Mechanical stimulation promotes human intestinal villus morphogenesis *in vivo*

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INTRODUCTION

From *Xenopus* to *Mus musculus*, lower organisms have long been used to elucidate developmental mechanisms, but translating these findings into the human condition is not always straightforward. For example, in the context of the gut, the establishment of the crypt-villus axis occurs prenatally in humans, while it is a postnatal phenomenon in mice.[1,2] Several elegant studies on the development and form of the gut have been performed using a variety of observations from non-human model systems and mathematical modeling, pointing to the significance of developmental mechanical cues.[3–6] More recently, the establishment and characterization of the human intestinal organoid (HIO) model system have presented the field with new opportunities to begin to validate these findings in a human setting.[7,8] HIOs closely replicate key aspects of the tissue architecture and function of the human intestine, including the formation of villi and microvilli structures, which are essential for nutrient absorption from digested food. Recently, our group applied microengineered springs to apply mechanical strain to the luminal space, demonstrating that mechanical strain enhances enterogenesis and promotes the process of villus morphogenesis in transplanted HIOs.[9] In the accompanying figure, scanning electron microscopy of tissue engineered human intestine exhibits both circular and longitudinal smooth muscle layers and, most importantly, the resolution of mucosal folding into discrete finger-like projections termed villi, whereas the unloaded condition exhibits mucosal folding (Fig. 1). This unique structural visualization highlights the marriage of mechanics and development within the field of human intestinal tissue engineering.

METHODS

Animals

Adult immune-deficient NSG mice were used as a bioreactor for the transplantation and engraftment of HIOs as previously described (PMID 30151330). All animal experiments were performed with the prior approval of CCHMC's Institutional Animal Care and Use Committee (Signaling Pathways associated with Intestinal Stem Cell Expansion, Protocol No. 2016-0014).

Generation of human intestinal organoids

HIOs were generated from the H1 embryonic stem cell line (WiCell Research Institute, Inc.) and maintained as previously described.[7,8,10] HIOs were utilized for surgical transplantation between days 28 and 36.

Generation of nitinol springs

Nitinol springs were fabricated as previously described.[11] Briefly, nitinol wires were wrapped around a mandrel and heat set to impart the spring's desired geometry (Nitinol Devices & Components). Then, they were cut down to have a relaxed length of approximately 12 mm. Springs were compressed to half their relaxed length and secured within a gelatin capsule (Torpac, Inc.). A double layer C-A-P (Eastman Chemical Company) coating was then applied as previously described.[12]

Transplantation of human intestinal organoids and implantation of springs

HIOs were prepared and transplanted as previously described.[8,13,14] Briefly, single Matrigel embedded HIOs were transplanted into the mesentery of the mice at the most distal arcade before the ileocecal junction. A midline incision was made, and the intestines eviscerated. A small pocket was created in the mesentery and the HIO placed within, then the intestine was placed back within the abdominal cavity. Finally, the mice were closed in a double layer

FIG. 1. Scanning electron micrographs of a transplanted human intestinal organoid that was sham operated (left) and exposed to strain for two weeks *in vivo* (right). Scale bars = 100 μm.

fashion and administered appropriate analgesics. Ten to twelve weeks following engraftment, the mice underwent a secondary surgery with similar preparations. During this procedure, the engrafted HIO was incised to gain access to the lumen and a compressed encapsulated spring was inserted taking care not to damage the organoid's mucosa. The engrafted HIO was then closed in a simple, interrupted fashion. Mice were sacrificed and tissue harvested 14d postoperatively.

Scanning electron microscopy

Engrafted organoids were harvested, dissected, and fixed overnight in 3% glutaraldehyde in 0.175 M sodium cacodylate buffer with pH 7.4. Samples were buffer rinsed and postfixed in 1% osmium tetroxide in 0.175 M cacodylate buffer for 1 h at 4 °C. After an additional buffer rinse, samples were put through a graded ethanol series (25%, 50%, 75%, 95%, and 3× 100%) for dehydration. Specimens were then critical point dried in a Leica EM CPD300, stub mounted, and sputter coated 10 nm thick with 60/40 gold palladium using a Leica EM ACE600. A Hitachi SU8010 transmission electron microscope was used to image samples.

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AUTHOR DECLARATIONS

Conflict of Interest

H.M.P., J.M.W., M.M.M., and M.A.H. have Patent WO/2018/200481 and US20210115366 in process.

Author Contributions

Holly M. Poling: Conceptualization (equal); Data curation (equal); Formal analysis (equal); Funding acquisition (equal); Investigation (equal); Methodology (equal); Visualization (equal); Writing – original draft (equal); Writing – review & editing (equal). **Nicole Brown:** Investigation (supporting). **James M. Wells:** Funding acquisition (equal); Supervision (equal); Writing – review & editing (equal). **Riccardo Barrile:** Data curation (equal); Supervision (equal); Writing – review & editing (equal). **Michael A. Helmrath:** Conceptualization (equal); Data curation (equal); Funding acquisition (equal); Methodology (equal); Supervision (equal); Writing – review & editing (equal). **Maxime M. Mahe:** Conceptualization (equal); Data curation (equal); Formal analysis (equal); Methodology (equal); Supervision (equal); Writing – review & editing (equal).

DATA AVAILABILITY

The data that support the finding of this study are available within the article.

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