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A novel approach to harvest human mucus on RepliGut® Planar platform for research related to Gastrointestinal tract and its associated disorders, along with drug delivery studies.

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INTRODUCTION

The mucus layer that lines the entirety of the gastrointestinal (GI) tract is a highly dynamic matrix and is essential for innate immunity. Mucus serves as the first line of defense by acting as a barrier against various pathogens, toxins, and foreign particles. Mucus thickness and rheology has been shown to be affected by various gastrointestinal, metabolic, and neurological disorders, including cystic fibrosis and Crohn's Disease [1,2,3,4], and plays a significant role in host defense, cancer progression, and bioavailability of therapeutics. Although a key component of many disease-states, the ability to study the role of mucus in these pathological and homeostatic conditions is hampered by the lack of availability of high-quality human GI mucus for experimentation.

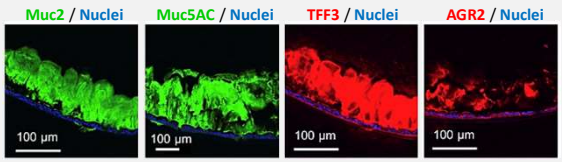
OBJECTIVES

Establish the first commercial source of native human-derived intestinal mucus for application in drug development and microbiome research. Specifically, the objectives focused on establishing (1) a robust manufacturing process; (2) commercial-scalability; and (3) to robust quality-control metrics to establish the first native human culture-derived GI mucus product that is both antibiotic- and bacteria-free. We envision that such a product would have broad applications for studies related to:

- Host-Microbe interactions and intestinal bacterial pathogenesis.
- GI immune regulation and innate immunity.
- Drug ADME/DMPK, including formulation compatibility testing.

METHODS

As described previously, human intestinal tissue was obtained post-mortem with informed consent under strict ethical guidelines established by the Organ Procurement Transplantation Network. Isolated crypts from the transverse colon were used to generate polarized GI cultures at an air-liquid interface. Immunohistochemistry was initially utilized to qualitatively assess mucus volume and composition (see figure below)[6].

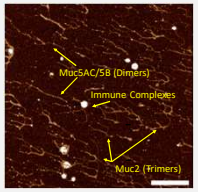


Mucus was directly harvested from the cultures without dilution, mucus was pooled from multiple plates to generate a 20 mL pooled collection, and samples were aliquoted and frozen. Detailed characterization of the banked aliquots included percent solids, proteomics, macro- and microscopic rheology, and atomic force microscopy were performed.

RESULTS

A.

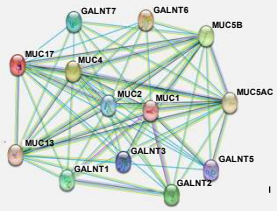
B.



C.

Rank	Protein	MW (kD)	Function
1	MUC-2	540	Mucin protein family, secreted mucin
2	MUC-5AC	586	Mucin protein family, oligomeric mucus / gel-forming
3	pIGR	83	Immunoglobulin superfamily, basal-to-apical transport
4	CECAM-5	77	Innate Immune System
5	CECAM-1	58	Innate Immune System
6	TFF3	9	Trefoil family, mucosal healing
7	MUC-5B	596	Mucin protein family, oligomeric mucus / gel-forming
8	Albumin	69	Found in pig gastric mucus
9	β2-microglobulin	14	Innate Immune System
10	Tetraspanin-8	26	Tetraspanin family, regulates biphasic release of mucus

D.



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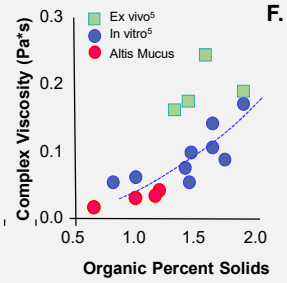

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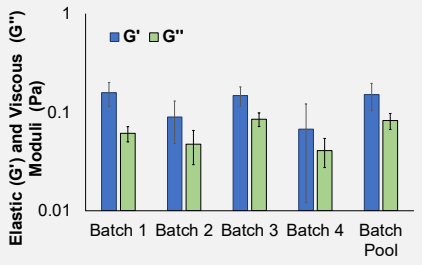

Human Mucus Harvested from ALI cultures of Intestinal Cells. (A) Sterile, pure, antibiotic-free viscous human mucus[6] from four different batches was collected from ALI cultures of human intestinal cells. **(B)** Atomic force microscopic images shows organization of the complex mucin polymeric network. **(C)** Mass spectrometry data showed that MUC2 and MUC5AC were the top two abundant proteins identified in the human mucus derived from intestinal cells. Other identified proteins contribute to complex glycosylation, innate immune/anti-microbial factors, and host-repair/homeostasis. **(D)** Interactome map of mucins and glycan-modifying enzymes from Altis mucus. **(E)** Bulk rheological data was utilized to benchmark mucus concentrations and complex viscosity in Altis samples compared to ex vivo human mucus scrapes and previously published data. **(F)** Macroscopic rheology studies determined the storage modulus (G') and loss modulus (G") at 1 hz for 4 individual batches plus the pooled batch of Altis mucus.

CONCLUSIONS

- Altis has developed a novel and reproducible process to commercially scale human intestinal mucus production using primary human stem-cell derived intestinal tissue cultures.
- Altis culture-derived mucus exhibited key hallmarks of human GI mucus inclusive of the composition, polymeric structure, proteome, and biophysical properties critical for the in vivo function of the intestinal mucus layer.
- Altis mucus can be generated in manufacturing runs of 4-6 mL per batch, with an initial pilot batch yielding 20 mL of neat mucus.
- Altis mucus has applications to characterize:
  - Formulation compatibility.
  - Drug binding, transport, and dissolution through the mucus layer.
  - Microbial growth and phenotypes
  - Mucus degradation from pathogenic bacteria
  - Drug pharmacology and target engagement

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