INNOVATION SESSION (CONT.)

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GENERATION OF AN ARTIFICIAL INTESTINE AND VALIDATION IN DOGS: A PROOF-OF -CONCEPT STUDY

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Purpose:
Short bowel syndrome remains a major pediatric problem for which intestinal transplantation represents definitive care but is fraught with complications. Investigators have turned to the development of tissue -engineered approaches to enhance absorptive capacity. We now sought to define the ability of intestinal stem cell precursors to form intact intestinal units on a novel scaffold, and then developed a large animal model to evaluate its regenerative properties *in vivo*.

Methods:
Intestinal stem cells were isolated from intestine resected from human neonates with NEC (n=5), and mice (6 weeks, n=10) expressing the stem cell reporter gene Lgr5, and were cultured in a collagen -coated scaffold derived from acellular porcine intestinal submucosa. To mimic the natural environment of the intestine, murine intestinal myofibroblasts and microbiome components were added. Whole mount confocal microscopy and mRNA expression were used to evaluate epithelial differentiation (MUC2, lys, e-cadherin, chromagranin), and recapitulation of a native intestinal architecture. We developed a canine model in which we performed rectal mucosectomy then implanted the novel scaffold into the mucosal defect (n=4), then evaluated the scaffold biweekly via colonoscopy and biopsy to determine neo-mucosal growth.

Results:
Isolated intestinal stem cells from both mice and humans formed donor-derived structures resembling villi and crypts within the collagen lined scaffold, and to differentiate into goblet, enteroendocrine, paneth and enterocytes based on immunofluorescence and RT -PCR. The addition of the microbiome and intestinal myofibroblasts induced increased differentiation into goblet and paneth cells (p=0.03). Strikingly, histologic analysis of implanted scaffold into dogs revealed a newly formed mucosal layer by six weeks with intact large intestinal crypt and villi structures on the scaffold.