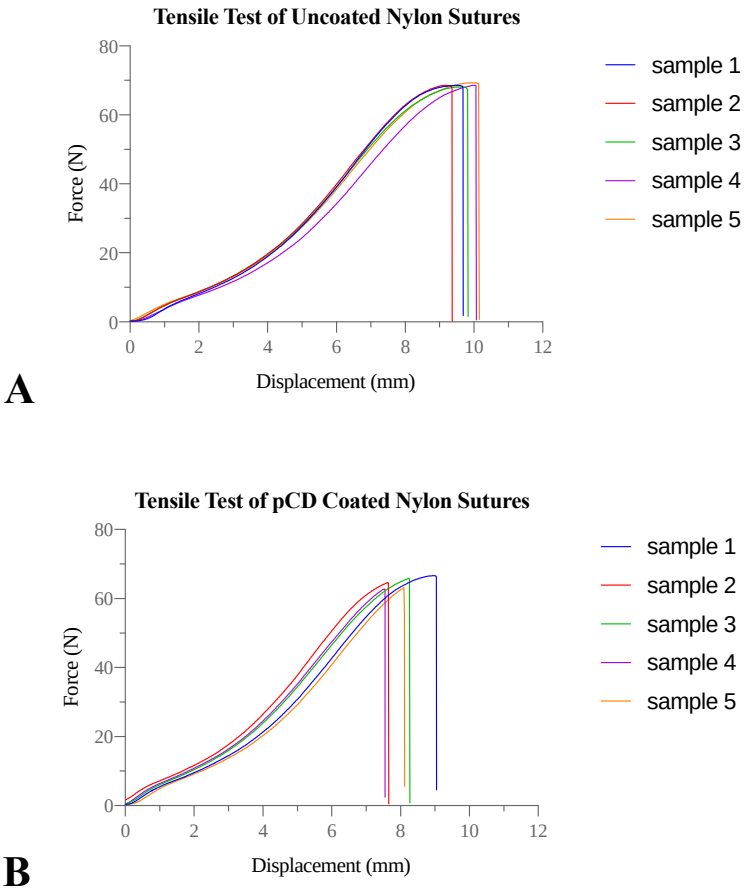


Figure 2.4. Force-Displacement curves produced during tensile testing of uncoated (A) and polymerized cyclodextrin (pCD) coated (B) nylon sutures. pCD coated sutures show decreased maximum displacement before failure, and less consistent mechanical behavior.

In pCD coated samples, the determined average values for max load, work-to-failure, and strain-to-failure of the coated sutures were 94.1%, 74.8%, and 84.1% respectively of the average uncoated suture sample values. There is no significant difference between determined stiffness values (Figure 2.5).

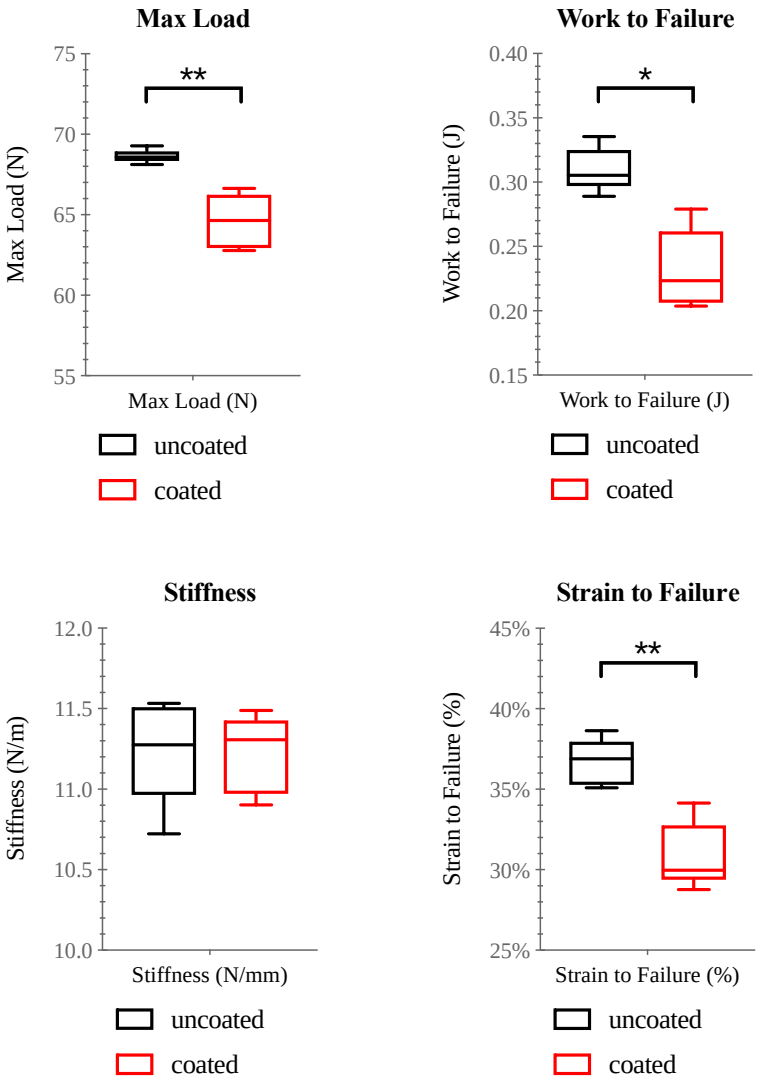


Figure 2.5. Tensile testing of polymerized cyclodextrin (pCD) coated and uncoated nylon sutures shows significant difference between Max Load, Work to Failure, and Strain to Failure of the two

sample conditions. (*p < 0.005 and **p < 0.001) There is no significant difference between Stiffness values.

Polyester and polypropylene sutures are used for many of the same procedures as nylon. However, they have been reported to exhibit values for max load which are 79% those for nylon sutures.[38] With this in mind, the significant differences in mechanical properties should prove acceptable in vivo, since the reduction is less than that which is clinically accepted in the case of polyester and polypropylene.

2.2.4 Evaluation of loading efficiency and total drug loading capacity of pCD suture coating – solvent extraction

From the extraction experiment, it was found that a 1 inch segment of pCD coated nylon suture loaded 71.06±6.03 and 68.7±12.94 μg of RMP and RSV respectively. These raw amounts can also be expressed in terms of weight percent of the coating (Figure 2.6): 11.84±1.01 and 11.45±2.16 wt% of RMP and RSV respectively. Both RMP and RSV load similarly under the 50mg/mL, 90% DMF loading solution.

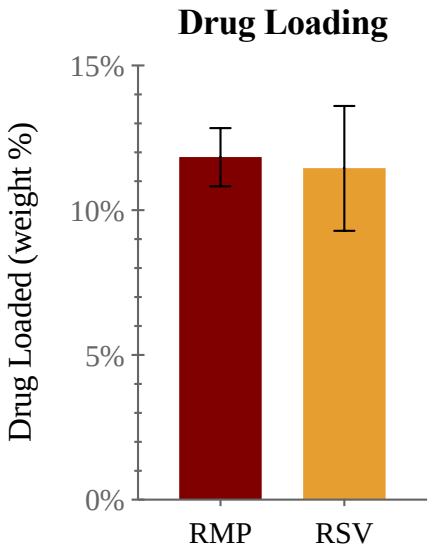


Figure 2.6. Leach study of polymerized cyclodextrin (pCD) coated sutures, dual-loaded with rifampicin (RMP) and resveratrol (RSV), averaged (n = 3) with error bars representing standard deviation. Statistically similar loading of each drug is seen under optimal loading conditions.

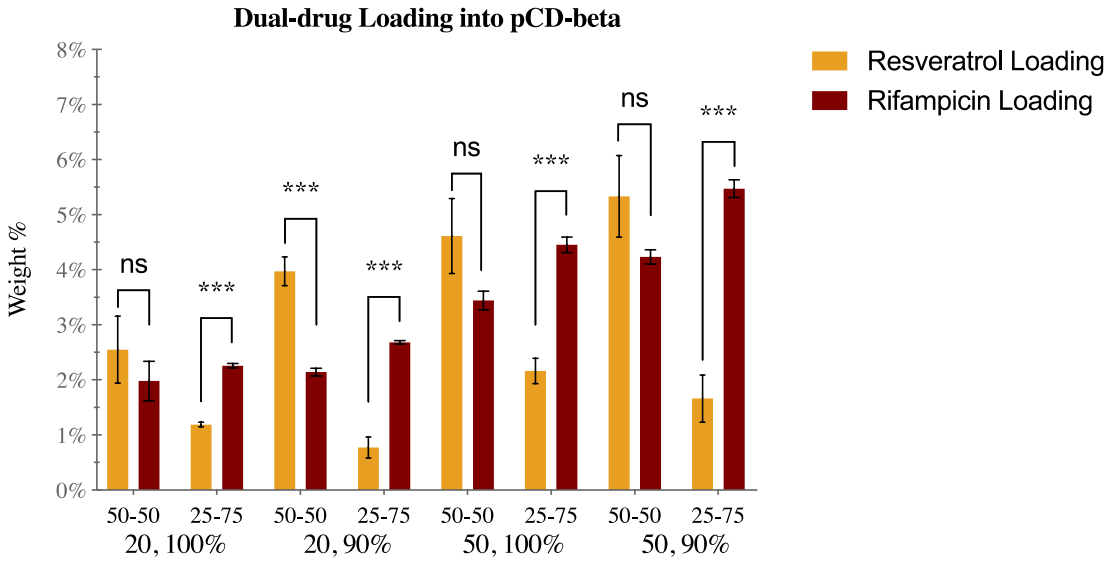


Figure 2.7. Weight percent loading of rifampicin (RMP) and resveratrol (RSV) into polymerized cyclodextrin (pCD) gel disks under various loading conditions (20mg/mL vs 50mg/mL

concentration, 100% DMF vs 90% DMF, and at varying ratios of drug). Optimal condition of 50:50, 50mg/mL, 90% DMF was used for studies in this manuscript due to high and fairly similar loading of both drugs.

However, in the preliminary loading studies (Figure 2.7), which used pCD disks to determine optimal loading conditions, weight % loading was significantly reduced (4.23±0.13 and 5.33±0.74 wt% of RMP and RSV respectively). This is likely due to the different surface to area ratio that is seen in the pCD disks used in the optimization studies when compared to the coated suture fibers. Disks are much thicker, and have overall more mass than the suture coating. Increased weight % loading is ideal, as it allows for more drug to be originally introduced to the same length of suture material. This, in turn, allows for a longer-term release profile.

In many clinical settings, larger wounds will require more stitches and therefore a greater length of suture material. This greater length will increase the raw weight of drug loaded and available for delivery. With increased suture material, the release profile should maintain the same shape, but with increased values, as there is more polymer and drug undergoing release.

2.2.5 Quantification of Rifampicin and Resveratrol release – Infinite sink model

A profile of *in-vitro* drug release over time can be seen in Figure 4. There is a small burst of drug release at the beginning of release, which can be seen as increased values in the daily release curve (Figure 2.8.a) or as an increased slope in the cumulative release curve (Figure 2.8.b). Specifically, 5.16±0.82 μg of RMP and 1.59±0.50 μg of RSV are released within the first hour, and 10.65±1.07 μg of RMP and 9.55±1.55 μg of

RSV are released within the first day. This small initial burst represents about 15 and 14% of total RMP and RSV release respectively overall. The rest of the curve appears linear, due to the consistent daily release of both drugs. A linear regression of the data shows that the RMP release profile after initial burst release can be fit to the line $y = 1.545x + 7.715$ with an R^2 value of 0.9916, and the RSV release can be fit to the line $y = 1.356x + 7.855$ with an R^2 value of 0.9596. This matches the average daily release of 1.56 μg and 1.37 μg of rifampicin and resveratrol respectively from the pCD coated suture segments, which has been recorded for nearly five weeks.

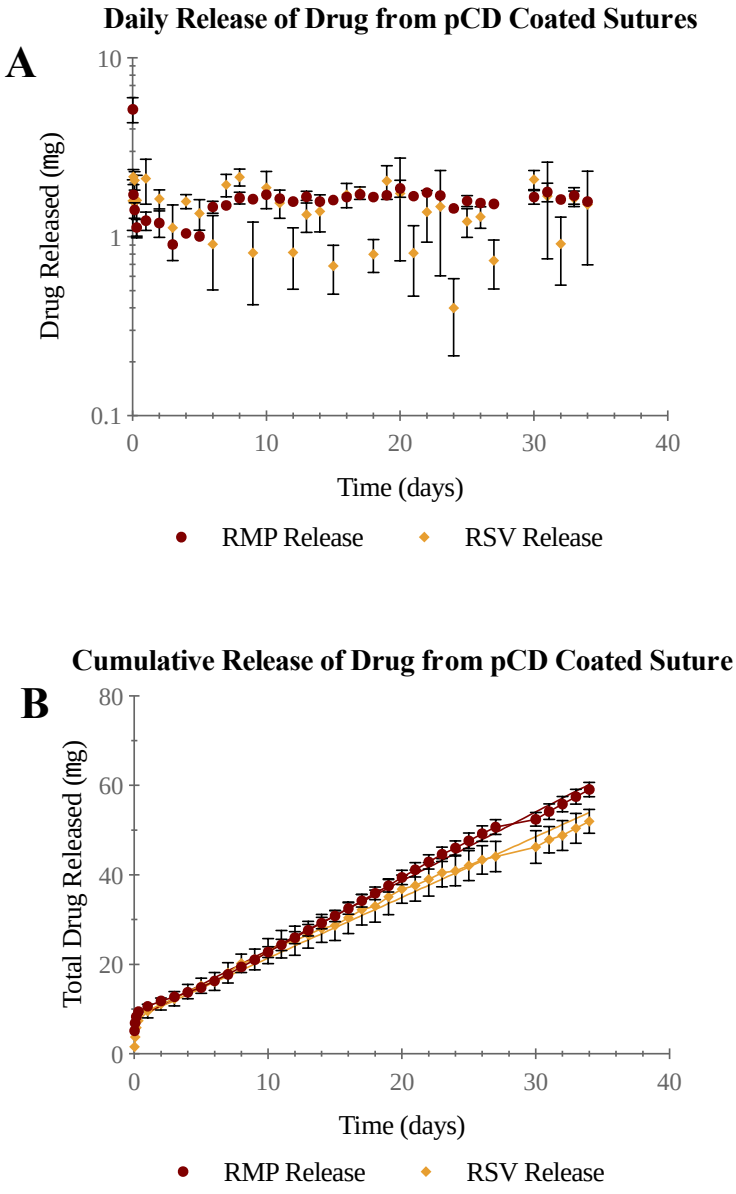
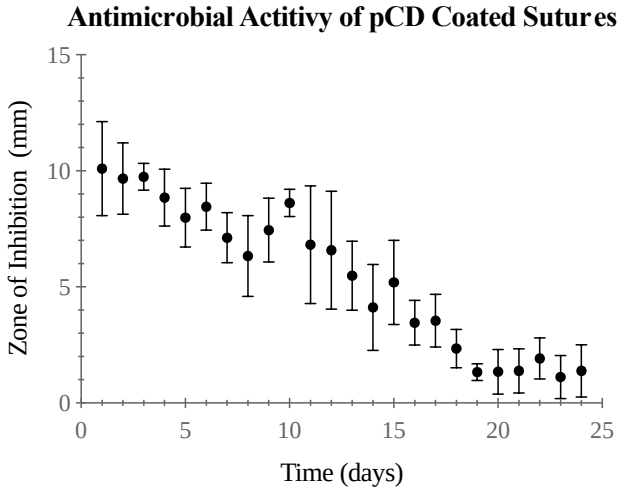


Figure 2.8. Infinite sink release study of polymerized cyclodextrin (pCD) coated sutures, dual-loaded with rifampicin (RMP) and resveratrol (RSV). Release points for each drug over time (t = 34 days) are averaged (n = 3) with error bars representing standard deviation, and plotted to visualize both daily (A) and cumulative (B) release. pCD coated sutures demonstrate consistent daily release for nearly 5 weeks, resulting in fairly linear release over time.

Spectroscopy confirms continued release of both RMP and RSV at fairly consistent levels throughout a five-week period. RMP has been shown to have a minimum inhibitory concentration of <0.06 μg/mL against *S. aureus*.[51] With the completed release showing an average daily release of 1.56 μg/mL (Figure 2.8.a) from pCD coated sutures, there should be more than enough RMP to prevent infection. In published literature, 5–25 μM (1.14–5.7 μg/mL) has been cited as an effective RSV dosage to modulate inflammation in neural tissue. [50] The completed release shows an average daily release of 1.37 μg/mL (Figure 2.8.a) from the sutures, which is appropriate for therapeutic effectiveness.

2.2.6 Evaluation of Anti-Bacterial Activity – in-vitro zone of inhibition study

To evaluate anti-microbial activity, dual-loaded pCD coated sutures were used in a zone of inhibition assay against *S. aureus*. Figure 5 shows that the fabricated dual-loading sutures are capable of eradicating *S. aureus* for at least 24 days.



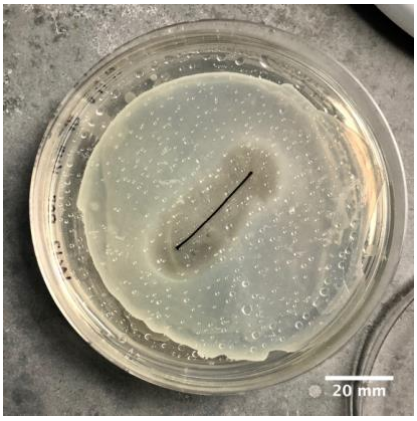


Figure 2.9. Zone of inhibition study of polymerized cyclodextrin (pCD) coated sutures, dual-loaded with rifampicin (RMP) and resveratrol (RSV), against *S. Aureus* (t = 24 days) averaged (n = 3) with error bars representing standard deviation. pCD coated sutures demonstrate antibacterial activity against *S. Aureus* for at least 24 days. ANOVA shows no statistically significant difference between adjacent time points.

The zone of inhibition measured shows a slow decrease in size, starting at 10.09±2.02 mm, but seems to hold steady at an average of 1.41 mm in the last week of testing. Linear regression of the data shows that the first phase of Figure 2.9 (days 1-18) can be fit to the line $y = 0.426x + 10.76$ with an R^2 value of 0.7882, while the second phase (days 19-24) does not fit to a significantly non-zero slope. ANOVA was also used to compare the day-to-day change in the zone of inhibition, but no significant statistical differences were found between adjacent time points. When all time points are compared against day 1, the first statistically significant difference is seen at day 7 ($p < 0.01$). In this study, both RMP and RSV are loaded and released. In the zone of inhibition study, dual drug delivering sutures are challenged against a fresh host of *S. aureus* bacteria every day, showing the efficacy of pCD coated sutures in a worst-case scenario, where there is continual introduction of healthy bacterial cells. The results of this study (Figure 2.9) confirm that the fabricated pCD coated sutures are capable of releasing a therapeutic dose of active antibiotic which is sufficient to inhibit the growth of bacteria for approximately four weeks.

2.2.7 Evaluation of Anti-inflammatory activity – DPPH Scavenging

To evaluate anti-inflammatory activity, release samples from dual-loaded pCD coated sutures were used in a DPPH scavenging assay. Figure 2.10 shows that the fabricated dual-loading sutures exhibit anti-inflammatory activity for approximately 14 days, with an average scavenging activity of 9.25±4.83%. Overall, the results of this study confirm that the fabricated pCD coated sutures are capable of releasing a measurable dose of an active anti-inflammatory for a two-week period. Analysis of the data shows that while Figure 2.10 has a clear trend, a linear fit does not represent the data well, with an R^2 value of only 0.6384. ANOVA was used to compare the day-to-day change in scavenging, but no significant statistical differences were found between adjacent time points.

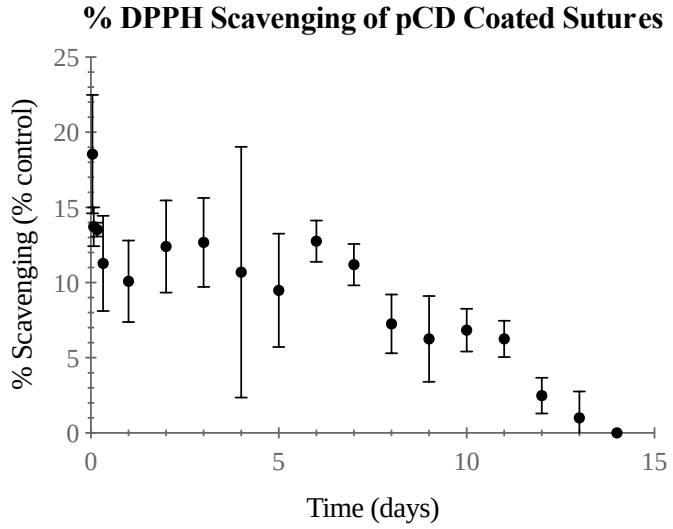


Figure 2.10. DPPH scavenging study of polymerized cyclodextrin (pCD) coated sutures, dual-loaded with rifampicin (RMP) and resveratrol (RSV) (t = 14 days) averaged (n = 3) with error bars representing standard deviation. pCD coated sutures demonstrate anti-inflammatory activity

through two weeks. ANOVA shows no statistically significant difference between adjacent time points.

Based on the release study and an experimentally determined standard for the anti-inflammatory activity of RSV (Figure 2.11), DPPH % scavenging was as expected. The average daily release of 1.37 µg (Figure 2.8.a) corresponds to a scavenging of ~12% on the standard curve. With an initial 18.56±3.94% scavenging within the first hour, and an average of 9.25% scavenging throughout the two-week period, the anti-inflammatory activity recorded is only slightly below expected values.

The timing of suture removal varies with wound location and patient, but in the majority of cases, non-absorbable suture removal occurs after 10 to 14 days. With this in mind, the two-week time frame of anti-inflammatory activity is acceptable for pain reduction and improved wound healing.

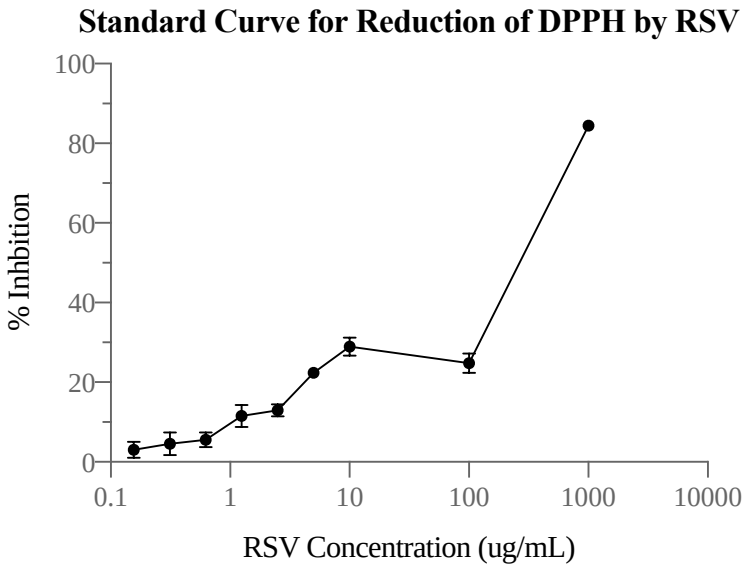


Figure 2.11. DPPH scavenging standard curve of resveratrol (RSV). Relationship between RSV concentration and *in-vitro* anti-inflammatory activity is neither linear nor truly logarithmic. Even at fairly high concentrations (100μg/mL) the activity of RSV is low, indicating that it is a weak anti-inflammatory.

2.3 Discussion

The dual loading of a pre-formed polymer is a fairly novel concept, both for our lab and the general scientific space. Most drug releasing polymers must be loaded with drug as they are formed, not after the polymerization/coating process. Our pCD coatings can be loaded after polymerization is complete, and can be loaded with drug cocktails when desired.

This manuscript shows a successful potential application where pCD coatings are necessary and advantageous. The polymer coating is required to successfully integrate with surgical sutures, possibly reducing the barrier to clinical entry by simplifying manufacture. Mechanical changes were found to be significant, and there is evidence that polymer coatings are vulnerable to cracking. Even though polymer cracking does not induce delamination, there is evidence based on previous work[47], that fragments of polymer coating (i.e. particles) remain equally good delivery vehicles as compared to coated materials.

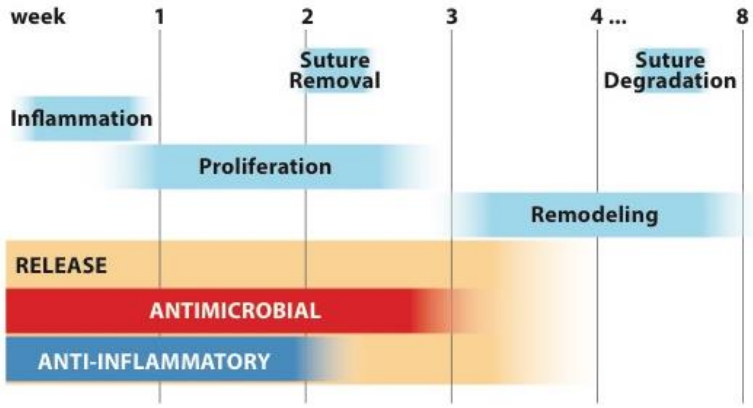


Figure 2.12. Schematic showing the general timing of wound healing, suture removal, and drug release from polymerized cyclodextrin (pCD) coated sutures. Release and activity match suture wound resolution, and goes out to the original 4 wk benchmark goal.

This system meets all desired properties as originally designed, with therapeutic time frames of 2 and 4 weeks for anti-inflammatory and anti-microbial drugs respectively – the times for removal and tissue remodeling/suture breakdown. If delamination or breakage of coating is found to still be a concern, our next steps would be a different way to further integrate pCD into the suture (e.g. chemical conjugation). For absorbable situations, a degradable version of the polymer would be an ideal way to adapt the system to a greater clinical space. Now that general loading and release kinetics have been quantified, different drugs could be used due to the simple nature of the loading process. An anti-inflammatory with higher activity (simvastatin) or an antibiotic with additional anti-inflammatory activity (minocycline) could be used just as easily. The dual-loading process could even be altered to allow for the loading of three drugs, to take advantage of

known synergistic antibiotic interactions (rifampicin/minocycline) in more extreme or at-risk suture sites.

Chapter 3 – Future Directions

The system explored in this thesis work was specifically optimized for the coating of larger (size 0) braided nylon sutures, which do not degrade, are generally non-reactive, and have significant surface texture. I see the future directions for this work going in two main directions: the improvement of nylon suture coatings and the expansion of coating to alternate suture types.

3.1 Improved Nylon Suture Coatings

3.1.1 Coating Methods for Improved Mechanics

Across disciplines, coatings are known for having delamination issues. While there is no indication that delamination will significantly affect the anti-microbial and anti-inflammatory properties of the pCD coated sutures developed in this work, this is an easy target for suture coating improvement, as there is clear cracking and delamination of the coating itself, especially after mechanical testing (Figure 3.1).

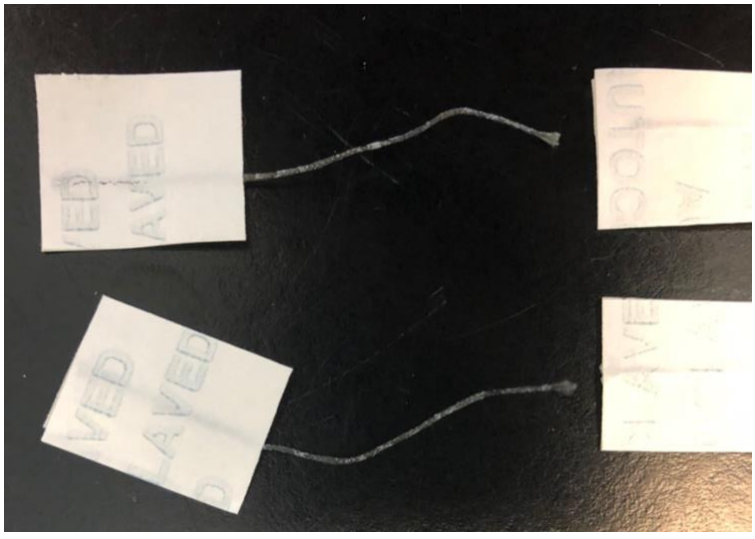


Figure 3.1 Digital camera images of polymerized cyclodextrin (pCD) coated sutures after undergoing mechanical testing (pull test) and failure. The nylon suture core is black, and the white surface is due to pCD which has partially lifted from the surface.

I believe that a significant amount of the adherence of the pCD coating to the suture surface is due to the surface roughness of the braided nylon fibers. If this method was to be implemented in thinner monofilament sutures, it is likely that delamination would occur differently, and could potentially have more significant effects. To improve adherence and reduce delamination, there are a few possible techniques that could be used.

For comparison, I would suggest three levels of interaction between pCD coating and nylon suture surface be used. The lowest level is that of the method outlined in this thesis work, where no surface treatments are done before dip coating. The second level would be chemical interaction, and could be achieved through plasma (N_2) treatment of the nylon surface to add some additional reactivity. The third, and highest level, of interaction would be chemically binding pCD to nylon. Based on work done by Jia et. al.[52], I believe that the crosslinker poly(ethylene glycol) diglycidyl ether (EGDGE) could be functionalized to the nylon surface through activation of the amide group. This is not the crosslinker used to produce pCD in this thesis work, but the lab as a whole commonly uses EGDGE to create pCD microparticles. Th crosslinker is bifunctional and fairly short, and should create functional sites for binding of CD and the greater pCD matrix to the nylon chemical backbone.

3.1.2 Quantifying Surface Modifications

To confirm that plasma (N_2) treatment creates an activated nylon surface, X-ray photoelectron spectroscopy (XPS) can be used to show an increase in Nitrogen. To quantify the number of functionalized EGDGE crosslinking molecules, a probe molecule with reactive amine chemistry can be used (BDP TMR amine) to tag molecules available for reaction.

3.1.3 Determination of Improved Mechanics

Once various levels of surface modification have been achieved, it would be important to verify whether there is any significant effect on the resulting mechanical properties. In addition to the pull test done in this work, T bend and lap shear tests can be explored. T bend tests can be used to determine the angle of bending at which failure (or surface cracking/delamination) occurs. This is likely more relevant to the type of mechanical usage that sutures see *in vivo*, as compared to the straight tensile pull test. The lap shear test is used to measure the strength of the bond between the pCD coating and the nylon surface. While this does not represent a clinical use case, it should show any differences in strength of surface association.

Additionally, there may be differences in mechanics between pCD coatings that are dry, rehydrated, and always kept aqueous. In this thesis work, the majority of tests were done using dry sutures, except for the drug release studies, which were done using rehydrated sutures. Since the pCD coating is a hydrogel, switching between dry and aqueous environments can cause significant swelling, which may be contributing to the cracking that occurs. To test this, sutures kept under various conditions could be tested for any changes in mechanics.

3.1.4 Impact on Drug Loading and Release

Finally, the last thing that may be affected by alternate coating techniques is the drug loading efficiency and overall release profile. I do not expect that the release profile will change shape. However, it is possible that by having more pCD adhered to the surface, or by having a more aqueous and swollen hydrogel, drug loading may be significantly affected. If this is the case, alternate methods may need to be employed to increase the efficiency of loading. Based on the data in Figure 2.7, increasing the water content of the loading solution could work to increase loading efficiency enough to offset any reductions that may occur.

3.2 Alternate Coated Suture Types

3.2.1 Absorbable Sutures

While non-absorbable sutures are commonly used, absorbable sutures represent a slightly larger market, and generally a more at-risk population. Most internal suturing is done with the use of absorbable sutures, as they do not necessitate repeat surgery for removal. In general, internal surgeries present patients with higher risk for infection and also delayed or improper healing, as they are overall more difficult procedures. Due to this, I believe that this dual drug delivering system could have significant clinical effect if it were to be modified for an absorbable version.

To do this, there would have to be studies done regarding the degradation profile of the absorbable suture in question. These studies would explore the effects of pCD

coating on the degradation profile of the suture material, and the effects of degradation on the drug release profile.

3.2.2 Degradable pCD Coatings

Using the current pCD dip-coating method would result in a suture core which is degradable, but an outer pCD coating which is not. This may or may not be an issue clinically, but if absorbable sutures were to be explored, I would think that a degradable form of the pCD hydrogel could be explored as well. The monomer form of CD can be functionalized with carboxylic acid, and this chemistry could potentially be utilized to created crosslinks that degrade in the body over time. Once reduce to a lower molecular weight polymer, and the CD monomer, the body should be able to break down these easily solubilized sugar compounds.

3.3 Integrated CD Fibers

The final formulation that I can see as a possibility for altering the pCD drug delivering suture is an integrated pCD suture fiber. Whether this fiber is based on nylon or something absorbable is yet to be determined, but it is possible that pCD microparticles or monomer units, could be integrated into the polymer backbone during electrospinning or extrusion. Using functionalized CDs would allow a number of chemistries to be used for this application, based on the chosen suture material.

In general, while I see a lot of potential future application in the suture space, pCD coatings as a whole have potentially wide-reaching applications. The coating process

outlined in this thesis work is simple, and could be adapted to a variety of biomedical devices or surfaces which have infection and inflammation concerns.

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