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TISSUE ENGINEERING FOR INTESTINAL FAILURE: *IN VITRO* RENEWAL OF THE INTESTINAL EPITHELIUM

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Study aims: Intestinal failure (IF) is a disabling condition that impairs quality of life in children. Our research aims to develop the biotechnology of intestinal tissue engineering to create a transplantable graft demonstrating structural and functional competence. Here, we present our work in mimicking the complex intestinal stem cell niche in vitro, in the endeavour to provide an alternative to the current available treatments for IF.

Methods: Following ethical approval (REC reference 04/Q0508/79), intestinal tissue was collected and epithelial stem cells (ISCs) were isolated to establish organoid cultures. Quantitative real-time PCR analysis was performed to optimise culture conditions. Tissue was processed using detergent enzymatic treatment to derive decellularized intestinal scaffolds. The expanded ISCs were seeded onto scaffolds. Cell survival, differentiation and morphology was analysed through histology, immunostaining and electron microscopy techniques.

Main results: Intestinal crypts from the small intestine were isolated and cultured to generate three-dimensional organoids from patient of both healthy and disease backgrounds, including intestinal failure. The presence of the small molecules Rho-associated protein kinase (ROCK) inhibitor Y-27632 and CHIR99021, a glycogen synthase kinase 3β (GSK3β) inhibitor assisted proliferation of crypt cells, thereby enabling rapid expansion of each patient's cells. After decellularization, scanning electron microscopy revealed preservation of the crypt-villus axis. Intestinal stem cells were seeded onto the epithelial surface of the acellular intestinal matrices in vitro. Intestinal stem cells were found to have differentiated to absorptive lineages on scaffolds derived from healthy donors. Whereas, cells seeded onto diseased scaffold exhibited a more disordered epithelium, indicating the importance of extracellular matrix topography on governing cell morphology.

Conclusion: This work demonstrates the potential of tissue engineering for intestinal failure by combining organoid culture technology with decellularized scaffolds. Our results confirm human intestinal organoids to be an ideal source of progenitor cells to regenerate the epithelial layer of intestinal matrices.