DDRC DIGEST
Digestive Disease Research Center
Medical University of South Carolina

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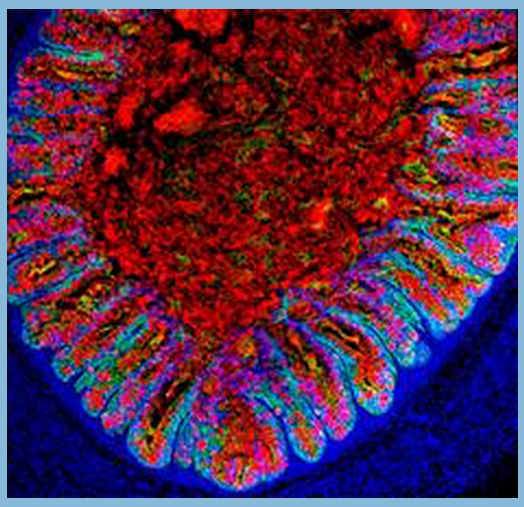


Transplanted human colonic organoid (Múnera Lab, *unpublished*) immuno-stained for E-cadherin (green), MUC2 (red) and DAPI (blue). More details about this technology can be found in the article about our new COBRE JI.

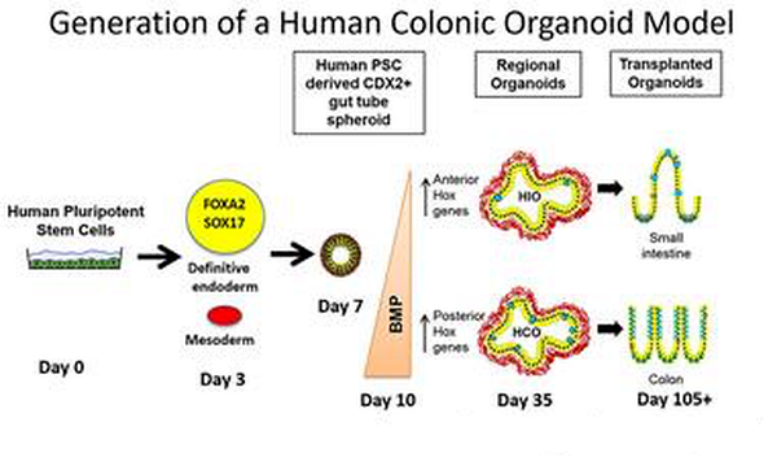
On the Inside

Welcome New CDLD Junior Investigator

This cycle, the Digestive Disease Research Core Center welcomed over a dozen new Full Members, including our new Junior Investigator from the COBRE in Digestive and Liver Disease. A listing of the current DDRCC Full Membership may be found on the **Member Directory** page of the **DDRCC Website**.

Jorge Múnera, PhD currently serves as an Assistant Professor in the Regenerative Medicine and Cell Biology department at MUSC. He received his PhD in Molecular Pathology from the University of California, San Diego. He joined the MUSC faculty following a postdoctoral fellowship in stem cell biology in the laboratory of Dr. Jim Wells at the Cincinnati Children's Hospital Medical Center.

The main focus of Dr Múnera's postdoctoral research was to identify the mechanisms that regulate embryonic development of the intestine and colon, and to apply this information towards his pioneering efforts to generate human colonic organoids from induced pluripotent stem cells (iPSC). His participation in a collaborative program combining the strengths of animal models with human pluripotent stem cell-derived gut tube cultures identified bone morphogenetic protein or BMP as an important factor specifying patterning of the gut tube. A BMP-patterning step was similarly found to be essential for the development of human colonic organoids (HCOs) containing cell types consistent with colonic identity. Following transplantation under the mouse kidney capsule, HCOs further matured into a colon-like structure with well-differentiated smooth muscle layers. These organoids represent an innovative and accessible new model for studying human colonic development and GI disease *in vitro*.



A summary of the culture process used by the Múnera Lab to generate HCOs. (from Múnera et al., *Cell Stem Cell*, 2017)

With the support of the DDRCC/CDLD Advanced Cell Models Core, the Múnera lab will focus on elucidating molecular mechanisms of congenital GI diseases that affect the colon using HCOs derived from either patient specific iPSCs, or iPSCs where CRISPR-Cas9 editing has introduced candidate disease-associated gene mutations. Additionally, their recent discovery that HCOs also contain co-developing immune cells will allow them to further study signaling networks between gut and immune cells in the setting of either normal function or pathology. This work is expected to lead to new insights into diseases such as polyposis syndromes and inflammatory bowel disease.

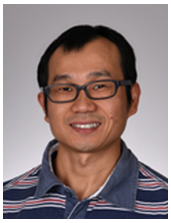
Dr. Múnera will join the current cohort of CDLD Junior Investigators (JIs). The overall goal of the CDLD JI program is to support their development into funded independent investigators. Upon achieving this goal, the CDLD JIs will then graduate to join the cadre of Full Member PIs supported by the P30-funded DDRCC. We look forward to the continued success of Drs. Múnera, Novince, Meissner and Kourtidis.

For further details about the Múnera Lab and opportunities for collaboration, please contact **Dr. Múnera**.

Congratulations Pilot and Feasibility Awardees

This cycle, following extensive review by intramural and extramural referees including the External Advisory Boards of the DDRCC and CDLD, the DDRC is pleased to announce the funding of the following P&F awards:

COBRE in Digestive and Liver Disease



Wenjian Gan, PhD Biochemistry and Molecular Biology

"Role of PRMT1 in regulating mTORC1 pathway and fatty liver disease."

Silvia Guglietta, PhD Microbiology and Immunology

"Impact of anaphylatoxin C3aR on immune responses and gut microbiota in IBD."

Digestive Disease Research Core Center

Seok-Hyung Kim, PhD Medicine

"Role of FAD in high fat diet induced non-alcoholic fatty liver disease."

Chad Novince, DDS, PhD Oral Health Sciences

"Bile acid regulation of antibiotic gut dysbiosis effects on metabolism and skeletal growth."

Jihad Obeid, MD Public Health Sciences

"Leveraging artificial intelligence for the identification and early detection of cirrhosis in electronic health records."

Along with their project funding and core support, our P&F investigators will be granted **Full Member** status for the one-year duration of their award.

Look for the upcoming request for applications for the 2021 Pilot and Feasibility Award cycle early in 2021.

DDRCC Core Profile: Advanced Imaging Core

Leadership

John J. Lemasters
Core Director

Anna-Liisa Nieminen
Assistant Director

Monika B. Gooz
Core Manager

Li Li
Core Technician

The Advanced Imaging Core (AIC) provides investigators with sophisticated optical imaging technologies, methods, project development and training required for successful, high-end cell and tissue-based imaging and analysis.

Services include consultation and assistance concerning experimental design, sample preparation, probe selection and data collection (assisted imaging) and analysis for imaging applications. Users can get in-depth training in multiple imaging modalities, and education in the fundamentals of imaging and image analysis technologies.

The following applications are available for users:

• **Fixed Cell & Tissue Imaging** using chromogenic and fluorescence techniques in combination with antibody-based methods to monitor expression, distribution and interaction of specific molecules.

• **Live Cell Imaging** of parameter-sensitive fluorophores to monitor ions, electrical potentials, oxygen and nitrogen radical generation, NAD(P)H, mitochondrial and plasmalemmal membrane permeability, cell viability (apoptosis and necrosis), fluorescent protein biosensors and other parameters.

• **Intravital Microscopy** to monitor microcirculation, leukocyte margination, invadopodia, mitochondrial polarization, membrane permeability, radical generation, gene expression, detection of collagen fibers and other factors in living animals and tissues.

• **Advanced Imaging Techniques**: Fluorescence resonance energy transfer (FRET) and fluorescence recovery after photobleaching (FRAP) to characterize and quantify interactions between specific molecules and their mobility; second harmonic generation (SHG) and polarization microscopy for label-free visualization of collagen; Fast Airyscan super-resolution imaging with high quantum efficiency GaAsP photomultipliers.

• **Image Analysis**: Three imaging workstations are dedicated for image processing and analysis. Site licenses are maintained for Zeiss Zen software, Imaris 3D/4D interactive microscopy image analysis software and for Huygens advanced deconvolution suite. Other available image analysis software includes Metamorph, Image J FIJI, Duolink ImageTool, Adobe Photoshop, Olympus Viewer, and IP Lab.

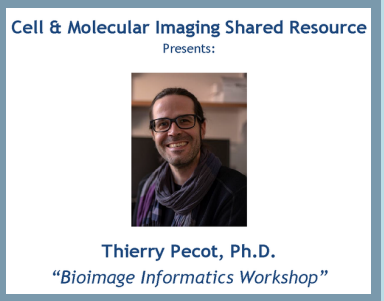
State-of-the-art Imaging Equipment Include:

AIC News: a newly purchased light-tight CO2/heating incubator for the Zeiss 880 system with movable observation window will enhance the ACI's live cell imaging capability.

- Zeiss LSM 880 NLO confocal/multiphoton microscope with Fast Airyscan super-resolution and Quasar spectral detectors
- Olympus FluoView 1200 MPE intravital multiphoton microscope
- Olympus FluoView Fv10i confocal microscope
- BD Biosciences CARV II real-time spinning disk confocal microscope
- Zeiss LSM 510 META confocal/multiphoton microscope
- Zeiss Axiovert 200M microscope
- **NEW:** A recently awarded NIH Shared Instrumentation Grant (Gooz, PI) provides funding for a BioTek "High-content, high-throughput imaging system" for drug discovery approaches.

A detailed description of equipment capability can be found at the **AIC website**.

Education: The AIC organizes the biennial **Charleston Workshop on Light Microscopy for the Biosciences (LMB)** that provides a solid introduction to the concepts and practical applications of light microscopy relevant to modern cell and molecular biology. The LMB workshop is designed for doctoral level scientists, pre-doctoral students, and high level technical personnel. No prior experience with microscopy is required.



2020 Fall Workshops include the "BioImage Analysis Workshop" by Thierry Pecot and the "Huygens Deconvolution Software" by Vincent Schoonderwoert (Scientific Volume Imaging).

Initiating a project with the Imaging Core:

An Infinity account is required for usage of this core.

The DDRC financially subsidizes the use of the AIC by its members. For details, please contact the DDRCC Center Manager, Kyu-Ho Lee.

For initial project **consultation**, please email Dr. Monika Gooz

email Dr. Gooz

Pricing details for the AIC may be found using the link below:

AIC Pricing

Equipment use can be **scheduled** using the link below:

AIC Scheduling

CDLD Core Profile: Animals Core

Suzanne Craig, DVM
Director

The CDLD Animal Models Core supports survival and non-survival surgeries for research as well as various teaching labs that include laparoscopic, endoscopic, endovascular and general surgeries. Additionally, surgical services also provide training in routine and specialized anesthetic services, such as cardiopulmonary bypass. Our main goal is to ensure that each animal receives proper care and is treated humanely, while still providing a constructive and successful research and teaching environment to DDRC investigators. Our veterinary faculty and staff can assist with all procedures required for research studies. We have experience and expertise in numerous device studies encompassing all systems.

Procedures and Services Offered by the Animals Core

General handling
Injections (multiple sites)
Surgical and aseptic techniques
Blood collection (multiple sites)
Gavage
Anesthesia– administration / support
Surgical support
Perioperative care
Xenografts
DSS administration
Training on animal handling
Colony management

· Physician training
· Conference wet labs
· Feasibility
· Product development
· Laboratory testing (hematology (CBC), blood chemistry, urinalysis, blood gas, bacterial cultures)
· Tissue sampling
· Complete Necropsies and histological analysis by a veterinary pathologist
· MRI support
· Fluoroscopy

The Gnotobiotic Animal Core

The Medical University of South Carolina Gnotobiotic Animal Core offers investigators a unique opportunity to address research questions that require the use of gnotobiotic (germfree or defined flora) and conventionalized animals. The ability to raise animals in the absence of microbes is a powerful tool to understand the relationships between animal hosts and their microbial residents. A large body of work has established that microbes shape human health by influencing biological processes such as development, physiology, immunity, and lifespan. The microbiome has also been linked to metabolic phenotypes and is intimately involved in nutrient acquisition and drug metabolism. Moreover, microbes have been implicated in the etiology of a number of diseases, including allergy, cancer, autoimmunity, and gastrointestinal disorders.

Standard Services

(covered by Facility IACUC Protocol):

- Provide germfree mice (e.g. BALB/c, C57BL/6)
- Collect tissue and/or fluid from mice that have not received treatment or manipulations
- Export germfree mice in sterile transporters to other institutions

Non-Standard Services

(must be approved under user IACUC Protocol):

- Maintain and breed newly derived germ-free mice
- Administer specialized diets
- Administer compounds (therapeutic or disease-inducing agents)
- Collect tissue and/or fluid from mice that have received treatment or manipulations

Additional Services

(subject to consultation with the Director):

- Associate germfree mice with single or multiple species or strains (commensal and/or pathogenic microbes)
- Maintain and breed defined-flora mice
- Provide quality control tests to confirm gnotobiotic status
-

For Consultations:

For further details, or to initiate a project with the Gnotobiotic Facility, please contact the core director,

Caroline Westwater, PhD

The Transgenic and Genome Editing (TGE) Core

The MUSC Transgenic and Genome Editing (TGE) Core

Alexander Awgulewitch PhD, Director

CRISPR/Cas-based genome editing technology in embryos forms the backbone for making most of these mice that included the following types of genomic modifications:

- simple KO alleles involving frameshifts through indels in targeted exons
- targeted deletions
- precise single-nucleotide/single amino acid exchanges
- generation of conditional KO (cKO) alleles by flanking a critical exon with loxP sites
- generation of cKO alleles involving distantly located loxP sites
- small reporter knock-in (KI)

The turn-around time for all of these genome editing projects is only 3-4 months at a highly competitive price ranging from $6,500 – $8,000 depending on the type of project.

The Core continues to make “conventional” transgenic mice that involve random genomic integration of diverse gene constructs, including inducible (Tet or Cre) transgenic systems.

Assisted Reproductive Technology Services (ARTS) provided by the Core include:

- sperm cryopreservation
- sperm resuscitation
- in vitro fertilization (IVF)
- embryo resuscitation
- strain rescue

For more details: visit:

Contact:
Dr. Awgulewitsch
Jan Guz
(core technician)

Enrichment Series: Upcoming Dates to Remember

October 14

Wayne Jonas, MD University of Turku, Finland

Seeing the invisible: adhesions and forces in cancer cell invasion and pluripotency.

DDRCC / CDLD / GI and Hepatology Grand Rounds: Wednesday, 7am EST

October 7

William Lee, MD University Texas Southwestern Medical Center

Update on Hepatitis E - 2020

October 21

GI and Hepatology Fellows MUSC

Clinical case presentations.

October 28

Anne Marie Lennon GI and Hepatology, Johns Hopkins Medical Institutions

Potential and challenges of screening for cancer with a blood test.

DDRCC/CDLD/ RMCB Virtual Seminar Series: Wednesday, 11 am EST

October 7

Johanna Ivaska, PhD University of Turku, Finland

Seeing the invisible: adhesions and forces in cancer cell invasion and pluripotency.

October 14

Bernd Schnabl, MD Gastroenterology, UCSD School of Medicine

Translating microbiota research into therapies for human liver disease.

October 21

Elda Grabokcka, PhD Thomas Jefferson University/Kimmel Cancer Center

Stress granules: a stress-adaptive mechanism in KRAS-driven pancreatic tumorigenesis.

October 28

Antonis Kourtidis, PhD Regenerative Medicine MUSC (works in progress)

Novel functions of cadherin complexes.

Notes from the DDRCC

A Note from the Center Manager: Citing Publications

Center Manager
Kyu-Ho Lee, MD-PhD

One of the bigger ongoing challenges for our center, particularly for our progress reports and applications for renewal, is the proper documentation of DDRC-supported papers. As Center Manager, I will be tracking publications by our Full and Associate Members. However, I need your help in ensuring that these papers cite our P30 and P20 awards.

This is most readily done **during the initial manuscript submission**. Whether or not you are the corresponding author, please make sure that the proper NIH grant numbers are entered (**P30 DK123704** or **P20 GM120475**).

Under some circumstances, grant support can be added retroactively using **MyBibliography** in NCBI. For details please contact **me** or **Teri-Lynn Herbert** at the MUSC Education Center.

Selected GI Publications by our Membership (July - Sept, 2020)

Dunbar E, Greer PJ, Melhem N, Alkaade S, Amann ST, Brand R, **Coté GA**, Forsmark CE, Gardner TB, Gelrud A, Guda NM, LaRusch J, Lewis MD, Machicado JD, Muniraj T, Papachristou GI, Romagnuolo J, Sandhu BS, Sherman S, Wilcox CM, Singh VK, Yadav D, Whitcomb DC. Constant-severe pain in chronic pancreatitis is associated with genetic loci for major depression in the NAPS2 cohort. J Gastroenterol. 2020 Jul 17. PubMed PMID: 32681239.

Sun Y, Song L, Zhang Y, **Wang H**, Dong X. Adipose stem cells from type 2 diabetic mice exhibit therapeutic potential in wound healing. Stem Cell Res Ther. 2020 Jul 17;11(1):298. PubMed PMID: 32680569; PubMed Central PMCID: PMC7368682.

Daulagala AC, Yost J, Yeganegi A, Richardson WJ, Yost MJ, **Kourtidis A**. A simple method to test mechanical strain on epithelial cell monolayers using a 3D-printed stretcher. Methods Mol Biol. 2020 Aug 14. PubMed PMID: 32789778.

Zhang L, Townsend DM, Morris M, **Maldonado EN,** Jiang YL, Broome AM, Bethard JR, **Ball LE**, Tew KD. Voltage-dependent anion channels influence cytotoxicity of ME-344, a therapeutic isoflavone. J Pharmacol Exp Ther. 2020 Aug;374(2):308-318. PubMed PMID: 32546528; PubMed Central PMCID: PMC7372917.

Pachera E, Assassi S, Salazar GA, Stellato M, Renoux F, Wunderlin A, Blyszczuk P, Lafyatis R, Kurreeman F, de Vries-Bouwstra J, Messemaker T, **Feghali-Bostwick CA**, Rogler G, van Haaften WT, Dijkstra G, Oakley F, Calcagni M, Schniering J, Maurer B, Distler JH, Kania G, Frank-Bertoncelj M, Distler O. Long noncoding RNA H19X is a key mediator of TGF-β-driven fibrosis. J Clin Invest. 2020 Aug 17. PubMed PMID: 32603313.

Blaschke CRK, Black AP, Mehta AS, **Angel PM**, **Drake RR**. Rapid N-glycan profiling of serum and plasma by a novel slide-based imaging mass spectrometry workflow. J Am Soc Mass Spectrom. 2020 Aug 18. PubMed PMID: 32809822.

Engevik MA, Banks LD, Engevik KA, Chang-Graham AL, Perry JL, Hutchinson DS, Ajami NJ, Petrosino JF, Hyser JM. Rotavirus infection induces glycan availability to promote ileum-specific changes in the microbiome aiding rotavirus virulence. Gut Microbes. 2020 Sep 2;11(5):1324-1347. PubMed PMID: 32404017.

Song L, Gou W, Wang J, Wei H, Lee J, Strange C, **Wang H**. Overexpression of alpha-1 antitrypsin in mesenchymal stromal cells improves their intrinsic biological properties and therapeutic effects in nonobese diabetic mice. Stem Cells Transl Med. 2020 Sep 18. PubMed PMID: 32945622.

CITE OUR GRANTS

FOR THE DDRCC:

P30 DK123704

This project was supported in part by NIH P30 DK123704 (*core facility*) at the MUSC Digestive Disease Research Core Center.

This project was supported in part

For queries regarding DDRCC news, membership and cores, please contact the Center Manager:

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Gastroenterology and Hepatology
Department of Medicine
CSB HE903B
96 Jonathan Lucas St
Charleston, SC 29425
(843) 792-1689
Email Dr. Lee

For queries regarding the COBRE in Digestive and Liver Disease, please contact the COBRE PI:

Stephen Duncan, DPhil
Department Chair
Regenerative Medicine and Cell Biology
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Charleston, SC 29425
(843) 792-9104
Email Dr. Duncan

Visit the DDRCC Website:
https://medicine.musc.edu/departments/dom/divisions/gastroenterology/research/labs-and-centers/ddrcc

Visit the CDLD Website:
https://medicine.musc.edu/departments/regenerative-medicine/cobre-digestive-liver-disease