“A Novel Organ-on-Chip Approach for Replicating Small Intestine Architecture in Celiac Disease Research with Norbornene-modified Hydrogels”

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Celiac Disease (CD) is a common autoimmune enteropathy which is triggered by wheat gluten and prolamins of rye and barley. It can lead to villous atrophy and severe comorbidities, such as lymphoma, non-alcoholic hepatitis, and infertility. The only effective treatment is lifelong adherence to a gluten-free diet. Currently, the early pathological events of CD onset are not entirely revealed partially due to the limited intestinal *in vitro* models and by a lack of reproducible animal models. Recent studies have demonstrated that the dimensionality of small intestinal villi and crypts is crucial for the differentiation of intestinal stem cells. Advances in Organ-on-Chip (OoC) technologies have created opportunities for significant breakthroughs.

Our objective is to develop a villi-mimicking OoC with relevant biomimetic architecture and functionality to advance CD research.

To achieve this, we employed 3D-printed, photocrosslinkable hydrogels obtained from norbornene-modified collagen, gelatin, and PEG-thiol as scaffolds for culturing human Caco-2 cells. These hydrogels were crosslinked using thiol-ene click chemistry and designed to replicate the high-resolution, biomimetic structure of the small intestine. The morphology and functionality of these cultures were evaluated to validate our model. Additionally, the rheological and printing properties of our modified hydrogels are assessed.

Initial cytocompatibility assessments indicated that hydrogel properties, such as stiffness, greatly influence cell proliferation and functionality, highlighting their importance for future applications. Although our modified hydrogels are compatible with Caco-2 cultures, no tight junction markers were observed in immunofluorescence assays, and their softness impaired cellular adhesion.

Using the bioorthogonal thiol-ene click chemistry to modulate the stiffness of our hydrogels we could improve cell adhesion and promote mature marker expression, facilitating the development of a vascularized villi-mimicking OoC. This advancement would enhance CD research, as most current models fail to accurately replicate the small intestine's architecture, limiting their use in studying CD pathology and potential treatments.

Celiac Disease, Hydrogels, Organ-on-Chip, Small Intestine, Villi