PI: **Allbritton, Nancy L**	Title: Development of Human Intestinal Simulacra	
Received: 10/09/2014	FOA: RM14-003	Council: 05/2015
Competition ID: FORMS-C	FOA Title: NIH TRANSFORMATIVE RESEARCH AWARDS (R01)	
1 R01 DK109559-01	Dual: AT,ES,OD,RM	Accession Number: 3746003
IPF: 578206	Organization: UNIV OF NORTH CAROLINA CHAPEL HILL	
Former Number: 1R01OD020527-01	Department: Chemistry	
IRG/SRG: ZRG1 BCMB-A (51)	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: [redacted] Year 2: [redacted] Year 3: [redacted] Year 4: [redacted] Year 5: [redacted]	Animals: Y Humans: N Clinical Trial: N Current HS Code: 10 HESC: N HFT: N	New Investigator: N Early Stage Investigator: N
Senior/Key Personnel:	*Organization:*	*Role Category:*
Nancy Allbritton	University of North Carolina at Chapel Hill	PD/PI
Scott Bultman	University of North Carolina at Chapel Hill	MPI
Shawn Gomez	University of North Carolina at Chapel Hill	MPI
Scott Magness	University of North Carolina at Chapel Hill	MPI

OMB Number: 4040-0001
Expiration Date: 06/30/2016

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	State Application Identifier

1. TYPE OF SUBMISSION*
❍ Pre-application ● Application ❍ Changed/Corrected Application

4.a. Federal Identifier

b. Agency Routing Number

2. DATE SUBMITTED 2014-10-09

Application Identifier

c. Previous Grants.gov Tracking Number

5. APPLICANT INFORMATION

Organizational DUNS*: [redacted]

Legal Name*: University of North Carolina at Chapel Hill
Department:
Division:
Street1*: [redacted]
Street2: [redacted]
City*: [redacted]
County: [redacted]
State*: [redacted]
Province:
Country*: [redacted]
ZIP / Postal Code*: [redacted]

Person to be contacted on matters involving this application
Prefix: First Name*: [redacted] Middle Name: [redacted] Last Name*: [redacted] Suffix:
Position/Title: Grants/Contracts Specialist
Street1*: [redacted]
Street2:
City*: [redacted]
County: [redacted]
State*: [redacted]
Province:
Country*: [redacted]
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: [redacted] Email: [redacted]

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** [redacted]

7. TYPE OF APPLICANT* H: Public/State Controlled Institution of Higher Education

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*
● New ❍ Resubmission
❍ Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).
❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Development of Human Intestinal Simulacra

12. PROPOSED PROJECT
Start Date* 08/01/2015
Ending Date* 07/31/2020

13. CONGRESSIONAL DISTRICTS OF APPLICANT
NC-004

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: First Name*: Nancy Middle Name: Lynn Last Name*: Allbritton Suffix:

Position/Title: Dist Prof, Prof

Organization Name*: University of North Carolina at Chapel Hill

Department: [redacted]

Division:

Street1*: [redacted]

Street2:

City*: [redacted]

County: [redacted]

State*: [redacted]

Province:

Country*: [redacted]

ZIP / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number: Email*: [redacted]

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested* [redacted]

b. Total Non-Federal Funds* [redacted]

c. Total Federal & Non-Federal Funds* [redacted]

d. Estimated Program Income* [redacted]

16.IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ○ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR

○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: Dr. First Name*: [redacted] Middle Name: Last Name*: [redacted] Suffix: [redacted]

Position/Title*: Vice Chancellor for Research

Organization Name*: University of North Carolina at Chapel Hill

Department: [redacted]

Division: [redacted]

Street1*: [redacted]

Street2: [redacted]

City*: [redacted]

County: [redacted]

State*: [redacted]

Province:

Country*: [redacted]

ZIP / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number: [redacted] Email*: [redacted]

Signature of Authorized Representative* [redacted]

Date Signed* [redacted]

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:

424 R&R and PHS-398 Specific Table Of Contents

OMB Number: 4040-0010
Expiration Date: 06/30/2016

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: The University of North Carolina at Chapel Hill
Duns Number: [redacted]
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: [redacted]
Zip / Postal Code*: [redacted]
Project/Performance Site Congressional District*: NC-004

File Name

Additional Location(s)

Funding Opportunity Number: RFA-RM-14-003. Received Date: 2014-10-09T16:14:54.000-04:00

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ● **Yes** ○ **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ● Yes ○ No

If YES, check appropriate exemption number: — 1 — 2 — 3 ✓ 4 — 5 — 6

If NO, is the IRB review Pending? ○ **Yes** ○ **No**

IRB Approval Date:

Human Subject Assurance Number [redacted]

2. Are Vertebrate Animals Used?* ● **Yes** ○ **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ● Yes ○ No

IACUC Approval Date:

Animal Welfare Assurance Number [redacted]

3. Is proprietary/privileged information included in the application?* ○ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ○ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ○ Yes ○ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ○ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ○ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Abstract1018940352.pdf
8. Project Narrative*	Project_narrative1018940353.pdf
9. Bibliography & References Cited	Literature_Cited1019088511.pdf
10.Facilities & Other Resources	Facilities_and_Other_Resources1018940356.pdf
11.Equipment	

Funding Opportunity Number: RFA-RM-14-003. Received Date: 2014-10-09T16:14:54.000-04:00

ABSTRACT

In the current application, a collaborative, multidisciplinary research project is proposed that has the potential to create a new paradigm for the study of human physiology in health and disease. A state-of-the-art microfabricated platform will be developed to create a functional, *in vitro* replica, *i.e.* simulacrum, of the human colonic epithelium and its associated microbiome. This new technology will be used to perform novel studies and hypothesis testing of intestinal physiology that cannot currently be performed. Furthermore, the technology and protocols developed here will establish the basis for creating organ simulacra from normal and diseased primary human tissues that will change the manner in which studies of tissue function, microbiome influence, and drug effect are performed. Recent progress in organotypic culture of colonic epithelial stem cells has made it possible to create long-lived spheroids in a gelatinous matrix. However, the absence of chemical gradients as well as patterned physical support results in a disorganized and chaotic conformation that poorly mimics the structure and function of the colon, and furthermore is not amenable to microbiome co-culture. We propose to develop a novel microfabricated system that will enable *ex vivo* culture of human colonic epithelium and overlying microbiota that recapitulates the 3D structure and environment of the colonic mucosa in a setting which lies between an unpolarized cell culture system and the complexity of the intact human organism. Recent technical advances from our labs in sustained monolayer culture of colonic epithelial stem cells will be integrated with microfabricated scaffoldings and devices to create the colonic simulacrum. A number of innovations will be incorporated into the microengineered system with the goal of recapitulating the colonic stem-cell niche, the differentiated intestinal mucosa, the microbiota, and the dynamic information flow between these compartments. The platform will permit tight control of the luminal and basal crypt environments by providing independent fluidic and gaseous access to these compartments. The platform will support formation of mitogen, morphogen, differentiation-factor, dietary-compound and gaseous gradients to enable unprecedented investigations into colonic physiology. A number of hypotheses related to the interplay of dietary factors, the microbiome and the colonic epithelium will be tested to demonstrate the power and broad applicability of this transformative technology.

PROJECT NARRATIVE

The goal of this project is to develop a platform that enables a transformative approach to the study of the colon in health and disease. Assays of dietary and other factors in the setting of human tissue and intestinal bacteria will enable study of stem-cell self-renewal, differentiation and colon physiology using cells derived from human tissue. This technology will establish a new paradigm to understand the influence of diet and bacteria on intestinal and digestive diseases enhancing our understanding of the growth and development of the colon.

RESOURCES- Allbritton

FACILITIES: Specify the facilities to be used for the conduct of the proposed research. Indicate the performance sites and describe capacities, pertinent capabilities, relative proximity, and extent of availability to the project. Under "Other," identify support services such as machine shop, electronics shop, and specify the extent to which they will be available to the project. Use continuation pages if necessary.

Laboratory: At UNC, Dr. Allbritton has 4500 ft^2 of custom-designed lab space including wet benches, cabinets, sinks, two 5 ft and one 8 ft fume hoods. 1200 ft^2 of the space is dedicated to optical work with 4 optical tables (5X8 ft) as well as 3 smaller tables (3X4 ft). The lab contains a variety of standard equipment- two -20 °C freezers, two -80 °C freezers, liquid nitrogen cell storage dewars, refrigerators, balances, etc. Also available in the lab are facilities for bacterial culture and analysis (heated shakers, incubators, etc.), molecular biology protocols (thermocycler for PCR, electrophoresis supplies, etc.), and synthetic organic and polymer chemistry, including an automated peptide synthesizer. Two fully-equipped tissue culture hoods and three CO_2 incubators are present for mammalian cell culture. The fully equipped tissue culture facility also includes an inverted microscope, water baths and centrifuges. A Hewlett Packard HPLC with fluorescence and absorbance detector and a Molecular Devices multi-well plate reader for measurement of luminescence, fluorescence and absorbance are available in the lab. Two Eppendorf microinjectors are present in the lab as well as a lyophilizer and autoclave. Five laser-integrated microscope platforms, each custom-designed as specific experimental workstations for single-cell analysis by capillary electrophoresis or microfluidic chip, and cell manipulation using a microfabricated array platform, are housed in the lab. Two of these experimental workstations include stage incubators for prolonged cell-based experiments requiring controlled CO_2, temperature and humidity. These systems are also equipped with a Nd:Yag or solid state pulsed laser, argon or solid-state CW laser, and a variety of fluorescence detection systems (PMTs or CCD cameras). Two Beckman P800 CE instruments with fluorescence detection are available for automated capillary electrophoresis measurements. Two custom-built, high-sensitivity CE-LIF systems are housed in the optical room.

In-lab facilities are available for microfabrication of devices composed of PDMS, SU-8, 1002F, and other polymers. Equipment present include: a Nicolet IS5 FT-IR spectrophotometer, Thwing Albert goniometer, Newport collimated UV exposure system with optical aligner, two spin coaters, three 10-inch digitally controlled hot plates, two plasma oxidizers, N_2 gun, rotary shakers, laminar flow hood, and an automated CNC machine. Equipment for a variety of types of polymer grafting on the microdevices is also available.

Computer: Each experimental system in the lab employs a PC computer. There are also three shared computers for word processing and routine lab needs. Databases for biomedicine (Pubmed, etc.), physics and engineering (Inspec, SciFinder), and chemistry (Chem Abstracts, SciFinder) are accessible from within the lab. Accounts are also available on various workstations in the University.

Office: The students and fellows all have desk space at the end of their designated wet bench space. Dr. Allbritton has 150 ft^2 of office space.

Other: A large number of multi-user facilities at UNC (and North Carolina State) are available to Dr. Allbritton. A sampling follows.

Mass Spectrometry Core- The UNC Chemistry Dept. operates a mass spectrometry core facility with tandem MS, high-resolution MS, and LC-MS all available.

Electrical and Machine Shop- The UNC Physics/Chemistry Dept. provides core support in the form of an electrical shop for circuitry design, fabrication, and testing. A machine shop is available for parts and instrument fabrication. Both facilities are managed by professional staff.

Microfabrication Facilities-

1. UNC- In addition to the in-lab microfab facilities, the Allbritton Group is adjacent to the UNC clean room which comprises 2500 ft^2 of specifically designed space for fabrication and assembly of microfabricated devices. This includes approximately 300 ft^2 of class 100 work space and 700 ft^2 of class 1000 work space. Also available is 14 linear feet of chemical fume hood space for fabrication operations including photoresist application, developing, baking and removal, substrate cleaning, wet chemical etching, etc. The clean room laboratory includes a 5" contact photoexposure/alignment system, SF-100 maskless photoexposure system, spin coater, KLA/Tencor P-15 stylus profilometer, ultrapure water systems, and an array of programmable ovens for processing and bonding microfluidic devices. The facility has also recently installed an EVG520HE embossing system capable of micron-scale resolution in a variety of polymers. A dicing saw and powder blaster are also available for device fabrication. Thin film deposition equipment includes a South Bay Technology Model IBS/e ion beam sputter with sub-nanometer deposition precision, a Kurt J. Lesker PVD 75 magnetron sputtering system for metal and insulating thin film deposition, and STS Vision MkII dual frequency PECVD system for low stress deposition of SiO2, SiN, SiOxNy, pSi and doped pSi. An Alcatel AMS 100 SDE deep reactive ion etch system capable of both Si and SiO2 etching is available for creating high aspect ratio features on the submicron scale. Device metrology and diagnostics can also be provided by a Hitachi 4700 SEM, which additional includes an Oxford INCA Energy 250 energy dispersive x-ray microanalysis system. In addition, an ultraviolet photoelectron spectrometer as well as a microscale XPS system is available. An FEI Helios 600 NanoLab Dual Beam System is present for simultaneous SEM imaging and ion beam patterning (selective removal or addition of material). In addition, five optical inspection microscopes are distributed throughout the laboratory space for device inspection and diagnostics. A hot embosser and mulit-wavelength laser cutter are also available in CHANL.

2. NC State- UNC-CH faculty members have full access to user resources at the North Carolina State University (NCSU) campus. The Nanofabrication Facility and Triangle National Lithography Center (Prof. Carl Osburn) occupy a 7400 square feet class 100 and class 1000 cleanroom. The facility has a full range of micro and nano-fabrication capabilities including: photolithography, reactive ion etching (RIE), deep RIE, low pressure chemical vapour deposition (LPCVD), plasma enhanced CVD, rapid thermal anneal, thermal oxidation, solid source diffusion, thermal and e-beam evaporation, sputtering, chemical mechanical polishing, various wet etching and cleaning processes, along with various characterization tools. A broad range of substrates such as semiconductor glass, ceramics, and plastics with sizes from small pieces to 6" wafers can be utilized in the facility. Professional, full-time staff are available for consultation and teaching purposes.

Other Common Use Facilities- Other facilities at UNC include centrifuges, autoclaves, a lyophilizer, a cold room, and multiple other items.

Facilities and Other Resources:

Laboratory: The Bultman lab has >1,000-sq ft of wet lab space located in the Genetic Medicine Building (GMB). There are 9 individual bench spaces and each bench space has a desk nearby. Additionally, the lab has a 200-sq ft cell culture room, a 200-sq ft dissection and microscopy room, and a 200-sq ft microbiological lab, which includes an anaerobic chamber for cultivation of anaerobic bacteria.. There is also a walk-in cold room and additional space for major equipment, histology, refrigerators, and freezers.

Animal Resources: The Bultman lab has space for 400+ cages of mice in the SPF area of the AAALAC-approved animal resources unit located in the upper basement of the GMB. The lab also has 3 isolators in the Gnotobiotoic Facility located in the lower basement of the GMB.

Computer: Each person in the Bultman lab has their own Mac or PC laptop. In addition, the lab has 2 PCs dedicated for operation of a gel documentation system and real-time PCR machine.

Office: The PI's office is 100-sq ft and located adjacent to the lab.

Major Equipment: Molecular biology equipment include two conventional PCR machines (including an MJ tetrad) and real-time (ABI 7300) instruments, gel electrophoresis chambers, a Fotodyne gel documentation system, a sonicator, a polytron homogenizer, western blot apparatuses, a NanoDrop, waterbaths, centrifuges, microfuges, etc. There are two 4°C refrigerators, two -20°C freezers, and one -80°C freezer. The tissue culture room has two 4 ft tissue culture hoods and two water-jacketed CO_2 incubators. The microscopy room includes two Leica MZFLII fluorescent dissection scopes and a Leica DMIL inverted scope. The microbiology room includes conventional incubators, a shaking incubator, and an anaerobic chamber. Additional common equipment is available on the same floor (Department of Genetics) including ultracentrifuges, centrifuges, spectrophotometers, a dark room with film developer, and a histology room with a paraffin embedding station, microtome, and cryostat. The Lineberger Comprehensive Cancer Center (LCCC) has additional common equipment including instruments.

Core Facilities: The School of Medicine operates 31 core facilities led by Ph.D. staff scientists and faculty members (www.med.unc.edu/corefacilities, including the Microbiome Core Facility that will be used for 16S sequencing in this proposal. Other cores commonly used by the Bultman lab include: The High-Throughput Sequencing Core Facility operates Illumina [HiSeq2000 (n=8), HiSeq2500 (n=2), GAII (n=2)], Life Technologies (Ion Torrent), and PacBio RS instruments. The LCCC Genomics Core can assist investigators with library construction for next-generation sequencing projects (or perform the service for a fee) and performs array CGH. The Mouse Histopatholgy Core Facility performs all aspects of histopathology including embedding, sectioning, and H&E staining.

Facilities and Other Resources:

GOMEZ

Laboratory: The Gomez laboratory has approximately 500 sq. ft. of modern research space suitable for all computational, "dry lab" efforts as well as 120 sq. ft of wet lab space for basic cell preparation and microfluidic work (e.g. thermocycler for PCR, gel electrophoresis, freezer, microcentrifuge, spin coater, plasma oxidizer, digitally controlled hot plates, etc.).

Clinical: N/A

Animal: N/A

Computer: The laboratory has 4 Apple dual processor workstations (G5 or Intel; 4,8, or 12-cores), 3 Apple Intel iMacs, a Linux-based workstation (dual processor, 8 core), and color & bw laser printers. See Major Equipment for additional resources.

Office: The PI has approximately 120 sq. ft. of personal office space. All personnel have desk space and personal computers in the connected laboratory.

Major Equipment: We have an Apple Xserve G5 Server connected to a 2 TB XRAID system and another Intel Xserve connected to a Drobo Elite Raid system with and 7 TB of protected data storage on our private network. In addition, we have 5, dual-processor workstations/XServes (2.3 or 2.66 GHz) that are set up in a grid network to support compute-intensive jobs. We have an additional Xserve dedicated to web-related services including software dissemination. All necessary software packages and compilers are available.

Other:
We have access to all high-performance computing facilities at both UNC-Chapel Hill and NC State Universities, including:
UNC Clusters: The Research Computing division of Information Technology Services delivers a 772 node (9152 core) Dell Linux cluster, "Kill Devil," with QDR Infiniband interconnect and a minimum of 4 GB memory per core; a smaller 2300-core HP Linux cluster with QDR Infiniband interconnect and at least 6GB of memory per core; and two 32-core hosts with one terabyte of memory each to accommodate codes that require extremely large amounts of RAM. The Kill Devil Cluster also includes 64 NVidia Tesla GPUs (M2070). The first 200,000 hours are provided free of charge to each investigator.

The NCSU Henry2 cluster: 1149 dual socket servers with Intel Xeon processors having up to eight cores, minimum of 4 GB memory per core.

RESOURCES- Magness

FACILITIES: Specify the facilities to be used for the conduct of the proposed research. Indicate the performance sites and describe capacities, pertinent capabilities, relative proximity, and extent of availability to the project. Under "Other," identify support services such as machine shop, electronics shop, and specify the extent to which they will be available to the project. Use continuation pages if necessary.

Laboratory: The laboratory of Dr. Magness occupies 570 square feet in the Medical Biomolecular Research Building at the University of North Carolina at Chapel Hill in an open space configuration. In addition, Dr. Magness has a 200 sq. ft. tissue culture room, near his main laboratory, that contains all of the necessary equipment for the proposed cell culture work, including an Olympus IX71 and IX81 inverted phase/fluorescent (UV, EGFP, Cherry,Tomato filters) tissue culture microscope with a digital camera, 6 ft. laminar flow hood, and a dual chamber tissue culture incubator. The IX81 microscope is housed in Dr. Magness' lab. The IX81 has a physiologic chamber attached to the mechanical stage that can be utilized for long term live image capture. Adequate power, gas, vacuum, water, and safety equipment (e.g. fume hoods and eye wash stations) are available to carry out the procedures and accommodate personnel described in this proposal.

Animal: The Medical Biomolecular Research Building (MBRB) possesses an entire floor devoted to animal care, including facilities for treating animal with biohazardous chemicals. Facilities were constructed in strict compliance with AAALAC standards and have special isolation and biohazard facilities. Dr. Magness has 94 cage spaces available for his use.

Computer: Dr. Magness has a personal IBM-compatible and Macintosh computers that will do all the necessary word processing, slide-making, data entry, and statistical work. A laser jet printer, a LaserJet 5SiMx, and photo-quality inkjet printer is networked to Dr. Magness' computers.

Office: Dr. Magness has a 100 sq. ft. office adjacent to his laboratory.

CORE Facilities: UNC houses a Mutant Mouse Regional Resource Center and UNC Transgenic CORE facility which has conducted the pronuclear injections to generate the two novel mouse models described in this proposal. The UNC Center for GI Biology and Disease (CGIBD) house several COREs including an animal models CORE, histology CORE, and Biostatistics CORE, which will be extensively utilized. The UNC flow cytometry CORE facility has 6 tabletop flow cytometers (CyAn, FACscan, FACScaliber, DAKO cytomation) with 6 color capability that Dr. Magness has authorization to operate. Additionally, the facility possesses a MoFlo FACS sorter which is able to sort up to 50,000 cells/second. Dr. Magness has also acquired and directs the Center for GI Biology and Disease high-throughput gene expression analysis core that runs the Fluidigm Biomark HD.

Other: There is 1000 sq. ft. of common laboratory space available for use. This space contains an ultracentrifuge, two superspeed centrifuges, liquid scintillation counter, bacterial shaker, digital imaging system, phosphorimager, luminometer, plate reader, and chemical storage space. Shared space also includes two 200 sq. ft. cold rooms, one 100 sq. ft. cold room, and a 150 sq. ft. radiation room.

MAJOR EQUIPMENT: Major equipment items either in his laboratory or adjacent shared user space available to Dr. Magness for this project. Fluidigm Biomark HD, Beckman System Gold HPLC, Beckman LS6000SC Beta Counter, Perkin Elmer LS50B Luminescene Spectrometer, Savent Speed Vac SC100 Refrigerated Dryer, 2 Perkin Elmer Gene Amp PCR 9600 Systems, Innova-4300 Incubator/Shaker, Analytical Luminescence Monolight 2010 Luminometer, MVE XL440 Liquid Nitrogen Storage, Cyclone Plus DNA Synthesizer, Revco –800C Freezers,

AlphaImager 2000 System, Kodak 440 Imaging System, Kodak RP X-Omat Processor – Model M6B, Beckman DU640 Spectrometer, Robbins Scientific 400 Microhybridization Oven, MD Typhoon 8600 Phosphorimager, Beckman L80 ultracentrifuge, Sorval RC5C and RC5B Centrifuges, NuAir 6 ft. Laminar Flow Tissue Culture Hood, Fisher CO2 Tissue Culture Incubator, Roche LightCycler PCR Amplification System, Molecular Device SpectraMAX Plate Reader, Nanodrop Spectrophotometer, Olympus IX81 inverted fluorescent microscope with physiologic chamber for live imaging.

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: First Name*: Nancy Middle Name Lynn Last Name*: Allbritton Suffix:

Position/Title*: Dist Prof, Prof
Organization Name*: University of North Carolina at Chapel Hill
Department: [redacted]
Division:
Street1*: [redacted]
Street2:
City*: [redacted]
County: [redacted]
State*: [redacted]
Province:
Country*: [redacted]
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number: E-Mail*: [redacted]

Credential, e.g., agency login: [redacted]

Project Role*: PD/PI Other Project Role Category:

Degree Type: PhD Degree Year: 1987

	File Name
Attach Biographical Sketch*:	Biosketch_Allbritton1018940504.pdf
Attach Current & Pending Support:	

Funding Opportunity Number: RFA-RM-14-003 . Received Date: 2014-10-09T16:14:54.000-04:00

PROFILE - Senior/Key Person

Prefix: First Name*: Scott Middle Name J Last Name*: Bultman Suffix:

Position/Title*: Asst Prof
Organization Name*: University of North Carolina at Chapel Hill
Department: [redacted]
Division:
Street1*: [redacted]
Street2:
City*: [redacted]
County: [redacted]
State*: [redacted]
Province:
Country*: [redacted]
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number: [redacted] E-Mail*: [redacted]

Credential, e.g., agency login: [redacted]

Project Role*: PD/PI Other Project Role Category:

Degree Type: PhD Degree Year: 1994

File Name

Attach Biographical Sketch*: Biosketch_Bultman1018940509.pdf

Attach Current & Pending Support:

PROFILE - Senior/Key Person

Prefix: First Name*: Shawn Middle Name M Last Name*: Gomez Suffix:

Position/Title*: Adj Asst Prof, Asst Prof
Organization Name*: University of North Carolina at Chapel Hill
Department: [redacted]
Division:
Street1*: [redacted]
Street2:
City*: [redacted]
County: [redacted]
State*: [redacted]
Province:
Country*: [redacted]
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number: [redacted] E-Mail*: [redacted]

Credential, e.g., agency login: [redacted]

Project Role*: PD/PI Other Project Role Category:

Degree Type: PhD Degree Year: 1999

File Name

Attach Biographical Sketch*: Biosketch_Gomez1018940512.pdf

Attach Current & Pending Support:

PROFILE - Senior/Key Person

Prefix: First Name*: Scott Middle Name T Last Name*: Magness Suffix:

Position/Title*: Research Asst Prof
Organization Name*: University of North Carolina at Chapel Hill
Department: [redacted]
Division:
Street1*: [redacted]
Street2:
City*: [redacted]
County: [redacted]
State*: [redacted]
Province:
Country*: [redacted]
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number: [redacted] E-Mail*: [redacted]

Credential, e.g., agency login: [redacted]

Project Role*: PD/PI Other Project Role Category:

Degree Type: PhD Degree Year: 1994

	File Name
Attach Biographical Sketch*:	Biosketch_Magness1018940513.pdf
Attach Current & Pending Support:	

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Allbritton, Nancy L.	Professor
eRA COMMONS USER NAME [redacted]	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Louisiana State University- Baton Rouge, LA	B.S.	1979	Physics
Johns Hopkins University- Baltimore, MD	M.D.	1985	Medicine
Massachusetts Inst. of Tech.- Cambridge, MA	Ph.D.	1987	Medical Physics/ Medical Eng.

A. Personal Statement

Research in my laboratory focuses on the development of novel methods to answer fundamental questions in biology & medicine. Much of biology & medicine is technology limited in that leaps in knowledge follow closely on the heels of new discoveries and inventions in the physical and engineering sciences; consequently, interdisciplinary groups which bridge these different disciplines are playing increasingly important roles in biomedical research. My lab has developed partnerships with other investigators in the areas of biology, medicine, chemistry, physics, and engineering to design, fabricate, test, and utilize new tools for biomedical research. Technology from the lab has formed the basis for 3 spin-out companies with a total of >30 issued or pending patents. By virtue of the highly collaborative nature of my research, I have the expertise to drive a multi-investigator research project such as is needed for the development and application of the human colonic simulacrum. For the past decade, my lab has been continuously funded by 3-to-5 NIH R01 grants with each grant supporting a multidisciplinary collaborative team of investigators. I am also the Chair of the UNC/NC State Department of Biomedical Engineering, a position that has provided me with the experience and skills to form effective working groups. With the imminent expiration of three R01 grants on which I am the PI, I have the available effort and time commitment to serve as the coordinating PI on this multi-PI grant.

B. Positions and Honors

Positions and Employment

1988	Postdoctoral Fellow with Dr. Herman Eisen, Dept. of Biology, Massachusetts Institute of Technology
1989 – 1994	Postdoctoral Fellow with Dr. Lubert Stryer, Dept. of Cell Biology and Neurobiology, Stanford University
1994 – 2000	Assistant Professor, Dept. of Physiology and Biophysics, University of California, Irvine
2000 – 2004	Associate Professor, Dept. of Physiology and Biophysics and Dept. of Biomedical Engineering, University of California, Irvine, CA
2004 – 2007	Professor, Dept. of Physiology and Biophysics, and Dept. of Biomedical Engineering, University of California, Irvine, CA
2005 – 2007	Professor, Dept. of Chemistry, and Dept. of Chemical Engineering and Material Science, University of California, Irvine, CA
2007 – present	Debreczeny Distinguished Professor, Dept. of Chemistry, University of North Carolina, Chapel Hill, NC
2008 – present	Professor, Dept. of Pharmacology, University of North Carolina, Chapel Hill, NC

2009 – present Professor and Chair, UNC/NCSU Dept. of Biomedical Engineering, University of North Carolina, Chapel Hill, NC and North Carolina State University, Raleigh, NC
2010 – present Professor, Dept of Materials Science and Engineering, North Carolina State University, Raleigh, NC

Other Experience and Professional Memberships

2000 – 2003 Ad-Hoc Member of NCI Study Section: Innovative Molecular Analysis Technologies
2003 Ad-Hoc Member of NIH Study Section: Bioanalytical Engineering & Chemistry
2004 – 2009 Permanent Member, NIH Study Section: Enabling Bioanalytical and Biophysical Technologies (EBT)
2007 – 2009 Chair, NIH EBT Study Section
2010 – 2013 National Council, Biophysical Society
2012 – 2015 Analytical Chemistry Editorial Advisory Board

Society Memberships:
Biophysical Society
American Chemical Society
American Association for the Advancement of Science
International Society for Analytical Cytometry

Awards and Honors

1995 Searle Scholar Award
1995 Beckman Young Investigator Award
2003 UCI Mid-Career Research Award
2004 College of Medicine Excellence in Teaching Award
2009 Mary K. and Velmer A. Fassel Award, Dept. of Chemistry, Iowa State University
2010 Fellow, American Institute for Medical & Biological Engineering
2010-13 Member, Biophysical Society Council
2013 Member, Scientific Advisory Committee, Beckman Foundation

C. Ten Selected Publications

1. Allbritton, N.L., Meyer, T., and Stryer, L. 1992. Range of Messenger Action of Calcium Ion and Inositol 1,4,5-Trisphosphate. *Science.* 258: 1812-1818.

- This paper demonstrated that inositol-1,4,5-trisphosphate acts as a global messenger in cells, whereas calcium acts in restricted domains demonstrating fundamental cellular signaling mechanisms. This breakthrough was enabled by the design and fabrication of a microscale device to directly measure the diffusion coefficients of the second messengers inositol-1,4,5-trisphosphate and calcium in cell cytoplasm.

2. Meredith, G., Sims, C.E., Soughayer, J.S., Allbritton, N.L. 2000. Measurement of Kinase Activation in Single Mammalian Cells. *Nature Biotech.* 18: 309-312.

- The first simultaneous measurement of the activation of multiple key regulatory enzymes within single cells was demonstrated. A novel laser-based technique to sample single, adherent, mammalian cells and perform high temporal resolution biological measurements was developed and served as the initial basis for a startup company recently valued at over $300 million.

3. Hu, S., Ren, X., Bachman, M., Sims, C.E., Li, G.P., Allbritton, N.L. 2002. Surface Modification of Poly(dimethylsiloxane) Microfluidic Devices by Ultraviolet Polymer Grafting. *Anal. Chem.* 74: 4117-4123.

- This highly cited paper was the first demonstration of polymer graft placement onto the surface of poly(dimethylsiloxane) (PDMS) microdevice channels enabling facile surface customization. PDMS with a polymer coating is now used throughout microdevice fabrication.

4. Salazar, G., Wang, Y., Young, G., Bachman, M., Sims, C.E., Li, G.P., Allbritton, N.L. 2007. Micropallet Arrays for the Separation of Single, Adherent Cells. *Anal. Chem.* 79: 682-687.

- This paper pioneered a new class of cell sorting technologies with attributes complementary to that of flow cytometry. This platform technology enables cell sorting based on complex cell phenotypes such as shape, size, matrix invasion, cell-killing, calcium signaling and other dynamic cellular attributes. The technology is licensed to a multi-billion dollar electronics company.

5. Phillips, K.S., Kang, K.M., Licata, L., Allbritton, N.L. 2010. Air-Stable Supported Membranes for Single Cell Cytometry on PDMS Microchips. *Lab Chip*. 10:864-870. PMCID: PMC2992470

- Rugged coatings withstanding electric fields, prolonged dry storage, and protein adsorption yet providing excellent separation performance were developed for microfluidic devices. These coatings permitted high-throughput measurements of enzymatic activity in hundreds of single cells, measurements not possible with any other method including microscopy.

6. Wang, Y., Phillips, P., Pai, J.H., Xu, W., Sims, C.E., Allbritton, N.L. 2010. Micromolded Arrays for Separation of Adherent Cells. *Lab Chip.* 10:2917-2124. PMCID: PMC2994190.

- An inexpensive and robust method to clone cells from a microscopy-compatible array with 100% efficiency was described. The technology is the basis of a startup company now selling the product globally. The product is in use as a sample-handling and cell-selection method for single-cell PCR.

7. Wang, Y., Balowski, J., Phillips, C., Phillips, R., Sims, C.E., Allbritton, N.L. 2011. Benchtop Micromolding of Polystyrene by Soft Lithography. *Lab Chip.* 11:3089-3097. PMCID: PMC3454527

- Until this point in time, PDMS was the only material used for soft lithography, a technology in widespread use for microdevice fabrication. This paper demonstrated that other materials with improved bulk and surface properties such as polystyrene could be used for rapid bench top-based fabrication greatly broadening the range of applications and materials compatible with this popular fabrication method.

8. Wang, Y., Sims, C.E., Allbritton, N.L. 2012. Dissolution-Guided Wetting for Microarray and Microfluidic Devices. *Lab Chip.* 12: 3036-3039. PMCID: PMC3422633

- Eliminating air bubbles from the small cavities of lab-on-chip devices is extremely challenging but required for proper device function. A simple, water-soluble, biocompatible coating that guides wetting of microchannels or microwells was invented enabling bubble-free filling of the devices simply by adding water or aqueous buffer to a device. The technology is licensed to a startup company.

9. Balowski, J.J., Wang, Y., Allbritton, N.L. 2013. Fabrication of 3-D Microstructures from the Interactions of Immiscible Liquids with a Structured Surface. *Advanced Materials*. 10.1002/adma.201301658. PMCID: PMC3783858

- Liquid lithography is a new alignment-free fabrication method rapidly producing arrays of intricate microstructures. This highly parallel, "low-tech" method requires no dedicated equipment and easily produces curved, hollow and/or multi-level microstructures out of a variety of photoactive and non-photoactive materials opening the door tothe facile fabrication of exciting new microstructures for a variety of applications.

10. Wang, Y., Ahmad, A.A., Sims, C.E., Magness, S.T., Allbritton, N.L. 2014. In vitro generation of colonic epithelium from primary cells guided by microstructures. *Lab on Chip*. 14: 1622 - 1631. PMCID: PMC4037563.

- This innovative combination of microengineered substrates and state-of-the-art long-term culture protocols for gut-derived stem cells is the first demonstration of a 2D/3D hybrid culture to form a continuous, millimeter-scale colonic epithelial tissue *in vitro*, recapitulating the polarized architecture (*i.e.* distinct proliferative and non-proliferative zones) of the colonic epithelium *in vivo*.

D. Research Support

Ongoing

NIH R01 EB011763 Allbritton (P.I.) Waters, Voorhees (co-I's)
Protectides: A tool for drug target assays in myeloma 4/01/10-3/31/15
This project develops small stably-folded proteins as substrates for protein kinase B, protein kinase C and the proteosome. The goal is to measure the response of a patient's multiple myeloma cells to clinically relevant drugs.

NIH R01 CA139599 Allbritton (P.I.) Lawrence, Der, Yeh (co-I's)
Multiplexed Measurement of Kinase Activity in Single Cancer Cells 3/01/09-1/31/15
The grant proposes to develop reporters for EGFR, Her2, and S6K for intracellular measurements of kinase activity in single pancreatic tumor cells.

NIH R01 EB012549 Allbritton (P.I.) Cox, Sims (co-I's)
Arrays for Cloning Growth Suppressed Cells 2/01/11-1/31/15
Large scale arrays of micromolded rafts are to be developed to identify extremely rare cell cells with a growth disadvantage relative to their normal counterparts. The goal is to generate cell lines that are engineered to express tumor supressors as well proteins inhibiting the cell cycle, proteins important in the genesis of tumors.

NIH R01 ES013611-07S1 Jaspers (P.I.) Allbritton, Noah, Zhang (co-I's) 8/01/13-7/31/16
Diesel-induced Alterations of Influenza Infectivity
The goal of the consortium is to develop novel technologies and integrated complementary experimental approaches to investigate how exposure to diesel exhaust modifies innate immune responses in humans.

NIH R01 CA177993 Allbritton (P.I.) Yeh, Zhang (co-I's) 8/01/14-7/31/19
Single-Cell Measurement of Lipid Signaling in Colorectal Cancer
PI3 kinase activity and metabolites of phosphatidyl inositide 4,5-bisphosphate will be measured in single tissue-cultured colorectal cancer cells. PI3 kinase activity will also be measured in single cells from colorectal cancer specimens from humans.

NIH R01 EY024556 Allbritton (P.I.) Ramsey, Cowley (co-I's) 9/1/14 – 8/31/19
Generation of a Gene-Targeted Human iPS Cell Library for Macular Degeneration
The project develops a platform for rapid generation and testing of iPS cell lines carrying mutations associated with development of dry macular degeneration.

Recently Completed

NIH R01 HG084843 Allbritton (P.I.) Ramsey, Cowley (co-I's)
Rapid Genetic Engineering of Stem Cells 2/23/09-1/31/14
This grant focused on the development of large scale arrays of 1002F pallets and PCR microwells to rapidly engineer and select mouse embryonic stem cells to produce genetically engineered mice.

NIH R01 CA140173 Lawrence (P.I.) Allbritton, Whang (co-I's)
Signaling Network Dynamics in Metastatic Prostate Cancer 7/01/09-4/30/14
The grant proposed to develop reporters for src, Ack1 and mTOR for intracellular measurements of kinase activity in single prostate cancer cells.

NIH R01 EB007612 Allbritton (P.I.) Sims (co-I)
Micropallet Arrays for Separation of Single Cells and Colonies 4/01/07-1/31/12
This grant focused on the development of SU-8 pallets for separating tumor cells with comparisons to the separation efficiency of flow cytometry and limiting dilution methods.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Scott Bultman	Assistant Professor
eRA COMMONS USER NAME (credential, e.g., agency login) [redacted]	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Wisconsin, Stevens Point, WI	B.S.	1988	Biology/Chemistry
University of Tennessee, Knoxville, TN	Ph.D.	1994	Genetics
California Institute of Technology, Pasadena, CA	Postdoctoral	1995	Neuroscience
Case Western Reserve University, Cleveland, OH	Postdoctoral	2000	Genetics

A. Personal Statement

My lab is primarily interested in the role of chromatin-modifying factors and epigenetics in mammalian development and disease states. We are particularly interested in a molecule called butyrate, which is produced by bacterial fermentation of dietary fiber in the colon, and functions as an energy source and a histone deacetylase (HDAC) inhibitor in colonocytes of the host. Our previous work has utilized both cell culture models and gnotobiotic mouse models to investigate the role of butyrate in homeostasis and tumor suppression. My role in this project is to investigate the effect of altered Schaedler flora, butyrate-producing bacteria, and purified butyrate gradients on fundamental processes such as cell proliferation and apoptosis in human colonic simulacra. Not only is this an ideal proof-of-principle for the application of this technology, but this experimental platform will be crucial to advance our knowledge of butyrate function in the crypt because endogenous butyrate cannot be visualized or precisely manipulated in the mouse colon and other cell-culture models are not anatomically or physiologically relevant.

B. Positions and Honors

Professional Positions:

2000-2006 Research Associate, Department of Genetics, University of North Carolina at Chapel Hill
2006-present Assistant Professor, Department of Genetics, University of North Carolina at Chapel Hill
2006-present Member, Lineberger Comprehensive Cancer Center (LCCC)
2006-present Member, UNC Center for Gastrointestinal Biology and Disease (CGIBD)
2006-present Member, UNC Nutrition Obesity Research Center (NORC)

Other Experience & Selected Professional Memberships (past two years):

2014 Member (Ad Hoc) United States Department of Agriculture (USDA) Grant Review Panel, Function and Efficacy of Foods Study Section.
2014 Member (Ad Hoc) American Cancer Society (ACS) Advisory Committee for Extramural Grants Department
2014 Member (Ad Hoc) World Cancer Research Fund (WCRF) International Regular Grant Program Review Panel
2014 Member (Ad Hoc) NIH Scientific Review Committee, NIEHS Center for Translational Environmental Health Research
2014 Member (Ad Hoc) University of Toledo Biomedical Innovation Research Program Review Panel.
2014 Member (Ad Hoc) Cancer Research United Kingdom Grant Council Review Panel.
2014 Member (Ad Hoc) National Science Foundation (NSF) Review Panel.

2014, 2013 Member (Ad Hoc) American Institute of Cancer Research (AICR) Grant Review Panel Study Section.
2014, 2013 Member (Ad Hoc) Nutrition Obesity Research Center of University of Alabama at Birmingham Review Panel.
2013 Member (Ad Hoc) National Science Foundation (NSF) Review Panel.
2013 Member (Ad Hoc) NIH Scientific Review Committee, Chemo-Dietary Prevention (CDP) Study Section.
2013 Member (Ad Hoc) Medical Research Council (MRC) Review Panel.
2013 Member (Ad Hoc) Children's Tumor Foundation Review Panel.
2013 Member (Ad Hoc) American Cancer Society (ACS) Advisory Committee for Extramural Grants Department
2006-present Member (Ad Hoc) Hong Kong Research Grants Council Review Panel.
2006-present Member, Coordinating Committee, Mutant Mouse Regional Resource Centers
2006-present Reviewer for the following journals: *Mammalian Genome; Development; Mechanisms of Development; Genetics; Genesis; Proc. Natl. Acad. Sci. USA; Cancer Research; Oncogene; Human Molecular Genetics; Molecular and Cellular Biology; Current Biology; Nature Reviews Genetics; Genome Research; Molecular Cancer; Genes & Cancer; Developmental Biology; Inflammatory Bowel Diseases; Gene; Journal of Molecular Cell Biology; Genes, Chromosomes and Cancer; Developmental Cell; PLoS ONE*, *Journal of Cellular Physiology*; *Nucleic Acids Research; PLoS Genetics; Cell Host Microbe, Current Drug Metabolism; Physiological Genomics; Nucleus; Carcinogenesis; Stem Cell Reports; F1000 Primer Reports, Cell Metabolism*.
1996-present Member, American Association for the Advancement of Science (AAAS).

Honors, Awards, Fellowships:
1993 Chancellor's Award for extraordinary professional promise, University of Tennessee, Knoxville
1994 Oak Ridge National Laboratory Publication Award
1997-2000 Recipient of American Cancer Society Postdoctoral Fellowship (NIH NRSA declined)

C. Selected Peer-Reviewed Publications (all in high-visibility journals with impact factors > 11)

1. Donohoe, D.R., Montgomery, S.A., Collins, L.B., Curry, K.P., Holley, D., Renner, S.W., Tillotson, C., Ryan, E.P., Godfrey, V., Han, A., Swenberg, J.A., Threadgill, D.S., Threadgill, D.W., and **Bultman, S.J.** (2014). A gnotobiotic mouse model demonstrates that dietary fiber protects against colorectal tumorigenesis in a microbiota- and butyrate-dependent manner. *Cancer Discovery. In Press.*
   -Demonstrates that dietary fiber protects against colorectal cancer but in a microbiota- and butyrate-dependent manner. Due to the Warburg effect, butyrate accumulates in tumors and acts as an HDAC inhibitor. This mechanism is relevant to human cancer prevention because human colorectal adenocarcinomas have elevated butyrate and histone acetylation levels compared to normal colonic tissue. Identifies microbial butyrate as a tumor-suppressive metabolite.
2. Donohoe, D.R., Collins, L.B., Wali, A., Bigler, R., Sun, W., and **Bultman, S.J.** (2012). The Warburg effect dictates the mechanism of butyrate-mediated histone acetylation and cell proliferation. *Molecular Cell* 48, 612-626. PMCID:PMC3513569.
   -Discovery of the Warburg effect as a mechanism for how a bacterial metabolite (butyrate) exerts opposite effects on the proliferation of normal versus cancerous colonocytes. Relevant to diet-microbiome interactions in cancer prevention. Also describes a novel mechanism for butyrate-mediated histone acetylation independent of HDAC inhibition. Highlighted in *Nature Reviews Cancer*, *Nature Chemical Biology*, *Cancer Discovery*, and NCI newsletter *Nutrition Frontiers*.
3. Willis, M.S, Homeister, J.W., Rosson, G.B., Annayev, Y., Holley, D., Holly, S.P., Madden, V.,Godfrey, V., Parise, L.V., and **Bultman, S.J.** (2012). Functional redundancy of SWI/SNF catalytic subunits in maintaining cardiac vascular endothelial cells in the adult heart. *Circulation Research* 111, e111-e122. PMCID:PMC3501986.

Discovery that two chromatin remodelers, BRG1 and BRM, are functional redundant in an adult tissue (heart), whereas only BRG1 is utilized during development. Characterization of BRG1 and SWI/SNF chromatin remodeling in vascular endothelial cell viability.

4. Donohoe, D.R., Garge, N., Zhang, X., Sun, W., O'Connell, T.M., Bunger, M.K., and **Bultman, S.J.** (2011). The microbiome and butyrate regulate energy metabolism and autophagy in the mammalian colon. *Cell Metabolism* 13, 517-526. PMCID:PMC3099420.
   -Discovery that the microbiome is particularly important for energy homeostasis of colonocytes compared to other tissues. Identified butyrate as the causative factor where it functions as an energy source rather than an HDAC inhibitor to promote oxidative metabolism and prevent autophagy. Highlighted in *Cell Metabolism*, *Nature, Nature Medicine*, and Faculty of 1000. Cited>100 times.
5. **Bultman, S.J.**, Herschkowitz, J., Godfrey, V., Gebuhr, T.C., Yaniv, M., Perou, C., and Magnuson, T. (2008). Characterization of mammary tumors from *Brg1* heterozygous mice. *Oncogene 27,* 460-468. PMID: 17637742 [PubMed - indexed for MEDLINE]
   -Characterization of BRG1-mediated tumor suppression in a genetically-engineered mouse model. The first demonstration that BRG1 is a tumor suppressor. Highly relevant to the clinic because *BRG1* is mutated in ~4% of human primary tumors, and at least one SWI/SNF subunit is mutated in ~19% of human primary tumors. This makes BRG1 and SWI/SNF one of the most prevalent tumor suppressors.
6. **Bultman, S.J.**, Gebuhr, T.C., Pan, H., Svoboda, P., Schultz, R.M., and Magnuson, T. (2006). Maternal BRG1 regulates zygotic genome activation in the mouse. *Genes & Development* 20, 1744-1754. PMID: 16818606.
   -One of the first demonstrations of a mammalian "maternal-effect" gene in mammals where maternal genotype influences embryonic phenotype. BRG1 is required for zygotic genome activation where the genome goes from transcriptional silence to the establishment of a totipotent transcriptome profile in the pre-implantation embryo, which is necessary for the differentiation of all subsequent cell lineages during embryogenesis. Featured by Faculty of 1000. Cited>150 times.
7. **Bultman, S.J.,** Gebuhr, T.C., and Magnuson, T. (2005). A *Brg1* mutation that uncouples ATPase activity from chromatin remodeling reveals an essential role for SWI/SNF-related complexes in β-globin expression and erythroid development. *Genes & Development* 19, 2849-2861. PMID: 16287714.
   -Identification and characterization of an essential role for BRG1 and SWI/SNF in β -globin gene regulation and red blood cell development, which is relevant to thalassemias. First demonstration of a chromatin-modifying factor being required for the formation of chromatin loops between a distal enhancer and a promoter many kb away, which is a key part of the mechanism. Cited>100 times.
8. **Bultman, S.J.,** Gebuhr, T.C., Yee, D., Nicholson, J., La Mantia, C., Gilliam, A., Randazzo, F. Metzger, D., Chambon, P., Crabtree, G., and Magnuson, T. (2000). A *Brg1* null mutation in the mouse reveals functional differences among mammalian SWI/SNF complexes. *Molecular Cell* 6, 1287-1295. PMID: 11163203.
   -Knockout of a highly studied chromatin remodeler that is a catalytic subunit of SWI/SNF complexes. Homozygous *Brg1* mouse embryos die at an early stage of development (blastocyst), which is compatible with its role in maintenance of ES cell pluripotency. Also revealed functional heterogeneity of SWI/SNF complexes with BRG1-catalyzed complexes being essential while BRM-catalyzed complexes are dispensable. Cited>550 times.
9. *Rinchik, E.M., ***Bultman, S.J.,** Horsthemke, B., Lee, S.-T., Strunk, K.M., Spritz, R.A., Avidano, K.M., Jong, M.T.C., and Nicholls, R.D. (1993). A gene for the mouse pink-eyed dilution locus and for human type II oculocutaneous albinism. *Nature* 361, 72-76. PMID: 8421497.
   -Characterization of a pigmentation gene mutated in a classical mouse mutant and in human patients. Cited>350 times.
10. **Bultman, S.J.,** Michaud, E.J., and Woychik, R.P. (1992). Molecular characterization of the mouse agouti locus. *Cell* 71, 1195-1204. PMID: 1473152.
    Characterization of the mouse classical coat-color gene, agouti, which is responsible for the wild-type coat-color of mice and other mammals. Because certain mutants are obese and diabetic, it also represents the first obesity gene to be cloned. Cited>750 times.

D. Research Support

Active

NIH 8-U42-OD10924-13 Bultman, S. (co-I); Magnuson, T. (PI)
09/30/99 – 02/28/15; $1,443,739 current year total costs; 10% effort (1.20 Calendar Months)
Agency: OD (previously NCRR)
Title: A Carolina Center to Characterize and Maintain Mutant Mice
The major goal of this project is to import, phenotype, and archive mutant mice produced throughout the country.

Completed:

[redacted]

NIH 1-RO1-CA125237-05 Bultman, S. (PI)
09/01/07 – 07/31/13; $190,000 annual direct costs; 35% effort (4.2 Calendar Months)
Agency: NCI
Title: Dietary Fiber, Gut Microflora, & Epigenetic Events that Prevent Colorectal Cancer
The major goal of this project is to understand the role of dietary fiber, gut microflora, and butyrate in epigenetic changes and gene expression that protect against colorectal cancer in mouse models.

[redacted]

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Gomez, Shawn Michael	Associate Professor, Joint Department of Biomedical Engineering, UNC-Chapel Hill
eRA COMMONS USER NAME (credential, e.g., agency login) [redacted]	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of Colorado, Boulder, USA	BS	06/90	Aerospace Engineering
University of Colorado, Boulder, USA	MS	06/93	Aerospace Engineering
Columbia University, New York City, USA	Eng.Sc.D	12/99	Biomedical Engineering
Columbia University, New York City, USA	PostDoc	1999-2002	Computational Biology
Institut Pasteur, Paris, FRANCE	PostDoc	2002-2005	Computational Biology

A. Personal Statement

My lab is focused on issues in computational systems biology and bioinformatics, with particular focus on understanding the architecture and dynamics of protein-signaling, metabolic and gene-regulatory networks in human disease. A significant portion of our work centers on the analysis and modeling of biomolecular systems at multiple scales, using statistical and machine learning techniques as well as more classical modeling approaches. Similarly, we are also actively involved in the development of novel, network-focused, computational algorithms, tools and methodologies that complement these research efforts. Recent work has led us into the development of computer vision tools and methodologies for the analysis of live-cell imaging data acquired through optical microscopy, with an initial focus on subcellular structures such as adhesions as well as cellular-scale behaviors like cell motility. Longer-term, these imaging efforts will provide the basis for inferring the dynamics of the underlying cell/molecular networks that underlie normal physiology as well as disease.

B. Positions and Honors

Positions and Employment

1987 – 1990 Intern, Martin Marietta, Astronautics Group, Denver, CO
1990 – 1994 Research Assistant, BioServe Space Technologies, University of Colorado, Boulder, CO
1991 Intern, Lockheed Missiles & Space Company, Bioastronautics Division, Sunnyvale, CA
1995 – 1999 Research Assistant and Eng.Sc.D. Candidate, Biomedical Engineering, Columbia University, New York, NY
1999 – 2002 Postdoctoral Fellow, Columbia Genome Center, Columbia University, New York, NY
2002 – 2005 Postdoctoral Fellow, Institut Pasteur, Paris, France
2005 – 2012 Assistant Professor, Joint Department of Biomedical Engineering, UNC-Chapel Hill and NC State University
2009 – 2012 Joint Assistant Professor, Department of Pharmacology, UNC-Chapel Hill
2009 – present Adjunct Assistant Professor, Department of Computer Science, UNC-Chapel Hill
2012 – present Associate Professor, Joint Department of Biomedical Engineering, UNC-Chapel Hill and NC State University
2012 – present Joint Associate Professor, Department of Pharmacology, UNC-Chapel Hill

Other Experience and Professional Memberships

International Society for Computational Biology
Biomedical Engineering Society

Honors

1986 National Hispanic Scholar

1986	University of Colorado Engineering Scholarship
1989	Martin Marietta/Inroads Distinguished Intern
1990	NASA/USRA Summer Conference Leader
1991-1994	NASA Fellow, Graduate Student Researchers Program
1999-2001	Research Foundation for Mental Hygiene Fellow
2002-2005	Florence Gould Scholar, Pasteur Foundation Fellow
2006	UNC Junior Faculty Development Award
2008	Carl Storm URM Fellowship
2011	UNC Research Council Award
2013	ACCLAIM Scholar – UNC School of Medicine/UNC Hospitals

C. Ten Selected Peer-reviewed Publications

Berginski ME, Vitriol EA, Hahn KM and **Gomez SM**. (2011) High-Resolution Quantification of Focal Adhesion Spatiotemporal Dynamics in Living Cells. *PLoS ONE*. 2011;6(7):e22025. PMCID: PMC3136503. *This publication describes the groundwork for a novel, fully automated image analysis system for the quantitative analysis of focal adhesion static, dynamic and spatial behaviors within single cells. To date, the web-version of this system has processed 385,502 images encompassing 9920 experiments that have been submitted from over 350 outside researchers.*

Wu C, Asokan SB, Berginski ME, Sharpless NE, Griffith JD, **Gomez SM** and Bear JE. (2012) Arp2/3 complex is critical for lamellipodia and organization of cell-matrix adhesion but dispensable for fibroblast chemotaxis. *Cell.* 2012 Mar 2;148(5):973-87. PMCID: PMC3707508. *My group developed and performed the automated image analysis that quantitatively demonstrated how global focal adhesion spatial alignment within the cell is dependent on lamellipodia as well as alterations in adhesion properties due to Arp2/3 deletion. This work further demonstrates that haptotaxis is dependent on the existence of lamellipodia and that chemotaxis can function despite loss of Arp2/3.*

Gomez SM, Noble WS and Rzhetsky A. (2003) Learning to predict protein-protein interactions from protein sequences. *Bioinformatics.* Oct 12;19(15):1875-81. *This paper described a novel Bayesian approach for the prediction of protein-protein interaction networks. Based on an "attraction-repulsion" model, this approach was shown to outperform other state-of-the-art machine learning approaches including support vector machines. This approach is capable of readily integrating heterogeneous data types as well as being able to truly scale to "big data" sized applications.*

Choi K and **Gomez SM**. (2009) Comparison of phylogenetic trees through alignment of embedded evolutionary distances. *BMC Bioinformatics.* 10:423. PMCID: PMC3087345. *This work describes several novel algorithms for the comparison of tree structures, such as phylogenetic trees. Based on the alignment of high-dimensional embeddings of the underlying data, these methodologies were shown to outperform the current state-of-the art as well as provide novel capabilities such as horizontal gene transfer event detection and the prediction of interaction specificity.*

Doolittle JM and **Gomez SM**. (2011) Mapping protein interactions between Dengue virus and its human and insect hosts. *PLoS Neglected Tropical Diseases*. 5(2):e954. PMCID: PMC2877021. *Utilizing machine learning approaches, this work provided the first ever prediction of the protein interaction interface that exists between the Dengue virus and both its human and insect hosts. With previously only 19 known protein interactions documented, this work provided hundreds of high probability therapeutic targets. This article is in the top 10% of all articles ever tracked by Altmetric.*

Abell AN, Jordan NV, Huang W, Prat A, Midland AA, Johnson NL, Granger DA, Mieczkowski PA, Perou CM, **Gomez SM**, Li L and Johnson GL. (2011) MAP3K4/CBP-Regulated H2B Acetylation Controls Epithelial-Mesenchymal Transition in Trophoblast Stem Cells. *Cell Stem Cell*. 6;8(5):525-37. PMCID: PMC3091002. *My group performed comprehensive bioinformatics analyses of gene expression of EMT within trophoblast stem cells. This work showed that the expression profile of MAP3K4-deficient TS cells defines an H2B acetylation-regulated gene signature that overlaps that of human breast cancer cells. This further defined*

an epigenetic switch maintaining the epithelial phenotype and new genes potentially contributing to triple negative breast cancer.

Rzhetsky A, **Gomez SM**. Birth of scale-free molecular networks and the number of distinct DNA and protein domains per genome. *Bioinformatics*. 2001 Oct;17(10):988-96. *This work explores statistical properties of real biomolecular networks based on a basic evolutionary principles-based stochastic model. The model correctly predicted that the frequencies of distinct DNA and protein domains follows a power-law distribution and led to a simple equation linking the total number of dna/protein domains in a genome with the total number of genes and network topology.*

Xu K, Morgan KT, Elston TC and **Gomez SM**. (2011) A whole-body model for glycogen regulation reveals a critical role for substrate cycling in maintaining blood glucose homeostasis. *PLoS Computational Biology*. 7(12):e1002272. PMCID: PMC3233304. *This work describes a multi-organ model describing glucose/glycogen metabolism in the liver as well as muscle, adipose and blood compartments. With extensive detail on the glycogen futile cycle, this model provided novel insight as to the physiologic role of glycogen cycling and the first quantitative predictive description of a previously only hypothesized metabolic behavior.*

Duncan JS, Whittle MC, Nkamura K, Abell AN, Midland AA, Zawistowksi JS, Johnson NL, Granger DA, Jordan NV, Darr D, Usary J, Major B, He X, Hoadley K, Sharpless NE, Perou CM, **Gomez SM**, Jin J, Frye SV, Earp HS, Graves LM and Johnson GL. (2011) Dynamic reprogramming of the kinome in response to targeted MEK inhibition in triple negative breast cancer. *Cell*. 2012, 149(2):307-21. PMCID: PMC3328787 *This work demonstrated a mechanism for tumor cell reprogramming in response to treatment with a single inhibitor and the ability to block this effect through the use of specific drug combinations. My group's work encompassed the bioinformatics efforts, which suggests a potential strategy for the design of rational combination therapies that improve clinical response.*

Berginski ME, Creed SJ, Cochran S, Roadcap DW, Bear JE and **Gomez SM**. Automated analysis of invadopodia dynamics in live cells. *PeerJ.* 2014 2:e462 2014. PMCID: PMC4103095. *Focuses on the description of the first described automated image analysis system quantifying matrix degradation behaviors at both the single cell and cell-population levels. Methods developed allowed for the simultaneous capture of intracellular protein dynamics along with quantitative extracellular behaviors such as matrix degradation rates and allowed for the identification of distinct metastatic-relevant cellular subpopulation phenotypes.*

D. Research Support

Ongoing Research Support

1U01GM094663, (Subcontract to The Burnham Institute) NIH
9/30/2010 – 6/30/2015
Assembly, dynamics and evolution of cell-cell and cell-matrix adhesions
The Consortium will leverage high-throughput expression and structures of large sets of target families of proteins and signaling networks to understand the structural and functional organization of cell-cell and cell ECM adhesion complexes.
Role: Co-Investigator (Klaus Hahn-Subcontract PI)

R01-GM101141 NIH
4/15/2012 – 1/31/2016
Kinome reprogramming in response to targeted kinase inhibitors
The goal of this project is to investigate the reprogramming of the breast cancer kinome in response to combinatorial kinase inhibitor therapy.
Role: Co-Investigator (Gary Johnson PI)

R01-CA177993 NIH
8/01/14 - 7/31/18
Single-Cell Measurement of Lipid Signaling in Colorectal Cancer
PI3 kinase activity and metabolites of phosphatidyl inositide 4,5-bisphosphate will be measured in single tissue-cultured colorectal cancer cells. PI3 kinase activity will also be measured in single cells from a mouse xenograft model of human colorectal cancer with and without pharmacologic intervention.
Role: Co-Investigator (Nancy Allbritton PI)

Recently Completed Support

R01 HL092544-01 4/15/2010 – 2/28/2014 NIH/NHLBI
CIB 1 regulation of endothelial function
Many people suffer from illnesses related to abnormally increased or decreased blood vessel growth, e.g. in cancers, retinopathies, rheumatoid arthritis, psoriasis and heart disease. In this proposal we seek to better understand how endothelial cells, which line all blood vessels, control blood vessel growth, by studying a protein called CIB1, which appears to regulate endothelial cell function.
Role: Co-Investigator (Leslie Parise - PI)

R01-GM084071 1/01/2009 – 12/31/2012 NIH/NIGMS
MAP kinase regulation of cell-fate transitions in yeast
The major goals of this project are to learn how differences MAPK activation profiles translate into alternative transcription programs and to uncover how the two programs differentially affect the physiological and phenotypic potential of the cell.
Role: Co-PI (Beverly Errede and Timothy Elston, Co-PIs)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
SCOTT T. MAGNESS	ASSOCIATE PROFESSOR
eRA COMMONS USER NAME (credential, e.g., agency login) [redacted]	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of California at San Diego	B.S.	1987-1992	Chemistry/Biochemistry
University of North Carolina at Chapel Hill	Ph.D.	1994-1999	Genetics & Molecular Biol.
University of North Carolina at Chapel Hill	Post-doc	1999-2003	Molecular Pathology
University of North Carolina at Chapel Hill	Post-doc	2003-2006	Stem Cells

A. Personal Statement

I have had a long-standing scientific interest in genetic mechanisms that underlie tissue regeneration with a particular focus on stem cells and fibrosis. My current research focuses on understanding factors that control the maintenance and differentiation of intestinal and colonic epithelial stem cells (ISCs). Early work from my laboratory demonstrated that distinct levels of the *Sox9* transcription factor differentially mark 'active' ISCs that primarily contribute to homeostatic renewal. High levels of *Sox9* also mark 'reserve' ISCs that respond to injury. These studies were important to the field because they established *Sox9* as one of the first ISC biomarkers and provided an early foundation to develop stem cell isolation and culture for translational research. To our knowledge my lab was the first to pioneer and validate the *in vitro* culture system defined by Sato et al, and since then we have made a number of modifications to increase efficiency, reproducibility and utility of ISC culture. Much of the basic understanding of ISC behavior in Sox9 mouse models led directly to development of biomarkers for human intestinal ISC isolation from bariatric surgery remnants. This work established a minimal cell surface signature that could be used to FACS enrich ISCs consistent with 'active' and 'reserve' characteristics. Our unique expertise in isolation and culture of mouse and human ISCs led to a study to develop a pig model as a translational species to develop novel cell-based therapies for a number of conditions that afflict GI health in humans. An immediate focus of my lab is to build on this previous work to develop simulacra of the small intestine and colon. My collaboration with Dr. Allbritton has led to three foundational studies that marry cell biology with microfabricated platforms to begin to build a colon-on-a-chip using primary human tissues. While normal human tissue is extremely difficult to obtain for most investigators, we are in a unique position by having an established relationship with Carolina Donor Services (CDS) to collect full-length donor quality intestine and colon. Stem cells from 6 different regions (from a goal of 50 donors) will be ex vivo expanded and banked for establishing simulacra. Because the colon is typically discarded, we anticipate more than sufficient supply to meet this goal.

B. Positions and Honors

- 1994-1999 Graduate Student (Dr. David Brenner, Dept. of Genetics, UNC Chapel Hill)
- 1999-2000 Visiting Lecturer (Dept. Biology, UNC Chapel Hill)
- 1999-2003 Post-doctoral fellow (Dr. David Brenner, Dept. of Medicine, GI Division, UNC Chapel Hill)
- 2003-2006 Post-doctoral fellow (Dr. Larysa Pevny, Dept. of Genetics, UNC Chapel Hill)
- 2006-2014 Assistant Professor (Depts. of Medicine, GI Division, Cell biology & Physiology, and Biomedical Engineering, UNC Chapel Hill/NC State University)
- 2014-present (Depts. of Medicine, GI Division, Cell biology & Physiology, and Biomedical Engineering, UNC Chapel Hill/NC State University)

C. Selected Peer-reviewed Publications Most Relevant to the Current Application (10 of 36)

1. Formeister E.J., Sionas A.L., Lorance D.K., Barkley C.L., Lee G.H., **Magness ST** - Distinct SOX9 Levels Differentially Mark Stem/Progenitor Populations and Enteroendocrine Cells of the Small Intestine Epithelium. *Am J Physiol Gastrointest Liver Physiol. 2009, May; 296(5):G1108-18. Epub 2009 Feb 19.* PMC2696217. This study developed the Sox9EGFP mouse model to facilitate identification of putative ISCs.
   - The study was initiated prior to any validated genetic biomarkers for ISCs and when published established Sox9 levels as a biomarker for cells consistent with ISCs (Sox9low) and enteroendocrine cells (Sox9high). The contribution to the field was a new mouse model for intestinal stem cell studies.

2. Gracz, A.D., Ramalingam, S., **Magness ST** – Sox9-expression marks a subset of CD24-expressing small intestine epithelial stem cells that form organoids *in* vitro. 2010; 298 (5): G590-600 *Am J Physiol Gastrointest Liver Physiol. Epub 2010 Feb 25.* PMC2867430
   - This study validated Sox9 as one of the first biomarkers for ISCs. This work also established that CD24 could be used to enrich for ISCs from non-transgenic sources. The contribution to the field was a new ISC biomarker and identification of the first cell surface protein that could be used to enrich for ISCs.

3. Ramalingam, H., Daughtridge, G.W., Johnston, M.J., Gracz, A.D. **Magness, ST** - Distinct levels of Sox9 expression mark colon epithelial stem cells that form colonoids in culture. *American Journal of Physiology Gastro, 2012 Jan;302(1):G10-20. Epub 2011 Oct 13.* PMC3345960
   - This study demonstrated that Sox9 was a marker of colonic epithelial stem cells (CSCs) and that Wnt3a and myofibroblasts were required for growth of single colonic stem cells. This work establishes our expertise in isolation and culture of colonic stem cells and colonoids in defined conditions. The contribution to the field was a novel biomarker and culture conditions for long-term culture of primary colonic epithelium.

4. VanDussen, K.L., Carulli, A.J., Keeley, T.M., Patel, S.R., Puthoff, B.J., **Magness, S.T.**, Tran, I.T., Maillard, I., Siebel, C.W., Kolterud, A., Grosse, A.S., Gumucio, D.L., Ernst, S.A., Tsai, Y-H, Dempsey, P.J., and Samuelson, L.C. Notch Signaling Modulates Proliferation and Differentiation of Intestinal Crypt Base Columnar Stem Cells; *Development*. 2012;139(3):488-97. PMC3252352
   - Our contribution to this study was to manipulate Notch signaling in enteroid cultures to demonstrate that Notch signaling was required for ISC proliferation. This work establishes our ability to modulate Notch signaling to impact proliferation and secretory lineage differentiation in culture.

5. Van Landeghem, L.M., Santoro, A., Krebs, A., Mah, A.T., Dehmer, J.J., Gracz., A.D., Scull, B.P., McNaughton, K., **Magness, ST**, and Lund, P.K., - Activation of two distinct Sox9-EGFP expressing intestinal stem cell populations during crypt regeneration after irradiation. Am J Physiol Gastrointest Liver Physiol. 2012 May 15;302(10):G1111-32. Epub 2012 Feb 23. PMC3362093
   - This study demonstrated that cells expressing high levels of Sox9 marked a facultative ISC population that responded to radiation injury. The contribution to the field is that an endocrine-like cell type can act as a stem cell under certain conditions.

6. Gracz AD*, Fuller MK*, Wang F, Li L, Stelzner M, Dunn JCY, Martin M, **Magness ST** – Brief Report: CD24 and CD44 mark human intestinal epithelial cell populations with characteristics of active and facultative stem cells. *Stem Cells. 2013 Sep;31(9):2024-30.* *Authors contributed equally to this work. PMC3783577
   - This study defined the first minimal cell surface marker signature to isolate two different functional states of human intestinal stem cells. This work establishes expertise in isolation of human stem cells and epithelial cell culture. The contribution to the field is a combinatorial cell surface protein signature that can be used to enrich for human ISCs.

7. Gonzalez LM, Williamson IA, Piedrahita JA, Blikslager AT, **Magness ST** - Cell lineage identification and stem cell culture in a porcine model for the study of intestinal epithelial regeneration*. PLoS One. 2013 Jun 28;8(6).* PMC3783577
   - This study was the first to generate pig enteroids. It established new reagents to study pig intestinal epithelium. The contribution was new methodology for using pig as a translational model for intestinal stem cell studies aimed toward regenerative medicine. This establishes further expertise in enteroid culture, and isolation of ISCs from full-length intestine similar in size to human.

8. Mah AT, Van Landeghem L, Gavin HE, **Magness ST**, Lund PK. - Impact of diet-induced obesity on intestinal stem cells: hyperproliferation but impaired intrinsic function that requires insulin/IGF1. *Endocrinology, 2014, Jun 10:en20141112.* PMC4138564
   - This work uses the Sox9EGFP reporter gene model to monitor stem and progenitor cells in vivo. It establishes expertise in using reporter gene models to assess physiologic changes associated with normal and abnormal gut function.

9. Ding S., Walton, K.L.W., Blue, R.E., MacNaughton, K., **Magness, S.T.**, Lund P.K. Mucosal healing and fibrosis after acute or chronic inflammation in wild type FVB-N mice and C57BL6 procollagen 1(I)-promoter-GFP reporter mice. *PLosOne*. 2012;7(8):e42568. Epub 2012 Aug 3. PMC3411826
   - In this study we used a dual reporter gene mouse, smooth-muscle actin DsRED and Collagen-EGFP, I generated in previous work to identify and monitor populations of fibrotic cell populations following a fibrotic stimulus. It establishes further expertise in reporter gene models, gut physiology and immune mediated responses.

10. **Magness ST**, Jijon H, Van Houten Fisher N, Sharpless, NE, Brenner DA, Jobin C. *In vivo* pattern of LPS and anti-CD3-induced NF-kB activation using a novel gene targeted EGFP reporter gene mouse. *Journal of Immunology,* 2004;173:1561-70.
    - In this study I generated an EGFP reporter mouse to monitor the activation of NF-kB in vivo. It establishes expertise in reporter gene mouse models, intestinal epithelial cell biology and immune cell mediated inflammation.

D. Research Support

ACTIVE

[redacted]

NIH R01 DK091427 Magness (PI) 09/1/11– 08/31/16

Genetic Control of Intestinal Epithelial Stem Cell Maintenance & Differentiation.

The focus of this study is to investigate the intrinsic and extrinsic roles of the transcription factor, Sox9, in intestinal epithelial stem cell behavior.

NIH R01 DK091427 Lund (PI), Magness (co-I) 08/01/12– 06/30/17

Aging intestinal stem cells and the insulin/IGF system.

The goal of this project is to understand how aging impacts intestinal epithelial stem cells from an insulin/IGF perspective.

NIH R01 DK040247 Lund (PI), Magness (co-I) 05/01/12– 06/30/15

Intestinal adaptation – Role of hormones and growth factors.

The goal of this project is to understand how the insulin/IGF signaling axis impacts stem cell behavour.

RECENTLY COMPLETED

NIH R03 EB013803 Wang (PI)/Magness (co-I) 7/2011-6/2013

Microengineered systems for *ex vivo* culturing of intestinal crypts. The goal of this project is to develop systems to facilitate long-term culture of small intestine tissues.
NIH P30 DK34987 Pilot Feasibility Magness (PI) 7/2011-6/2012
Intestinal tissue engineering: bioengineered crypts as an *ex vivo* model of the intestine and a translational approach to stem cell-based therapies. The goal of this project is to develop biodegradable crypt scaffolds for transplantation of small intestine epithelial stem cells.

[redacted]

NIH R03 DK089126 Magness (PI) 7/2010-6/2012
Sox9, Enteroendocrine and Intestinal Epithelial Stem Cells: A Study of Gene Networks and Cellulary Hierarcy in the Intestainl Crypt. The goal of this project is to investigate the role of crypt-based enteroendocrine cells on the intestinal epithelial stem cells.
NIH U01 DK085547 (pilot project) Henning (PI)/Magness (co-I) 9/2011-8/2012
Isolation and characterization of stem cells from the normal mouse colon . The aims of this project are to isolate and characterize colonic epithelial stem cells via: a) positive/negative FACS, and b) culture of putative CESCs marked by CD24, and c) generate the transcriptome of CESCs
NIH U01 DK085547 (pilot project) Wong (PI)/Magness (co-I) 9/2011-8/2012
Lineage tracing in intestinal organoids harnessing photoconvertible technology. The aims of this project are to a) To establish the hierarchical relationship among progenitors within the intestinal crypt, and b) to determine if mTert cells are capable of asymmetric division.
NIH K01 DK080181-03 Magness (PI) 04/01/08 – 03/31/11
Intestinal Stem/Progenitor Cell Competence: A Study of SOX-factors, Genetic Markers and Lineage Tracing. The goal of this project is to identifiy putative intestinal epithelial stem cell markers and genes that control stem cell maintenance and differentiation.

[redacted]

BUDGET JUSTIFICATION

YEAR 1

PERSONNEL

Nancy Allbritton, M.D., Ph.D., Principal Investigator
Dr. Allbritton, Professor, Departments of Biomedical Engineering and Chemistry, will devote 2 calendar months per year to the project. Her areas of expertise include cellular signal transduction and the development and application of new analytical and microengineered technologies for cell-based assays, cell manipulation and cell signaling. She is an expert on microfluidic and microarray devices, particularly in the area of polymer-based tools. Dr. Allbritton will be responsible for the overall coordination of the research project and will take the lead role in experimental design and analysis.

Scott Bultman, Ph.D., Principal Investigator
Dr. Scott Bultman, Assistant Professor, Department of Genetics, will devote 2 calendar months to the project. His areas of expertise include mouse models of cancer including dietary intervention. He is an expert on studying the metabolic and epigenetic properties of butyrate in the colon using mouse models and tissue-culture models. He will be responsible for the experimental design, troubleshooting any technical issues that may arise involving the microbiota component of simulacra-bacteria co-cultures. He will also be responsible for overseeing the experiments that will interrogate the function of butyrate-producing bacteria and butyrate on epithelial homeostasis. He will train and oversee other personnel in these experiments.

Shawn Gomez, Eng.Sc.D., Principal Investigator
Dr. Gomez, Associate Professor, Department of Biomedical Engineering, will devote 1 calendar month per year in years 1-2 and 2 calendar months in years 3-5 as the imaging and bioinformatics needs of the project ramp up. His areas of expertise are in the areas of computational biology and bioinformatics with extensive experience in image analysis and machine learning. He will actively manage the image analysis efforts as well as be involved in all aspects of experimental design and interpretation.

Scott T. Magness, Ph.D., Principal Investigator
Dr. Magness, Assistant Professor, Departments of Medicine, and Cell and Molecular Physiology will devote 2 calendar months per year to the project. His areas of expertise include epithelial stem cell biology, molecular pathology, gene-expression profiling, and developing transgenic mouse models that facilitate cell sorting. Dr. Magness will be responsible for the coordination of the biological aspects of this proposal involving tissue procurement, tissue dissociation, cell isolation, biomarker analysis, maintenance of mouse models, and the experimental design and analysis of Objective 1b. Dr. Magness will also be responsible for obtaining human tissue and genetic modifications to that tissue. Dr. Magness will also train and manage personnel related to the biological experiments conducted in his laboratory.

Christopher E. Sims, M.D., Co-Investigator
Dr. Sims, Research Professor in the Department of Chemistry, will devote 2 calendar months per year to the project. Dr. Sims has extensive experience in cell biology, bioassays, single-cell assays as well as application of microfabricated devices for cell manipulation and analysis. Dr. Sims will also oversee the performance of culture techniques and cell-based experiments in the Allbritton lab and will coordinate the day-to-day interactions among the laboratories.

Yuli Wang, Ph.D., Research Associate
Dr. Wang, currently a research associate in the Allbritton Group, is an expert in polymer and surface chemistry possessing a B.S. in Chemical Engineering, M.S. in Polymer Chemistry and Ph.D. in Materials Science. Dr. Wang will devote 12 calendar months of his time to the project per year. Dr. Wang has extensive experience in microfabrication methods, surface modifications and interfacing microdevices with biological systems and assays. He has worked extensively in the creation of microdevices for directed differentiation and maintenance of primary stem cells. Dr. Wang developed and optimized the collagen-based scaffold described in the preliminary data. He will optimize the materials, surface properties, fabrication, and microstructural design of the arrays and work with the postdoctoral fellows in overall design and implementation of the scaffold within the microfluidic and gas exchange compartments. His primary efforts in the current work will be as follows:

- Design, fabricate, and optimize the 3D substrate and growth of epithelial cells (Objective 1a).
- Design, fabricate, and optimize the device for chemical gradients application in Objective 1a
- Perform the assays on the tissue simulacrum for validation of the device function

- Supply the biologists with mouse and human simulacra for experiments with chemical gradients for Objective 1b
- Optimize and characterize chemical gradient formation in the devices
- Design, fabricate, and optimize the 96-well device for chemical gradients in Objective 3a
- Optimize the materials, surface properties, and liquid handling aspects for both of these low- and high-throughput platforms
- Develop, fabricate and optimize the standard, sampling and TEER lids in Objective 3b.
- Interface with the biologists for redesign of the chemical gradient device to enhamce properties for ease of use for non-engineers/non-chemists.
- Translate the technology into the Magness and Bultman Labs

Dulan B. Gunasekara, Ph.D., PGYI Postgraduate Researcher
Dr. Gunasekara, currently a postdoctoral fellow in the Allbritton Lab, obtained his Ph.D. in analytical chemistry at the University of Kansas under the mentorship of Susan Lunte. He has extensive experience in microengineered systems and the use of analytical devices for bioanalytical assays. His experience includes in-depth training in electrochemistry, fabrication of ultramicroelectrodes and thin film electrodes and integration of electronics systems for both on-chip control and analysis applications. He will be supported at a 12 calendar month effort per year. His primary effort in the current work will be the design, testing, integration and automation of the gaseous gradient devices. Specifically, his duties will be:

- Design, fabricate, and optimize the device for gas gradients in Objective 2a
- Supply the biologists with mouse and human simulacra for experiments with gas gradients for Objective 2b
- Optimize and characterize gas gradient formation on the devices
- Incorporate O_2 sensors into the devices to profile O_2 gradients
- Perform the assays on the tissue simulacrum for validation of the device function.
- Design, fabricate, and optimize the 96-well device for chemical gradients in Objective 3c
- Optimize the materials, surface properties, and liquid handling aspects for both of these low- and high-throughput platforms
- Interface with biologists to culture all simulacra with microbiota
- Interface with the biologists for redesign of the gas gradient device to enhance properties for ease of use by non-engineers/non-chemists
- Translate the technology into the Magness and Bultman Labs

PGYI, Postgraduate Researcher TBN
A postgraduate research fellow with a Ph.D. in biomedical engineering or related field who has extensive experience in imaging and instrument automation will be hired at 12 calendar month effort per year under the supervision of Dr. Allbritton. This postdoc will be responsible for the development of the hardware and software for the automated image analysis of simulacrum. The postdoc will work closely with Drs. Wang, Gunasekara, and the collaborating labs in the optimization and validation of software and integrating the hardware (motorized microscope) for the assays. Her/his role will be to accomplish the following tasks:

- All microscopy hardware modifications including stage redesign for device accommodation
- Construction and implementation of heating/CO_2/humidity and other control systems for the microscope incubator system
- All hardware automation and integration with software
- Development of front end GUIs for the software:human interface
- Integration and testing of the software with the array platform in collaboration with Wang, Gunasekara, and collaborators
- Comsol models of chemical gradient devices
- Comsol models of gas gradient devices
- Acquire images for assays and handoff of image data to the Gomez Lab
- Interfacing with biologists to solve all signal/noise challenges with assay readout
- Solve challenges related to assay multiplexing

PGYI, Postgraduate Researcher Magness TBN
A postgraduate research fellow with a Ph.D. in cell biology or related field will be hired at 12 calendar month effort per year under the supervision of Dr. Magness. The post-doctoral fellow will devote 12 calendar months to this project. Their expertise will include molecular and cell biology, cell culture and reporter gene mouse models. They will be required to organize, implement and execute the objectives described in the proposal. They will be responsible for design of breeding schemes for animal models, development of SOP for human tissue dissociation, culture, transfection, cryopreservation and banking of human organoids. This fellow will

also develop all of the transgenic reporter cell lines of the human colonic epithelium. The post-doctoral fellow will be assisting Dr. Magness with experimental planning and design and data analysis and interpretation, as well as with the training and coordination of the graduate student with Dr. Magness.

PGYI, Postgraduate Researcher TBN
A postgraduate research fellow with a Ph.D. in molecular biology who has extensive experience in host-microbe interactions will be hired at 12 calendar month effort per year under the supervision of Dr. Bultman. This post-doc will be responsible for carrying out assays related to co-culturing human simulacra with microbiota. He/she will monitor these bacteria using the assays listed in Table 5, which include live imaging of fluorescent ASF bacteria and Gram staining of simulacra cryosections. In addition, for co-cultures with complex human microbiota, the postdoc will work with the Microbiome Core Facility to monitor bacteria by 16S sequence analysis. He/she will also be responsible for monitoring the effect of each microbiota and FOS/inulin treatment group on crypt homeostasis of human simulacra. He/she will perform all genetic engineering to develop fluorescent ASF or luminal bacteria. This will involve performing the assays listed in Table 2 including EdU proliferation assays, cleaved caspase 3 apoptosis assays, mitotracker metabolic assays, and histone acetylation assays.

Graduate Researcher- Gomez, TBN
A graduate student with a background in engineering, computer science or related field will be hired at 12 calendar months per year on this project. He/She will be under the supervision of Dr. Gomez. Primary responsibilities will be on the development of novel algorithms, methodologies and tools for the computational image analysis of the data. He/She will also be actively involved in the integration of the developed hardware and software systems as well as interfacing with other team members on data analysis and interpretation.

Graduate Researcher- Magness, TBN
A graduate student will be hired at 12 calendar month effort per year under the supervision of Dr. Magness. The graduate student will be responsible for maintenance/husbandry and breeding of the project's mice and all genotyping of the transgenic mice used in the project. They will be responsible for obtaining mice crypts by performing surgical excision of small intestine followed by crypt isolation and single cell dissociation and all steps required to generate crypts for the project and will be responsible for all quality control of the isolated crypts as well as aliquoting and maintaining the roughly 10 different proteins/small molecules required for organoid formation and growth. The student will assay various parameters described on the simulacrum. They will be required to independently develop and optimize protocols for Objective 1b. The graduate student will be trained and supervised directly by Drs. Magness and post-doc TBN.

Darcy Holley
Ms. Holley, B.S, is a lab manager/technician who has been a member of the Bultman lab for 5 years. She will devote 6 calendar months per year to this project under the supervision of Dr. Bultman. She has experience culturing the anaerobic ASF and B. *fibrisolvens* bacteria that will be used in this study. She also has extensive experience performing PCR and a variety of molecular biology assays as demonstrated by a recent first-author publication and several other publications. She will be responsible for culturing ASF and *B. fibrisolvens* bacteria in an anaerobic chamber in advance of simaculra co-cultures. She will also be responsible for monitoring each of these bacteria in the co-cultures by qPCR. She will work with The Biomarker Mass Spectrometry Facility to quantify the production of butyrate and other SCFAs because she has performed this task in previous projects. In addition, she will assist the Bultman lab postdoc with many of the proposed assays.

EQUIPMENT

Year 1
Olympus Microscope ([redacted]) Funds are requested for an Olympus IX83 inverted fluorescence microscope which will be required for all imaging aspects of the project. The price listed includes the base microscope including fluorescence, DIC optics, 96-well-plate accessories, high numerical aperture, long working distance objectives [redacted], ASI piezo motorized stage ([redacted]), and Tokai Hit stage top incubator ($16,420). Initial imaging does not need to be confocal in year one as the project is in the early stages of development with respect to the scaffold, chemical-gradient device and gaseous-gradient device. We expect the purchase of this automated microscope to fill early needs as well as to permit basic instrumentation automation. Occasional needs for confocal imaging will be addressed by using microscopy core facilities. However by year 2, we expect to be occupying a confocal scope full time either with automated measurements or hardware/software improvements. This intensive usage plus the need to extensively modify hardware and software preclude the

use of a confocal microscope housed in a core facility. In year two, we will request the confocal unit that will mount on this Olympus microscope.

Andor iXon ULTRA 888BV EMCCD Camera ([redacted]) Funds are requested in year 1 for a high-sensitivity CCD camera for use in imaging on the Olympus IX83 inverted fluorescence microscope. This particular camera is selected because it is compatible with the spinning disc laser confocal system (purchased in Year 2) and is expected to provide sensitivity, speed and imaging area most suitable for imaging the simulacra. This back-illuminated, deep-cooled EMCCD camera is capable of 26 frames-per-second acquisition with linear EM Gain and Gain Calibration. Chip specifications are 1024×1024 13-um pixels, 16 bit digitization, 30, 20, 10, 1 MHz readout.

Image analysis desktop workstation ([redacted]) Funds for a dedicated image analysis workstation with multicore processors, 64 GB of memory, AMD FirePro graphics cards with Dual GPUs and 27 inch monitors will be required. The workstation will be devoted to the image analysis and reconstruction, algorithm development, data analysis and visualization work that are the focus of this proposal. A second workstation is requested in Year 3 in to handle the need for the increased image analysis and bioinformatics requirements as the high-throughput 96-device array is implemented.

MATERIALS AND SUPPLIES

Allbritton - Materials and supplies costs are estimated based on spending on related activities over the past year for the project. The microfluidic platform will be housed in the Allbritton Lab so that the experiments using this device will continue to utilize laboratory supplies, equipment and tissue culture facilities throughout the project. Fabrication of the devices will occur in all years. Funds are requested for a PC workstation for image/data collection and analysis including a PC computer, A/D board, interface and cable, and software. Numerous biological assays will be performed throughout the grant period. Included in this category is the cost for consumables and reagents for the biological assays to be performed as controls and include such items as fluorescent indicators, viability dyes, and cell biology reagents. Also included are the cost of the factors/morphogens/differentiating factors grown on the devices and in standard culture in the Allbritton Lab. Chemicals for 1002F photoresist synthesis (masters and molds), surface modification as well as standard chemicals for buffers, and general laboratory needs will be required. Glassware for chemical syntheses as well as beakers, flasks, graduated cylinders, pipets, and other general labware are requested. Tissue culture supplies such as media, serum, PBS, trypsin, and tissue culture-related consumables including sterile serologic pipets, sterile flasks, conical tubes, Petri dishes, compressed CO_2, anaerobic gas mixture, and autoclave supplies will be needed to carry out the proposed experiments. Fabrication projects as proposed tend to be intensive with regards to materials and supplies costs. Fabrication substrates, photolithographic masks and chemicals are requested in addition to chemical and biochemical reagents related to the cassette and 96-well scale-up efforts. The supplies budget for the first year is subdivided into categories below. The supplies budget for the first year is subdivided into categories below.

Category	Budget
Computer system	[redacted]
Gas & temperature control supplies	
Bioassay supplies	
Extracellular matrices and cross-linkers	
Lab chemicals/scaffolding reagents	
Tissue culture supplies	
Glass- and plastic-ware	
Fabrication chemicals/solvents	
Polymers and plastics for fabrication	
Fluorescent antibodies/indicators	
Fabrication substrates, mold materials	
Electronic supplies	
Optical and filter supplies	
Factors/morphogens/mitogens	
Total	

Bultman - Materials and supplies costs are estimated based on spending on related activities over the past few years. Primary cost points for the Bultman lab will be materials related to the reagents needed for assays and controls, specifically EdU for proliferation assays, apoptosis assays, Mitotracker for metabolic assays, and

antibodies (H3ac, H3) for histone acetylation assays, as well as general lab chemicals and labware. The following bioassays will be performed: EdU incorporation for cell proliferation, phospho H3 staining for cell cycle, cleaved caspase 3 apoptosis kit, Mitotracker assays for oxidative metabolism, analysis of H3ac and total H3 expression to analyze histone acetylation, Rhodamine 123 for epithelial transport. N= 8 bioassays. Objective 4a has 6 treatment groups, and each treatment group will be represented by 5 replicates for a total of 30 samples. 8 bioassays × 30 samples = 240 experiments. Performed on 50 independent simulacra. 240 × 50 =12,000 assays. Objective 4b has 2 treatment groups. 8 bioassays x 2 treatment groups × 5 replicates = 80 experiments. Performed on 50 independent simulacra. 80 × 50 = 4,000 assays. These are feasible because bioassays will be performed in a 96-well format: 16,000 total assays/96 = 167 96-well plates. 167/5 = 33 96-well plates per year.

Category	Budget
Microbiology	[redacted]
EdU	
Mitotracker	
Antibodies	
Cleaved Caspase-3 kit	
Molecular Biology Reagents/PCR	
Total	

Gomez - Funds are requested for software license purchases and renewals (compilers, specialized MATLAB toolboxes, development tools, software maintenance fees, etc.) as well as back up hardware and maintenance for storage of images/movies and additional 200,000 CPU hours of compute time on the UNC Killdevil cluster.

Category	Budget
Software	[redacted]
Killdevil cluster time	
Total	

Magness - Materials and supplies costs are estimated based on spending on related activities over the past few years. Primary cost points for the Magness lab will be materials related to tissue isolation, cell culture and gene expression studies. The growth factors and morphogens are critical to successful long-term growth of colonic crypts and represent a significant cost due to their high price point. A computer is requested for data analysis, data storage and manuscript preparation.

Category	Budget
Gene expression (Taqman probes)	[redacted]
Standard tissue culture supplies	
Growth Factors	
Antibodies/Immunostaining	
General Lab Supplies (Chemicals, plastic/glassware)	
Computer	
Total	

TRAVEL COSTS
Travel costs ([redacted]) for investigators to each make one trip per budget year to present and discuss project findings at national meetings. The cost of travel includes airfare on domestic U.S. flag carrier, ground transportation, lodging, meeting registration and per diem. Travel costs were estimated based on historical costs for past trips.

PUBLICATION COSTS
Publications costs ([redacted]). We anticipate on average 2 publications per year that will have multiple color images. The budget will pay for the additional costs of color figures that will be necessary to represent the data.

OTHER EXPENSES
Maskmaking services ([redacted]). Funds are requested for high resolution chrome/glass masks for the design and fabrication of arrays. Mask costs are typically ~[redacted] mask and numerous designs will be needed for experiments described in Objectives 1-3 as well as optimization as the work proceeds.

Machining services ([redacted]). Funds are requested for 200 hours of machining services in the Physical Sciences Machine Shop at [redacted] for custom fabrication of components needed for various custom made plastic cassette) or metal (microscope) parts.

Chemical and biohazardous waste disposal [redacted]. Funds are requested for the disposal of hazardous wastes per University regulations.

Maintenance costs [redacted]. Service contract for lab equipment include maintenance on laboratory equipment such as hoods, balances, centrifuges, cryostat as well as small percentage of the cost of service contracts for departmental common equipment.

Institutional High-Performance Computing Resources [redacted]. High-throughput applications will benefit from additional computational throughput. The Research Computing division of Information Technology Services at UNC delivers a 772 node (9152 core) Dell Linux cluster, "Kill Devil," with QDR Infiniband interconnect and a minimum of 4 GB memory per core; a smaller 2300-core HP Linux cluster with QDR Infiniband interconnect and at least 6GB of memory per core; and two 32-core hosts with one terabyte of memory each to accommodate codes that require extremely large amounts of RAM. The Kill Devil Cluster also includes 64 NVidia Tesla GPUs (M2070). UNC-Chapel Hill recognizes that computational research varies with respect to its data and processing demands, and also with respect to the need to compute, modify theories/codes, re-compute, etc. UNC-Chapel Hill is also committed to providing a base computational resource both to help build research programs, to extend the value of extramural contracts/grants/awards, and to help sustain programs. The university acknowledges, too, that some projects may take weeks to realize, some may take decades to realize. Scientific problems are not one-sized; therefore, computational demands are not one-sized. Accordingly, the university supports UNC-Chapel Hill investigators with direct investment for computational cores: (i) by delivering a free annual allocation of 200,000 core-hours at no cost to each investigator; (ii) for annual consumption above the free allocation, by covering a portion of the cost of the investigator's core-hours, passing on only $.005 per core-hour (a half-cent per core-hour). The value of UNC-Chapel Hill's investment is intended to cover at least 70% of the total cost of owning and operating the computational cores resource. The free allocation and charge for additional core-hours is reviewed on an annual basis. The additional core-hour charge is a standardized institutional use charge for high performance computing use: it has been developed by an official university committee and in accordance with approved recharge center policies. As part of this resource, funds are requested for the acquisition of Patron status on the killdevil cluster. This provides dedicated access to 16 cores on the cluster at all times (beyond, priority queue status as well as no limit on the time for jobs to execute.

Microbiome Core Facility ([redacted]). This core will be used for 16S sequencing of the microbiome collected from the intestinal simulacrum.

LC-MS Biomarker Core Facility ([redacted]) .This core will be used for quantification of butyrate and other short chain fatty acids.

Advanced Analytics Core ([redacted]). Funds are requested for high-throughput qPCR to assess gene expression patterns on the simulacra. The Center for Gastrointestinal Biology and Disease's Advanced Analytics Core offers high-throughput real-time qPCR service on its Fluidigm BioMark HD system, allowing users to simultaneously measure as many as 96 samples for up to 96 genes.

BAC Transgenic Core ([redacted]). Funds are requested to develop a total of 10 BAC transgenic reporter genes. The reporter gene constructs will be generated by the UNC BAC transgene core. Funds for two extra BAC transgene are included in the event that the original transgenes do not express properly and need to be re-designed. These BAC constructs will be transfected into human colonoids to create the transgenic human simulacra used in the project.

Carolina Donor Services ([redacted]). Funds are requested to procure human colonic tissue from 12 cases per year. Carolina Donor Services fees are $800 per case for OR time and tissue storage reagents.

Animal per diem charges ([redacted]). Mice strains are currently established in Dr. Magness' lab. All mice for this project will be bred in house so that no purchase of new strains are expected to be required. We anticipate using 18 cage spaces annually for breeding and aging of mice (18 cages at [redacted]).

YEAR 2

PERSONNEL
See Year 1

EQUIPMENT
Andor REVOLUTION 600 Confocal System ([redacted]) Funds are requested for an Andor REVOLUTION 600 series spinning disc confocal microscope possessing six solid state laser modules with an operating range of 400 – 650 nm. This confocal system will mate with the Olympus IX83 inverted fluorescence microscope to provide the imaging system possessing the necessary capabilities for carrying out the imaging experiments on the simulacra described in the grant. By year 2, we expect to be occupying a confocal scope full time either with automated measurements on the simulacra or with the time needed for hardware/software modifications. This intensive usage plus the need to extensively modify hardware and software preclude the part-time use of a confocal microscope in a core facility. The project requests a confocal unit for full-time usage by the investigators.

MATERIALS AND SUPPLIES
Same as Year 1 less [redacted] for computer system requested in Year 1.

TRAVEL COSTS
See Year 1

OTHER EXPENSES
Same as year 1 less [redacted] for one-time Institutional High-Performance Computing Resource charge

YEAR 3

Year 3 will see a significant increase in the image analysis/bioinformatics needs of the grant as the high-throughput 96-device array is implemented. For this reason additional funds are requested for the Gomez Lab to meet the added work load. A robotic pipettor is also requested for daily fluidic replenishment and removal from the 96-well array as described below.

PERSONNEL
Same as Years 1-2 with the increase in effort for Dr. Gomez from 1 to 2 calendar months and the addition of a postdoctoral fellow as justified below:

Postdoctoral Researcher- Gomez, TBN. A postdoctoral fellow will be hired with a background in image analysis, engineering, computer science or related field at 12 calendar months per year. He/She will be under the supervision of Dr. Gomez. Primary responsibilities will be the development and application of image analysis and machine learning tools and methodologies. He/She will also be actively involved in HW development as it impacts imaging efforts as well as in the design, data analysis and interpretation of experiments.

EQUIPMENT
8-Channel reagent dispenser ([redacted]). Funds are requested for a Biotek Precision Microplate Pipetting System. This is an 8-channel reagent dispenser to be used in the daily preparation of the 96-device array making up the high-throughput system. This work will take place in the Allbritton Lab and will require extensive pipetting to fill and withdraw fluid from the reservoirs of the devices during the development work and for the assays to be carried out on the arrays. The Biotek Precision is one of the few automated 8-channel pipetting systems capable of both delivery and removal of fluid on a 96-well format.

Image analysis desktop workstation ([redacted]). Funds for a dedicated image analysis workstation with multicore processors, 64 GB of memory, AMD FirePro graphics cards with Dual GPUs and 27 inch monitors will be required. The workstation will be devoted to the image analysis and reconstruction, algorithm development, data analysis and visualization work that are the focus of this proposal. This second workstation is requested in Year 3 for the increased image analysis and bioinformatics needs as the high-throughput 96-device array is implemented.

MATERIALS AND SUPPLIES
See Years 1-2.

TRAVEL COSTS
See Year 1

OTHER EXPENSES
See Years 1-2

YEARS 4-5
PERSONNEL
Same as in Year 3

EQUIPMENT- none

MATERIALS AND SUPPLIES
Same as in Year 3

TRAVEL COSTS
Same as in Year 1

OTHER EXPENSES
Same as in Year 3

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

1. Project Director / Principal Investigator (PD/PI)

Prefix:
First Name*: Nancy
Middle Name: Lynn
Last Name*: Allbritton
Suffix:

2. Human Subjects

Clinical Trial? ● No ○ Yes
Agency-Defined Phase III Clinical Trial?* ○ No ○ Yes

3. Permission Statement*

If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?

● Yes ○ No

4. Program Income*

Is program income anticipated during the periods for which the grant support is requested? ○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

Budget Period*	Anticipated Amount ($)*	Source(s)*

PHS 398 Cover Page Supplement

5. Human Embryonic Stem Cells

Does the proposed project involve human embryonic stem cells?* ● No ○ Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Cell Line(s): Specific stem cell line cannot be referenced at this time. One from the registry will be used.

6. Inventions and Patents (For renewal applications only)

Inventions and Patents*: ○ Yes ○ No

If the answer is "Yes" then please answer the following:

Previously Reported*: ○ Yes ○ No

7. Change of Investigator / Change of Institution Questions

❑ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:

First Name*:

Middle Name:

Last Name*:

Suffix:

❑ Change of Grantee Institution

Name of former institution*:

PHS 398 Research Plan

Please attach applicable sections of the research plan, below.

OMB Number: 0925-0001

1. Introduction to Application (for RESUBMISSION or REVISION only)	
2. Specific Aims	Challenge_Innovation1019088512.pdf
3. Research Strategy*	Approach1019088513.pdf
4. Progress Report Publication List	
Human Subjects Sections	
5. Protection of Human Subjects	HUMAN_SUBJECTS1018940501.pdf
6. Inclusion of Women and Minorities	
7. Inclusion of Children	
Other Research Plan Sections	
8. Vertebrate Animals	ANIMAL_JUSTIFICATION1018940503.pdf
9. Select Agent Research	
10. Multiple PD/PI Leadership Plan	Project_Leadership_Plan1018940500.pdf
11. Consortium/Contractual Arrangements	
12. Letters of Support	
13. Resource Sharing Plan(s)	Resource_Sharing_Plan1018940502.pdf
Appendix (if applicable)	
14. Appendix	

Challenge, Innovation, and Impact Statement: Humans and their resident gut microbiota have co-evolved to form a symbiotic relationship that is essential for health, yet little is known about how each of the thousands of bacterial species impacts epithelial biology.[1, 2] A number of human diseases are directly associated with a dysbiosis of bacteria, and while Genome Wide Association Studies (GWAS) have just begun to make links between an individual's genetic predisposition, microbiome, and disease, it is widely accepted that there exists a technical gap to experimentally validate cause and effect.[2-8] The confluence of cutting-edge microengineered systems and advances in expansion and long-term culture of primary human stem cells and their progeny make it possible to conceive of creating functional tissues *in vitro*.[9, 10] In the current work, a human tissue possessing the microarchitecture and physiologic behavior of the intestinal epithelium will be developed and combined with gut microbiota to form a tissue/microbiome environment recapitulating that of the human gut (a simulacrum). The simulacrum can be created directly from stem cells from normal individuals or patients to transform the field of intestinal biology and medicine by enabling unprecedented studies of cellular physiology, stem-cell dynamics, and disease mechanisms on primary tissues, including those obtained by patient biopsies.

Rationale: The advent of protocols for long-term *in vitro* culture of colonic stem cells and organoids has significantly advanced our understanding of stem cell-driven epithelial renewal.[11] While organoids contain self-renewing stem cells and all differentiated colonic cells, they lack organized stem and differentiated cell compartments and the orderly spatial and temporal transitioning of cells between these regions (Fig. 1).[11-13] Since 2009, the field has remained at this impasse unable to recapitulate *in vitro* a fully organized epithelium possessing crypts.[12-14] The critical missing step has been the ability to recreate a 2D monolayer with stem and differentiated cells segregated within a planar layer much as would be found if the lining of the colon were flattened out. To date, all planar culture systems have resulted in rapid cell differentiation followed quickly by cell death.[15-17] The collaborative group of investigators has met this challenge by developing a 2D culture system for colonic epithelium which supports the growth of both stem and differentiated cells. The focus of this proposal is in essence to now refold this planar epithelial system into the architecture of a functioning colonic epithelium with all appropriate temporal and spatial cellular relationships. A 3D scaffold on which the monolayer is cultured will recapitulate the physical geometry of the crypt while appropriate chemical gradients across the tissue layer will support the stem and differentiated cell compartments and facilitate their dynamic relationships. Technical achievements in hydrogel microfabrication will provide scaffolding with optimized composition, porosity, stiffness and geometry while microengineering innovations will create stable, robust chemical gradients across the simulacra and enable long-term health of the tissue within a controlled environment.

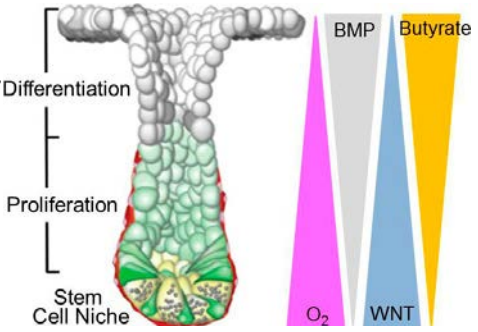


Fig. 1. *Crypts are tube-like invaginations lining the colon and form the basic functional subunit of the colon. Gradients of growth factors, mitogens, morphogens, food metabolites and gases across the luminal-to-basal epithelial surfaces regulate nearly every aspect of colonic physiology.[18, 19] WNT, BMP (bone morphogenic protein), butyrate (a dietary metabolite), and other molecules are thought to participate in crypt polarity by regulating cell position and proliferation. The O_2 gradient spanning the normoxic crypt base to anoxic lumen is critical for proper microbiota diversity and function. The stem cells (dark green) reside within the stem-cell niche at the crypt base. The rapidly dividing transit-amplifying cells (light green) are positioned between the stem cells and the differentiated epithelial cells (white) at the luminal surface. Typical crypt dimensions are: mouse crypts- length 250 μm, diameter 50 μm, human crypts- length 500 μm, diameter 100 μm.[20, 21]*

The colonic microbiota have a profound impact on non-intestinal processes including autism, immune tolerance, cardiovascular disease and diabetes, but the metabolic factors and modulators mediating this influence are as yet unknown.[22-26] The development of model systems to understand how bacteria localized to a single organ yield system-wide changes in organismal physiology has proven technically daunting. Metabolomic, metagenomic and metatranscriptomic characterization of the colonic microbiota are all currently aggressively pursued.[23] However, none of these tool sets reveal the intricate interplay within the living community comprised of the colonic epithelium and its oxygen-sensitive microbes. Gnotobiotic (germ-free) mouse models that can be colonized with known bacterial communities have provided insight into the role of microbiota in certain diseases; however, there are relatively few gnotobiotic mouse facilities, and significant logistical hurdles pose major bottlenecks impeding progress in this area.[27, 28] While *in vitro* culture systems would greatly enhance these studies, substantial obstacles have prevented such systems from filling this technological gap due to the challenges of the organoid culture system discussed above, as well as the need to create an anaerobic environment for the colonic microbiota adjacent to a near-normoxic epithelium. In this proposal, microengineering advances will be used to create a co-culture system with a steep O_2 gradient so that the luminal environment of the simulacra is anaerobic enabling a physiologic bacterial community to thrive and participate in metabolic activity within the simulacrum. These technical advances will permit novel studies of colon physiology in health and disease that cannot currently be performed.

1. Approach:

Project Overview- We propose to develop a novel microfabricated platform that will enable *ex vivo* culture of colonic epithelium. The envisioned platform will provide the means to recapitulate the 3D structure of the colonic mucosa and its microbiota in a setting that lies between an unpolarized cell culture system and a complex intact animal. Recent technical advances from our labs in sustained monolayer culture of colonic epithelial stem cells (Preliminary Data) will be integrated with microfabricated scaffoldings and devices to create the colonic simulacrum. A number of innovations will be incorporated into the microengineered system with the goal of recapitulating the colonic stem-cell niche, the differentiated intestinal mucosa, the microbiota, and the dynamic information flow between these compartments. The platform will permit tight control of the luminal and basal crypt environments by providing independent fluidic and gaseous access to these compartments. The platform will support formation of mitogen, morphogen, differentiation-factor, dietary-compound and gaseous gradients enabling unprecedented investigations into colonic physiology. After validation, the microfluidic device will be parallelized as an array amenable to high-throughput screening of different combinations of chemical conditions, microbiota and microbial metabolites. A series of hypotheses with butyrate-producing bacteria will be tested to demonstrate the device's potential in transforming our understanding of dietary metabolites on colonic epithelium.

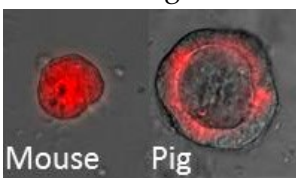


Fig. 2 *Primary organoids from mouse or pig intestinal epithelium constitutively expressing a DsRed reporter gene that was stably introduced using established methods. The same strategy can be used to generate transgenic human organoids with lineage-specific reporter genes (Table 1).*[29] *The Magness Lab has extensive experience in the culture and handling of colon organoids.*

Objective 1- Design and build a colon simulacrum for investigation of basic colon crypt physiology.

In this objective, *in vitro*-crypt construction and simulacrum analysis will be optimized followed by fundamental biological experimentation. Initial studies will use transgenic mice that provide powerful tools to monitor cell behavior in constructing the colonic simulacra.[14, 30-32] An example is the CAG-DsRed/Lgr5-EGFP mouse which displays constitutive DsRed expression in all cells, but EGFP only in stem cells.[13, 33, 34] Information gleaned from the mouse studies will be utilized to develop the human epithelium, a strategy that has proven key in developing useful methods to identify and culture human stem cells and differentiated epithelium from other organ systems. There are currently no transgenic reporter cell lines for human colonic epithelium, so we will develop new transgenic human-colon-derived lines (Table 1[32, 35]) by stably integrating stem and lineage restricted reporter genes in human colonic stem cells as previously reported.[29] We have already done so with other mammalian species (Fig. 2). These lines will be propagated as organoids in a Matrigel patty.[12, 13, 36] These organoids can be interconverted to the planar culture system used in this project (Fig. 3). Fluorescent reporter transgenes will provide robust read-outs for stem-cell activity and lineage specification in simulacra.

Table 1- Transgenic Human Colonoid Lines

Cell Lineage	Biomarker	Transgenic Reporter
Stem Cell	Lgr5	Lgr5nucEGFP-BAC
Transit Amplifying Cell	Sox9sublow	Sox9nucCherry-BAC
Enterocytes	Carbonic Anhydrase II	CAIIECFP-BAC
Goblet Cell	Mucin 2	MUC2EYFP-BAC
Enteroendocrine Cells	Chromogranin A	CHGAmemCherry-BAC
cKIT+niche cell	cKit	cKITmemEGFP-BAC
Proliferative/Nuclear	Histone H2B	H2B-Tg
Stem Cell Lineage Tracer	Lgr5	Lgr5nucEGFP-CreERT2:Confetti

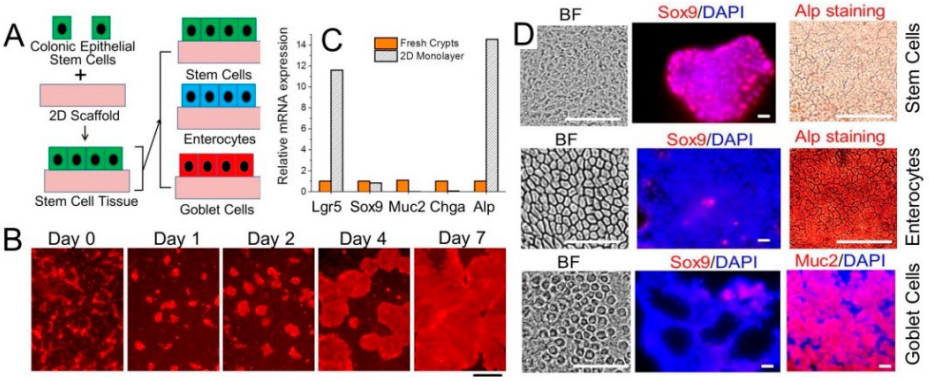


Fig. 3. *Monolayer culture of colonic epithelial cells on a planar biomimetic scaffold. A) Schematic of cell growth. Epithelial stem cells are plated on a scaffold to form a continuous monolayer consisting predominantly of stem/transit-amplifying cells. Cells can be either maintained as stem cells or differentiated to specialized cell types such as goblet cells or enterocytes. B) Culture of colonic epithelial cells expressing DsRed on a flat collagen hydrogel scaffold. The cells proliferated on the surface forming a contiguous layer by day 7. Scale bar = 500 μm. C) Relative mRNA expression of the stem-cell marker (Lgr5), a stem/transit amplifying cell marker (Sox9), and differentiated cell markers (Alp [alkaline phosphatase], an enterocyte marker; Chga [chromogranin A], an enteroendocrine cell marker; muc2 [mucin 2], a goblet cell marker) for a monolayer cultured in the presence of Wnt-3a, Noggin, R spondin-1 and EGF for 7 days. Freshly isolated colon crypts were used as a control. D) Differentiation of the monolayer on-demand. In all rows the left image is brightfield (BF), the middle image is immunostaining for Sox9 (pink) and nuclear staining with DAPI (blue) to show all cells. The presence of enterocytes was confirmed by Alp staining (upper and middle rows). The presence of goblet cells was confirmed by Muc2 staining (lower row). <u>Upper row:</u> images of undifferentiated tissue; <u>Middle row:</u> images of tissue after withdrawal of Wnt-3A and addition of butyrate; <u>Lower row:</u> images of tissue after withdrawal of Wnt-3A and addition of a Notch inhibitor. Scale bars = 50 μm.*

<u>Preliminary data: Colonic epithelium can be generated on a 3D biomimetic scaffold.</u>

A monolayer culture system was developed to support colonic stem cells by presentation of soluble growth factors and provision of a hydrogel scaffold mimicking the *lamina propria* in terms of permeability, stiffness,

and ECM components (Fig. 3). Isolated crypts were loaded onto the scaffold to which the cells attached and formed densely packed monolayers (Fig. 3B). The cell islands merged to form a contiguous monolayer by day 7, which persisted up to 3 months in culture, the longest time tested. Expression analysis of mRNA (Fig. 3C) revealed high levels of the stem cell marker Lgr5 compared to that of freshly isolated crypts. The stem/transit amplifying-cell marker gene Sox9 was expressed at levels similar to that found in fresh crypts. The sustained tissue growth, high levels of Lgr5 mRNA, and the presence of Sox9 confirmed the presence of stem cells. Markers of differentiated cells such as Muc2 and Chga were down regulated relative to that found in fresh crypts. Cells in the monolayer were readily induced to differentiate to enterocytes simply by withdrawing Wnt-3A and adding butyrate to the culture medium (Fig. 3D). During differentiation, the cells became columnar in shape, lost Sox9 expression, and expressed large amounts of alkaline phosphatase (Alp), an enterocyte marker.[37] Cells in the monolayer were also readily differentiated to goblet cells by withdrawal of Wnt-3A and addition of a notch inhibitor (Fig. 3D). Secretory granules characteristic of goblet cells appeared within the cell cytoplasm. Sox9 expression was lost, but Muc2 (a goblet cell marker) was increased substantially. Thus, differentiated cells were readily formed from the monolayer under appropriate conditions.

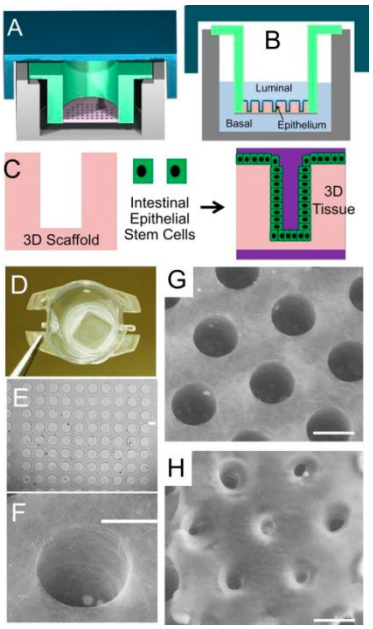


Fig. 4. *Fabrication of a 3D tissue construct. A) Cut away of the transwell device. Green = transwell insert forming the luminal reservoir. Violet/Pink = transwell filter with biomimetic/collagen scaffold. The transwell insert sits in a microwell that acts as the basal reservoir during cell culture. B) Schematic of the proposed device shown in (A) with scaffolding supporting an epithelial layer. C) Addition of epithelial cells to the 3D scaffold on the transwell filter produces a monolayer covering the scaffold surface. D) Photo of the prototyped transwell device with collagen scaffold. The collagen scaffold was molded onto a clear, porous PTFE membrane inside the insert. The central square portion (6×6 mm) contained 3,600 microwells (well diameter=60 μm, height=240 μm, center-to-center gap=100 μm). E) Brightfield image of the collagen scaffold showing a region of the microwell array. F) SEM image of a well in the scaffold on the transwell. G) SEM image of the scaffold with cells at day 0 of culture. H) SEM image of epithelial cells cultured on the 3D scaffold for 5 days. The diameter of the microwells is visibly narrowed relative to that in (G) and cells can be seen to extend down into the well lumen. All scale bars are 50 μm.*

In this proposal, we will fold the cell monolayer into the shape of a crypt by growing an epithelial monolayer across a 3D scaffold. A stem-cell niche will be created through localized application of biochemical stimuli applied to the crypt base. The differentiated compartment of cells will be maintained *via* appropriate stimuli to the luminal epithelium. To test the feasibility of this concept, 3D scaffolds possessing arrays of deep microwell structures were molded into collagen placed on a permeable transwell surface (Fig. 4A-H). The optical transparency of the porous membrane and collagen permitted imaging of the scaffold by brightfield and fluorescence microscopy. A monolayer possessing stem cells was formed over the surface of the 3D scaffold after 5 days (Fig. 4H). *In vitro* crypt-shaped structures with open lumens were readily identified on the tissue covering the surface of the scaffold. After the tissue grew to cover the surface including that deep within the wells, a gradient of Wnt-3A was applied across the epithelial cells such that Wnt-3A was high on the basal surface, but low on the luminal surface (Fig. 5A). EGFP expression (driven by the Sox-9 promoter) was localized to the basal aspect of the tissue, *i.e.* present only deep within the microwells, demonstrating formation of a stem-cell compartment at the base of the *in vitro* crypt similar to the expression pattern found in freshly isolated crypts (Fig. 5B-E). EGFP was not expressed in the cells at the luminal surface suggesting formation of a differentiated luminal epithelial monolayer. These data are consistent with the known role of Wnt-3A in the maintenance of stem cells.

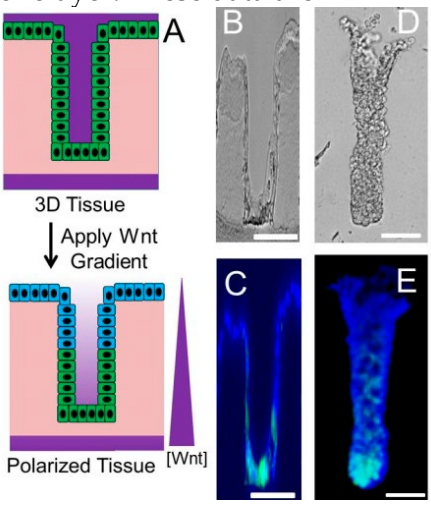


Fig. 5. *Polarization of the 3D tissue on the transwell surface. A) Removal of Wnt-3A from the luminal compartment leaving Wnt-3A only in the basal compartment creates a Wnt-3A gradient across the tissue construct. The Wnt-3A gradient is depicted by the triangle to the right of the lower device schematic. Green and blue squares are stem and differentiated cells, respectively. B,C) Colonic stem cells were cultured on the scaffold for 5 days to form a 3D tissue. Wnt-3A was then removed from the luminal compartment and cells cultured for a further 3 days. Shown is an image of a single well from a cross-sectioned scaffold. B) Brightfield image showing cells lining the artificial crypt. C) Fluorescence image of the crypt in (B) Blue = Hoechst 33342 nuclear stain. Green = EGFP expressed under the Sox9 promoter. The green-fluorescent stem/transit amplifying cells clearly occupy the base of the in vitro crypt. D,E) Images of a native, freshly isolated crypt from a mouse. D) Brightfield image of the entire crypt, i.e. not a cross-section. E) Fluorescence image of crypt in D. All scale bars are 50 μm.*

<u>*Objective 1.a. Optimize the basic simulacrum to mimic key intestinal features.*</u>

Optimize the properties of the epithelial monolayer. The goal of this objective is to generate a continuous cell monolayer across the entire scaffold including the interior surfaces of the wells while maintaining open lumens within the wells. The monolayer should be high in electrical resistance and impermeable to high molecular-

weight molecules.[16, 38, 39] The scaffold geometry and composition, and the culture conditions (cell plating density, culture time, and medium composition) will be optimized to provide a high quality, densely packed monolayer prior to application of gradients (Table 2[39-46]). Other chemical conditions will be similar to that in the Prel. Data. The monolayer will be assayed for surface coverage, integrity (transepithelial electrical resistance [TEER]), and permeability (Table 4[30, 31, 36, 47-51]). Unlike other mammals, the human colon lacks cyclic motility, thus mechanical forces (stretching, contraction) need not be applied to the simulacrum.[52, 53]

Table 2- Tailoring Scaffold Attributes

Property	Possible Modifications	Rationale	Assay
Composition	Collagen with vary amounts of laminin, heparin sulfate proteoglycan, fibronectin, entactin, gelatin, or thrombin	Optimize ECM contacts	--
Porosity	Vary collagen concentration, incorporate other methods (particle leaching, gas foaming)	Nutrient flow, gradient stability	Fluorescence dextran diffusion
Stiffness	crosslinking through ultraviolet, chemical, or transglutaminase enzymes	Impacts differentiation, controls scaffold-ing stability	Micro-indentation by AFM
Microwell geometry	crypt sized: length~250-500 μm, diameter ~50-100 μm, well spacing~100-200 μm	Impacts crypt dimensions & dynamics	SEM

Optimize chemical gradient formation across the simulacrum. Gradients of morphogens, differentiation factors and dietary metabolites participate in crypt polarity by regulating cell position and proliferation.[18, 19, 54-56] The scaffold on a transwell insert is convenient for generating sharp gradients in open-access culture. Building on our preliminary data, the *in vivo* biochemical microenvironment of crypts will be mimicked by applying chemical gradients along the basal-to-luminal axis. Conventional supplemented DMEM media will be placed in the luminal and basal device reservoirs while molecules hypothesized to exist in a gradient across crypts will be placed in either the basal or luminal reservoirs (Table 3[11-13, 17, 36, 48, 49]). COMSOL simulations solving Fick's Law with appropriate boundary conditions will supplement experiments to predict and optimize gradient profiles over time taking into account the different device layers and geometries.[57-60] The simulations will be verified by measuring the diffusion coefficient of fluorescent or colorimetric surrogate molecules across the different layers of the device. For ease of operation, the desired media-exchange frequency to maintain a stable gradient will be no more than once per 24 h. The impact of the chemical gradients on the simulacrum will be assessed by microscopy. Surveys of varying chemical concentrations and mixtures will be used to identify the basal and luminal components that yield a simulacrum most closely matching the features of native tissue, including a basal stem-cell compartment, luminal differentiated cells, and an open crypt lumen (Table 4). An important goal is formation of uniform, high-quality crypts across the scaffold to yield 3,600 crypts on this transwell device. More detailed tissue analyses will be conducted in Objective 1b.

Table 3- Physiologic Factors

Factor	[Basal]	[Lumenal]
Rspondin-1	50-200 ng/mL	0
Wnt3a	1-5 μg/mL	0
Noggin	60-300 ng/mL	0
Jagged	0.3-1.2 μg/mL	0
Gremlin	2-6 μg/mL	0
EGF	20-100 pg/mL	0
BMP	0	10-40 ng/mL
Butyrate	0-1 mM	1-10 mM
Propionate	0-1 mM	1-10 mM
Acetate	0-3 mM	3-30 mM

Infrastructure for crypt and simulacrum analyses. A robust, integrated hardware platform enabling continuous live tissue monitoring will be utilized for the proposed work. Screening will be implemented on an inverted confocal microscope (Andor Technology Revolution) with dual spinning discs housed in a CO_2 and temperature-controlled incubator. A highly sensitive camera such as a back-illuminated electron multiplying CCD will enable adequate light collection with minimal light toxicity. Up to 6 diode lasers will enable highly multiplexed measurements. The new generation of long-working-distance objectives (20×, Olympus) is expected to offer the optimal compromise between resolution (best theoretical ~0.32 μm in X-Y; ~3.5 μm in Z), field-of-view, light collection efficiency (0.45 numerical aperture) and working distance (6-8 mm). Expected imaging time for a single image plane is ~1 s for the entire simulacra. Images will be exported to a disk drive so that analyses can be performed in real time or off-line as required for the assay. This platform will also provide the needed throughput for the 96-well array to be developed later in Objective 3.

Assays and fluorescence image quantification. Standard assays in common use in the Magness and Bultman labs will be implemented to characterize global attributes of the simulacra and individual crypt properties (Table 4).[30, 31, 36, 47-51] The majority of these assays will use fluorescence-based readouts ensuring compatibility with the optically clear tissue construct. High-throughput, low-resolution image acquisition will be adequate for most assays in which regional fluorescence intensity and area are quantified. Low-throughput, higher-resolution imaging will be reserved for conditions of particular interest in which only a simulacrum sub-region is characterized. An automated pipeline for image analysis will be constructed to provide a procedural flow producing the most accurate tissue metrics with the highest throughput. During software development, success for each of the proposed steps will be assessed by manual re-analysis of the scanned simulacra.

Table 4- Crypt/Simulacrum Assays

Attribute	Reagent/Assay	Highlighted Features
Stem Cells	EGFR expression under Lgr5 promoter	Basal Stem niche
Cell Cycle	EdU pulse, phospho-H3 IHC*	Basal- proliferation, Luminal- no proliferation
Apoptosis	Assay for cleaved caspase-3	Rare luminal cell
Dead Cells	Propidium Iodide	Dead cells in luminal reservoir
Histone Acetylation (Transcriptional Activity)	pan-acetyl H3 and total H3 IHC*	Luminal HDAC inhibition by high butyrate
Oxidative Metabolism	MitoTracker Red CM-H2XRos + (Total mitochondria- MitoTracker Green FM)	Oxidative metabolism of butyrate by epithelium
Goblet Cells	Muc 2 IHC*	Located luminally
Enteroendocrine Cells	ChgA IHC*	Located luminally
Enterocytes	Alkaline Phosphatase IHC*	Located luminally
Monolayer Integrity	Transepithelial Resistance (TEER)	High resistance
Monolayer Permeability	Permeability Coefficient (Dextrans)	Low permeability to hi M.W.
Mucous Layer	Immunofluorescence of Mucins	Complete mucous coverage
Transport	^{22}Na, Rhodamine 123	Transport across monolayer
Drug Metabolism	Cyp3A4 (P450-Glo™) or Cyp1A1 (Ethoxyresorufin)	Inducible drug metabolism

*IHC- Immunohistochemistry

Most assays will require the definition and characterization of fluorescence within 6 distinct zones spanning the entire crypt structure. These zones are 1) the stem cell niche (crypt base), 2) the proliferation region (crypt central region), 3) the luminal crypt region, 4) upper differentiated epithelium, 5) luminal mucous layer, and 6) the luminal contents (bacteria in later objectives). Zones will be demarcated through a combination of both positional measures (*e.g.*, the crypt base is initially defined as the basal 70 μm of the crypt) that can be experimentally programmed, as well as marker-based approaches. *A priori* estimates of the

demarcations of these zones will be refined through either rapid course z-stack imaging of multiple samples and/or complete z-stack imaging of a small representative sample. For these assays (Table 4), every Z plane in the crypt need not be imaged, enhancing throughput. A judiciously selected series of Z-planes appropriate to the assay will be selected. During low-resolution imaging, crypts are expected to be readily identified since they are surrounded by an acellular scaffold and bounded by basal and luminal fluid reservoirs. When imaging crypts at high-resolution, *e.g.* for lineage tracing or for follow up investigations, a denser series of Z stacks can be acquired although at reduced throughput. Standard image analysis protocols already developed by the assembled investigators (*e.g.*, background correction, volume rendering, registration, segmentation, *etc.*) will be applied to the quantitative imaging required for this application and built on a combination of existing software platforms (*e.g.*, Fiji, CellProfiler, Icy, Vaa3D, BioimageXD, Matlab) along with custom code.[61-67]

Data Analysis. Each crypt can be thought of as a unique sample with a known physical array location and an extensive multiparametric data set collected over time (see Table 5 for examples). Each simulacrum can similarly be treated as the unit of measurement depending on the experimental question. Considering that the single simulacrum possesses 3600 crypts each assayed repeatedly over many days, a truly comprehensive characterization of our simulacrum will be achieved. Quantified parameters derived from all sources, imaging and otherwise, will be consolidated, normalized or otherwise preprocessed as appropriate. A range of statistical, machine learning and pattern recognition approaches will be used to assess variation in measured parameters and their association with functional readouts.[68-72] Initially, such analyses will focus on optimizing conditions for simulacrum establishment. Subsequently, downstream exploration of microbiota composition (Objective 2), butyrate, and similar studies will also utilize the developed analytical tools. Assay values are expected to vary both between individual crypts and simulacra under the same environmental conditions (*e.g.*, a single transwell) as well as under different environments. Depending on the experimental question, techniques such as dimension reduction, clustering, and feature selection, along with standard hypothesis testing will be used to define the parameter space that leads to desired simulacra properties. In many instances, we will have measurements on a range of variables along with an associated outcome measure(s) such as proliferation rates or cellular life-span. In such instances, multivariate regression approaches (*e.g.*, partial least squares regression (PLS-R), ridge regression, LASSO or similar techniques) will be used to identify "driver" parameters that seem to dictate outcome measures.[68, 73-75] Cross-validation, multiple test correction, FDR and related techniques will be utilized throughout the validation process. The Gomez lab has extensive experience with the development and application of statistical, pattern recognition and machine learning approaches to the analysis of high-throughput, multi-dimensional data sets derived from images, single-cell analyses and genomic data and will adapt appropriate methods as needed.[68, 73-76]

Table 5 – Image-Derived Crypt Parameters

Crypt Property	Example Metrics
Crypt Dimensions	Height, diameter
Crypt Polarization	Fluorescence intensity, presence/absence of markers
Zone Dimensions	Length, fraction of crypt length
Cell Lineage	Immunofluorescence intensity and area, cell segmentation
Cell Lineage Spatial Distribution	Immunofluorescence intensity and area per unit length; per zone
Cell Proliferation	Rate of cell division/turnover via fluorescence and/or segmentation
Bacterial Species Distribution	Average fluorescence intensity per species
Bacterial Spatial Distribution	Fluorescence intensity per unit length; per zone

Objective 1.b. Comparison of the simulacrum to in vivo colonic epithelium.

Maintenance of colonic epithelium *in vivo* is controlled by the coordinated regulation of the stem and differentiated cell compartments to balance proliferation, differentiation, lineage ratios and programmed cell death. Success results in a contiguous monolayer of cells that carry out absorption of nutrients, translocation of ions and water, and maintenance of a physical barrier against luminal contents and microbes. Our goal will be to achieve many of the temporal features of cell proliferation rates, migration times and life-span within the simulacrum. The lack of a model that replicates any of these basic functions has up to now precluded meaningful *in vitro* studies of epithelial self-renewal.

Proliferative and differentiated compartments. The differentiated colonic epithelium at the luminal surface is maintained by stem cells located at the base of the crypts. We hypothesize that directed mitogen and morphogen gradients can establish these compartments in the simulacrum. Long-term, stable establishment of proliferative and differentiated cell compartments in the simulacrum will be assessed by 3D confocal microscopy. Proliferation and differentiation will be monitored by EdU-assays and immunostaining for lineage-specific biomarkers, and live-cell imaging of stem/transit amplifying cells and differentiated compartments.[31, 36] We predict stable maintenance of a stem-cell compartment in the crypt base and a luminal differentiated cell compartment. We also predict regional boundaries between the two compartments can be controlled by modulating the mitogen and morphogen gradients across the simulacrum.[37]

Differentiation. The colonic epithelium is composed of absorptive and secretory colonocytes maintained in known ratios. We hypothesize that modulation of Wnt and Notch gradients will control their lineage ratios in the simulacrum.[37, 54-56, 77] The presence of these differentiated cells will be monitored in both mouse and human simulacra by transgenic reporters and by whole mount immunostaining for lineage restricted markers (Table 4). The benchmark will be the same ratios of differentiated lineages in the simulacra compared to those *in vivo*. If differences are observed, Wnt and Notch pathway signaling will be modified to bias lineage allocation.[37]

Migration. Colonic epithelium is renewed *in vivo* approximately every 5-7 days by cells migrating from the crypts to the luminal surface.[11, 18, 19] Organoids cultured within a Matrigel patty demonstrate intrinsic

properties of controlled migration toward the surface of the 'pseudolumen'. We hypothesize that modulation of Wnt and EGF gradients will allow control of migration rates in the simulacrum. Migration rates will be quantified by EdU pulse-chase experiments and lineage tracing using Lgr5-CreERT2:confetti reporter mouse. Live imaging will be used to demonstrate migration of marked cells out of crypts.[35] Transit times will be quantified. We predict that optimization of stem-cell numbers and proliferation rates will produce sufficient cell numbers to replace all differentiated lineages every 5-7 days. Migration rates can be fine-tuned by changing growth factor concentration gradients.

Apoptosis. Epithelial replacement is controlled in part by sloughing of cells at the luminal surface by apoptosis.[11, 18, 19] Colon-derived organoids grown within Matrigel demonstrate controlled apoptosis at the surface of the pseudolumen, thus we hypothesize that these intrinsic properties can be established in the simulacrum. Cleaved-caspase-3 assay will be used to quantify apoptotic cells in the crypt base and luminal surface. We predict low apoptotic events in the base of the crypt with a larger number of events at the luminal surface. The incidence of apoptosis can be fine-tuned by changing luminal properties such as [butyrate].

Long-term stable culture. Colon-derived organoids grow indefinitely within a Matrigel patty when conditions are optimized.[11-13] We hypothesize that a simulacrum that exhibits the steady state attributes described above can be cultured long-term. Assays described above will be used to monitor the proliferation, differentiation, and migration over weeks to months. Like colon-derived organoids in Matrigel, we predict stable maintenance of the epithelium and gene expression signatures in the simulacrum. If bias in any parameter is observed, factor gradients will be changed to modulate the bias.

Clonal crypt units. In vivo crypts are thought to be clonal units derived from a single stem cell that possesses a competitive advantage over other stem cells.[78, 79] We hypothesize that a similar clonal drift will occur in the simulacrum. This property will be tested using the Lgr5-CreERT2:confetti reporter mouse which allows random and permanent temporal marking of stem cells with up to 4 different fluorescent signatures.[78] We predict that after lineage marking of stem cells, each crypt will originally have heterogeneous colors of stem cells, but over time each crypt will undergo neutral drift to single-color descendants. If the alternative occurs, gradients can be modified to evaluate the impact of growth and morphogen factors on this property.

Mucous layer: A thin mucous layer (~50 μm) produced by goblet cells covers the epithelium, functions as a protective barrier, and has physiologic consequences related to nutrient absorption.[80] We will establish whether the simulacrum possesses a mucous layer and quantify the thickness of the layer using immunostaining for mucin (Muc2, Muc5). Because goblet cells have been observed to exist in colon-derived organoids cultured within Matrigel and to produce a layer of mucous in the organoid pseudolumen,[11-13] we predict that a mucous layer will form on the simulacrum. Unlike the spherical organoids that have an enclosed pseudolumen, which causes a mucous build-up, we predict the open luminal surface in the simulacrum combined with robust proliferation, migration and sloughing of epithelium will produce a physiologically relevant mucous layer. The thickness of the mucous layer will be modulated by perturbing Notch signaling to modulate goblet cell number.[77]

Objective 2- Optimize the colon simulacrum for investigation of the colonic microbiome.
The platform will be adapted to enable gaseous gradients across the simulacra in order to create the necessary anaerobic luminal compartment required for colonic bacteria. This co-culture system will revolutionize microbiome research by enabling the function of host-microbe interactions to be interrogated.

Objective 2.a. Construct a basic simulacrum co-cultured with microbiota.

Adapt the microdevice housing to implement gaseous gradients. As *in vivo* crypts are positioned between an anaerobic lumen and a highly vascularized *lamina propria*, a steep O_2 gradient (-0.065 Torr/μm) exists along the basal-to-luminal axis.[84-86]

Each component can be readily fabricated in multiple layers using methods commonly in use in the Allbritton Lab, such as hot embossing, precision CNC milling, molding and lithography. In order to mitigate risk, designs will be carefully simulated in COMSOL by solving the governing equations for both steady and transient oxygen tensions to optimize the O_2 gradient profile upon changing various parameters, such as gas flow rates, PDMS-membrane thickness, and tissue-PDMS membrane gap.[88-90] Under optimized parameters, we expect a steep gradient of O_2 will be generated across the tissue and *in vitro* crypts. To confirm the O_2 gradient, fluorescent O_2-sensitive microbeads will be embedded in the scaffold to allow *in situ*, 3D measurement of [O_2] by confocal microscopy.[91] A MitoXpress O_2-sensitive probe will be used to confirm that the luminal medium is anaerobic.[92] Fluorescence-based assays will be used to assess the impact of O_2 gradient on cell wellbeing (Table 4).

Bacterial co-culture. The microbiota that reside in the colonic lumen are highly diverse and abundant, with hundreds of bacterial species and ~10^{12} viable bacteria/gm of luminal contents.[93-95] A mixed population model of intestinal flora, the altered Schaedler flora (ASF) which consists of 8 bacterial species representative of diverse taxa, were developed for colonizing germfree rodents with a standardized microbiota (Table 6).[96-98] To mimic host-microbe interactions, we will co-culture the 8 ASF bacteria with the simulacra. After establishing the chemical and O_2 gradients, the ASF bacteria will be added to the luminal compartment. It is anticipated that the growth of facultative anaerobes in the lumen will consume residual O_2 to diminish the O_2 tension to near zero just as these bacteria do *in vivo*.[99, 100]

Table 6- ASF Bacteria

Name	Description	Anaerobe Type*	Enriched in Colon
ASF360	Lactobacillus	FA	No
ASF361	Lactobacillus	FA	No
ASF519	Bacteroides	A	No
ASF457	Flexistipes	A	No
ASF356	Clostridium	EOS	Yes
ASF492	Eubacterium	EOS	Yes
ASF500	Clostridium	EOS	Yes
ASF502	Firmicutes	EOS	Yes

*FA- Facultative Anaerobe; A- Anaerobe; EOS- Extremely O_2 Sensitive

Colonic luminal contents are relatively energy deprived because most nutrients are absorbed in the small intestine.[49, 99-101] The contents consist primarily of salt water, which is compatible with the function of the colon in water/electrolyte absorption. The other major luminal constituents are microbiota and dietary fiber (undigested complex carbohydrates derived from plants), which serves as a fermentation substrate and an energy/carbon source for the microbiota. O_2-depleted media of 2 types will be tested in the luminal compartment. Initially, we will use ASF broth supplemented with 5% FBS because it is the preferred media for cultivating ASF bacteria. We will also test phosphate-buffered saline (PBS) supplemented with 5% FBS because it more closely resembles the bland, salt water mixture in the lumen of the colon. Each medium will possess 6% fructo-oligosaccharides (FOS)/inulin as a highly fermentable fiber source. The osmolarity of these media will be adjusted to be equivalent to the DMEM basal media. We will determine the longevity of the co-culture system and monitor the growth of bacteria for potential microbial overgrowth, which could lead to loss of epithelial cell viability. To prevent overgrowth, bacterial density can be reduced by replacing the culture fluid in the luminal compartment as needed.

Table 7- Crypt/Simulacrum Assays for Microbes

Procedure/Assay	Measurement/Objective
SEM	Survival and distribution of bacteria
Spectrophotmetry of luminal media	Turbidity as measure of bacterial growth
qPCR to quantify bacteria in media	Abundance of each ASF bacterium using strain-specific primers
Gram stain of simaculra cyrosections	Bacterial mucosal adherence, invasion, translocation, and biofilm formation
Immunofluorescence of ASF bacteria	Live imaging of each ASF bacterium to evaluate mucosal adherence, position along crypt base-lumen axis, and invasion
Fluorescence of genetically engineered bacteria	Identification of ASF bacteria expressing different fluorescent proteins
LC-MS of luminal media to detect bacterial metabolites	Quantification of bacterial fermentation products- acetate, propionate, and butyrate SCFAs

<u>*Objective 2b. Characterization of the simulacrum microbiota.*</u>

Imaging the simulacrum in the gas-gradient device. The device height and component interfaces below the tissue must not significantly impair tissue imaging. The new generation of long-working distance objectives (6-8 mm working distance for a 20× objective) compatible with confocal microscopy should readily meet this goal. The simulacrum will be ≤4 mm from the lower surface of the gas gradient device. While the best theoretical image quality will likely not be attainable due to the multiple refractive-index-mismatches at interfaces, we anticipate that image quality will be more than sufficient to achieve our required resolution of less than one cell diameter. Reflections and distortions will be minimized to the degree possible by implementing nonreflective surface coatings and incorporating materials with high optical quality and flatness into the device. We will also consider using liquids with high gas solubility such as perfluorocarbons to create the anaerobic and aerobic conditions in the channels of the top and bottom compartments to decrease the largest air-liquid refractive-index-mismatches.[102, 103]

Assays of the bacterial community. SEM imaging will document the survival and distribution of bacteria in simulacrum co-cultures (Table 7). The abundance of all 8 ASF bacteria will be quantified by qPCR using strain-specific primers.[104] Cryosection and Gram staining will be used to assay microbial adherence, invasion,

translocation, and biofilm formation. A major function of colonic bacteria is to ferment dietary fiber into the short-chain fatty acids (SCFAs) acetate, propionate, and butyrate.[99, 105-108] LC-MS will be performed to quantify each of these SCFAs in media from the luminal compartment. The Bultman lab has extensive experience in quantifying the SCFAs from small-scale samples.[109]

Hypotheses. Four **hypotheses** will be tested using data from the assays proposed above. **1)** There will be significant differences in the abundance of individual ASF bacteria in the simulacrum co-cultures based on qPCR. When all 8 ASF are introduced into germ-free mice, 4 of the strains (ASF356, ASF492, ASF500, ASF502) are highly abundant in the lumen of the colon; therefore, we expect these strains to be the most abundant ASF in colonic simulacra.[98] However, these colonic ASF are extremely O_2 sensitive (EOS, Table 6) so they will be underrepresented if O_2 levels are too high in the luminal media. We will use the relative abundance of these ASF as one read out to evaluate the O_2 gradient in our co-cultures. We anticipate that this will complement O_2 sensor measurements and can be used to fine-tune the O_2 gradient if necessary. **2)** Because facultative anaerobes consume residual O_2, the 4 ASF EOS strains will not grow as well in the absence of the other 4 ASF strains. **3**) Gram staining of simulacrum cryosections will show mucosal adherence, but the ASF bacteria will not translocate through the crypt wall if the epithelial cells are continuous and form tight junctions as expected. **4)** Acetate, propionate, and butyrate will be present in the luminal media as assessed by LC-MS, and this will be dependent on the FOS/inulin fiber source being added to the luminal media. Because SCFAs are responsible for the mildly acidic conditions of the colon, the pH of the luminal media will be < 7 (pH ~6) in the presence of FOS/inulin, but be pH 7-7.5 in the absence of FOS/inulin.[99]

Objective 2c. Characterization of the effect of microbiota on the simulacrum.

All animals co-evolved with microbiota so it is not surprising that gut bacteria have positive effects on crypt homeostasis and function. This has been documented in studies showing germ-free mice have significantly decreased cellular turnover within the crypt and impaired absorption of nutrients and water/electrolytes compared to conventional mice possessing microbiota.[110, 111] Based on these observations, we **hypothesize** that the ASF bacteria will increase cellular turnover and improve crypt function of human simulacra based on the bioassays listed in Table 4. **First**, the ASF will increase the proliferation of stem/transit amplifying cells near the crypt base as measured by EdU incorporation. The ASF will concomitantly increase apoptosis of differentiated cells near the lumen based on cleaved-caspase-3 staining. It is expected that increased cell proliferation near the crypt base will be counterbalanced with increased apoptosis near the lumen. As a result, cellular turnover should be increased, but the total number of cells per crypt should not change. **Second**, ASF will increase electrolyte transport as measured by ^{22}Na assays. The experimental design will compare colonic simulacra cultured without any bacteria to co-cultures with the 8 ASF bacteria.

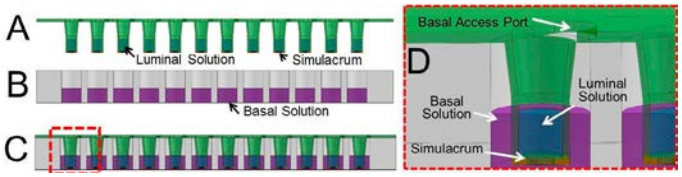
Objective 3- Array the colonic simulacra for high-throughput investigation.

Development of the simulacrum in a single transwell device as described above will enable rapid iteration through a multiplicity of designs to achieve the most robust platform and to accommodate initial biological experiments. However, the numbers of simulacra needed for thorough hypothesis testing in Objectives 1b and 2c are expected to exceed the capabilities of the single-simulacrum platform, particularly when replicate experiments are performed with the range of conditions and readouts described above as well as tissue samples from many humans (n=50). The optimal design established for the single transwell in Objectives 1 and 2 will be transitioned to a 96-well array format to increase assay throughput and greatly expand the range of experiments and testable hypotheses. The standardized spacing and positioning of a 96-well plate will ensure compatibility with robotic pipettors and automated imaging platforms. Based on manufacturer-supplied metrics, expected imaging times for 96 transwells with living cells expressing a fluorescent protein is ~2 min (single Z-plane). Each well of the 96-well plate will contain 2,500 *in-vitro*-crypts (100 crypts/mm^2) ensuring adequate crypt numbers to create meaningful data for each experimental condition. Image acquisition and processing will build on that of the prior objectives, and is expected to be readily adapted to the larger numbers of crypts and simulacra. System scaling will be implemented in a step-wise fashion with successive addition of device functionalities to enable careful optimization and ease of use by the biological community.

Objective 3a. Scale the simulacra with chemical gradients from 1 to 96 wells.

Design and fabricate a 96-well cassette to hold simulacra. A 3-component cassette with a lid, a transwell array, and basal microwell array in the geometry of a 96-well plate will be developed to support an array of 96 intestinal simulacra (Fig. 7). The lid is described in Objective 3b. The luminal compartment above the simulacra will be formed from a commercially available 96-transwell array. As in Objective 1, the transwell upper surfaces will support the simulacra and present luminal solutions to the colonic tissue. The bottom component supports the basal-fluid reservoirs. The simulacra will not share basal or luminal solutions so that a unique gradient may be applied across each epithelial layer on the array. Small ports or vias incorporated into the upper surface of the bottom component will allow ready access to the basal compartment when the

transwell top is in place facilitating basal media replenishment. The base of the bottom piece will be permanently bonded to a thin glass plate to facilitate confocal microscopy. The bottom piece will be fabricated from PDMS or polystyrene by soft lithography to allow rapid re-design and optimization. The Allbritton Lab has extensive experience fabricating microdevices from PDMS and pioneered polystyrene soft-lithography-based fabrication.[20, 112-117] Surface adsorption of biological molecules by PDMS or polystyrene has not affected epithelial-cell culture to date; however if needed, the interactions of the polymer with biological molecules can be greatly reduced by plasma or UV/ozone treatment or previously developed surface modifications, such as polymer grafts or sol gels.[114, 118-124]

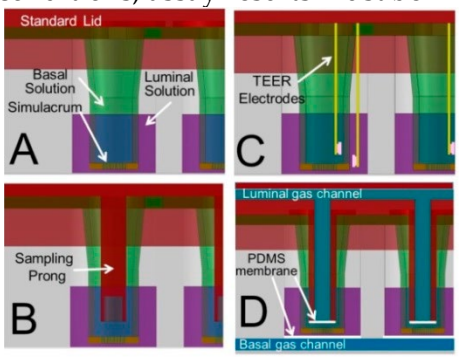


The size, volume, and shape of the luminal and basal compartments will be adjusted so that media need not be replaced more than once per day to maintain both the chemical gradients and support overall tissue health. A robotic dispensing system will be used for all sterile media exchanges and washes for the arrays. All washing and filling steps on the microdevice will be optimized to minimize bubble entrapment and cross-contamination between wells.

Placement of hydrogel scaffolding. To form the hydrogel scaffolding for the simulacra, scaffolding material will be dispensed into the individual transwells of the 96-well cassette and then molded into the appropriate shape using a PDMS stamp fabricated by multilayer soft lithography.[125-127] The stamp will possess an array of 96 posts with the top surface of each post itself covered by a 2,500-element micropost array. Proper weighting across the stamp as it is pressed onto the collagen will imprint uniform microwells across the collagen contained within each well. The hydrogel scaffolding and imprinted microwells will be examined for uniformity by light and electron microscopy. Hydrogel porosity and stiffness will be evaluated at the different sites of the 96-well plate to insure uniform scaffold production (Table 2).

Formation of simulacra in the 96-well cassette. Colonic epithelial cells will be seeded onto the scaffolding and cultured as in Objective 1. These initial efforts will focus on attaining uniform, high-quality epithelial growth in all regions of the device rather than applying gaseous and chemical gradients. Epithelial monolayer growth in each transwell will be examined by microscopy to optimize uniformity between wells and within a well. Standard control assays will be performed to assess growth rate and health (Table 4). Non-uniform cell growth within a well or between wells will initiate reexamination of the scaffolding quality, cell-dispensing reproducibility, environmental controls, and media exchange protocols. All results will be compared to that of a simulacrum in a single transwell described in Objective 1 under identical conditions.

Implementation of chemical gradients across simulacra in the 96-well cassette. Chemical gradients across the tissue will be initiated by placing the optimal reagents identified in Objective 1 into the luminal and basal chambers. Gradient-formation time, shape and stability will be measured across the wells in the array using the methods of Objective 1. When cells are grown under identical gradient conditions, assay results must be similar in all wells and recapitulate the tissue phenotype displayed on the single-well simulacrum. Non-uniform gradients or cell responses within a well or between wells on the array will trigger assessment of well fabrication quality, consideration of surface tension effects, and scrutiny of environmental impacts (evaporation/humidity/temp).



<u>*Objective 3b. Develop analytical tools for the arrayed simulacra.*</u>

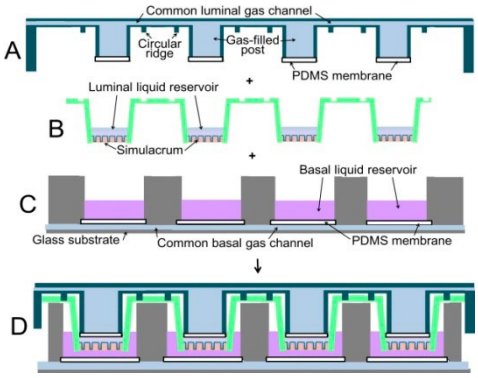
While the 96 simulacra of Objective 3a can be employed immediately for the experiments described in Objective 1b using a simple commercially available lid, we will also develop a series of assay lids (Fig. 8A-C) to facilitate quality control and additional assays.

Development of a 96-well fluid-sampling lid.

for sampling reliability, repeatability and cross-contamination.

Development of a 96-well TEER lid.

ontrol impedance spectra (acquired before cell seeding) will be subtracted from that with tissue to control for the electrolyte properties, transwell/collagen membrane resistance, and electrode-electrolyte interface effects. The data will be fit using customized software to an equivalent circuit model.[131] Controls measuring dynamic impedance changes in response to epithelial growth or death and defined-resistance solutions or membranes will be used to optimize device accuracy and reproducibility.



<u>*Objective 3c. Construct a gaseous gradient across 96 simulacra simultaneously.*</u>

The design for the single transwell/simulacrum will be recapitulated in a 96-transwell array format. Design will be guided by assay accuracy, ease of use, and manufacturability. The device will be fabricated in a manner analogous to the single-well device: a lid with a common luminal gas channel and inlet/outlet gas ports (Fig. 8D, 9A), a cassette with 96 transwells each housing a simulacrum (Fig. 9B), and a bottom piece with a basal gas channel and an inlet/outlet gas port (Fig. 9C).[83] The components will be reversibly mated to produce the final assembly (Fig. 9D). While the simulacra will not share basal or luminal solutions and may each possess a unique chemical gradient and microbiome, all simulacra will experience the same gaseous gradient. O_2 sensor wells (with beads or a chemical sensor) will be interspersed throughout the array to monitor the uniformity of gas delivery and gradient formation. The diameter and volume of the gas channels and gas flow rates will be optimized to achieve consistent delivery across the array. Bacterial cross contamination between wells must not occur, and the assembly will be designed with this in mind. The gas-filled posts on the lid can be shaped, sized, and coated to form a seal with the transwell cassette to eliminate inter-well cross contamination. Arrays with bacteria placed in every other luminal well will be assayed to determine whether these select wells remain bacteria-free. ASF bacteria will be cultured on the tissue and assayed as described in Objective 2. When bacteria are placed under identical conditions across the array, the bacterial community is expected to evolve in the same manner across all wells and similar to that on a single simulacrum.

Objective 4- Demonstrate the utility of the human colonic simulacra in microbiome research.

The 96-well array will be used to investigate the effect of butyrate-producing bacteria on crypt homeostasis. Certain clades of bacteria (*e.g., Clostridia* clusters IV and XIVa) ferment dietary fiber into acetate, propionate, and butyrate which are highly abundant (mM) in the lumen of the colon.[132] Butyrate is noteworthy because its bioavailability is primarily restricted to the colon where it serves as the primary energy source for colonocytes[101] and functions as a histone deacetylase (HDAC) inhibitor to epigenetically regulate gene expression.[133] Butyrate's protective effect with respect to colorectal cancer (CRC) is thought to be due to HDAC inhibition and identified target genes include cell-cycle regulators and pro-apoptotic genes.[48, 49, 100, 101, 134, 135] We will interrogate butyrate function in crypt homeostasis by adding butyrate-producing bacteria to human simulacra.

<u>*Objective 4.a. Co-culture of simulacra with bacteria to evaluate the effect of butyrate on crypt homeostasis.*</u>

Choice of microbiota. Human simulacra will be co-cultured with *Butyrivibrio fibrisolvens*. This particular butyrate-producing bacterium will be utilized because wild-type and mutant strains have been characterized that are commercially available and cultivatable. The naturally occurring mutant strain has a 0.8-kb deletion that ablates the crotonase gene within the butyryl-CoA synthesis operon and produces 5- to 10-fold less butyrate in culture.[136, 137] Because bacteria produce many metabolites, a comparison of the wild-type and mutant *B. fibrisolvens* strains will be crucial to demonstrate that butyrate is a causal factor for crypt homeostasis. The Bultman Lab has compared germ-free mice colonized with wild-type *B. fibrisolvens* (monoassociation) to germ-free controls. *B. fibrisolvens* stimulated oxidative metabolism in colonocytes and significantly diminished autophagy.[49] In a second series of experiments, a gnotobiotic mouse model of colorectal cancer was polyassociated with 4 ASF strains plus or minus wild-type or mutant *B. fibrisolvens* and provided high- or low-fiber diets.[109] Mice with a high-fiber diet and wild-type *B. fibrisolvens* yielded higher

levels of butyrate in their colons than the other treatment groups. More importantly, the combination of wild-type *B. fibrisolvens* and high fiber conferred a potent tumor-suppressive effect with decreased tumor number, size, and histopathologic progression (Fig. 10). These results validate functional differences between the wild-type and mutant butyrate-producing bacteria in mice and increase the likelihood of success for the proposed experiments with human tissue.

Hypotheses. Our **overarching hypothesis** is that bacterial-derived butyrate promotes colonic homeostasis in humans, which leads to the following **specific hypotheses** that will be tested on human simulacra. **1)** The combination of a wild-type butyrate-producing bacterium and fiber will yield higher butyrate levels, as quantified by LC-MS, than the control groups. **2)** Considering that butyrate is the preferred energy source of colonocytes and undergoes mitochondrial oxidation, butyrate will increase oxidative metabolism of human simulacra. **3)** Butyrate will increase the proliferation of stem/transit amplifying cells near the crypt base. **4)** Butyrate will concomitantly increase apoptosis of differentiated cells near the lumen. It is expected that increased cell proliferation near the crypt base will be counterbalanced with increased apoptosis near the lumen. As a result, cellular turnover should be increased, but the total number of cells per crypt should not change. **5)** Because butyrate is an HDAC inhibitor, it will increase global histone acetylation. **6)** Considering that H3ac is an epigenomic marker of actively transcribed genes, butyrate-induced H3ac will increase the expression of many genes. **7 & 8)** Butyrate will improve colonic function by improving barrier function and salt absorption.

Table 8- Human simulacrum groups for each bioassay

Microbiota	FOS/inulin	Group
None	+	Control
None	-	Control
Wild-type B. fibrisolvens + 4 ASF	+	Experimental
Wild-type B. fibrisolvens + 4 ASF	-	Control
Mutant B. fibrisolvens + 4 ASF	+	Control
Mutant B. fibrisolvens + 4 ASF	-	Control
4 ASF	+	Control
4 ASF	-	Control

Experimental design. To test these hypotheses, we will expose human simulacra to 8 different treatments (Table 8). *B. fibrisolvens* will be polyassociated with 4 ASF because bacteria do not function in isolation, but undergo myriad cooperative interactions. In fact, butyrate production occurs more efficiently when butyrate-producers are crossfed by certain clades of bacteria including *Bacteroidetes*.[132] ASF519 corresponds to *Bacteroides distasonis* and is therefore apropos. The 3 other non-butyrate producing ASF bacteria (ASF360, ASF361, ASF457) will also be polyassociated. ASF butyrate-producers will be excluded so that they do not mask the effect of *B. fibrisolvens.* qPCR will be performed to detect the abundance of wild-type and mutant *B. fibrisolvens* and the 4 ASF strains, which will allow us to determine the influence of FOS/inulin on bacterial growth and to control bacterial number in each experiment. Fifty independent simulacra will be analyzed to increase statistical power and evaluate human genetic background differences. Our **hypothesis** is that wild-type *B. fibrisolvens* in the presence of FOS/inulin will yield significantly higher butyrate levels than the other 5 treatment groups and will be the only treatment group to exert a significant effect in the assays. These experiments are expected to demonstrate that butyrate production and its biological effects are dependent on FOS/inulin as a fermentation substrate and the bacterial butyryl-CoA synthesis operon, respectively. In the unlikely event that the mutant *B. fibrisolvens* in the presence of FOS/inulin has a robust effect, fermentation products other than butyrate might be important with acetate and propionate being the leading candidates.[99]

Objective 4.b. Co-culture of simulacra with complex gut microbial communities from human colorectal cancer cases.

Rationale. The final step will be to co-culture human colonic simulacra with complex microbial communities obtained from human colorectal cancer (CRC) cases and controls. The objective will be to determine whether the dysbiosis that has been documented in CRC cases, which includes a significant decrease in butyrate producers, confers different effects on simulacra than the microbial communities from controls.[27] These experiments will leverage resources available to us through the UNC Gastrointestinal Cancer SPORE. First, we have access to archives of frozen fecal microbiota obtained from colorectal adenoma cases and controls.[5, 6, 138] The advantage of using benign adenoma cases instead of malignant adenocarcinoma cases is that microbiome changes at early stages of tumorigenesis are more likely to be causative than at late stages. Second, we will work with the UNC Microbiome Core Facility to perform 16S rDNA sequencing, which will allow us to assess how the composition of the microbial communities change when they are co-cultured with human colonic simulacra for different times.

Hypotheses and experimental design. Our **overarching hypothesis** is that the microbiota from adenoma cases will be compromised in their ability to promote crypt homeostasis compared to controls. The same **specific hypotheses** and assays described in Objective 4.a. and Table 4 are applicable here. To test these hypotheses, frozen fecal microbiota from 10 cases and 10 controls will be co-cultured with human simulacra in the presence or absence of FOS/inulin as a fiber source. The fecal microbiota samples in the repository have been characterized by 16S rDNA sequencing, and butyrate producers (*e.g.*, *Clostridia* clusters IV and XIVa) are depleted in the disease cases, but enriched in the controls.[139, 140] If the data support our hypothesis, then fecal microbiota from cases *vs*. controls could be transplanted into gnotobiotic mouse models to evaluate *in vivo* differences. As such, we anticipate that an experiment using simulacra will not necessarily be an end in and of itself, but will also be a useful screening tool for designing and prioritizing very-expensive and low-throughput gnotobiotic mouse experiments.

2. Appropriateness for the Transformative Research Awards Initiative:

Why is the proposed research uniquely suited to the goals of the Transformative Research Awards initiative? Perhaps the best answer to this question is provided by an NIH study section that reviewed our NIH R01 application based on this research program. The review stated: "This is a bold and far-reaching proposal … providing an extraordinarily powerful tool." "If successful, this study would demonstrate significant new bioengineering methods…while offering new insights into underlying biological processes…" "The work has the potential to exert a sustained and powerful influence in the fields of organ research and tissue engineering with subsequent implications for understanding disease processes." Despite this enthusiasm and potential for a transformative impact, the reviewers felt that the project remained too high risk: "barriers might hinder this work, including the assembly of crypts … into a functional model. So a successful outcome is not certain." The review comments reveal that while challenges remain to be overcome before success is guaranteed, the research has the potential for far-reaching and significant impact. These characteristics make this proposal an ideal example of the type of work the Transformative Research Awards initiative wishes to support.

How does the proposed research significantly differ from mainstream science? In the human body, there are 10-fold more microbial cells than all of our somatic and germ cells combined.[93-95] Furthermore, our microbiome encodes 100-fold more genes than the human genome. These microbial communities in their normal and diverse state are believed to facilitate homeostasis of the human host and decrease the risk of certain diseases including colorectal cancer.[141-143] The functional importance of our gut microbiota is supported by microbiome studies where metagenomic sequencing (*i.e.*, next-generation sequencing followed by computational analysis *via* bioinformatics) of stool samples or luminal contents obtained via endoscopy has identified altered compositions of microbial communities in individuals with various disease states.[2, 4-8, 139, 140] Although these are important association studies, they are at best correlative. Consequently, it is difficult or impossible to assess whether a particular change in the abundance of microbiota is a cause or a consequence of a disease state.

Functional studies can be performed using gnotobiotic mouse models maintained germfree or colonized with defined microbiota in specialized facilities.[27, 28] Bacteria for colonization can be derived from human disease states to interrogate the role of human microbiota in disease predisposition and etiology. Gnotobiotic mouse models have demonstrated the importance of gut microbiota in host energy homeostasis and have begun to provide insight into the role of microbiota in certain disease states. However, there are very few gnotobiotic mouse facilities, and each facility contains a small number of sterile isolators so the number of mice and microbiota conditions that can be analyzed is extremely limited. This logistical hurdle represents a major bottleneck that will impede microbiome research for the foreseeable future. To advance our knowledge of microbial function in human health and disease, the limitations of metagenomic sequencing and gnotobiotic mouse models must be overcome.

The ability to monitor and control the environment at the cellular and tissue level is one of the most promising applications for innovative microengineered systems integrated with stem-cell biology.[144-146] Recent advances have made it possible to conceive of physiologically functional devices reconstituting whole organ systems, but these "organ-on-a-chip" devices have yet to establish the tissue anatomy, structure and cellular compartments necessary for organ-level function except in very rudimentary ways.[9] This is particularly true with respect to intestine-on-chip where virtually all devices employ tissue-cultured tumor cell lines growing on a flat surface with little to no resemblance to a functional epithelium.[147-153] In contrast, the proposed platform will recreate a 3D, *ex vivo* colonic epithelium from primary tissue with precisely controlled environmental parameters, such as growth-factor and oxygen gradients. This human simulacrum will recapitulate key anatomical and physiologic features of the colon including the polarized structure of the crypt. The small-sample size requirements of the array will permit the use of endoscopic biopsy samples to populate the device and reconstruct human disease phenotypes *in vitro*. Since an immune cell component underlies a number of diseases of the gut and the subepithelial myofibroblasts play a role in epithelial regeneration, the open scale architecture will make possible inclusion of these and other cell types along with luminal microbiota to study the integrated function of the intestine and the impact of specific perturbations. The arrayed nature of the device will facilitate mechanistic studies and screening assays with sufficient replicates and statistical power to test hypotheses or survey large numbers of compounds for their impact.

What innovations in technology will be necessary to attack the problem? The fundamental technical innovation in this project is the creation of a living, functional human tissue and its associated microbiome in a setting where direct and real-time studies can be carried out under highly controlled conditions. To accomplish this, a number of challenges will be overcome. The approach will require long-term viability of stem cells along with directed differentiation and polarization of the *in vitro* tissue. Interfaces must be created and maintained between the tissue and microdevice as well as the tissue and microbiome. This will be accomplished through innovations in design and materials along with precision environmental control on the microscale. These advances will be implemented in such a way that the platform remains compatible with a large variety of biological assays for temporal as well as single-time point measurements. Based on our preliminary work and the expertise of the team, we are confident that these challenges will be met to achieve a successful outcome.

1. Years 0 to 1.5- Device with a single simulacrum is under construction.

Objectives	Example Measurable Outcomes
Single simulacrum with chemical gradient	Linear chemical gradient across simulacra ≤3% variability in gradient profile over time
Single simulacrum with gas gradient	EOS bacteria grow in luminal compartment Appropriate epithelial cells grow and divide
Formation of *ex vivo* crypt within an epithelium	≥90% of crypts possess 3 distinct cell zones (stem, intermediate, differentiated) High resistance epithelial layer (>4000 Ohms cm^2)
Library of human colonic organoids with reporter gene BACs	≥50 banked human organoids ≥20 banked organoid lines incorporating BACS
Optimize assay reagents	All assays validated on test simulacra
Optimize the ASF bacteria for the project	Identify two luminal broths with 6% FOS/inulin Engineer >6 ASF strains with fluorescent proteins Effective qPCR protocol for all 8 ASF stains

All device designs are based on published reports of functioning devices as well as our design presented in the Prel. Data, thus we anticipate that the devices will be developed with the specifications required by the project. A plethora of alternative designs for chemical and gaseous gradient devices are available in the literature and we will use these resources as needed.[57-60, 81-83, 87-89, 154-157] Formation of the polarized simulacrum with appropriate cell compartments and physiology is the most high-risk component. We have overcome the greatest challenge, conversion of the Matrigel-embedded organoid culture system into a monolayer with long-lived, proliferating stem cells (Prel. Data). We have also demonstrated successful polarization of the monolayer grown over a 3D scaffold with formation of stem and differentiated-cell zones. The proposed devices will enable rapid screening to identify optimal conditions for the functional simulacrum. Should development of the simulacrum be slower than anticipated, we can perform rapid screening and assay development on the monolayer with gas and chemical gradients applied along the X-axis of the 2D surface or across Matrigel-embedded organoids in microfluidic device adjuncts in order to speed development on the 3D system. Finally, BACs can be redesigned as needed to include additional regulatory regions and altered fluorescent reporter genes to improve expression efficiency in creating the transgenic human lines. Conventional staining strategies, while low in throughput, can also be used as alternatives to reporter genes if necessary.

2. Years 1.5 to 3.0- Single-simulacrum device available to biologists & 96-simulacra design initiated.

Objectives	Example Measurable Outcomes
Optimize culture of simulacra.	≥90% of seeded wells grow simulacra living for >1 wk
Initiate experiments in Objective 1b	Mucous layer >30 μm thick Appropriate cell migration within a crypt (basal to luminal)
Optimize tissue-bacterial co-culture system	Simulacra with replicating EOS in the luminal compartment
96 simulacra with chemical gradient	Linear chemical gradient across all simulacra ≤3% variability over time across wells
96 simulacra with gas gradient	≥90% of wells possess functioning simulacra with EOS bacteria growing in luminal compartment
Optimize assay systems for 96 simulacra	Assays yield results with a ≤5% variability across the array
Initiate experiments in Objective 2b	Temporal characterization of ASF bacteria on the simulacra
Initiate experiments in Objective 2c	Identified impact of the ASF on the simulacra

If O_2 tension is too high in the luminal compartment to support the growth of ASF bacteria that are EOS, then we will focus on the facultative anaerobes. This co-culture system would still be completely novel and provide unprecedented insight into host-microbe interactions.

3. Years 3.0 to 5.0- Device with 96 simulacra is available to biologists.

Objectives	Example Measurable Outcomes
Complete experiments in Objective 1b.	Simulacra growing under fully optimized conditions with known behavior relative to that of *in vivo* tissue
Complete experiments in Objective 2b & 2c.	Improved understanding of the interaction between the ASF and epithelium in the presence of dietary factors
Initiate and complete experiments in Objective 4.	Hypothesis testing completed

If the composition of the human fecal microbiota communities displays too much variability in co-cultures, we will simplify the experimental design by co-culture with select bacteria that are among the most enriched in patient cases vs. controls. This alternative experiment has the advantage of being more rigorously controlled and would still provide insight into the dysbiosis associated with human colorectal cancer.

HUMAN SUBJECTS

A. Risks to human subjects

Sources of materials: [redacted] Last year [redacted] reported 384 donors that entered a consent agreement. The race and gender makeup of the donor closely mirrors the demographics in the state of North Carolina and the U.S. Dr. Magness applied and was approved by [redacted] for intestinal and colonic tissue procurement.

Genetic background and sample size. Each of the hypotheses in the grant will be tested using human tissue. To ensure reproducibility and detect statistically significant differences, 5 replicates will be performed on the same simulacra for each bioassay (*i.e.*, technical replicates). Because different individuals are known to have different biological responses, we will address genetic background by repeating all of the proposed experiments on human simulacra derived from 50 individuals. According to [redacted], we will have access to >300 colons per year so 10 per year or n=50 over a 5-year period is a conservative number. These independent simulacra (*i.e.*, biological replicates) will increase the statistical power of our experiments considerably and allow us to detect modest differences.

B. Adequacy of protection against risks

Recruitment and informed consent: All donors have been consented by [redacted]. [redacted] transplant teams will collect the intestinal tissue from the deceased donors. All tissue have been de-identified by [redacted]. A separate IRB (Institutional Review Board) application was submitted to the UNC Office of Human Research Ethics to obtained de-identified tissue. The Office of Human Research Ethics reviewed the human subject activities described below (study number [redacted]). This authority has determined that these activities do not constitute human subjects research as defined under federal regulations [45 CFR 46.102 (d or f) and 21 CFR 56.102(c)(e)(l)] and does not require IRB approval. All personnel that have access to the tissue have HIPPA certification. No personal identifiers are distributed to the investigator. Tissue is distributed only to those investigators whose studies have been approved by the [redacted] and the Office of Human Research Ethics. If the study protocol changes, the investigators will contact the UNC IRB before making change.

C. Potential Benefits of the proposed research to the subjects and others: There are no direct benefits to the de-identified donors. New information derived from these studies could benefit patients with a number of conditions that impair intestinal/colonic epithelial renewal or loss of intestine.

D. Importance of the knowledge to be gained: These studies will increase our understanding of human intestinal/colonic epithelial stem cells biology. These studies will provide new models of the colonic epithelium to develop new therapies and determine how a number of environmental conditions are impacting the colonic stem cells.

E. Data and safety monitoring plan: N/A

VERTEBRATE ANIMALS

1. Description of Use and Animal Numbers that will be used for this study.

Mice must be used for this project because there are no computer models or cell culture models that can substitute the need for primary cell types. Both male and female mice will be used for tissue collection. We will collect tissues from adult mice following euthanasia. Colonic tissue will be used for crypt dissociation, FACS-isolation, culture on the simulacra. A **total of 5,555 mice** will be used for this study. A detailed description of the justification for these animal numbers is provided in the following section. Underlined numbers indicate values used for calculations.

2. Justification of animal numbers

Lgr5EGFP-CreERT2$^{+/-}$
All mice for this project will be bred on site. Since these mice will be used to generate a reagent, there are no control study groups. All mice with the target and non-target (littermates) genotypes are included in the numbers below. Trio breeding (1 male × 2 females) will be used to generate mice. The Lgr5EGFP-CreERT2 transgene is homozygous lethal, and therefore, breeders must be kept in a heterozygous state. Breeder mice (Lgr5EGFP-CreERT2$^{+/-}$) will generate 50% pups possessing the target genotype (Lgr5EGFP-CreERT2$^{+/-}$). Lgr5EGFP-CreERT2$^{+/-}$ breeder mice will be replenished every three months. This will require crossing each strain to itself producing 50% Lgr5EGFP-CreERT2$^{+/-}$. So, 8 pups w/genotype × 4 times/year × 5 years = **160**. For production phase breeding, four crosses (cages) of 2 females and 1 male will be in constant breeding rotation. This strategy will produce 2 litters of approximately 8 pups every week providing sufficient numbers of mice for the experiments described. The animal use number was estimated based on using 8 mice with target genotype (Lgr5EGFP-CreERT2$^{+/-}$) per week for single cell culture. Offspring numbers (assuming average of 8 pups/litter × 2 litters): 16 pups/week × 52 weeks/year × 5 years = **4160 mice**. An additional 7% will be added to total estimate to account for breeder ramp-up, unexpected death, low litter numbers = grand total = (160+4160) + 7% additional = **4640**.

Confetti:Lgr5EGFP-CreERT2$^{+/-}$
All mice for this project will be bred on site. Since these mice will be used to generate a reagent, there are no control study groups. All mice with the target and non-target (littermates) genotypes are included in the numbers below. Trio breeding (1 male × 2 females) will be used to generate mice. The Lgr5EGFP-CreERT2 transgene is homozygous lethal, and therefore, breeders must be kept in a heterozygous state. The confetti mouse is also maintained on a heterozygous background. Breeder mice (Lgr5EGFP-CreERT2$^{+/-}$ X Confetti$^{+/-}$) will generate 25% pups possessing the target genotype (Lgr5EGFP-CreERT2$^{+/-}$:Confetti$^{+/-}$). Lgr5EGFP-CreERT2$^{+/-}$ and Confetti$^{+/-}$ breeder mice will be replenished every three months. This will require crossing each strain to itself producing 50% target genotypes. So, 8 pups w/genotype × 4 times/year × 5 years × 2 strains = **320**. For production phase breeding, 2 crosses (cages) of 2 females and 1 male will be in constant breeding rotation. This strategy will produce 1 litters of approximately 8 pups every week providing sufficient numbers of mice for the experiments described. The animal use number was estimated based on using 8 mice with target genotype (Lgr5EGFP-CreERT2$^{+/-}$:Confetti$^{+/-}$) per week for single cell culture. We anticipate experiments conducted with this mouse line will be concluded in 2 years. Offspring numbers (assuming average of 8 pups/litter): 8 pups/week × 52 weeks/year × 2 years = **832 mice**. An additional 10% will be added to total estimate to account for breeder ramp-up, unexpected death, low litter numbers = grand total = (832) + 10% (83) additional = **915**.

3. Veterinary Care:

All mice at UNC-CH are housed in modern animal quarters under the supervision of the
UNC Division of Laboratory Animal Medicine. Veterinarians are on-site within walking distance to the animal facility. A veterinarian is available 24-hours. Mice will be checked on a daily basis by our trained laboratory staff, animal husbandry staff, veterinarians or veterinarian technicians. All animal care and veterinary health care is under the direct supervision of the veterinary staff of the Division of Laboratory Animal Medicine, [redacted], DVM, Director.

4. Provisions to minimize discomfort, stress, pain and injury:

All mice at UNC-CH are housed and fed in modern animal quarters under the supervision of the UNC Division of Laboratory Animal Medicine. None of the transgenic mouse models described in this application exhibit any deleterious phenotype. All mice breed normally and not exhibit any abnormal health issues. All animal handlers

are well trained to minimize stress associated with handling the mice. No procedure will result in more than momentary discomfort, distress, pain or injury. Prior to euthanasia, all mice are anesthetized with isoflurane followed by cervical dislocation to minimize any stress or pain. In the event that any accidental discomfort, distress, pain or injury occurs that is more than momentary, the laboratory personnel are instructed to either humanely euthanize the mouse or contact UNC veterinary services for assistance. Veterinary staff will also be notified in the case that any animal appears to be sick based on fur ruffling, hunched back, soars, or an aberrant gate. All protocols associated with this grant proposal are approved by the UNC IACUC.

Project Leadership Plan

Drs. Allbritton, Bultman, Gomez and Magness will serve as PIs for the project. Allbritton will be responsible for the analytical and microengineered technology to enable creation of the human colonic epithelium in both low- and high-throughput formats along with the imaging hardware technologies to be implemented on the platforms. Bultman will be responsible for the overall coordination of the biological aspects of this proposal related to experimental design, troubleshooting biological issues that may arise related to the gut microbioata and butyrate studies. Gomez will oversee all bioinformatics work related to the image analysis and genetics data and will work with the other PIs on all data analysis. Magness will be responsible for the overall coordination of the biological aspects of this proposal related to human and murine tissue procurement, tissue dissociation, cell isolation, biomarker analysis, maintenance of mouse models, and trouble-shooting cellular reconstitution of the *in vitro* epithelial tissue. The PIs will form a Steering Committee that will manage the oversight and coordination of project management, research administration, publications and data sharing, and integration of all resources needed for the project. The Institution will subdivide the award funds and each PI will be responsible for his own budget. The Steering Committee will oversee decisions on minor changes in research direction and have the authority to reallocate funds and resources between PIs. Allbritton will serve as Chair of the Steering Committee and be responsible for communication among PIs, including meeting schedules and agendas and will be designated the contact PI. Responsibilities for preparing necessary documents to the NIH, including approvals for mouse and human studies, and annual progress reports will be shared by the PIs based on their respective roles in the grant with Allbritton responsible for the annual submission process.

Intellectual Property

The PIs are all employed by the University of North Carolina at Chapel Hill, and all work will take place at this performance site. The University retains all rights to pre-existing patents and the patents potentially generated within the frame of this project and will grant access for the purpose of this research project to all PIs on a non-exclusive royalty-free basis for the purpose of this research. Any intellectual property generated by this work will be the property of the University and subject to the relevant administrative and managerial rules of the organization.

Conflict Resolution

If a potential conflict develops, the appropriate Departmental administrators representing the PIs shall meet and attempt in good faith to settle any dispute, claim or controversy arising out of or relating to the interpretation, performance or breach of this disagreement. However, if the Departmental administrators fail to resolve the disagreement within thirty business days, then such disagreement shall be referred for resolution to a designated senior executive of the parties who has the authority to settle the disagreement but who is not directly involved in the disagreement.

Change in PI Location

If one of the PIs moves to a new institution, attempts will be made to transfer the relevant portion of the grant to the new institution. In the event that a PI cannot carry out his/her duties, a new PI will be recruited as a replacement, subject to the approval of a majority vote of the Steering Committee and the University.

Literature Cited

1. Blaser, M.J. The microbiome revolution. *J Clin Investigation* **124**, 4162-4165 (2014).
2. Flint, H.J., Scott, K.P., Louis, P. & Duncan, S.H. The role of the gut microbiota in nutrition and health. *Nat Rev Gastroenterol Hepatol* **9**, 577-589 (2012).
3. van Baarlen, P., Kleerebezem, M. & Wells, J.M. Omics approaches to study host-microbiota interactions. *Curr Opin Microbiol* **16**, 270-277 (2013).
4. Karlsson, F., Tremaroli, V., Nielsen, J. & Bäckhed, F. Assessing the human gut microbiota in metabolic diseases. *Diabetes* **62**, 3341-3349 (2013).
5. Nugent, J.L., McCoy, A.N., Addamo, C., Jia, W., Sandler, R.S. & Keku, T.O. Altered tissue metabolites correlate with microbial dysbiosis in colorectal adenomas. *J Proteome Res* **13**, 1921-1929 (2014).
6. Arthur, J.C., Perez-Chanona, E., Mühlbauer, M., Tomkovich, S., Uronis, J.M., Fan, T.J., Campbell, B.J., Abujamel, T., Dogan, B., Rogers, A.B., Rhodes, J.M., Stintzi, A., Simpson, K.W., Hansen, J.J., Keku, T.O., Fodor, A.A. & Jobin, C. Intestinal inflammation targets cancer-inducing activity of the microbiota. *Science* **338**, 120-123 (2012).
7. Ferguson, L.R. Nutrigenetics, nutrigenomics and inflammatory bowel diseases. *Expert Rev Clin Immunol* **9**, 717-726 (2013).
8. Lozupone, C.A., Stombaugh, J., Gonzalez, A., Ackermann, G., Wendel, D., Vázquez-Baeza, Y., Jansson, J.K., Gordon, J.I. & Knight, R. Meta-analyses of studies of the human microbiota. *Genome Res* **23**, 1704-1714 (2013).
9. Bhatia, S.N. & Ingber, D.E. Microfluidic organs-on-chips. *Nature Biotech* **32**, 760-772 (2014).
10. Lancaster, M.A. & Knoblich, J.A. Organogenesis in a dish: Modeling development and disease using organoid technologies. *Science* **345**, DOI: 10.1126/science.1247125 (2014).
11. Sato, T. & Clevers, H. Growing self-organizing mini-guts from a single intestinal stem cell: mechanism and applications. *Science* **340**, 1190-1194 (2013).
12. Sato, T., Stange, D.E., Ferrante, M., Vries, R.G., Van Es, J.H., Van den Brink, S., Van Houdt, W.J., Pronk, A., Van Gorp, J., Siersema, P.D. & Clevers, H. Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. *Gastroenterology* **141**, 1762-1772 (2011).
13. Jung, P., Sato, T., Merlos-Suarez, A., Barriga, F.M., Iglesias, M., Rossell, D., Auer, H., Gallardo, M., Blasco, M.A., Sancho, E., Clevers, H. & Batlle, E. Isolation and in vitro expansion of human colonic stem cells. *Nature Med* **17**, 1225-1227 (2011).
14. Sato, T., Vries, R.G., Snippert, H.J., van de Wetering, M., Barker, N., Stange, D.E., van Es, J.H., Abo, A., Kujala, P., Peters, P.J. & Clevers, H. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature* **459**, 262-U147 (2009).
15. VanDussen, K.L., Marinshaw, J.M., Shaikh, N., Miyoshi, H., Moon, C., Tarr, P.I., Ciorba, M.A. & Stappenbeck, T.S. Development of an enhanced human gastrointestinal epithelial culture system to facilitate patient-based assays. *Gut* **ePub**, doi: 10.1136/gutjnl-2013-306651 (2014).
16. Moon, C., Vandussen, K.L., Miyoshi, H. & Stappenbeck, T.S. Development of a primary mouse intestinal epithelial cell monolayer culture system to evaluate factors that modulate IgA transcytosis. *Nature Mucosal Immunol* **7**, 818-828 (2014).
17. Ahmad, A.A., Wang, Y., Gracz, A.D., Sims, C.E., Magness, S.T. & Allbritton, N.L. Optimization of 3-D organotypic primary colonic cultures for organ-on-chip applications. *J Biol Eng* **8**, 1754-1611 (2014).
18. Clevers, H. The intestinal crypt, a prototype stem cell compartment. *Cell* **154**, 274-284 (2013).
19. Clevers, H. Stem Cells: A unifying theory for the crypt. *Nature* **495**, 53-54 (2013).
20. Wang, Y., Dhopeshwarkar, R., Najdi, R., Waterman, M.L., Sims, C.E. & Allbritton, N. Microdevice to capture colon crypts for in vitro studies. *Lab Chip* **10**, 1596-1603 (2010).
21. Dame, M.K., Jiang, Y., Appelman, H.D., Copley, K.D., McClintock, S.D., Aslam, M.N., Attili, D., Elmunzer, B.J., Brenner, D.E., Varani, J. & Turgeon, D.K. Human colonic crypts in culture: segregation of immunochemical markers in normal versus adenoma-derived. *Laboratory Investigation* **94**, 222-234 (2014).
22. Kabat, A.M., Srinivasan, N. & Maloy, K.J. Modulation of immune development and function by intestinal microbiota. *Trends Immunol* **epub**, doi: 10.1016/j.it.2014.1007.1010 (2014).
23. Consortium, T.I.H.i.R.N. The Integrative Human Microbiome Project: Dynamic Analysis of Microbiome-Host Omics Profiles during Periods of Human Health and Disease. *Cell Host Microbe* **16**, 276-289 (2014).
24. Konrad, D. & Wueest, S. The Gut-Adipose-Liver Axis in the Metabolic Syndrome. *Physiology (Bethesda)* **29**, 304-313 (2014).
25. Douglas-Escobar, M., Elliott, E. & Neu, J. Effect of intestinal microbial ecology on the developing brain. *JAMA Pediatr* **167**, 374-379 (2013).

26. Clemente, J.C., Ursell, L.K., Parfrey, L.W. & Knight, R. The Impact of the Gut Microbiota on Human Health: An Integrative View. *Cell* **148**, 1258-1270 (2012).
27. Bultman, S.J. Emerging roles of the microbiome in cancer. *Carcinogenesis* **35**, 249-255 (2014).
28. Yi, P. & Li, L. The germfree murine animal: an important animal model for research on the relationship between gut microbiota and the host. *Vet Microbiol* **157**, 1-7 (2012).
29. Schwank, G., Andersson-Rolf, A., Koo, B.-K., Sasaki, N. & Clevers, H. Generation of BAC transgenic epithelial organoids. *PLoS ONE* **8**, e76871 (2013).
30. Formeister, E.J., Sionas, A.L., Lorance, D.K., Barkley, C.L., Lee, G.H. & Magness, S.T. Distinct SOX9 levels differentially mark stem/progenitor populations and enteroendocrine cells of the small intestine epithelium. *Am J Physiol-Gastroint Liver Physiol* **296**, G1108-G1118 (2009).
31. Gracz, A.D., Ramalingam, S. & Magness, S.T. Sox9 expression marks a subset of CD24-expressing small intestine epithelial stem cells that form organoids in vitro. *Am J Physiol-Gastroint Liver Physiol* **298**, G590-G600 (2010).
32. Schepers, A.G., Snippert, H.J., Stange, D.E., van den Born, M., van Es, J.H., van de Wetering, M. & Clevers, H. Lineage Tracing Reveals Lgr5(+) Stem Cell Activity in Mouse Intestinal Adenomas. *Science* **337**, 730-735 (2012).
33. Barker, N., Huch, M., Kujala, P., van de Wetering, M., Snippert, H.J., van Es, J.H., Sato, T., Stange, D.E., Begthel, H., van den Born, M., Danenberg, E., van den Brink, S., Korving, J., Abo, A., Peters, P.J., Wright, N., Poulsom, R. & Clevers, H. Lgr5(+ve) stem cells drive self-renewal in the stomach and build long-lived gastric units in vitro. *Cell Stem Cell* **6**, 25-36 (2010).
34. Fan, Y.-Y., Davidson, L.A., Callaway, E.S., Goldsby, J.S. & Chapkin, R.S. Differential effects of 2- and 3-series E-prostaglandins on in vitro expansion of Lgr5+ colonic stem cells. *Carcinogenesis* **35**, 606-612 (2013).
35. Ritsma, L. & al, e. Intestinal crypt homeostasis revealed at single-stem-cell level by in vivo live imaging. *Nature* **507**, 362–365 (2014).
36. Ramalingam, S., Daughtridge, G.W., Johnston, M.J., Gracz, A.D. & Magness, S.T. Distinct levels of Sox9 expression mark colon epithelial stem cells that form colonoids in culture. *Am J Physiol-Gastroint Liver Physiol* **302**, G10-G20 (2012).
37. Yin, X.L., Farin, H.F., van Es, J.H., Clevers, H., Langer, R. & Karp, J.M. Niche-independent high-purity cultures of Lgr5(+) intestinal stem cells and their progeny. *Nature Meth* **11**, 106-112 (2014).
38. Spiller, R.C. Intestinal absorptive function. *Gut* **35(1 Suppl)**, S5-9 (1994).
39. Mizutani, T., Nakamura, T., Morikawa, R., Fukuda, M., Mochizuki, W., Yamauchi, Y., Nozaki, K., Yui, S., Nemoto, Y., Nagaishi, T., Okamoto, R., Tsuchiya, K. & Watanabe, M. Real-time analysis of P-glycoprotein-mediated drug transport across primary intestinal epithelium three-dimensionally cultured in vitro. *BBRC* **419**, 238-243 (2012).
40. Annabi, N., Nichol, J.W., Zhong, X., Ji, C.D., Koshy, S., Khademhosseini, A. & Dehghani, F. Controlling the Porosity and Microarchitecture of Hydrogels for Tissue Engineering. *Tissue Eng Part B-Rev* **16**, 371-383 (2010).
41. Chau, D.Y.S., Collighan, R.J., Verderio, E.A.M., Addy, V.L. & Griffin, M. The cellular response to transglutaminase-cross-linked collagen. *Biomaterials* **26**, 6518-6529 (2005).
42. Hovakimyan, M., Guthoff, R.F. & Stachs, O. Collagen Cross-Linking: Current Status and Future Directions. *J Ophthalmology* **2012**, 406850 (2012).
43. Lew, D.H., Liu, P.H.T. & Orgill, D.P. Optimization of UV cross-linking density for durable and nontoxic collagen GAG dermal substitute. *J Biomed Mater Res Part B-Appl Biomater* **82B**, 51-56 (2007).
44. Nakaji-Hirabayashi, T., Kato, K. & Iwata, H. Improvement of Neural Stem Cell Survival in Collagen Hydrogels by Incorporating Laminin-Derived Cell Adhesive Polypeptides. *Bioconjugate Chem* **23**, 212-221 (2012).
45. Reiser, K., McCormick, R.J. & Rucker, R.B. Enzymatic and nonenzymatic cross-linking of collagen and elastin. *Faseb J.* **6**, 2439-2449 (1992).
46. Yang, C.R. Enhanced physicochemical properties of collagen by using EDC/NHS-crosslinking. *Bull Mater Sci* **35**, 913-918 (2012).
47. Barry, E.R., Morikawa, T., Butler, B.L., Shrestha, K., de la Rosa, R., Yan, K.S., Fuchs, C.S., Magness, S.T., Smits, R., Ogino, S., Kuo, C.J. & Camargo, F.D. Restriction of intestinal stem cell expansion and the regenerative response by YAP. *Nature* **493**, 106-110 (2013).
48. Donohoe, D.R., Collins, L.B., Wali, A., Bigler, R., Sun, W. & Bultman, S.J. The Warburg effect dictates the mechanism of butyrate-mediated histone acetylation and cell proliferation. *Mol Cell* **48**, 612-626 (2012).
49. Donohoe, D.R., Garge, N., Zhang, X., Sun, W., O'Connell, T.M., Bunger, M.K. & Bultman, S.J. The microbiome and butyrate regulate energy metabolism and autophagy in the mammalian colon. *Cell Metabol* **13**, 517-526 (2011).

50. Donohoe, D.R., Wali, A., Brylawski, B.P. & Bultman, S.J. Microbial regulation of glucose metabolism and cell-cycle progression in mammalian colonocytes. *PLoS ONE* **7**, e46589 (2012).
51. Van Landeghem, L., Santoro, M.A., Krebs, A.E., Mah, A.T., Dehmer, J.J., Gracz, A.D., Scull, B.P., McNaughton, K., Magness, S.T. & Lund, P.K. Activation of two distinct Sox9-EGFP expressing intestinal stem cell populations during crypt regeneration after irradiation. *Am J Physiol Gastrointest Liver Physiol*, G1111-1132 (2012).
52. Bassotti, G., Germani, U. & Morelli, A. Human colonic motility: physiological aspects. *Int J Colorectal Dis* **10**, 173-180 (1995).
53. Szmulowicz, U.M. & Hull, T.L. in The ASCRS Textbook of Colon and Rectal Surgery. (ed. D.E. Beck) 23-39 (Springer, 2011).
54. Krausova, M. & Korinek, V. Wnt signaling in adult intestinal stem cells and cancer. *Cellular Signalling* **26**, 570-579 (2014).
55. Schepers, A. & Clevers, H. Wnt Signaling, Stem Cells, and Cancer of the Gastrointestinal Tract. *Cold Spring Harbor Perspectives in Biology* **4**, a007989 (2012).
56. Scoville, D.H., Sato, T., He, X.C. & Li, L.H. Current view: Intestinal stem cells and signaling. *Gastroenterology* **134**, 849-864 (2008).
57. Wu, J., Wu, X. & Lin, F. Recent developments in microfluidics-based chemotaxis studies. *Lab Chip* **13**, 2484-2499 (2013).
58. Abhyankar, V.V., Lokuta, M.A., Huttenlocher, A. & Beebe, D.J. Characterization of a membrane-based gradient generator for use in cell-signaling studies. *Lab Chip* **6**, 389-393 (2006).
59. Abhyankar, V.V., Toepke, M.W., Cortesio, C.L., Lokuta, M.A., Huttenlocher, A. & Beebe, D.J. A platform for assessing chemotactic migration within a spatiotemporally defined 3D microenvironment. *Lab Chip* **8**, 1507-1515 (2008).
60. Cheng, S.Y., Heilman, S., Wasserman, M., Archer, S., Shuler, M.L. & Wu, M. A hydrogel-based microfluidic device for the studies of directed cell migration. *Lab Chip* **7**, 763-769 (2007).
61. Huang, K. & Murphy, R.F. From Quantitative Microscopy to Automated Image Understanding. *J Biomed Optics* **9**, 893-912 (2004).
62. Carpenter, A.E., Jones, T.R., Lamprecht, M.R., Clarke, C., Kang, I.H., Friman, O., Guertin, D.A., Chang, J.H., Lindquist, R.A., Moffat, J., Golland, P. & Sabatini, D.M. CellProfiler: image analysis software for identifying and quantifying cell phenotypes. *Genome Biol* **7**, R100 (2006).
63. de Chaumont, F., Dallongeville, S., Chenouard, N., Hervé, N., Pop, S., Provoost, T., Meas-Yedid, V., Pankajakshan, P., Lecomte, T., Le Montagner, Y., Lagache, T., Dufour, A. & Olivo-Marin, J.-C. Icy: an open bioimage informatics platform for extended reproducible research. *Nature Meth* **9**, 690-696 (2012).
64. Eliceiri, K.W., Berthold, M.R., Goldberg, I.G., Ibáñez, L., Manjunath, B.S., Martone, M.E., Murphy, R.F., Peng, H., Plant, A.L., Roysam, B., Stuurmann, N., Swedlow, J.R., Tomancak, P. & Carpenter, A.E. Biological imaging software tools. *Nature Meth* **9**, 697-710 (2012).
65. Kankaanpää, P., Paavolainen, L., Tiitta, S., Karjalainen, M., Päivärinne, J., Nieminen, J., Marjomäki, V., Heino, J. & White, D.J. BioImageXD: an open, general-purpose and high-throughput image-processing platform. *Nature Meth* **9**, 683-689 (2012).
66. Peng, H., Ruan, Z., Long, F., Simpson, J.H. & Myers, E.W. V3D enables real-time 3D visualization and quantitative analysis of large-scale biological image data sets. *Nature Biotechnol* **28**, 348-353 (2010).
67. Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.Y., White, D.J., Hartenstein, V., Eliceiri, K., Tomancak, P. & Cardona, A. Fiji: an open-source platform for biological-image analysis. *Nature Meth* **9**, 676-682 (2012).
68. Bishop, C.M. Pattern recognition and machine learning. (Springer New York, New York, USA; 2006).
69. Coelho, L., Glory-Afshar, E. & Kangas, J. Principles of bioimage informatics: focus on machine learning of cell patterns. *Lecture Notes Computer Sci* **6004**, 8-18 (2010).
70. Danuser, G. Computer vision in cell biology. *Cell* **147**, 973-978 (2011).
71. Jones, T.R., Carpenter, A.E., Lamprecht, M.R., Moffat, J., Silver, S.J., Grenier, J.K., Castoreno, A.B., Eggert, U.S., Root, D.E., Golland, P. & Sabatini, D.M. Scoring diverse cellular morphologies in image-based screens with iterative feedback and machine learning. *PNAS* **106**, 1826-1831 (2009).
72. Jones, T.R., Kang, I.H., Wheeler, D.B., Lindquist, R.a., Papallo, A., Sabatini, D.M., Golland, P. & Carpenter, A.E. CellProfiler Analyst: data exploration and analysis software for complex image-based screens. *BMC bioinformatics* **9**, 482 (2008).
73. Kyung, M., Gill, J., Ghosh, M. & Casella, G. Penalized regression, standard errors, and Bayesian lassos. *Bayesian Analysis* **5**, 369-411 (2010).
74. Wu, Y., Johnson, G.L. & Gomez, S.M. Data-driven modeling of cellular stimulation, signaling and output response in RAW 264.7 cells. *J Molecular Signaling* **3**, 11 (2008).
75. Zou, H. & Hastie, T. Regularization and variable selection via the elastic net. *J Royal Statistical Soc* **67**, 301-320 (2005).

76. Peng, H. Bioimage informatics: A new area of engineering biology. *Bioinformatics* **24**, 1827-1836 (2008).
77. Vandussen, K.L., Carulli, A.J., Keeley, T.M., Patel, S.R., Puthoff, B.J., Magness, S.T., Tran, I.T., Maillard, I., Siebel, C., Kolterud, A., Grosse, A.S., Gumucio, D.L., Ernst, S.A., Tsai, Y.H., Dempsey, P.J. & Samuelson, L.C. Notch signaling modulates proliferation and differentiation of intestinal crypt base columnar stem cells. *Development* **139**, 488-497 (2012).
78. Snippert, H.J., van der Flier, L.G., Sato, T., van Es, J.H., van den Born, M., Kroon-Veenboer, C., Barker, N., Klein, A.M., van Rheenen, J., Simons, B.D. & Clevers, H. Intestinal crypt homeostasis results from neutral competition between symmetrically dividing Lgr5 stem cells. *Cell* **143**, 134-144 (2010).
79. Vermeulen, L., Morrissey, E., van der Heijden, M., Nicholson, A.M., Sottoriva, A., Buczacki, S., Kemp, R., Tavaré, S. & Winton, D.J. Defining Stem Cell Dynamics in Models of Intestinal Tumor Initiation. *Science* **342**, 995-998 (2013).
80. Johansson, M.E.V., Larsson, J.M.H. & Hansson, G.C. The two mucus layers of colon are organized by the MUC2 mucin, whereas the outer layer is a legislator of host–microbial interactions. *PNAS* **108(suppl 1)**, 4659-4665 (2011).
81. Oppegard, S.C., Blake, A.J., Williams, J.C. & Eddington, D.T. Precise control over the oxygen conditions within the Boyden chamber using a microfabricated insert. *Lab Chip* **10**, 2366-2373 (2010).
82. Oppegard, S.C. & Eddington, D.T. A microfabricated platform for establishing oxygen gradients in 3-D constructs. *Biomed Microdev* **15**, 407-414 (2013).
83. Oppegard, S.C., Nam, K.H., Carr, J.R., Skaalure, S.C. & Eddington, D.T. Modulating Temporal and Spatial Oxygenation over Adherent Cellular Cultures. *Plos ONE* **4** (2009).
84. He, G., Shankar, R.A., Chzhan, M., Samouilov, A., Kuppusamy, P. & Zweier, J.L. Noninvasive Measurement of Anatomic Structure and Intraluminal Oxygenation in theGastrointestinal Tract of Living Mice with Spatial and Spectral EPR Imaging. *PNAS* **96**, 4586-4591 (1999).
85. Guebel, D.V. & Torres, N.V. A computer model of oxygen dynamics in human colon mucosa: Implications in normal physiology and early tumor development. *J Theoret Biol* **250**, 389-409 (2008).
86. Espey, M. Role of oxygen gradients in shaping redox relationships between the human intestine and its microbiota. *Free Radical Biol Med* **55**, 130-140 (2013).
87. Lo, J.F., Sinkala, E. & Eddington, D.T. Oxygen gradients for open well cellular cultures via microfluidic substrates. *Lab Chip* **10**, 2394–2401 (2010).
88. Brennan, M.D., Rexius, M.L., Elgass, L.J. & Eddington, D.T. Oxygen control with microfluidics. *Lab Chip* **14** (2014).
89. Funamoto, K., Zervantonakis, I.K., Liu, Y., Ochs, C.J., Kim, C. & Kamm, R.D. A novel microfluidic platform for high-resolution imaging of a three-dimensional cell culture under a controlled hypoxic environment. *Lab Chip* **12**, 4855-4863 (2012).
90. Mehta, G., Mehta, K., Sud, D., Song, J.W., Bersano-Begey, T., Futai, N., Heo, Y.S., Mycek, M.-A., Linderman, J.J. & Takayama, S. Quantitative measurement and control of oxygen levels in microfluidic poly(dimethylsiloxane) bioreactors during cell culture. *Biomed Microdev* **9**, 123–134 (2007).
91. Lambrechts, D., Roeffaers, M., Kerckhofs, G., Roberts, S.J., Hofkens, J., Van de Putte, T., Van Oostervvyck, H. & Schrooten, J. Fluorescent oxygen sensitive microbead incorporation for measuring oxygen tension in cell aggregates. *Biomaterials* **34**, 922-929 (2013).
92. Kondrashina, A.V., Ogurtsov, V.I. & Papkovsky, D.B. Comparison of the three optical platforms for measurement of cellular respiration. *Anal Biochem* **ePub**, doi: 10.1016/j.ab.2014.1009.1001 (2014).
93. Ley, R.E., Peterson, D.A. & Gordon, J.I. Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell* **124**, 837-848 (2006).
94. O'Hara, A.M. & Shanahan, F. The gut flora as a forgotten organ. *Embo Reports* **7**, 688-693 (2006).
95. Lozupone, C.A., Stombaugh, J.I., Gordon, J.I., Jansson, J.K. & Knight, R. Diversity, stability and resilience of the human gut microbiota. *Nature* **489**, 220-230 (2012).
96. Orcutt, R.P., Gianni, F.J. & Judge, R.J. Development of an "altered Schaedler flora" for NCI gnotobiotic rodents. *Microecol Ther* **17**, 59 (1987).
97. Dewhirst, F.E., Chien, C.C., Paster, B.J., Ericson, R.L., Orcutt, R.P., Schauer, D.B. & Fox, J.G. Phylogeny of the defined murine microbiota: altered Schaedler flora. *Appl Environ Microbiol* **65**, 3287-3292 (1999).
98. Alexander, A.D., Orcutt, R.P., Henry, J.C., Baker, J.J., Bissahoyo, A.C. & Threadgill, D.W. Quantitative PCR assays for mouse enteric flora reveal strain-dependent differences in composition that are influenced by the microenvironment. *Mammalian Genome* **17**, 1093-1104 (2006).
99. Lee, W.-J. & Hase, K. Gut microbiota–generated metabolites in animal health and disease. *Nature Chem Biol* **10**, 416-424 (2014).
100. Roediger, W.E. Role of anaerobic bacteria in the metabolic welfare of the colonic mucosa in man. *Gut* **21**, 793-798 (1980).
101. Roediger, W.E. Utilization of nutrients by isolated epithelial cells of the rat colon. *Gastroenterology* **83**, 424-429 (1982).

102. Waxman, K. Perfluorocarbons as blood substitutes. *Ann Emerg Med* **15**, 1423-1424 (1986).
103. Spahn, D.R. Blood substitutes Artificial oxygen carriers: perfluorocarbon emulsions. *Crit Care* **3**, R93–R97 (1999).
104. Ge, Z.M., Feng, Y., Taylor, N.S., Ohtani, M., Polz, M.F., Schauer, D.B. & Fox, J.G. Colonization dynamics of altered Schaedler flora is influenced by gender, aging, and Helicobacter hepaticus infection in the intestines of Swiss Webster mice. *Appl Environ Microbiol* **72**, 5100-5103 (2006).
105. Balamurugan, R., Rajendiran, E., George, S., Samuel, G.V. & Ramakrishna, B.S. Real-time polymerase chain reaction quantification of specific butyrate-producing bacteria, Desulfovibrio and Enterococcus faecalis in the feces of patients with colorectal cancer. *J Gastroenterology Hepatology* **23**, 1298-1303 (2008).
106. David, L.A., Maurice, C.F., Carmody, R.N., Gootenberg, D.B., Button, J.E., Wolfe, B.E., Ling, A.V., Devlin, A.S., Varma, Y., Fischbach, M.A., Biddinger, S.B., Dutton, R.J. & Turnbaugh, P.J. Diet rapidly and reproducibly alters the human gut microbiome. *Nature* **505**, 559-563 (2014).
107. Hamer, H.M., Jonkers, D., Venema, K., Vanhoutvin, S., Troost, F.J. & Brummer, R.J. Review article: the role of butyrate on colonic function. *Alimentary Pharmacol Therapeut* **27**, 104-119 (2008).
108. Topping, D.L. & Clifton, P.M. Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. *Physiological Rev* **81**, 1031-1064 (2001).
109. Donohoe, D.R., Montgomery, S.A., Collins, L.B., Curry, K.P., Holley, D., Renner, S.W., Ryan, E.P., Godfrey, V., Han, A., Swenberg, J.A., Threadgill, D.S., Threadgill, D.W. & Bultman, S.J. A gnotobiotic mouse model demonstrates that dietary fiber protects against colorectal tumorigenesis in a microbiota- and butyrate-dependent manner. *Cancer Discovery.* **In Press.** (2014).
110. Backhed, F., Ley, R.E., Sonnenburg, J.L., Peterson, D.A. & Gordon, J.I. Host-bacterial mutualism in the human intestine. *Science* **307**, 1915-1920 (2005).
111. Gordon, H.A. & Pesti, L. The gnotobiotic animal as a tool in the study of host microbial relationships. *Bacteriological Rev* **35**, 390-429 (1971).
112. Kovarik, M.L., Shah, P.K., Armistead, P.M. & Allbritton, N.L. Microfluidic chemical cytometry of peptide degradation in single drug-treated acute myeloid leukemia cells. *Anal Chem* **85**, 4991–4997 (2013).
113. Phillips, K.S., Lai, H.H., Johnson, E., Sims, C.E. & Allbritton, N.L. Continuous chemical analysis of single cells on a microfluidic chip. *Lab Chip* **11**, 1333-1341 (2011).
114. Wang, Y., Lai, H.-H., Bachman, M., Sims, C.E., Li, G.P. & Allbritton, N.L. Covalent micropatterning of poly(dimethylsiloxane) by photografting through a mask. *Anal Chem* **77**, 7539-7546 (2005).
115. Wang, Y., Phillips, C., Xu, W., Pai, J.H., Dhopeshwarkar, R., Sims, C.E. & Allbritton, N. Micromolded arrays for separation of adherent cells. *Lab Chip* **10**, 2917-2924 (2010).
116. Wang, Y., Shah, P., Phillips, C., Sims, C.E. & Allbritton, N.L. Trapping cells on a stretchable microwell array for single-cell analysis. *Anal Bioanal Chem* **402**, 1065-1072 (2012).
117. Wang, Y., Balowski, J., Phillips, C., Phillips, R., Sims, C.E. & Allbritton, N.L. Benchtop micromolding of polystyrene by soft lithography. *Lab Chip* **11**, 3089-3097 (2011).
118. Abate, A.R., Lee, D., Do, T., Holtze, C. & Weitz, D.A. Glass coating for PDMS microfluidic channels by sol-gel methods. *Lab Chip* **8**, 516-518 (2008).
119. Gomez-Sjoberg, R., Leyrat, A.A., Houseman, B.T., Shokat, K. & Quake, S.R. Biocompatibility and Reduced Drug Absorption of Sol-Gel-Treated Poly(dimethyl siloxane) for Microfluidic Cell Culture Applications. *Anal Chem* **82**, 8954-8960 (2010).
120. Hu, S., Ren, X., Bachman, M., Sims, C.E., Li, G.P. & Allbritton, N.L. Surface modification of poly(dimethylsiloxane) microfluidic devices by ultraviolet polymer grafting. *Anal Chem* **74**, 4117-4123 (2002).
121. Hu, S., Ren, X., Bachman, M., Sims, C.E., Li, G.P. & Allbritton, N.L. Tailoring the surface properties of poly(dimethylsiloxane) microfluidic devices. *Langmuir* **20**, 5569-5574 (2004).
122. Hu, S., Ren, X., Bachman, M., Sims, C.E., Li, G.P. & Allbritton, N.L. Surface-directed, graft polymerization within microfluidic channels. *Anal Chem* **76**, 1865-1870 (2004).
123. Roman, G.T., Hlaus, T., Bass, K.J., Seelhammer, T.G. & Culbertson, C.T. Sol-gel modified poly(dimethylsiloxane) microfluidic devices with high electroosmotic Mobilities and hydrophilic channel wall characteristics. *Anal Chem* **77**, 1414-1422 (2005).
124. Wang, L., Sun, B., Ziemer, K.S., Barabino, G.A. & Carrier, R.L. Chemical and physical modifications to poly(dimethylsiloxane) surfaces affect adhesion of Caco-2 cells. *J Biomed Mater Res Part A* **93A**, 1260-1271 (2010).
125. Whitesides, G.M., Ostuni, E., Takayama, S., Jiang, X.Y. & Ingber, D.E. Soft lithography in biology and biochemistry. *Ann Rev Biomed Eng* **3**, 335-373 (2001).
126. Golden, A.P. & Tien, J. Fabrication of microfluidic hydrogels using molded gelatin as a sacrificial element. *Lab Chip* **7**, 720-725 (2007).

127. Yajima, Y., Yamada, M., Yamada, E., Iwase, M. & Seki, M. Facile fabrication processes for hydrogel-based microfluidic devices made of natural biopolymers. *Biomicrofluidics* **8** (2014).
128. Narasimhan, J. & Papautsky, I. Polymer embossing tools for rapid prototyping of plastic microfluidic devices. *J Micromech Microeng* **14**, 96–103 (2004).
129. Worgull, M. Hot Embossing: Theory and Technology of Microreplication (Micro and Nano Technologies). (Wm. Andrew, 2009).
130. Ferrell, N., Desai, R.R., Fleischman, A.J., Roy, S., Humes, H.D. & Fissell, W.H. A Microfluidic Bioreactor With Integrated Transepithelial Electrical Resistance (TEER) Measurement Electrodes for Evaluation of Renal Epithelial Cells. *Biotechnol Bioeng* **107**, 707-716 (2010).
131. Douville, N.J., Tung, Y.-C., Li, R., Wang, J.D., El-Sayed, E.H. & Takayama, S. Fabrication of Two-Layered Channel System with Embedded Electrodes to Measure Resistance Across Epithelial and Endothelial Barriers. *Anal Chem* **82**, 2505-2511 (2010).
132. Pryde, S.E., Duncan, S.H., Hold, G.L., Stewart, C.S. & Flint, H.J. The microbiology of butyrate formation in the human colon. *FEMS Microbiol Lett* **217**, 133-139 (2002).
133. Davie, J.R. Inhibition of histone deacetylase activity by butyrate. *J Nutrition* **133**, 2485S-2493S (2003).
134. Sengupta, S., Muir, J.G. & Gibson, P.R. Does butyrate protect from colorectal cancer? *J Gastroenterology Hepatology* **21**, 209-218 (2006).
135. Fung, K.Y., Cosgrove, L., Lockett, T., Head, R. & Topping, D.L. A review of the potential mechanisms for the lowering of colorectal oncogenesis by butyrate. *Brit J Nutrition* **108**, 820-831 (2012).
136. Asanuma, N., Ishiwata, M., Yoshii, T., Kikuchi, M., Nishina, Y. & Hino, T. Characterization and transcription of the genes involved in butyrate production in Butyrivibrio fibrisolvens type I and II strains. *Curr Microbiol* **51**, 91-94 (2005).
137. Shane, B.S., Gouws, L. & Kistner, A. Cellulolytic bacteria occurring in the rumen of sheep conditioned to low-protein teff hay. *J Gen Microbiol* **55**, 445-457 (1969).
138. Araujo-Perez, F., McCoy, A.N., Okechukwu, C., Carroll, I.M., Smith, K.M., Jeremiah, K., Sandler, R.S., Asher, G.N. & Keku, T.O. Differences in microbial signatures between rectal mucosal biopsies and rectal swabs. *Gut Microbes* **3**, 530-535 (2012).
139. Dulal, S. & Keku, T.O. Gut microbiome and colorectal adenomas. *Cancer J* **20**, 225-231 (2014).
140. Sanapareddy, N., Legge, R.M., Jovov, B., McCoy, A., Burcal, L., Araujo-Perez, F., Randall, T.A., Galanko, J., Benson, A., Sandler, R.S., Rawls, J.F., Abdo, Z., Fodor, A.A. & Keku, T.O. Increased rectal microbial richness is associated with the presence of colorectal adenomas in humans. *ISME J* **6**, 1858-1868 (2012).
141. Iida, N., Dzutsev, A., Stewart, C.A., Smith, L., Bouladoux, N., Weingarten, R.A., Molina, D.A., Salcedo, R., Back, T., Cramer, S., Dai, R.-M., Kiu, H., Cardone, M., Naik, S., Patri, A.K., Wang, E., Marincola, F.M., Frank, K.M., Belkaid, Y., Trinchieri, G. & Goldszmid, R.S. Commensal Bacteria Control Cancer Response to Therapy by Modulating the Tumor Microenvironment. *Science* **342**, 967-970 (2013).
142. Hsiao, E.Y., McBride, S.W., Hsien, S., Sharon, G., Hyde, E.R., McCue, T., Codelli, J.A., Chow, J., Reisman, S.E., Petrosino, J.F., Patterson, P.H. & Mazmanian, S.K. Microbiota Modulate Behavioral and Physiological Abnormalities Associated with Neurodevelopmental Disorders. *Cell* **155**, 1451–1463 (2013).
143. Gilbert, J.A., Krajmalnik-Brown, R., Porazinska, D.L., Weiss, S.J. & Knight, R. Toward Effective Probiotics for Autism and Other Neurodevelopmental Disorders. *Cell* **155**, 1446-1448 (2013).
144. Sung, J.H., Esch, M.B., Prot, J.-L., Long, C.J., Smith, A., Hickmancd, J.J. & Shuler, M.L. Microfabricated mammalian organ systems and their integration into models of whole animals and humans. *Lab Chip* **13**, 1201-1212 (2013).
145. Capulli, A.K., Tian, K., Mehandru, N., Bukhta, A., Choudhury, S.F., Suchyta, M. & Parker, K.K. Approaching the in vitro clinical trial: engineering organs on chips. *Lab Chip* **14**, 3181–3186 (2013).
146. Chan, C.Y., Huang, P.-H., Guo, F., Ding, X., Kapur, Mai, J.D., Yuen, P.K. & Huang, T.J. Accelerating drug discovery via organs-on-chips. *Lab Chip* **13**, 4697–4710 (2013).
147. Kim, H.J., Huh, D., Hamilton, G. & Ingber, D.E. Human gut-on-a-chip inhabited by microbial flora that experiences intestinal peristalsis-like motions and flow. *Lab Chip* **12**, 2165-2174 (2012).
148. Kim, H.-J. & Ingber, D.E. Gut-on-a-Chip microenvironment induces human intestinal cells to undergo villus diff erentiation. *Integr Biol* **5**, 1130-1140 (2013).
149. Kim, J., Hegde, M. & Jayaraman, A. Co-culture of epithelial cells and bacteria for investigating host-pathogen interactions. *Lab Chip* **10**, 43-50 (2010).
150. Pfluger, C.A., Burkey, D.D., Wang, L., Sun, B., Ziemer, K.S. & Carrier, R.L. Biocompatiblity of plasma enhanced chemical vapor deposited poly(2-hydroxyethyl methacrylate) films for biomimetic replication of the intestinal basement membrane. *Biomacromolecules* **11**, 1579-1584 (2010).
151. Sung, J.H., Yu, J., Luo, D., Shuler, M.L. & March, J.C. Microscale 3-D hydrogel scaffold for biomimetic gastrointestinal (GI) tract model. *Lab Chip* **11**, 389-392 (2011).

152. Wang, L., Murthy, S.K., Barabino, G.A. & Carrier, R.L. Synergic effects of crypt-like topography and ECM proteins on intestinal cell behavior in collagen based membranes. *Biomaterials* **31**, 7586-7598 (2010).
153. Wang, L., Murthy, S.K., Fowle, W.H., Barabino, G.A. & Carrier, R.L. Influence of micro-well biomimetic topography on intestinal epithelial Caco-2 cell phenotype. *Biomaterials* **30**, 6825-6834 (2009).
154. Chang, C.-W., Cheng, Y.-J., Tu, M., Chen, Y.-H., Peng, C.-C., Liaoa, W.H. & TungY.-C. A polydimethylsiloxane–polycarbonate hybrid microfluidic device capable of generating perpendicular chemical and oxygen gradients for cell culture studies. *Lab Chip* **14**, 3762-3772 (2014).
155. Skolimowski, M., Nielsen, M.W., Emneus, J., Molin, S., Taboryski, R., Sternberg, C., Dufva, M. & Geschke, O. Microfluidic dissolved oxygen gradient generator biochip as a useful tool inbacterial biofilm studies. *Lab Chip* **10**, 2162–2169 (2010).
156. Wang, L., Liu, W., Wang, Y., Wang, J.-C., Tu, Q., Liu, R. & Wang, J. Construction of oxygen and chemical concentration gradients in a single microfluidic device for studying tumor cell–drug interactions in a dynamic hypoxia microenvironment. *Lab Chip* **13**, 695 (2013).
157. Marei, M.M., Roussel, T.J., Keynton, R.S. & Baldwin, R.P. Electrochemical Dissolved Oxygen Removal from Microfluidic Streams for LOC Sample Pretreatment. *Anal Chem* **86**, 8541-8546 (2014).

Resource Sharing Plan for Development of Human Intestinal Simulacra

What resources will be shared?
All data generated in this NIH funded project will be shared through publication in peer-reviewed scientific and engineering journals. Submitted data will conform to relevant data and terminology standards. Technical CAD drawings of devices that have been designed, fabricated and validated in this work will be made available. Investigators will be given access to the transgenic cell lines and transgenic bacteria developed in this project.

Who will have access to the data?
The data will be available to public and private individuals through electronic and standard subscriptions to the above mentioned journals. Cell lines will be made available to scientists with the appropriate biosafety training and equipment.

Where will the data be available?
The data will be provided in published journals with repository data access policies and procedures consistent with NIH data sharing policies. Technical CAD drawings of devices that have been designed, fabricated and validated in this work will be made available as Supplemental Data associated with the relevant publications or via electronic files by request of interested scientific investigators to Dr. Allbritton. Investigators will have access to the transgenic cell lines developed in this project through the Stem Cell Biobank (SCB) at the Coriell Institute for Medical Research or by request to Dr. Magness, and transgenic bacteria will be available through the American Tissue Culture Collection (ATCC) or by request to Dr. Bultman.

When will the data be shared?
All data will be provided in a timely manner as it is generated and published in scientific journals and cell lines deposited to the appropriate bank.

How will researchers locate and access the data?
The data will be locatable by using standard search engines, such as PubMed. Data will be accessed by qualified researchers under electronic and library subscriptions fully consistent with NIH data sharing policies and applicable laws and regulations. Cell lines will be obtained by orders placed through the Coriell Institute's Cell Repository and ATCC catalogs or via direct contact with the appropriate project investigator.