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Engineering a motile, controllable intestine with integrated electronics to study host-microbiome interactions

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14. ABSTRACT

Functional gastrointestinal disorders, including inflammatory bowel disease (IBD) and short bowel syndrome, may lead to chronic intestinal failure (IF), a complex medical condition that severely impacts the individual’s quality of life and is associated with high costs. Patients require long-term home parenteral nutrition (HPN), and frequent admissions for the underlying disease, contributing to high medical expenses, estimated at $150K per year per person. Moreover, liver and metabolic diseases are severe complications associated with HPN. The total annual financial burden of IBD alone in the United States was estimated at $31.6 billion in 2014. Currently, there is no permanent pharmaceutical cure for IF and some of the underlying conditions. Intestinal transplantation appears to be the most cost-effective treatment strategy. However, immune response issues and scarcity of donors hinder this approach. Tissue engineering evolved as a strategy to build replacement tissues for damaged organs. Current approaches to engineer intestinal tissues focus on recapitulating the absorptive function of the intestinal epithelial tissue. This is generally achieved by placing intestinal stem cells on absorptive scaffolds resembling native intestine. However, although such engineered tissues are capable of absorption, they lack peristaltic function, which is naturally obtained by the muscular layers and is critical for proper function. This project aims to develop a novel approach to engineer a smart, implantable intestine. The engineered intestine will integrate absorptive epithelial tissue together with a muscular bilayer and complex electronics.

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Title: "Engineering a motile, controllable intestine with integrated electronics to study host-microbiome interactions"

Principle Investigator: Prof. Tal Dvir

Period of Performance: 1/15/21 - 1/14/24

Report Abstract

Functional gastrointestinal disorders, including inflammatory bowel disease (IBD) and short bowel syndrome, may lead to chronic intestinal failure (IF), a complex medical condition that severely impacts the individual's quality of life and is associated with high costs. Patients require long-term home parenteral nutrition (HPN), and frequent admissions for the underlying disease, contributing to high medical expenses, estimated at $150K per year per person. Moreover, liver and metabolic diseases are severe complications associated with HPN. The total annual financial burden of IBD alone in the United States was estimated at $31.6 billion in 2014. Currently, there is no permanent pharmaceutical cure for IF and some of the underlying conditions. Intestinal transplantation appears to be the most cost-effective treatment strategy. However, immune response issues and scarcity of donors hinder this approach. Tissue engineering evolved as a strategy to build replacement tissues for damaged organs. Current approaches to engineer intestinal tissues focus on recapitulating the absorptive function of the intestinal epithelial tissue. This is generally achieved by placing intestinal stem cells on absorptive scaffolds resembling native intestine. However, although such engineered tissues are capable of absorption, they lack peristaltic function, which is naturally obtained by the muscular layers and is critical for proper function. This project aims to develop a novel approach to engineer a smart, implantable intestine. The engineered intestine will integrate absorptive epithelial tissue together with a muscular bilayer and complex electronics.

In the project's first year, a decellularization protocol was developed to establish a rat intestinal extracellular matrix (ECM) scaffold. Scaffold characterization demonstrated its biocompatibility and the maintenance of the unique crypt-villi topographical structure. Mouse LGR5 stem cells were successfully isolated and cultured into functional intestinal epithelial organoids to be used as the epithelial cell source for the final construct. Spontaneously contracting rodent smooth muscle cells were successfully isolated and aligned via gelatin micro-mold patterning to be used as the peristaltic layer of the final construct. Additionally, biocompatible microelectronics were designed and fabricated using AutoCAD software, photolithography, and wet-/ion- etching techniques.

In the project's second year, a technique for seeding the intestinal ECM scaffold with intestinal epithelial cells dissociated from organoids was developed. The re-epithelialized construct was cultured until a close-to-confluent tubular monolayer of the epithelium was achieved. Electrical stimulation to elicit contractions in the aligned smooth muscle cell construct was proving challenging, with variable stimulation parameters being explored. In parallel C2C12 cells (myoblast skeletal muscle cell line) were used to optimize the stimulation set-up. Finally, integration of the microelectronics with the ECM scaffold was achieved using gelatin crosslinked with microbial transglutaminase.

In the final year of the project, the re-epithelialised construct was further improved by increasing the seeding concentration of cells and increasing barrier integrity with the addition of dynamic culture (introducing sheer stress). Contractions in the smooth muscle constructs were successfully elicited via electrical field stimulation (EFS) on an improved scaffold: Aligned electrospun gelatin scaffolds presented less resistance than the micro-molded gelatin scaffold, allowing for contractions in response to EFS in 2D. Spatio-temporal control of the contractions was demonstrated, and the stimulation parameters influencing the contraction amplitude were evaluated. Experiments are currently underway, eliciting contractions in 3D and integrating the re-epithelialised construct with the smooth muscle cell -microelectronic hybrid construct.

Accomplishments

Research Objectives:

Aim 1: Create a biological scaffold to support intestinal tissue engineering that will recapitulate digestive function.

As described in previous reports, an enzymatic-detergent-based decellularization protocol on rat intestine was successfully developed. The resultant intestinal ECM scaffold was biocompatible and maintained both the intestinal micro and macro-structure, crucial for epithelial function (crypt-villi structure harbor LGR5 stem cell niches). Simultaneously, mouse LGR5+ intestinal crypts were isolated, cultured into intestinal primary organoids, and expanded in culture. Organoid characterization was done via immunostaining for different mature epithelial cell type markers and the forskolin-induced swelling assay, demonstrating functioning water channels and epithelial barrier integrity. The functional organoids are the cell source for re-seeding the intestinal epithelium. The organoids were dissociated and the cells seeded onto the pre-coated (with Cultrex® basement membrane extract) lumen of the ECM scaffold, forming a monolayer.

In this project's final year, the re-epithelialised construct was improved to reach a higher and more consistent epithelial confluency. This was achieved by increased organoid expansion and seeding higher cell densities. Figure 1A-D shows different planes of a Z stack confocal image, demonstrating epithelial cell presence along the crypt villi microtopography. This topography is crucial for long-term intestinal culture, as the epithelium completely regenerates every 5 days from stem cell niches, which are protected from the lumen contents and growth factor gradients at the base of the crypts. In addition, literature suggests that sheer stress significantly increases the epithelial barrier integrity, which is crucial to a healthy intestinal function by preventing leakage and reducing risk of infections. This was demonstrated by culturing a re-epithelialized construct for 26 days statically, then flushing the lumen with 10kDa FITC-dextran via a peristaltic pump and measuring the fluorescent leakage through the construct over 4 hours. The construct was then flushed and cultured under sheer stress (peristaltic pump flow) for an additional 4 days before the FITC-dextran perfusion was repeated. As Figure 1D shows, the addition of dynamic culture resulted in a significant increase in epithelial barrier integrity.

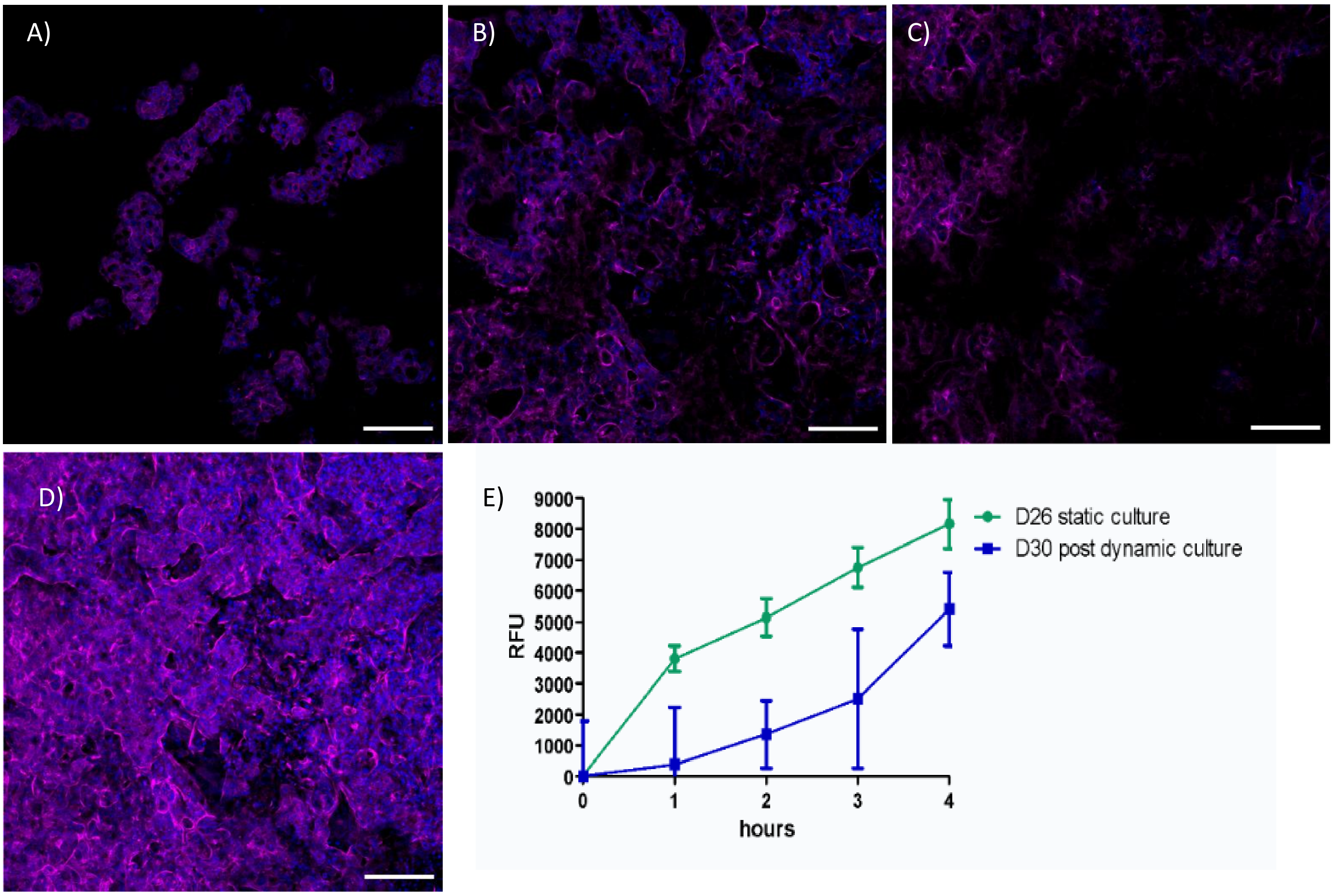

Figure 1. A-C) Individual planes of a Z stack of immune-stained re-seeded DC scaffold, after 14 days in culture, demonstrating cell presence from crypts to villi. Stained with Hoechst (blue) and phalloidin (purple). D) All planes of Z stack of immune-stained re-seeded DC scaffold, after 14 days in culture, superimposed, to show close to full confluency of epithelium. Stained with Hoechst (nuclei, blue) and phalloidin (f-actin, purple). All scale bars are 100um. D) FITC dextran (10kDa) perfusion assay showing barrier integrity inversely to FITC-dextran leakage from intestinal lumen, demonstrating significant increase in barrier integrity post 4 day additional dynamic culture.

***Aim 2: Construct monolayers of organized intestinal smooth muscle tissues which will be integrated onto the intestinal scaffold*.**

As described in previous reports, neonatal rat intestinal smooth muscle cells were isolated and expanded, and aligned on micro-molded soft gelatin hydrogel. Chemical stimulation

(15mM Acetylcholine) resulted in contractions of select areas. However, electrical field stimulation (EFS) of these samples did not render any conclusive results. C2C12 (mouse skeletal myoblast cell line) cells were differentiated in parallel and successfully produced aligned cellular sheets that were successfully stimulated to elicit uniform contractions via EFS after 27 days in culture (stimulation parameters: 1.5-3V, 10ms pulse width, 1Hz).

In this project’s final year, it was discovered that the thick patterned gelatin presented too much resistance for a single smooth muscle layer contraction. Thus, a protocol was developed to fabricate a thinner gelatin scaffold via electrospinning. Hexafluoro-isopropanol (HFP) was discovered as one of the few electro-spinnable gelatin solvents, and crosslinking was achieved via glutaraldehyde vapor to prevent fiber damage in the process. As crosslinking with glutaraldehyde raised concerns of cytotoxic remnants on the scaffold, the latter was vacuum dried, followed by treatment with multiple washes and glycine solution blocking overnight (Figure 2D). The electrospinning protocol allowed for aligned fiber formation, which is crucial for smooth muscle cell alignment and resultant maximum contractile force. The resultant scaffold presented less resistance to the smooth muscle cells, allowing for smooth muscle cells cultured on the scaffold to visibly contract spontaneously. Bidirectional layers were fabricated to mimic intestinal smooth muscle physiology by directly electrospinning one layer on top of the other before crosslinking. As a result of successfully having cultured smooth muscle sheets with contractile capacities, the C2C12 approach explored in previous years was dropped.

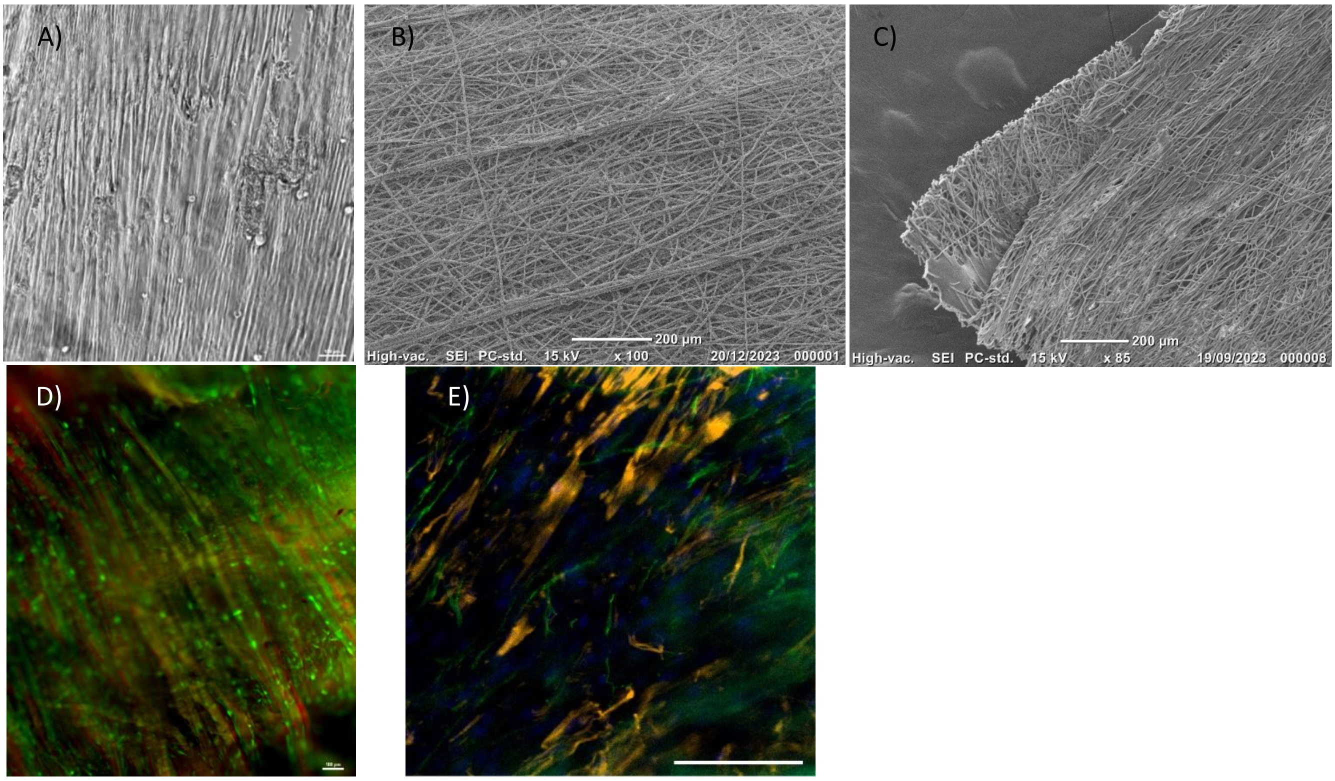

Figure 2. A) BF image of aligned differentiated C2C12 muscle fibers on micromolded gelatin scaffold demonstrating full confluency of muscle fibers. Scale bar is 100um. B-C) SEM image of aligned, bidirectional electrospun gelatin scaffold for intestinal smooth muscle cells. Scale Bar is 200um. D) Live/Dead assay after 48 hours, showing biocompatibility of electrospun gelatin scaffold after washing off glutaraldehyde remnants, and blocking with glycine, using C2C12 cells. Scale Bar is 100um. E) Confocal image of immune-stained smooth

muscle cells, showing alignment after 31-day culture on aligned gelatin scaffold. Stained with Hoechst (nuclei, blue), smooth muscle actin (orange) and Beta-tubulin III (green). Scale bar is 100um.

Aim 3: Design, build, and test flexible, stretchable electronics that will enable on-line monitoring, control, and regulation of the engineered intestinal graft.

As described in previous reports, freestanding, biocompatible, flexible biodevices were designed using AutoCAD and fabricated using metal sputtering, photolithography, and wet- and ion-etching. Devices were characterised and proved to withstand mechanical disruption typical of the intestinal environment via stress-strain experiments.

In this project's final year, these devices successfully delivered electrical field stimulation (EFS) to the smooth muscle cells seeded onto the electrospun gelatin scaffolds. Different EFS parameters were systematically explored, and 8V with 50ms pulses at 1Hz, showed clear contractions (Figure 3A). Interestingly, increasing the frequency to 0.1Hz resulted in a cumulative effect and increased contractile amplitude. This demonstrates control over temporal pacing and contractile magnitude. Increasing the pulse width has also largely impacted contraction amplitude (Figure 2B). Finally, the threshold for this cumulative response resulting in tonic contractions was determined to be at 0.33Hz (Figure 3C).

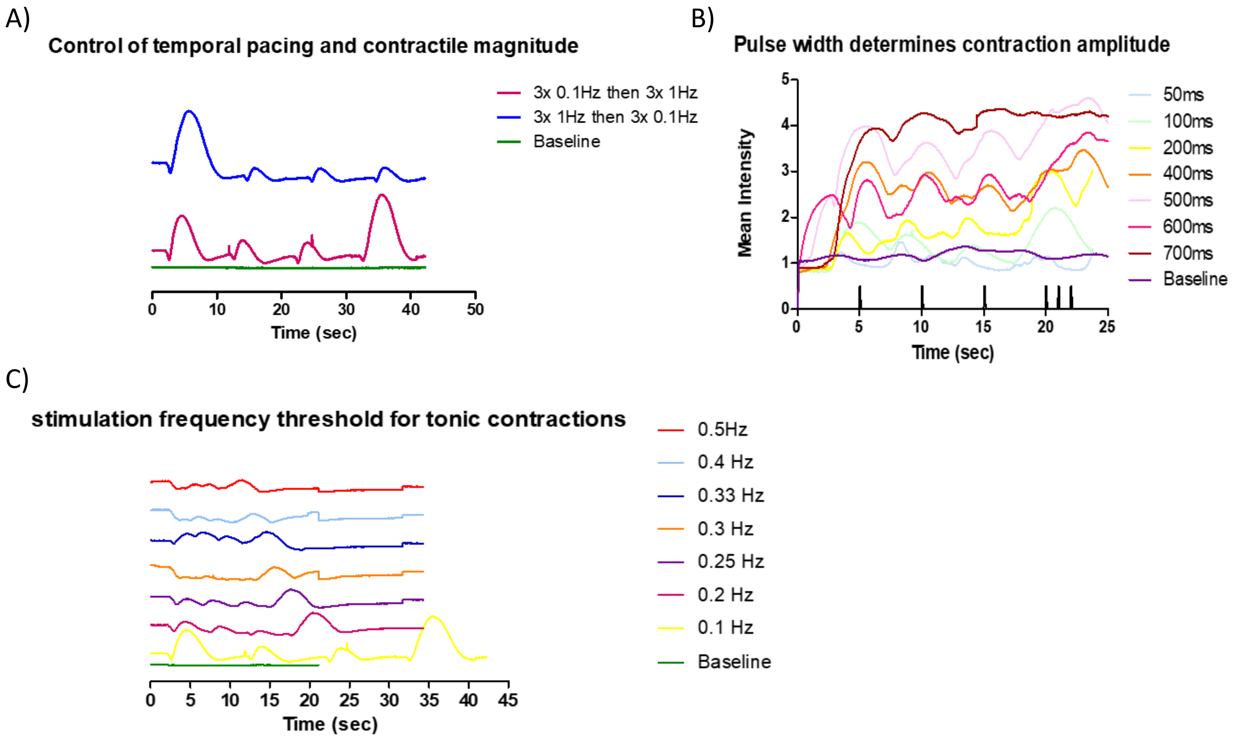

Figure 3. A) A chart showing control of temporal pacing and contractile magnitude of smooth muscle sheets using EFS via biodevices. Analysis from bright-field microscopy video, projecting change in median intensity over time using ImageJ software. B) Chart demonstrating effect of pulse width on contraction magnitude, at 8V, 50-700ms pulse width. Stimulation frequency represented by black peaks. Analysis from bright-field microscopy video, projecting change in median intensity over time using ImageJ software. C) A chart demonstrating stimulation frequency threshold for tonic contractions. All stimulation at 8V, 50ms pulse width, with frequency depicted by colour. Analysis from bright-field microscopy video, projecting change in median intensity over time using ImageJ software.

Aim 4: Integration of the electronic mesh with the second layer of aligned, laminar, smooth muscle tissue, and with the intestinal graft containing the circular muscle layer, to create a 3-parts complex tube capable of peristalsis.

In this project's final year, the bio-devices were successfully integrated with the seeded electrospun smooth muscle sheet using a fibrin glue. This glue mimics the blood clotting mechanism with thrombin converting the fibrinogen into fibrin, taking only seconds to gel and resulting in direct contact between the biodevice and the smooth muscle cells. This hybrid smooth muscle construct was successfully stimulated via EFS from the integrated biodevices from day 20 of culture (stimulation parameters: 8V, 0.1-1Hz, 50-700ms pulse width) in 2D. Further experiments are underway, integrating the biodevice-smooth muscle hybrid construct with the re-epithelialised scaffold and eliciting contractions with EFS in 3D to mimic peristaltic-like contractile waves.

Impacts

Development of the principal discipline(s) of the project

To our knowledge, this is the first time a system has been developed that allows for spatiotemporal contractile control of a tissue-engineered intestinal smooth muscle construct via electrical stimulation *in vitro*. This paves the way for modeling the entire functionality of the intestine and is key in future intestinal host-microbiome and muscle dysfunction experiments.

Describe the impact on teaching and educational experiences

N/A

Describe the impact in this reporting period on physical, institutional, and information resources that form infrastructure.

N/A

Impact on society beyond science and technology:

N/A

Changes

Changes in approach

N/A

Problems or delays

N/A

Expenditure Impacts

N/A

Significant changes in the use or care of human subjects, vertebrate animals and/or biohazards

N/A

Changes to the primary place of performance from that originally proposed
N/A

Technical Updates

N/A