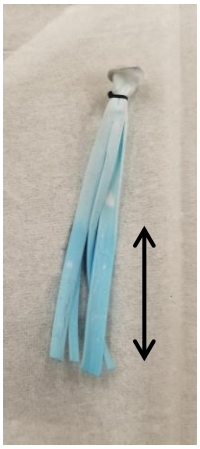
A. Perianal Fistula Plug

A1. *Electrospin fistula plug (pre and post surface modification)*

Fistula Plug Development: Several different constructs of fistula plugs have been developed. Our designs focus on using existing device structures made from electrospun materials (nondegradable). During this time, our group met with Dr. Vitaliy Poylin, who was brought onto the program as a consultant, to gain insight into existing devices (Cook and Gore). Dr. Poylin intimated that if healing occurs, should not be an issue for device to remain within the body. Two main polymer combinations selected (polyurethane (PU) and polyethylene terephthalate/polybutylene terephthalate (PET-PBT)).

One of the main challenges was to manufacture various-sized devices (one that could be used for the proof of concept studies in a rat fistula plug model and one that could be used for porcine and eventually human trials). We were able to form smaller versions of the clinically-relevant sized devices that could be implanted into the rat fistula tract model by altering electrospinning conditions and the collecting surface. For PET-PBT plug, we also prepared a thick nPET-PBT suture (60-minute electrospin) was used for the Jelly plugs to provide greater "tentacle" stability, resulting in a complete electrospun device. All devices were EtO-sterilized for implantation (currently evaluating E-beam sterilization as an alternative)



Fistula Plug Surface Modification: For PET-PBT plug, we evaluated creating a hydrolyzed surface to improve wicking capability in the "tentacle" area while reducing fluid movement toward the "head" of the device. Initially, the tentacles of the Jelly plugs were physically suspended in 0.5% NaOH for 30 minutes at 100°C followed by distilled water rinse. Plugs, which were evaluated for functional group formation via methylene blue dye uptake (provide visual assessment of greatest group creation), showed wicking located primarily on tentacles. A more automated process needed to be developed.

We then developed a novel 3D-printed chamber. Devices were inserted into the chamber. The height was adjusted to only target the tentacles of the device into the alkaline solution while each device "head" was placed under a cap snapped into the chamber to prevent exposure to the alkaline fumes. This process allowed us to modify 3 Jelly plugs at one time.

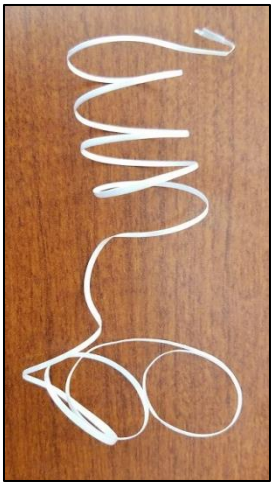
One set of nPET-PBT plugs were sent to BIDMC where Dr. Contreras cut an additional 4 "tentacles" into the plugs, resulting in a total of 8 tentacles. All devices were sterilized via ethylene oxide followed by implantation into tracts formed in the rat fistula plug model.

We have also begun to investigate developing a process in which the Jelly head will be pre-formed on the mandrel. This process will reduce the handling required to make the Jelly, providing a more consistent device that can be mass produced. We incorporated a 3D printed attachment that fits onto the mandrel, resulting in expansion of the electrospin that can be cut in half to provide the top of the Jelly. Several runs are being performed to determine reproducibility. We are also looking at varying the polymers comprising each device to provide flexibility with the design. We are currently evaluating using PU to create a Jelly and nPET-PBT to create a club. These studies will be ongoing over the next several months.

Regulatory Documentation: We also began the process of compiling the various properties of current fistula plug devices along with our prototype devices in anticipation of submitting this information during the regulatory approval process.

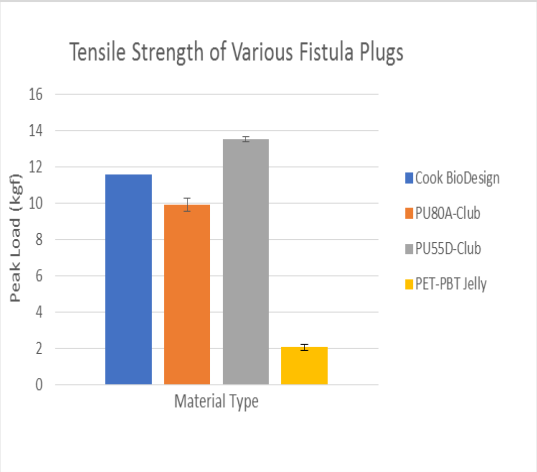
	Proposed BioSurfaces Anal Fitula Plugs			Cook		Gore
	Design A	Design B	Design C	BFP	BAFP	Bio-A Fistula Plug
						
Device Class	II	II	II	II	II	II
Degradable/NonDegradable	NonDegradable	NonDegradable	NonDegradable	Degradable	Degradable	Degradable
FDA Product Code	FTL	FTL	FTL	FTM	FTM	FTL
Indications for Use	Reinforcement of soft tissue for repair of anorectal fistulas	Reinforcement of soft tissue for repair of anorectal fistulas	Reinforcement of soft tissue for repair of anorectal fistulas	To reinforce soft tissue for repair of enterocutaneous fistulas	To reinforce soft tissue where a rolled configuration is reqd for repair of anal, rectal, and enterocutaneous fistulas	Reinforcement of soft tissue for repair of anorectal fistulas
510K number and year of approval	NA	NA	NA	K082682	K050337	K083266
Predicates	BFP, BAFP , Gore BAFP (Can we use FTM devices as predicates?)	BFP, BAFP , Gore BAFP (Can we use FTM devices as predicates?)	BFP, BAFP , Gore BAFP (Can we use FTM devices as predicates?)	1.BAFP (K050337)	1. Surgisis soft tissue graft (K980431) 2.Stratasis urethral sling (K992159)	1.Gore biosabsorbable mesh (K033671) 2. SIS fistula Plug (K050337)
Materials	PU 80A or PU 55D or blend of them	PU 80A or PU 55D or blend of them	PET	a. SIS, b.Cross linked SIS (X-SIS), c.Polyurethane, d.Suture (polydioxanone)	Porcine small intestinal submucosa (SIS) in lyophilized dried state.	Bioabsorbable Poly (glycolide:trimethylene carbonate) or PGA/TMC copolymer fiber
Physical Specs	6 mm x 95mm x 2mm	10mm disc x 9 mm long x 2 mm	10mm disc x 6 legs	15 mm x 6mm x 2 or 4 or 7mm	6 mm x 95mm x 2mm rolled and tapered	15mm disc x 100m long x 6 tubes (4-5 mm when squished together)
Packaging	Plastic thermoform, double bagged Tyvek and labelled. Outer cardboard package (PLANNED)	Plastic thermoform, double bagged Tyvek and labelled. Outer cardboard package (PLANNED)	Plastic thermoform, double bagged Tyvek and labelled. Outer cardboard package (PLANNED)	Plastic thermoform, double bagged Tyvek and labelled. Outer cardboard package	Plastic thermoform, double bagged Tyvek and labelled. Outer cardboard package	Double bagged with labelling on the clear plastic with minipax dessicant pouch in
Sterilization	EtO (PLANNED)	EtO (PLANNED)	EtO (PLANNED)	EtO	EtO	Gamma Sterilized
Biocompatibility	Permanent implant non-blood contacting	Permanent implant non-blood contacting	Permanent implant non-blood contacting	Permanent implant, degradable, non blood contacting	Permanent implant, degradable, non blood contacting	Permanent implant, degradable, non blood contacting
Price	TBD	TBD	TBD	$1469 (anal fistula plug set), with syringe, brush, suture, plug		Not Available
Reimbursement (need to confirm)	NA (to receive reimbursement from insurance need to show improvement over FLAP or LIFT procedure)	NA (to receive reimbursement need to show improvement over FLAP or LIFT procedure)	NA (to receive reimbursement need to show improvement over FLAP or LIFT procedure)	Patient out of pocket (need to confirm)	Patient out of pocket (need to confirm)	Patient out of pocket (need to confirm)
Animal Studies	Porcine (suggested)	Porcine (suggested)	Porcine (suggested)	Porcine (5 weeks)	Porcine	
Clinical trials	Vs FLAP for reimbursement vs BFP or BAFP for approval	Vs FLAP for reimbursement vs BFP or BAFP for approval	Vs FLAP for reimbursement vs BFP or BAFP for approval			
Anitbiottics used during surgery				1. Cipro + Flagell 2. Erithromycin +Neomoycin	1. Cipro + Flagell 2. Erithromycin +Neomoycin	
Sutures and needles used during surgery				a.2-0 UR-6 needles b.3-0 vicryl sutures	a.2-0 UR-6 needles b.3-0 vicryl sutures	
Procedures				Outpatient 20-25 min surgery (for Flap or LIFT add 10min)	Outpatient 20- 25min surgery	
End Point after surgery				Continence after 6months to yr with no adverse events. Secondary end point is quality of life and comfort	Continence after 6months to yr with no adverse events. Secondary end point is quality of life and comfort	Continence after 6months to yr with no adverse events. Secondary end point is quality of life and comfort

Seton Development: Electrospun setons were made from PET-PBT and ionic PU (polyurethane with carboxylic acid groups in the chain extender)-PET solutions. Setons with various widths (1mm, 2mm and 4mm) and thicknesses were made. Devices are made from tubular constructs which are extruded post-synthesis. Based on the idea of creating a surface functionalized seton material, we synthesized another seton made from a blend of ionic polyurethane (polyurethane that contains carboxylic acid groups within the polymer backbone) and PET. We prepared 1mm and 2mm setons using similar electrospinning conditions to the nPET-PBT seton, which also included a post-synthesis stretch. Methylene blue dye uptake confirmed the presence of the carboxylic acid functional groups within the polyurethane, with the intensity of the dye uptake much greater than the surface-hydrolyzed nPET-PBT setons.

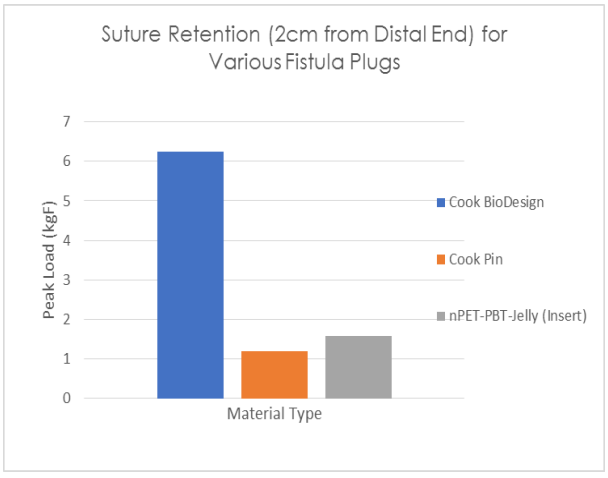


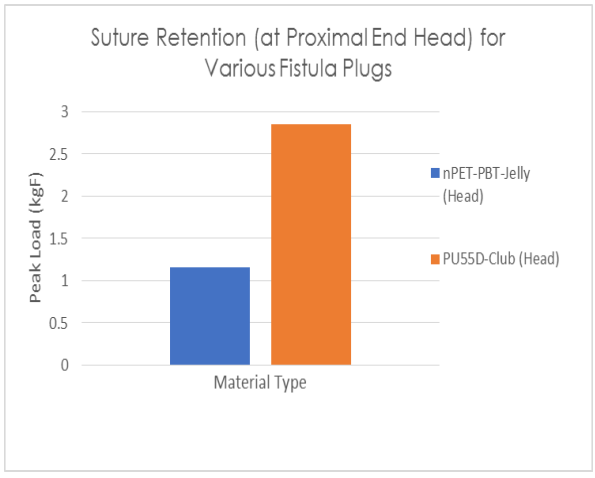
A2. *Physical testing/surface morphology of control plugs*

Fistula Plug Tensile Strength/Suture Retention: Several nanofibrous prototypes were randomly selected. Break strength and suture retention strength were evaluated at URI. These results were directly compared to current plugs using ISO parameters. Club strength (Type P) was similar to Cook BioDesign (similar solid construction). Reduction of strength within Jelly was expected due to hollowness of device since it is not solid throughout.

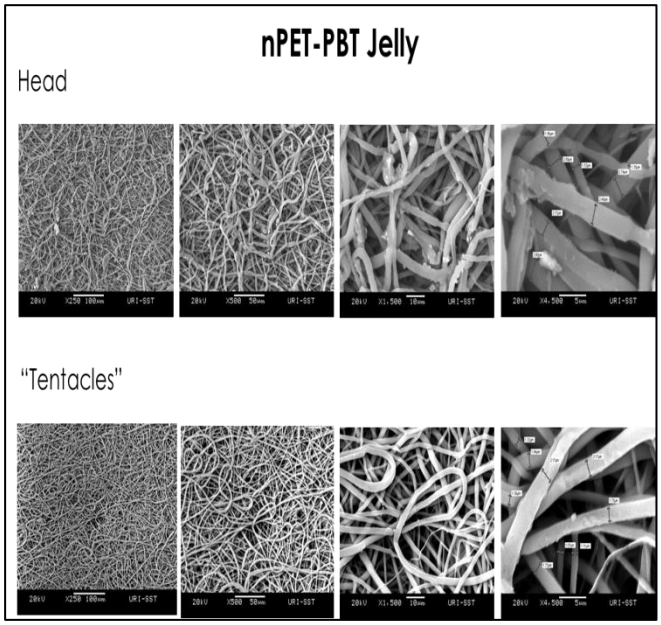


Suture retention toward the distal end of the Jelly plug was comparable to the Cook Pin. Both devices were lower than the Cook BioDesign, which is a solid device. All testing was performed without hydration, which is expected to significantly lower the strength of the Cook plugs and will not have an effect on our plugs. Suture retention at the "head" of our devices demonstrated the ability of these devices to be sewn into place. The Cook pin device uses a plastic head, which is not comparable to our fibrous device.

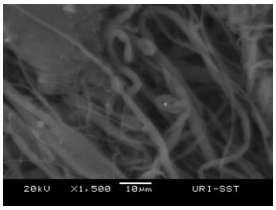


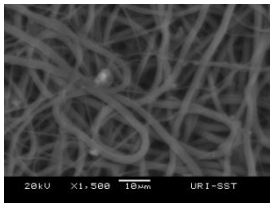


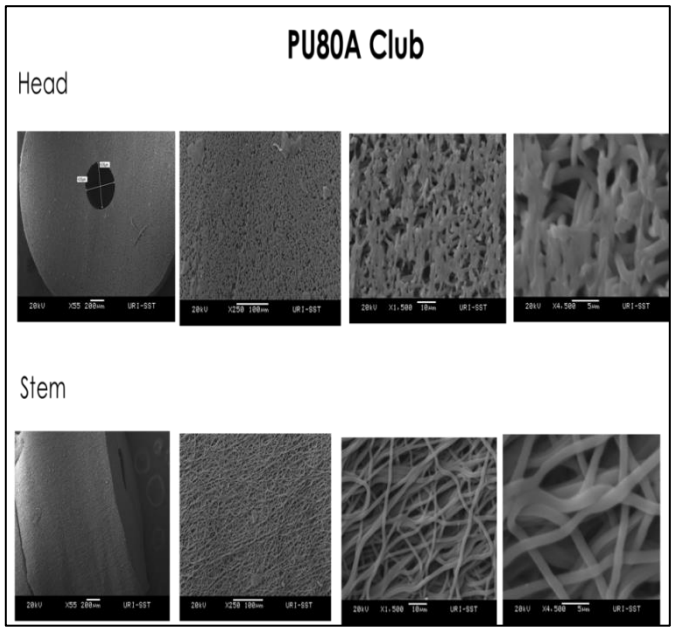
Fistula Plug Surface Morphology: We evaluated nPET-PBT Jelly plugs (both unmodified and hydrolyzed) as well as nPU club and nail devices via SEM. Hydrolysis of the Jelly plug did not have any gross differences in fiber morphology. However, tensile strength of the hydrolyzed flat materials appeared to be lower as compared to unmodified flat nPET-PBT materials (separate study). These flat materials will be used to assess differences in healing between control and surface modified materials. These materials were sent to Takeda after E-beam sterilization. We also prepared another group that was EtO-sterilized. From these materials, we were able to determine that there was no significant change in strength properties using either E-beam or EtO sterilization.

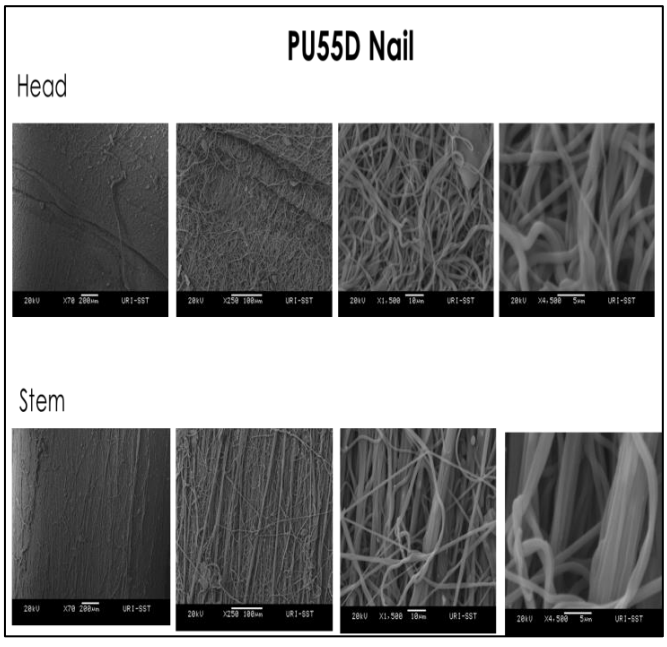


nPET-PBT Jelly (Hydrolyzed)

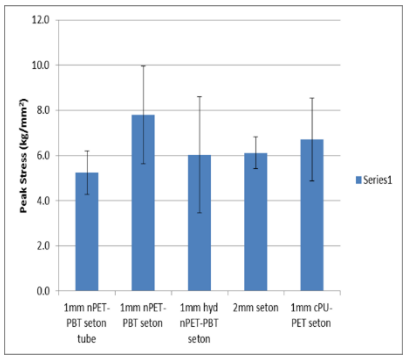




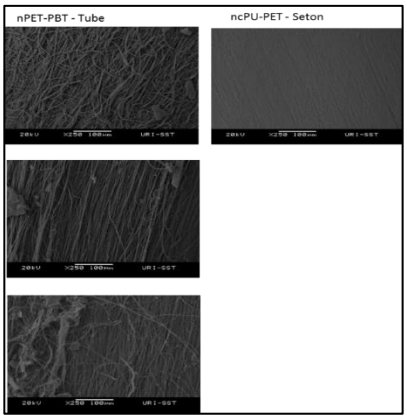




Seton Tensile Strength: nPET-PBT tubular constructs (1mm ID), extruded 1mm setons and hydrolyzed 1mm setons were prepared. Additionally, a 2mm nPET-PBT setons and ionic PU-PET setons were prepared. Peak stress (kgf/mm^2) for each material was determined. There was no significant difference between starting tubular construct and resulting seton. Additionally, hydrolysis did not adversely affect strength.

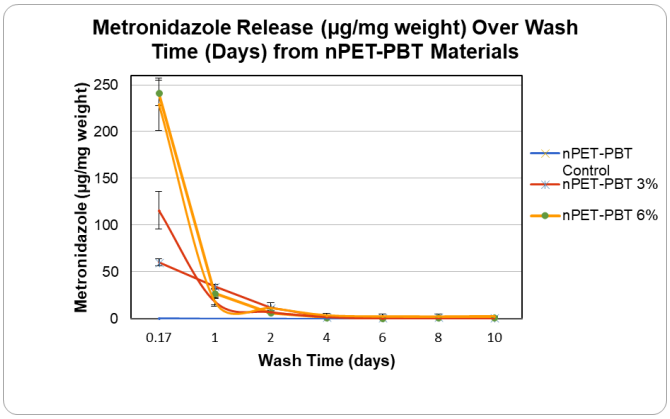


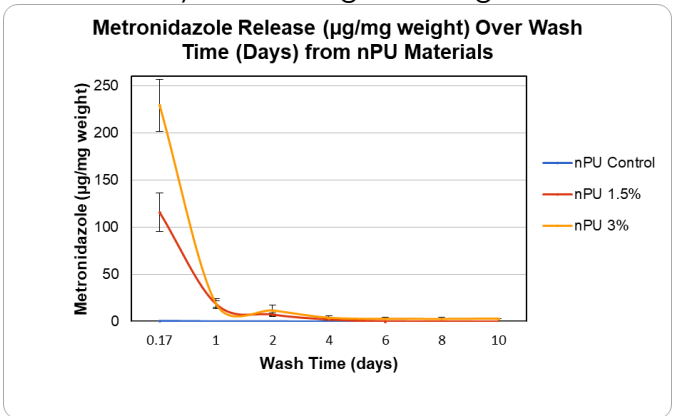
Seton Surface Morphology: Starting tubular nPET-PBT material has random nanofibrous distribution (typical morphology). Formation of the seton via extrusion provides directionality to the material. Surface hydrolysis does not alter the gross appearance of nanofibrous construct. Assessment of the ionic PU-PET material showed similar uni-directional orientation of the nanofibrous material.



A3. *Electrospin anti-infective fistula plug*

nPET-PBT materials containing 3% and 6% (w:v) metronidazole were synthesized. nPU-loaded metronidazole (1.5% and 3% w:v; respectively) materials were also prepared. Only vacuum-oven post treatment was carried out based on our previous short-term release studies. Three and ten day wash studies for control and the various metronidazole-loaded materials was performed. Metronidazole release occurred for 10 days for nPET-PBT materials. Initial metronidazole release from the nPET-PBT material was greatest from the materials with the higher initial metronidazole load, which was anticipated. However, drug release levels after 1 day of washing were higher with the 3%-loaded materials. The higher drug-loaded nPU material had release over 10 days. The 1.5% loaded material had no detectable release at 8 days. Antimicrobial activity of the washed segments will be assessed in the upcoming months. Control, unwashed and washed metronidazole-loaded materials were EtO-sterilized in preparation for the antimicrobial studies.



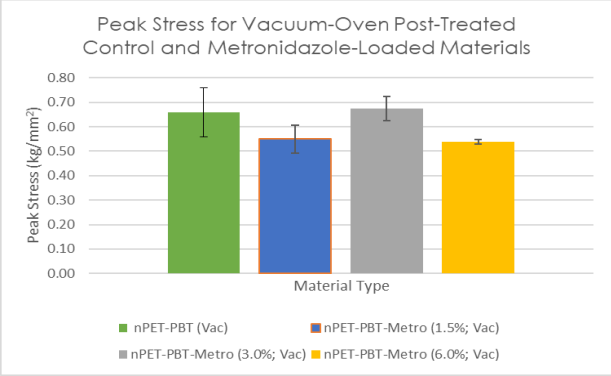


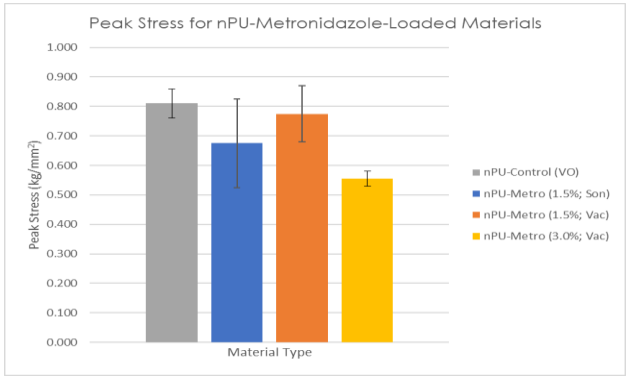
We also prepared our first metronidazole-loaded Jelly plug. The plug was made from 3% (w:v) metronidazole-loaded PET-PBT solution. Plug synthesis was comparable to non-drug loaded Jellys using this drug concentration. However, formation of a Jelly plug using 6% w:v metronidazole resulted in a stiffer structure which made it hard to form the head of the device.

After Metronidazole loading and release is characterized, ciprofloxacin will then be incorporated into the solution to provide full bacterial coverage. The initial amount of Ciprofloxacin loading will be derived from our historical work with the fluoroquinolone. Fluorescence could be used to differentiate between Ciprofloxacin and Metronidazole release.

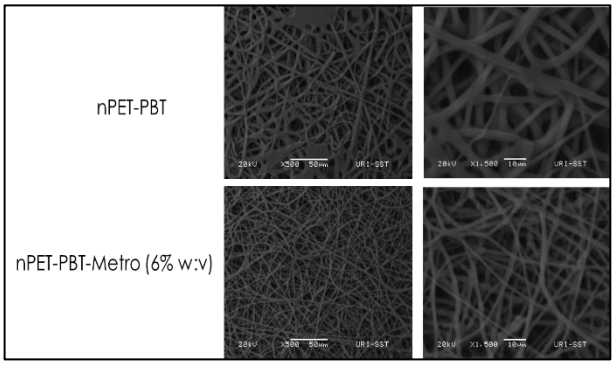
A4. *Physical testing/surface morphology of anti-infective plug*

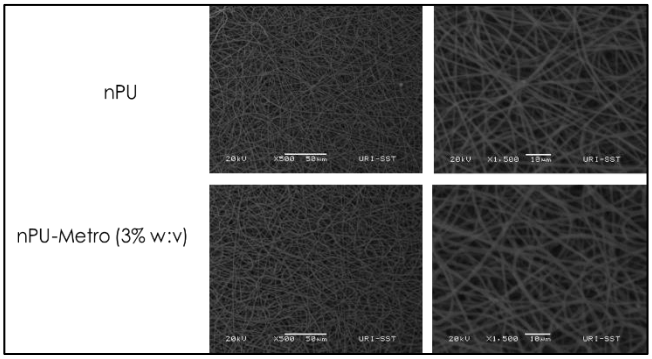
Physical Testing: nPU/nPET-PBT control and metronidazole-loaded materials were evaluated at URI. In both cases, materials with highest initial antibiotic loading concentrations did have decreased strength as compared to control materials. Plugs containing Metronidazole will be examined for physical testing and surface morphology in the upcoming months.



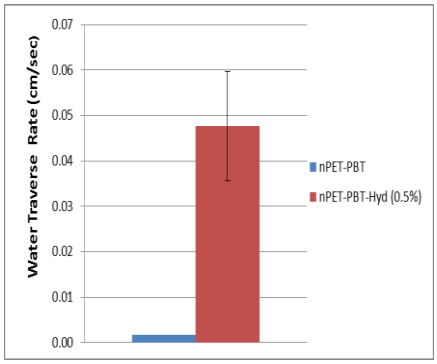


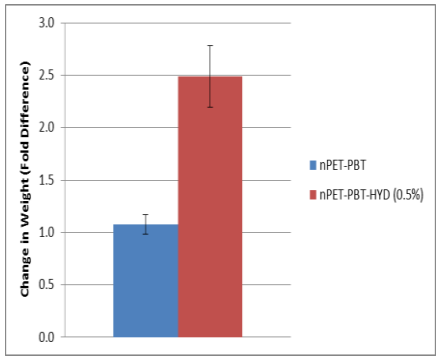
Surface Morphology: We randomly selected control and metronidazole-loaded materials to assess surface morphology via SEM. The highest loaded materials from each polymer type were evaluated to detect any potential changes in fiber morphology. There were no gross morphological changes in the drug-loaded materials as compared to their respective controls. The nPU fibers, as expected, were smaller in diameter as compared to the nPET-PBT fibers.



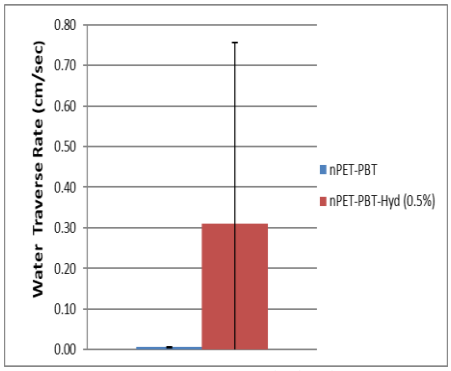


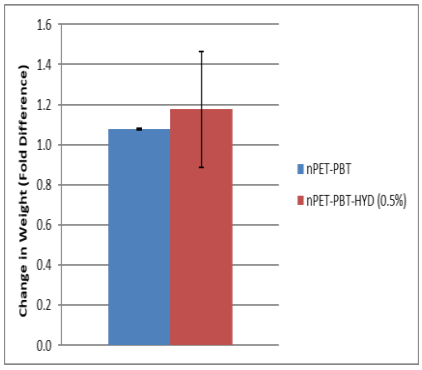
A5. *Benchtop antimicrobial/wicking testing*





Fistula Plug Wicking: Control and hydrolyzed nPET-PBT Jelly plugs were evaluated for solution wicking. Materials were suspended above an acid red 1 dye solution. Plug was then submerged into the dye solution, with dye migration visually monitored was over a 3-minute period (2cm length). From these studies, water traverse rate (cm/sec) was 28.6-fold greater with hydrolyzed plugs as compared to the control nPET-PBT plugs. Water uptake was 2.3X greater for the hydrolyzed plug as compared to the control.





Seton Wicking: Using the same process employed for the Jelly plugs, water traverse rate (cm/sec) was 54.8-fold greater with hydrolyzed setons than non-hydrolyzed seton. Water uptake was comparable between the setons. We did not immediately weigh these samples, so it is likely that water uptake will be greater with the hydrolyzed setons which we demonstrated using the hydrolyzed plugs. We will also assess wicking using ionic PU-PET setons.

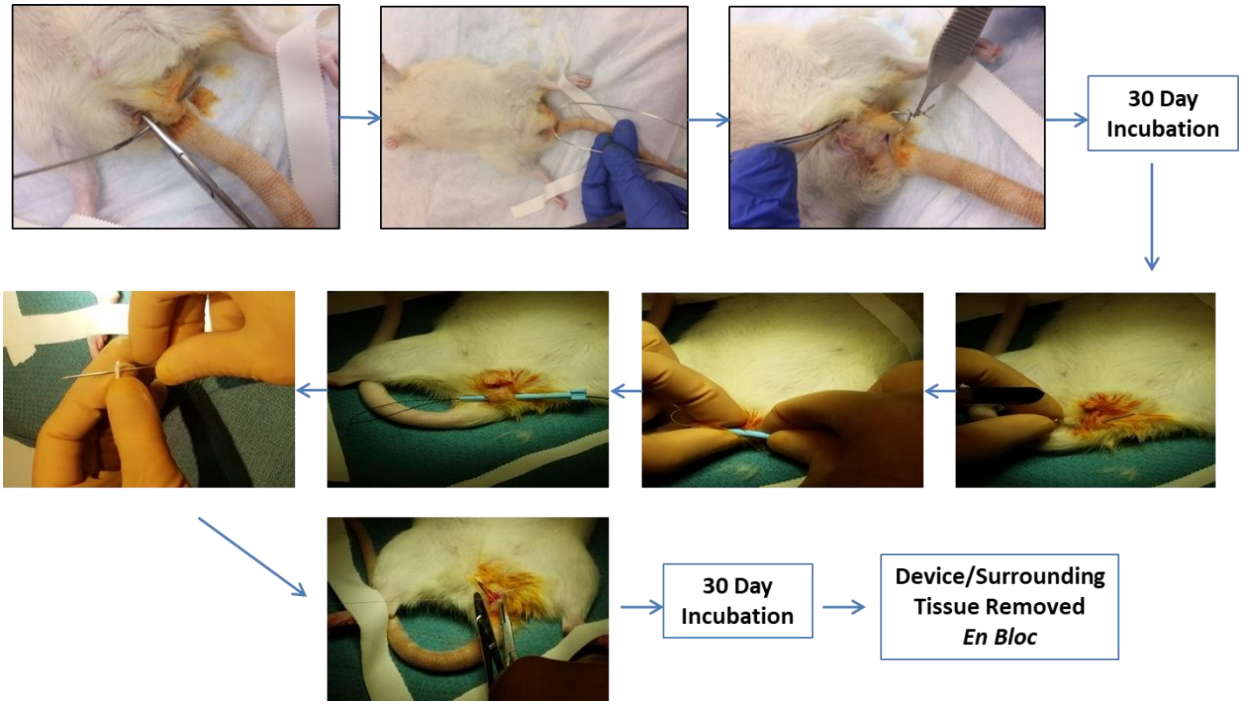
Antimicrobial Analysis: After a lengthy delay, anaerobic kits produced by Becton-Dickenson were received from Fisher Scientific. We have begun preparing broth and agar plates. The anaerobic bacteria *Bacteroides fragilis* will be used to evaluate antimicrobial properties of control and metronidazole-loaded materials. We plan on starting these experiments with simple growth and zone of inhibition studies using a Metronidazole Seni-Disc followed by evaluating Metronidazole-loaded materials for antimicrobial activity after undergoing stringent washing.

A6. *Biocompatibility of fistula plug*

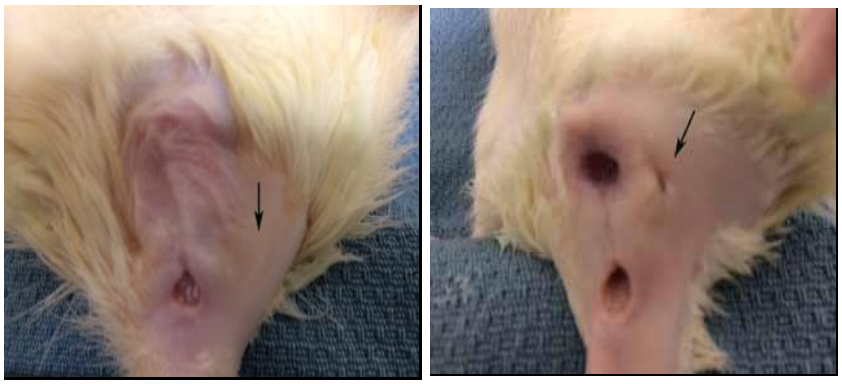
Biocompatibility (i.e. no toxicity, cellular healing) of the non-drug loaded fistula plugs will be assessed in the upcoming year using standard tissue culture techniques involving smooth muscle cells, which are essential in wound healing based on our data from the rat preclinical model. Materials will either be seeded directly with cells or placed into a tissue culture well containing smooth muscle cells to assess healing and toxicity, respectively. We have demonstrated from the drug release studies in Section B that fibroblasts readily attach and proliferation on control materials so we anticipate similar results with smooth muscle cells.

A7. Preclinical assessment of fistula plug

Fistula tract was created in Wistar rats using protocol by Arakaki *et. al.* Size 7 surgical steel suture was inserted for 30 days to create the tract (n = 8 rats). In one rat, suture was removed and followed by immediate sacrifice to evaluate tract formation. In another rat, suture removed and rat was allowed to continue in protocol for 30 days to assess tract healing. For remaining 6 rats, either a Cook BioDesign (Pin), PU55D Club and nPET-PBT Jelly plugs were implanted into the tract (n = 2 rats/plug design). Rats were sacrificed at 30 days, with gross assessment and histology performed.



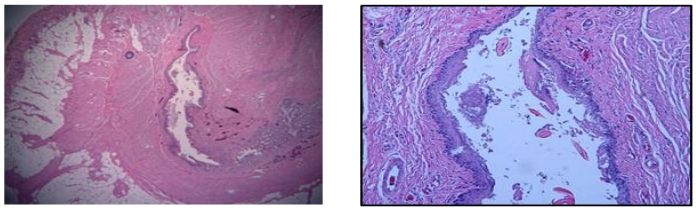
Wound healing along the outside of the tract was grossly apparent. There was no visual difference between the no implant, Cook BioDesign and electrospun plugs. Based on our surgeon's input, it was hard to feel plugs through the skin (only 1-2 could be detected). Tract area and surrounding tissue removed *en bloc*.



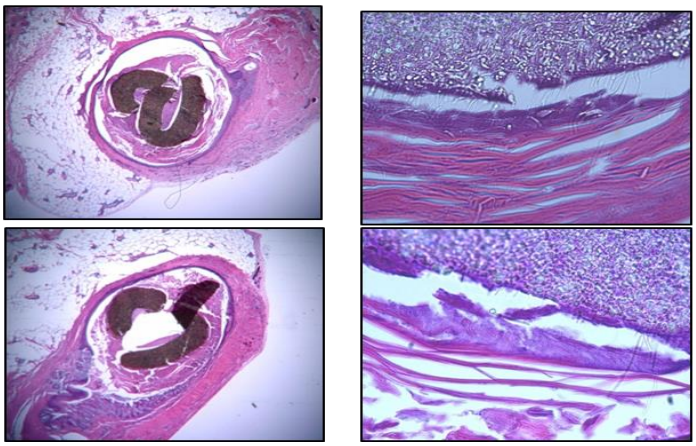
All plugs remained in place after implantation for 30 days. Tract was that was formed using the steel suture was detectable via histology. After removal of the steel suture and permitting the tract to remain unaltered for an additional 30 days, the tract did not heal. In contrast, healing around Jelly plug was observed with tissue ingrowth at the surface of the device.

Plug 1

Tract – 30 Days Post-Suture Removal

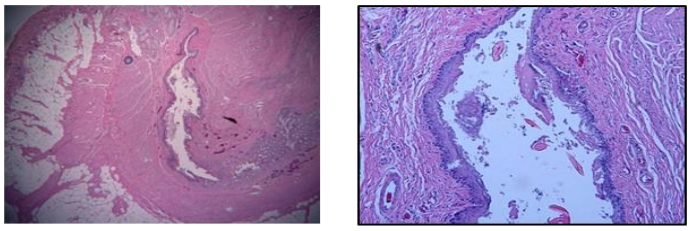


Jelly Plug – 30 Days Post-Suture Removal

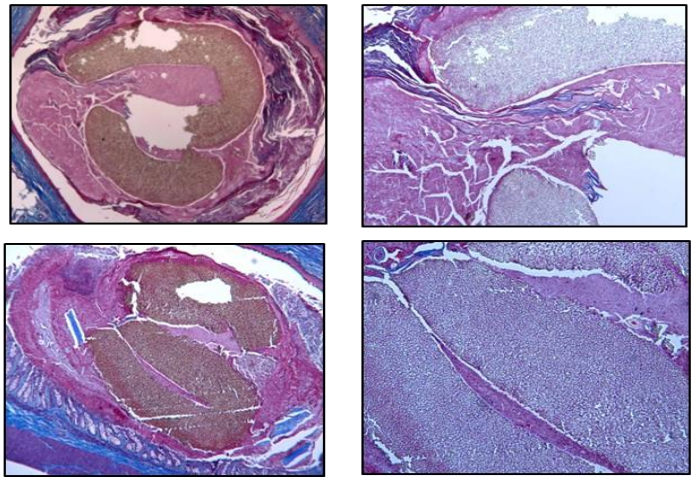


Plug 2

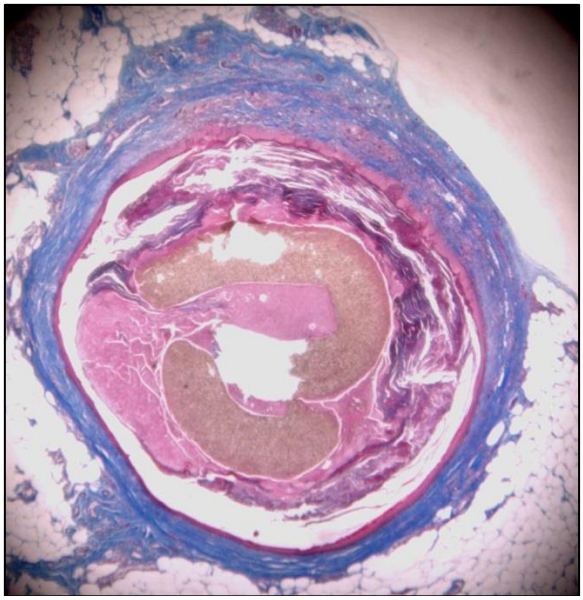
Tract – 30 Days Post-Suture Removal



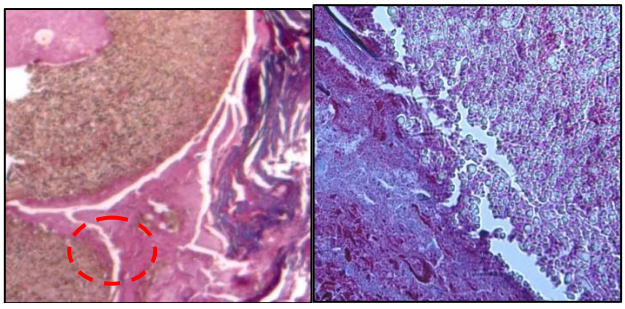
Jelly Plug – 30 Days Post-Suture Removal



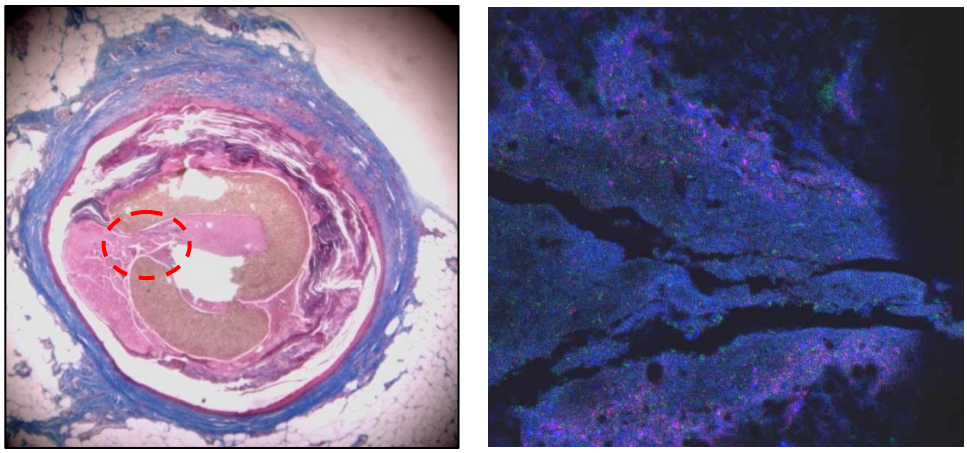
For Immunohistochemistry studies, areas of interest to be evaluated were the "interface" of the J-Plug and tissue to assess cellular type, density and cellular penetration into the material. Antibodies were labeled with fluorescent field labeled Abs, such as; Ab-CD31 (Endothelial cells/ECs), Ab-206 (Macrophage type II; M1), Ab-INOS (Macrophage type I; M1), Ab-SMA (SMCs/myofibroblasts), and Ab-Collagen (Type III). Slides were covered slip with mounting media and DAPI (cell Nuclei). Slides were analyzed under Confocal Fluorescent Microscope as well as Wide-Field Fluorescent Microscope. Different pseudo colors were attributed to each Ab specifically: Blue/DAPI to identify Nuclei; Fuscia/CD31 to identify ECs; Green/Collagen T-III; Orange/206 to identify M-T II; Grey/INOS to identify M-T I; Yellow/ SMA to identify Myofibroblasts. All slides had a negative control to confirm targeted binding of the antibody.



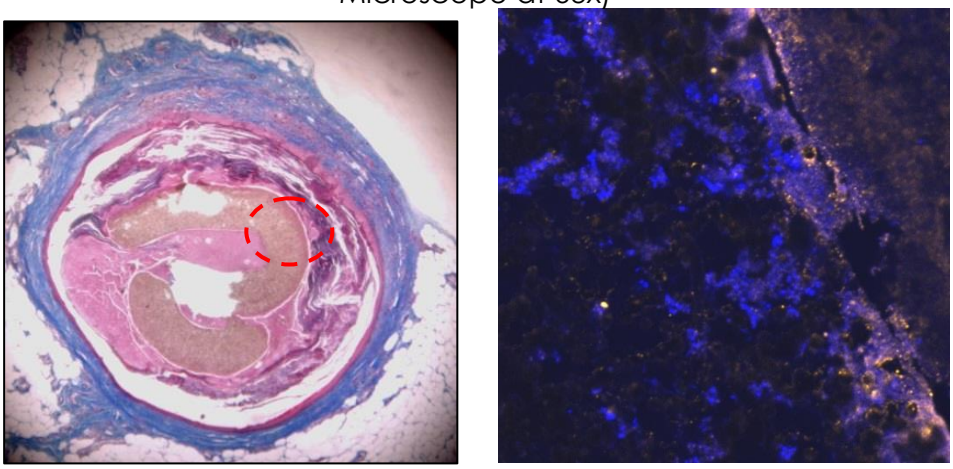
AF Tract with J-Plug (2-Arm Prototype); 30 Days Post-Implant
Specimen # 504772 (Cross-Section; Mason's Trichrome at 2X)



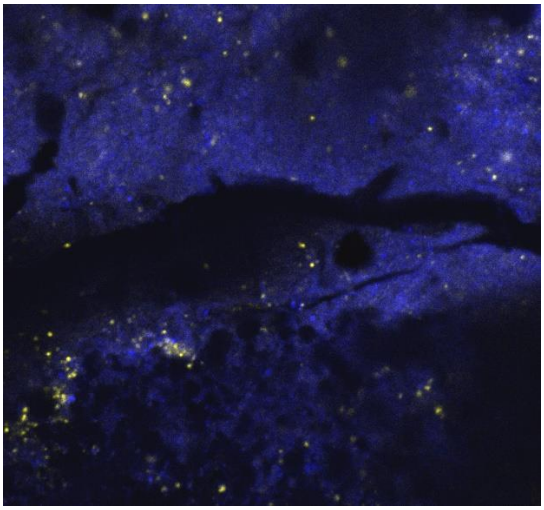
INTERFACE Between J-Plug Material (Grey) and Surrounding Tissue (Pink)
Specimen # 504772 (Cross-Section; Mason's Trichrome at 4x and 40x)



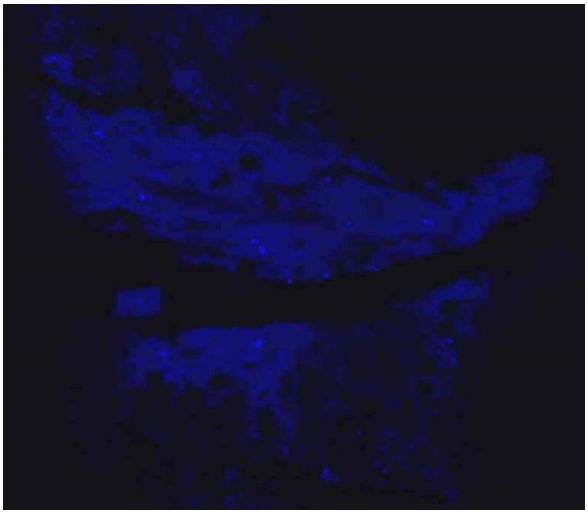
INTERFACE between J-Plug and Surrounding Tissue
(Cross-Section; CD-31/Fuscia, Collagen Type III/Green with DAPI/Blue Confocal Microscope at 63x)



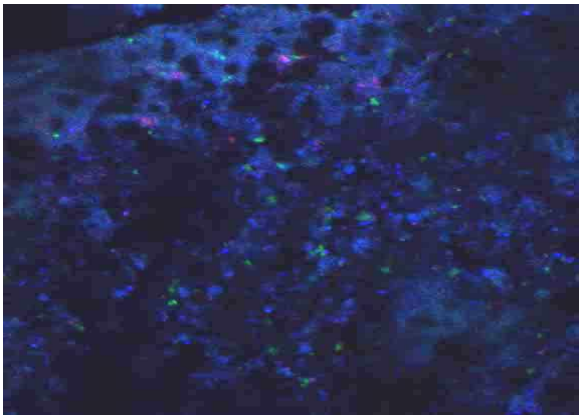
INTERFACE between J-Plug and Surrounding Tissue
(Cross-Section; 206/Macrophage M2/Orange with DAPI/Blue Confocal Microscope at 63x)



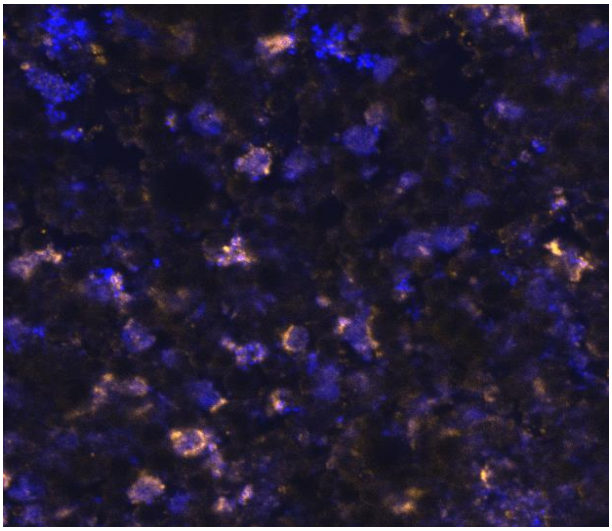
INTERFACE between J Plug and Surrounding Tissue
(Cross-Section ; SMA/Yellow with DAPI/Blue Confocal Microscope at 63x)



INTERFACE between J-Plug and Surrounding Tissue
(Cross-Section; Negative control (No Fluorescent Antibodies) with DAPI/Blue Confocal Microscope at 63x)



Interstices within the J-Plug Material
(Cross-Section; CD-31/Fuscia, Collagen Type III/Green with DAPI/Blue Confocal Microscope at 63x)

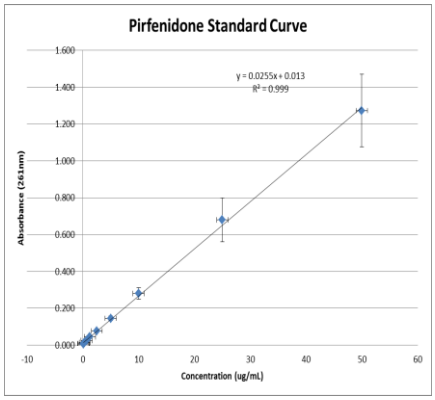


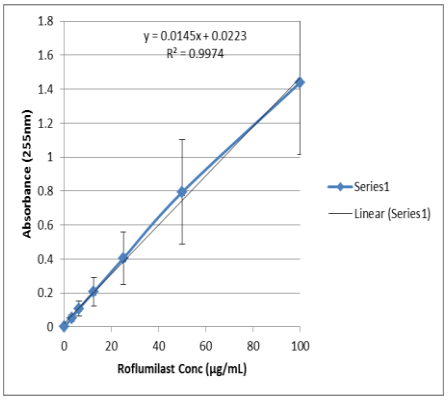
Interstices within the J-Plug Material
(Cross-Section; 206/Macrophage M2/Orange with DAPI/Blue Confocal Microscope at 63x)

The perianal fistula tract remained intact and was maintained following Jelly plug prototype implantation (at 30 days and harvest at 60 days). The electrospun material prompted the classic foreign-body response, with initial protein sheathing initiated by fibrinogen deposition. Binding macrophages then initiated the inflammatory and healing response, including formation of multinucleated giant cells. The ratio between Macrophages M1 and M2 could not be made because INOS Ab (marker of M1) was inconclusive and most likely did not work. However, Ab206 worked well (marker of M2), thus promoting cell proliferation and tissue repair, unlike M1, which inhibits cell proliferation and causes tissue damage. Positive staining for SMA and Collagen Type III confirmed the presence of active myofibroblasts, thus contributing to fibrous capsule formation. It was very interesting to see a vascular network not only at the interface between the Jelly plug material and the surrounding tissue, but within the interstices of the material as positive staining for CD31 Ab (specific EC marker). It remains to be seen if these immature/fragile micro-vessels can mature to more robust vessels that could support the cellular infiltrate/penetration into the material, which was quite intense as seen by positive nuclei staining (DAPI).

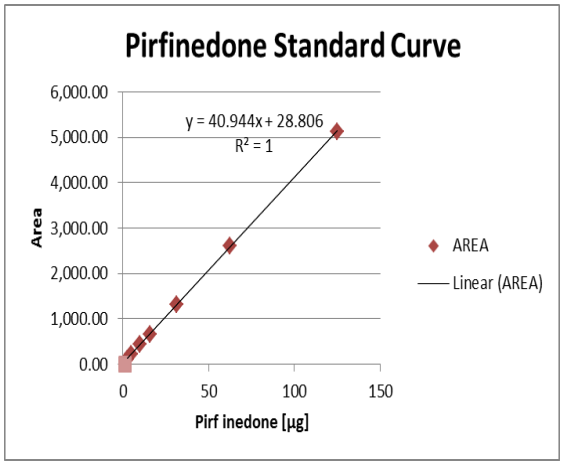
B. Gastric Wrap/Stent Sheath

B1. *Acquire target drug from Takeda*





Both pirfenidone and roflumilast were acquired from RIA International after discussions with Takeda. Both drugs were characterized for solubility and maximum absorbance profile. We have also procured tacrolimus as an anti-inflammatory agent to locally treat UC. We have begun initial characterization of the drug in terms of solubility, which will be completed in the upcoming year.



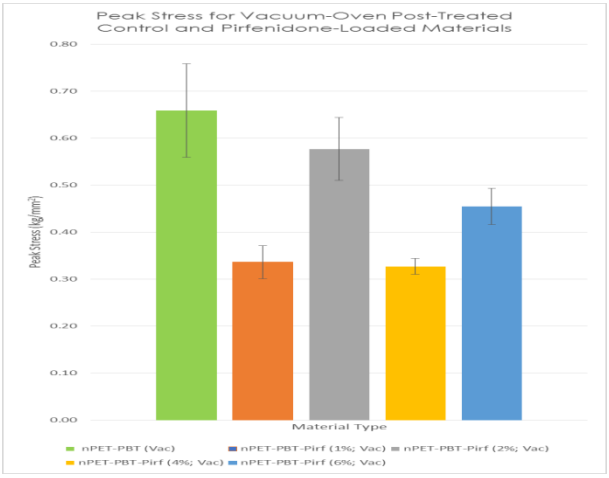
We also set up a HPLC provided by Takeda to assess drug stability after electrospinning. Analysis of unmodified pirfenidone and pirfenidone released from electrospun materials after washing is underway and will be completed in the upcoming year. We will also develop standard curves for roflumilast and tacrolimus to evaluate drugs being released from the respective materials. Additionally, the process will be used to assess metronidazole and ciprofloxacin release from the plug materials.

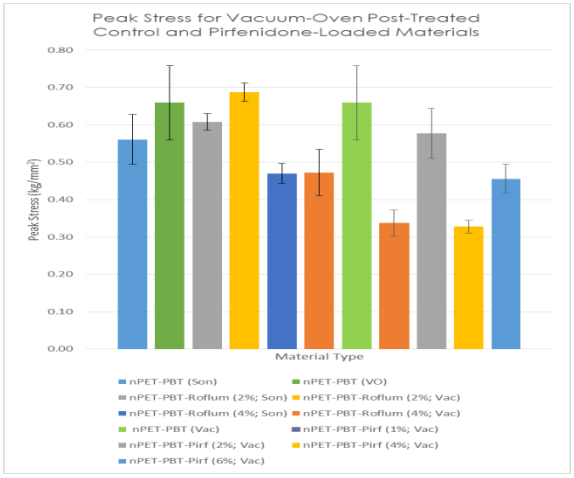
B2. *Electrospin control and drug-loaded flat sheets*

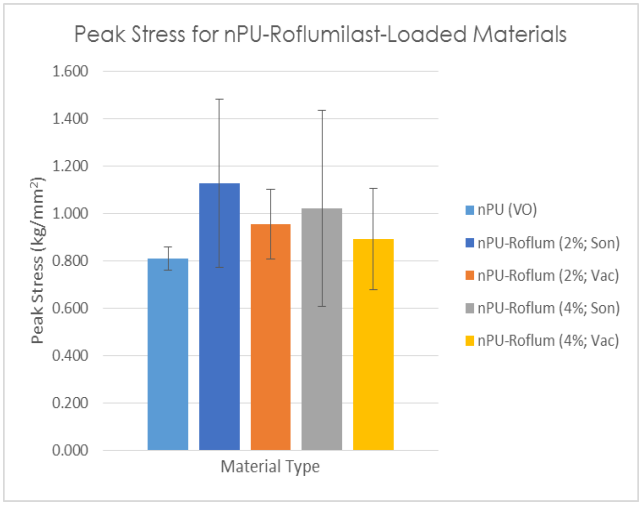
nPET-PBT materials with various pirfenidone concentrations (0%,1%,2%,4% and 6% w:v; post-treated with vacuum-oven) were synthesized. Additionally, nPET-PBT (0%, 2% and 4% w:v) and nPU (0%, 2% and 4% w:v) materials with roflumilast were synthesized. After examination of the materials for drug release, only vacuum-oven post-treated materials were assessed. All materials were cut and sterilized by either ethylene oxide or E-beam. Additionally, roflumilast-loaded nPET-PBT and nPU materials were synthesized. Materials were either ethylene oxide or E-beam sterilized (see Section B4).

B3. *Physical and chemical analysis of sheets*

Physical testing: Tensile testing for the materials described in Section B2 was performed at URI. For pirfenidone, material strength decreased as drug was added to the nPET-PBT polymer. For roflumilast-loaded materials, the highest drug loading resulted in a significant reduction in strength. Regardless of the strength reduction, all materials still possessed strength required for implantation since the proposed use for these materials is as a stent sheath or as a GI wrap, neither of which have extensive strength requirements.

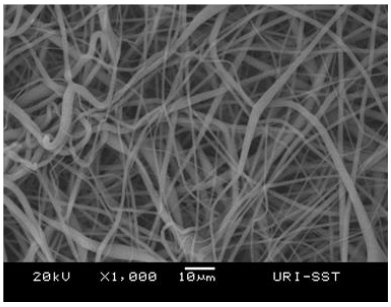


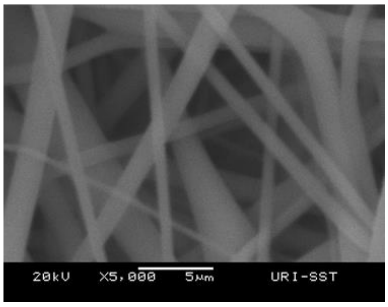




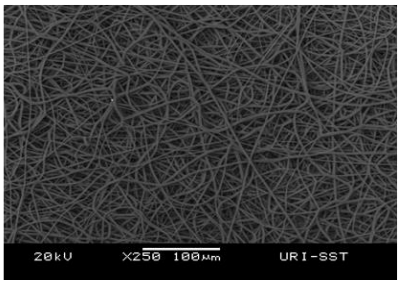
Surface Morphology: We evaluated pirfenidone and roflumilast-loaded electrospun materials via SEM. While physical testing demonstrated a reduction in material strength as drug-loading increased, there were no apparent changes in fiber morphology for the pirfenidone or roflumilast-loaded nPET-PBT materials. In contrast, increasing roflumilast loading in the nPU materials appeared to allow the drug to localize on the outer surface of the fibers (appearance of drug deposits).

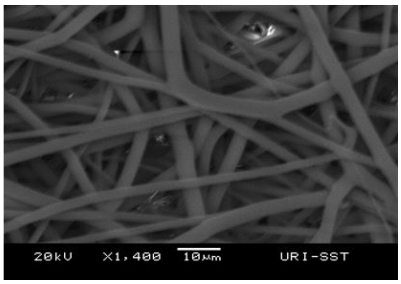
nPET-PBT (VO)

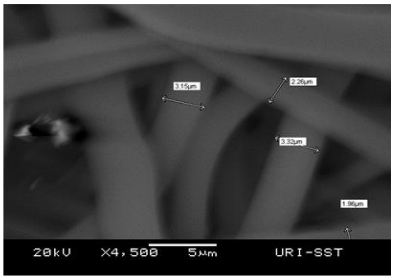




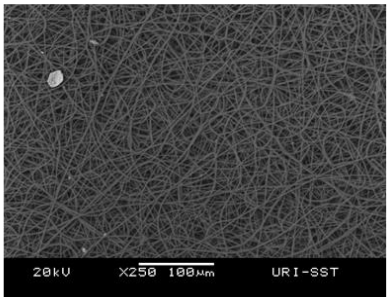
nPET-PBT-Roflumilast (2%; VO)

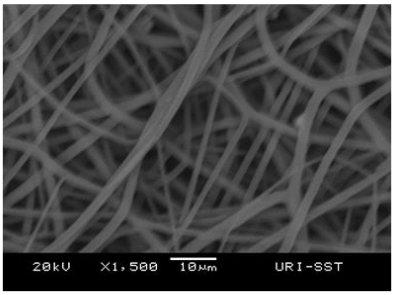


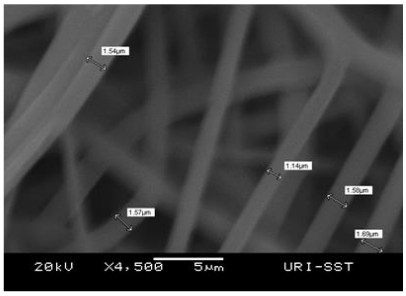




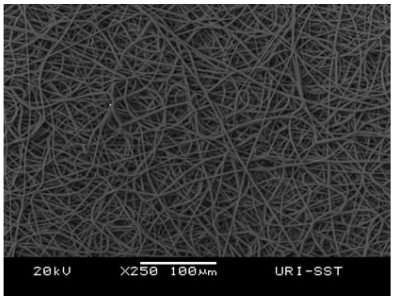
nPET-PBT-Pirfenidone (4%; VO)

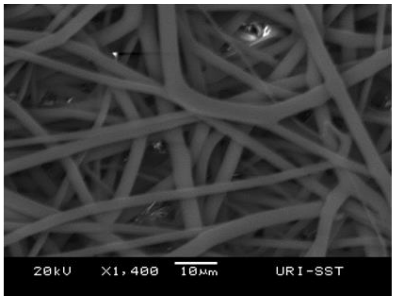


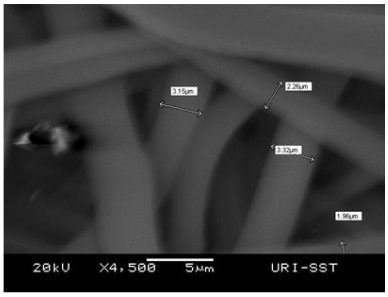




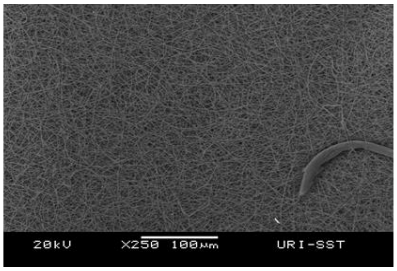
nPET-PBT-Roflumilast (2%; VO)

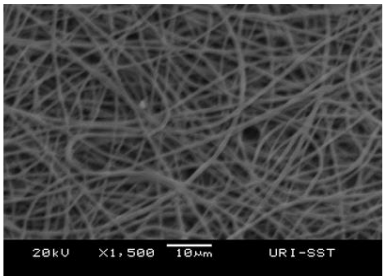


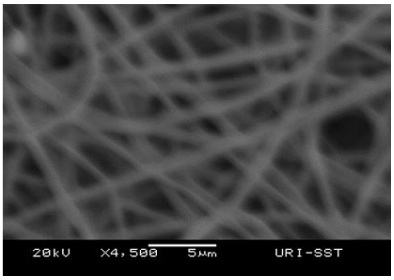




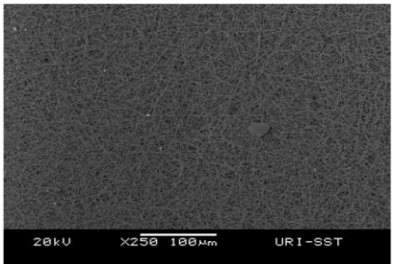
nPU-Roflumilast (2%; VO)

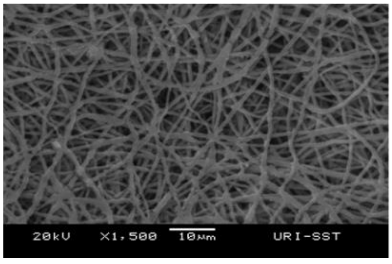


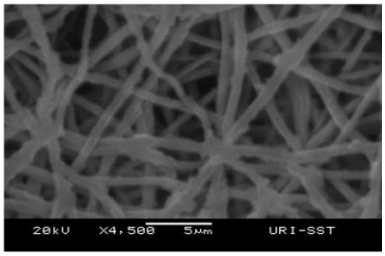




nPU-Roflumilast (4%; VO)

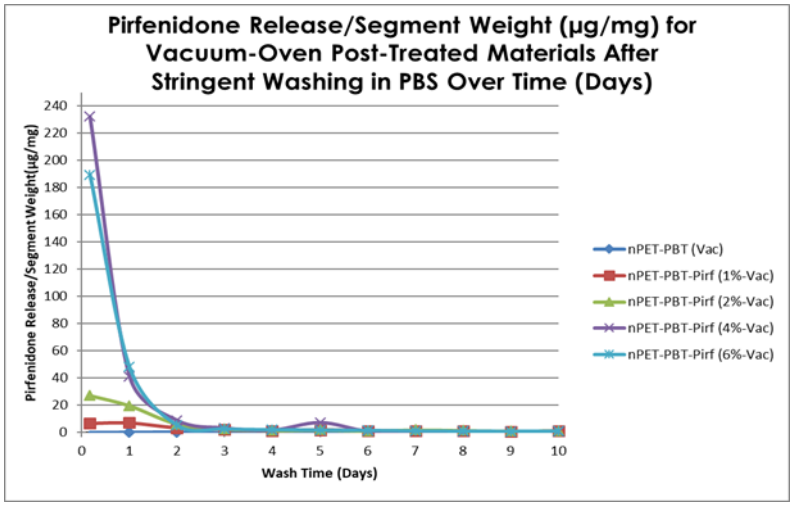




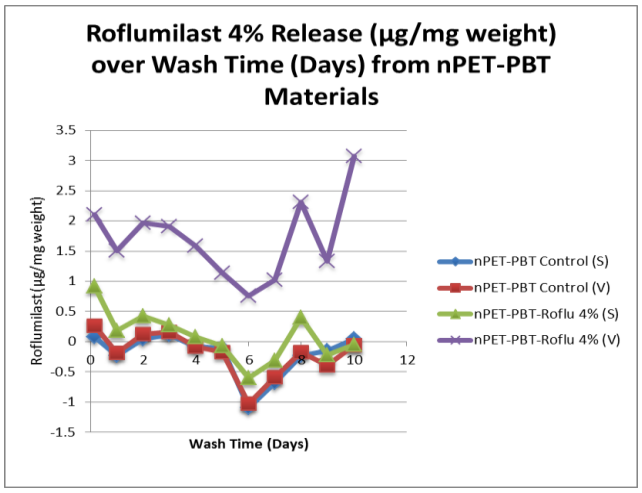


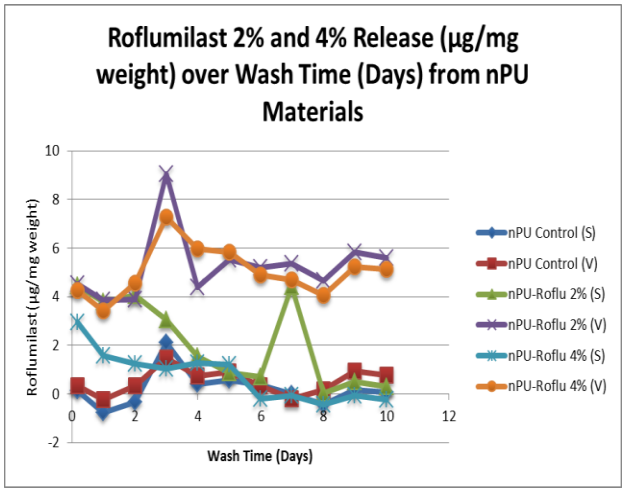
B4. *Examine drug release pharmacokinetics*

Initial pirfenidone release from the nPET-PBT materials was greatest using the 6% w:v loading concentration. Similar to the metronidazole results, release after 2 days leveled off between the highest concentrations, with the 4% loaded having higher release as the wash study progressed. This could be due to more rapid release of the surface bound drug, which could expedite release of the drug from deeper within the fiber.



Also similar to the metronidazole study, roflumilast release from the nPU materials was greater as compared to the nPET-PBT-Roflumilast materials at a similar concentration. This is a result of the openness of the PU polymer, which allows great wettability.



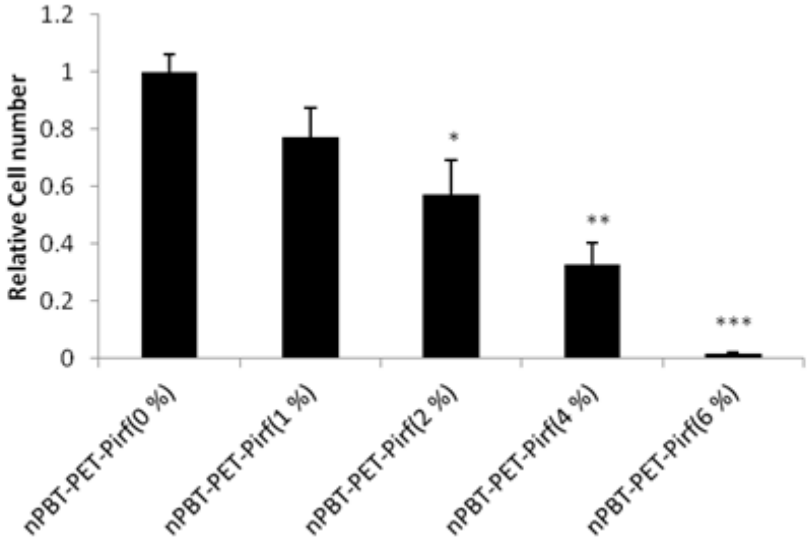


After washing, we sterilized materials using either ethylene oxide (EtO) or via E-beam. We were concerned about using EtO materials with providing enough time to fully degas to remove residuals. From our previous tissue culture studies, allowing materials to sit for a minimum of 30 days post-EtO sterilization results in a majority of residual EtO release from the surface. We determined that E-Beam sterilization at either 35 or 45 kGy does not affect pirfenidone activity or tensile strength of the material, providing us with a sterilization technique that does not require any extended shelf storage prior to use.

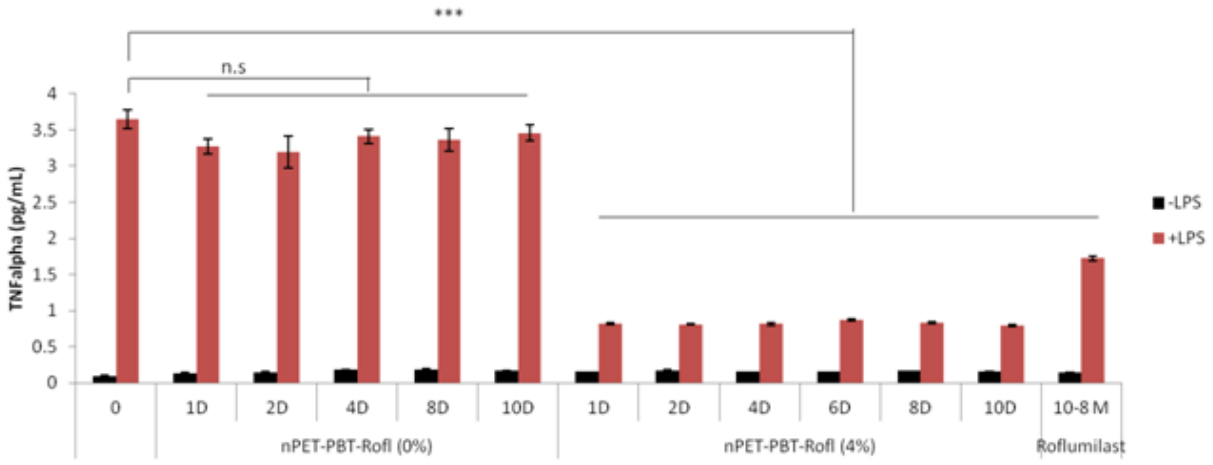
B5. *Evaluate bioactivity of released drug*

The rat cardiac fibroblast proliferation assay performed by Takeda to assess pirfenidone activity has shown that pirfenidone released from the vacuum-oven treated materials arrests proliferation in a dose-dependent fashion. Several pirfenidone concentrations were evaluated (0 - 4mg/mL), with the highest concentration resulting in cell death. Control and various-loaded nPET-PBT pirfenidone, sterilized by E-beam, had

comparable activity to non-sterilized samples, indicating that this sterilization process does not result in any residuals.



Testing of the nPU-Roflumilast materials (aerated for 40 days post-EtO sterilization, showed significant inhibition TNF-alpha production after washing for periods of up to 10 days as compared to controls and non-drug loaded nPU materials.



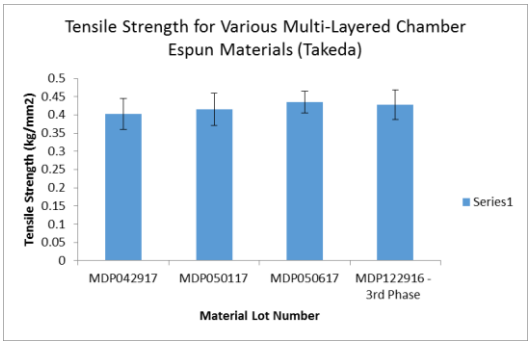
B6. ***Assess bioactivity in preclinical gastric wrap model***

Dr. Mauricio Contreras at BIDMC submitted a rat heterotopic transplantation of small bowel resections as described by Hausmann. This surgical protocol will be used to assess bioactivity of the pirfenidone/roflumilast-loaded materials. We are anticipating approval of the surgical protocol at his institution's animal care and use committee this month.

C. Cell Biofactory Chamber

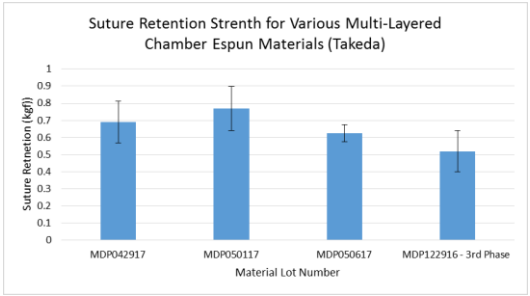
C1. Electrospin bilayered material components of chamber

Bilayered and quad-layered materials were electrospun into flat sheets and tubular constructs using processes developed in our initial contract work. We also were able to incorporate pirfenidone into outer nPET-PBT layer, which will be evaluated for protein permeation and cellular growth in the upcoming year.

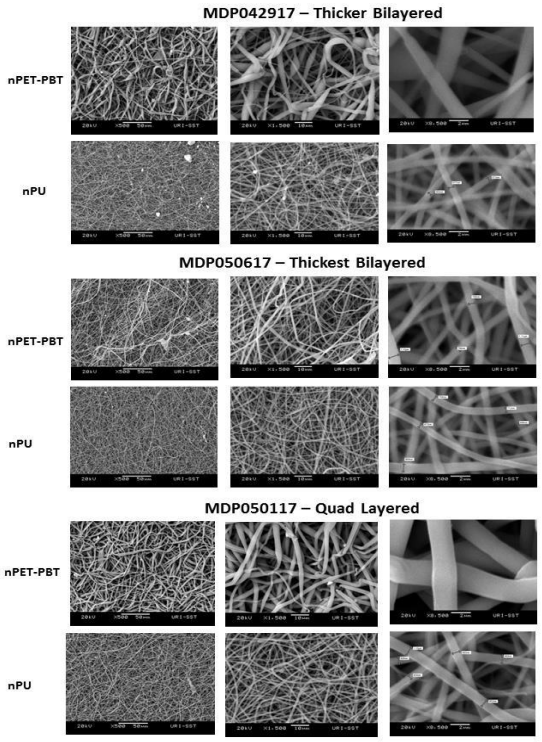


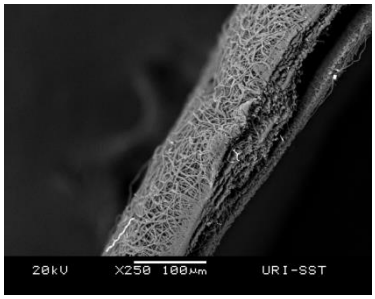
C2. Physical and surface morphological assessment

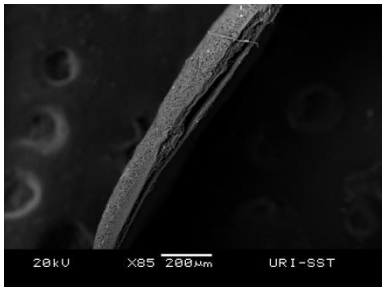
Physical Testing: Random sections of the layered materials were selected. Break strength and suture retention strength were evaluated at URI. These results, which were obtained in the contract work, showed no significant difference in tensile strength or suture retention between the various layered materials.



Surface Morphology: We evaluated layered materials on the surface as well as in a cross sectional area via SEM. These results showed that a fibrous composition on both sides of the layered materials (left image). Additionally, assessing a cross-section of the bilayered material showed two distinct layers (right images)

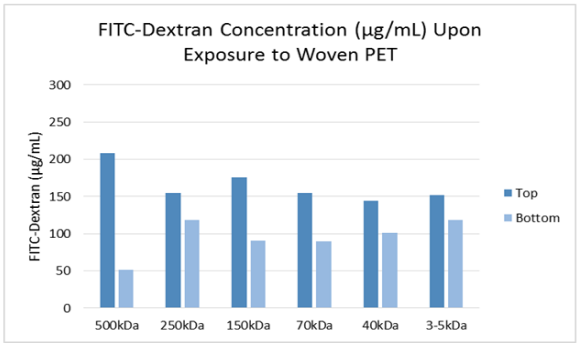


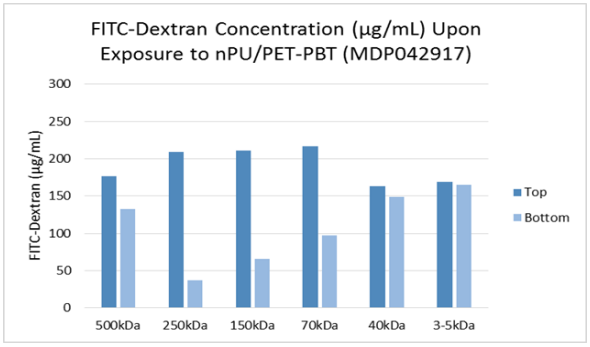


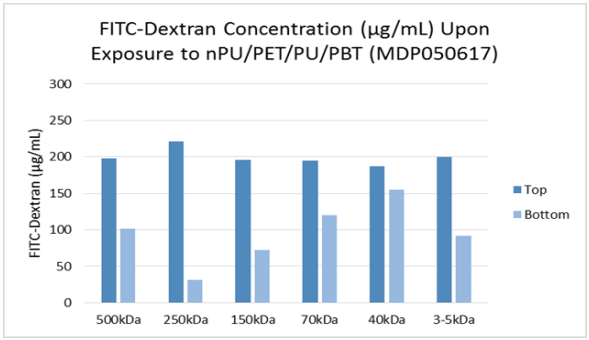


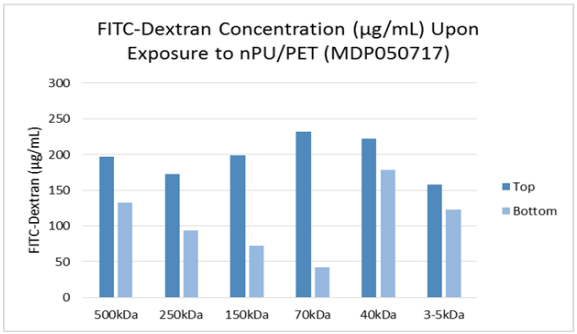
C3. *Tissue culture assessment of cell growth/protein production/release (Takeda/BioSurfaces)*

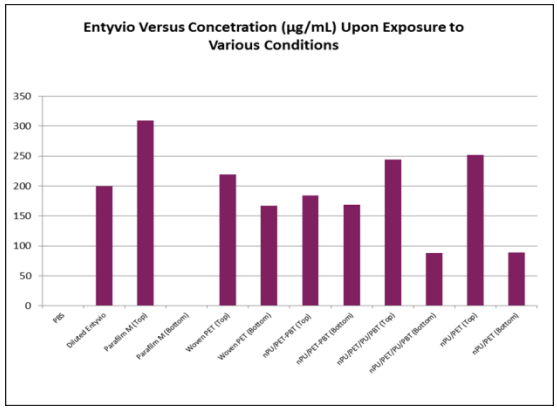
Protein Permeation: For the permeation studies, bilayered materials with different thicknesses and quad-layered materials were into circular segments. Assessment of Entyvio and fluorescently-tagged dextran was performed using our custom holding chamber. A SOP was developed for the process. These studies showed that altering the thickness and layers directly affected permeation. Entyvio permeation occurred through all materials, which is important to provide a local therapeutic dose.









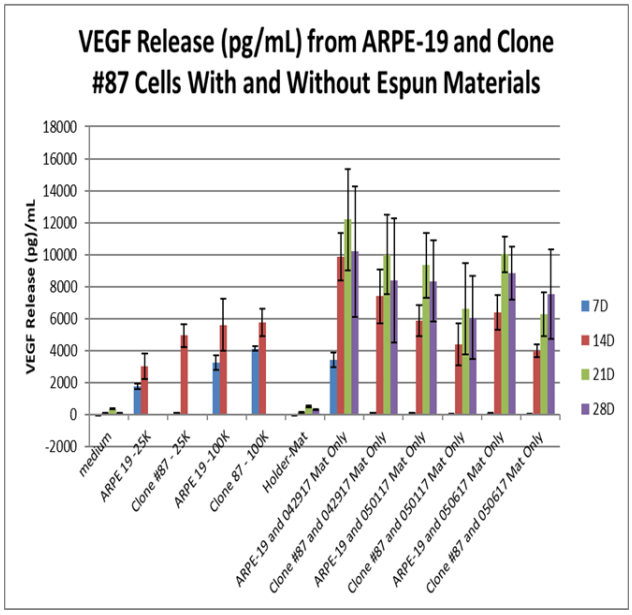


Cells were grown onto the nPU side of the material, with cell growth and protein production over time was examined. VEGF and Entyvio production was evaluated using ELISAs. SOPs for both processes were developed.

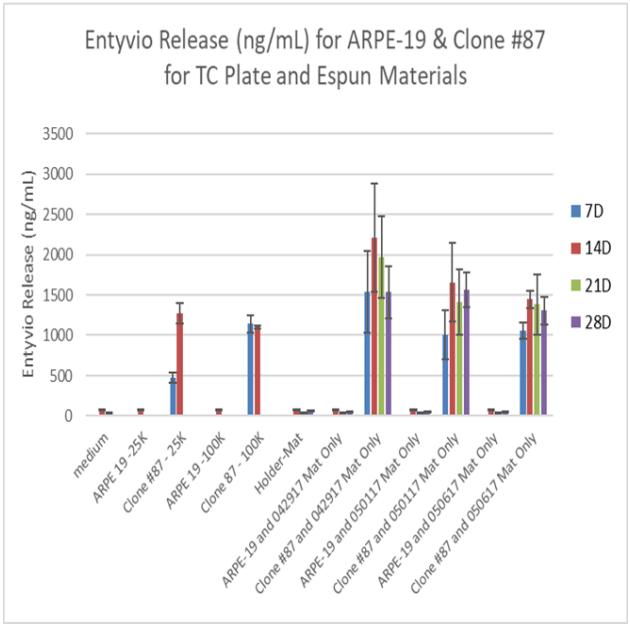
After confirmation that cells were viable and producing the target protein (ELISA), two segments with cells growing on the nPU side were sandwiched together with cells

facing each other and placed into a BioSeeder holding device. Unfortunately, cells were not confined to the sandwich area so data is not included.

Layered Materials



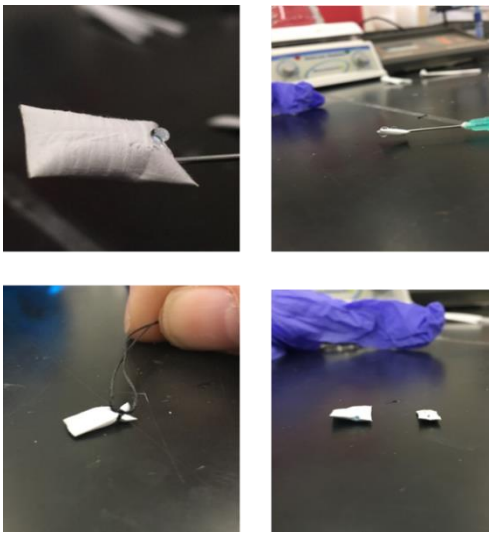
Layered Materials



VEGF production was persistent over 28 days from ARPE-19 or Clone #87 cells. Entyvio production also occurred over the 28 day study. Entyvio levels remained elevated on the flat sheets throughout the 28 day study.

C4. Develop cell chamber prototype

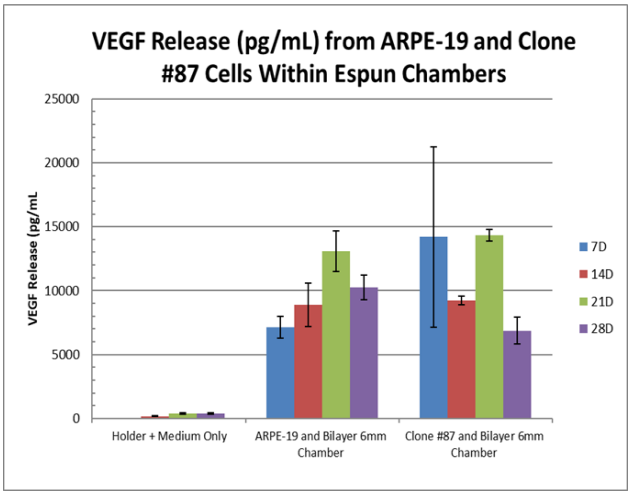
Chamber Formation: Tubular constructs were cut and ultrasonically welded to form chambers. Infusion of dye showed sealing of chambers with ultra-filtration permitted. Our biggest challenge will be sealing the infusion area.



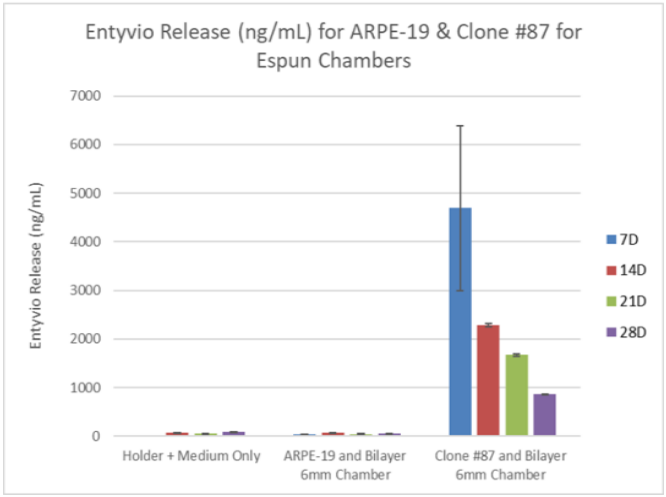
C5. Evaluate cell seeding, protein transport and viability within chamber

Cell Culture Assessment: EtO-sterilized bilayered and quad-layered materials were pre-soaked prior to seeding. Materials were held in place using custom holder. ARPE-19 and Clone #87 (Entyvio producing) were seeded onto the materials. Chambers were then infused with respective cells and sealed with Prolene suture tie. VEGF and Entyvio (custom assay developed by Sachiko) production by cells over 28 days examined via ELISA assays.

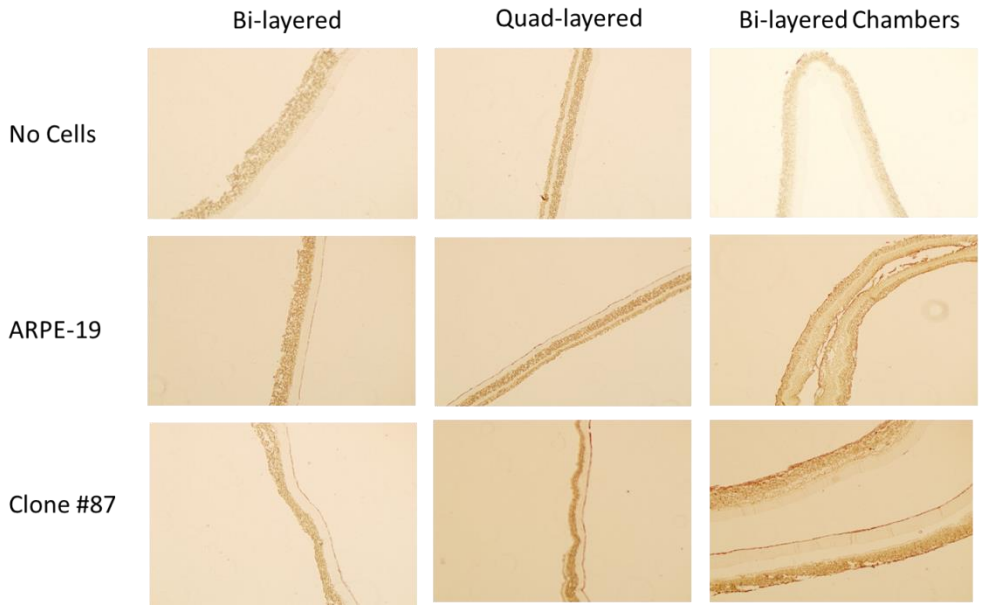
Chamber Materials



Chamber Materials



VEGF production from cells in the chamber decreased by 28 days, which could be due to cells growing into the material thereby reducing porosity. Entyvio production also occurred over the 28 day study. Entyvio levels reduced over time from the Clone #87 cells in the chamber. Again, this could be the result of reduced porosity as cell growth occurs within the chamber.

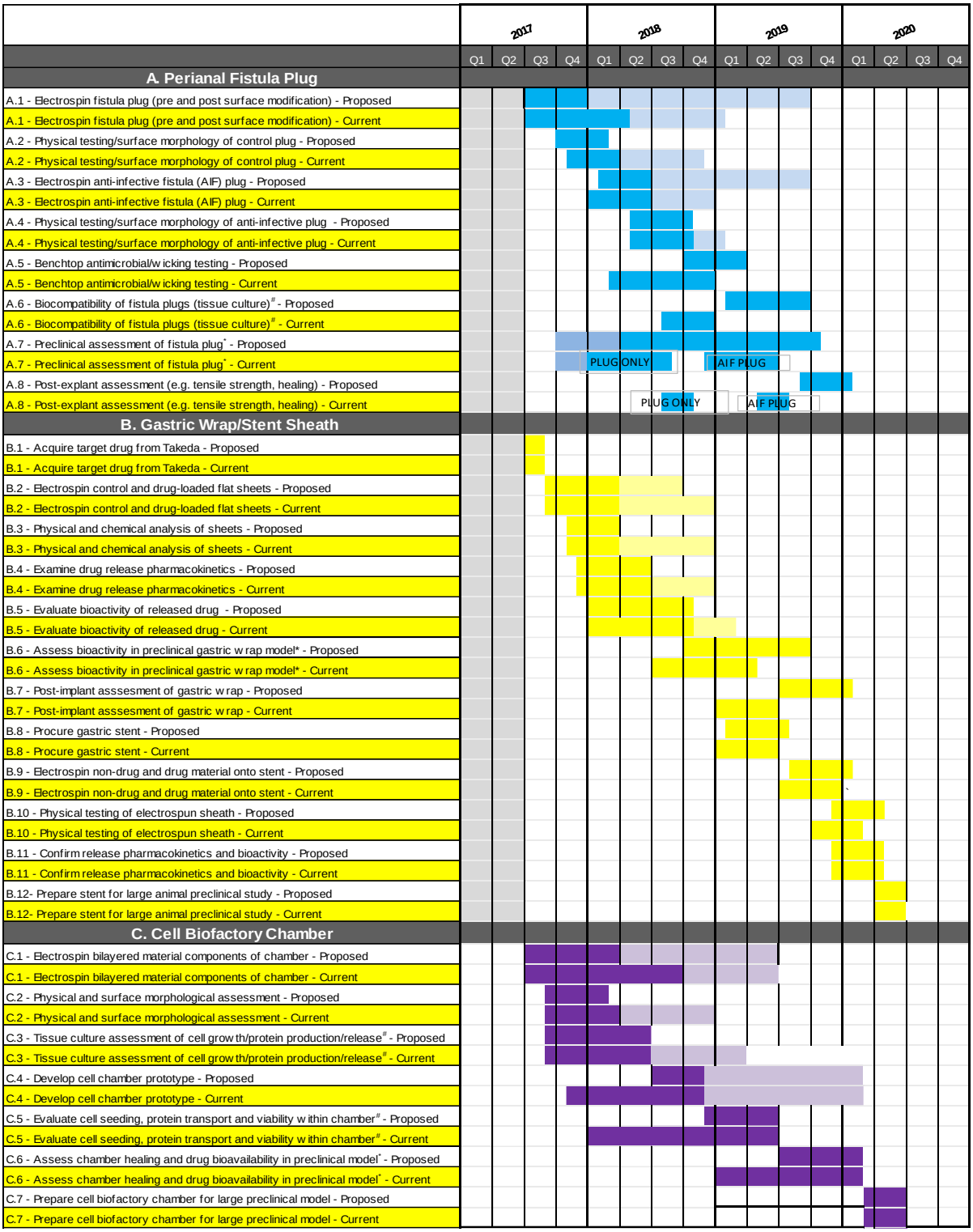


Histological assessment of the materials after the tissue culture study showed that cell growth occurred across all electrospun materials. This growth in the chambers could have slightly reduced permeation from inside the chamber.

Program Timeline

BioSurfaces-Takeda R&D Program

BioSurfaces' Fiscal Year (Jan - Dec)



Small animal preclinical development of the Jelly fistula plug is nearing completion. A decision by Takeda will need to be made to advance this technology toward large animal assessment followed by commercialization.