Synthetic Hydrogels to Study ECM Dynamics of Intestinal Organoids

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Statement of Purpose: Organoids are uniquely powerful *in vitro* models of development and disease, owing to their biomimetic 3D architecture and capacity to match tissue and organ-level functionality on the benchtop. Perhaps the best characterized is the intestinal organoid, which has seen transformative advances in an understanding of the biochemical cues that drive self-organization[1]. However, notably absent from these studies has been a thorough investigation of the role of extracellular matrix (ECM) dynamics and composition in driving organoid formation.

Studies in intestinal developmental biology have revealed a highly dynamic ECM that changes over time and with respect to cell type and tissue region[2]. Owing to difficulties in imaging tissue samples, these studies have been relatively cursory in nature. Fortuitously, concurrent to advances in organoids and 3D cell culture, significant progress has been made in high resolution 3D imaging, which has enabled characterization of cell composition and fate with spatiotemporal precision. Additionally, advances in metabolic labeling and visualization of newly synthesized proteins in 3D cell culture have recently been shown as a powerful indicator of the critical importance of cell-secreted ECM in governing cell fate and morphology[3].

Translating these imaging advances to organoid characterization has been met with unique challenges because of their relatively large size and z-depth. As such, signal attenuation is severe and requires sectioning or optical clearing. To account for these limitations and take advantage of advancements in image characterization and superresolution techniques, we have developed protocols for both metabolic labeling of newly synthesized proteins and for optical clearing and imaging through expansion microscopy (ExM)[4].

Methods: Murine intestinal organoids from Lgr5^{eGFP} mice were cultured in both natural hydrogels (Matrigel) and synthetic PEG hydrogels. Metabolic labeling was performed by culturing organoids in methionine-free media containing non-canonical amino acid (ncAA) substitutes AHA or HPG. ncAAs were incorporated into newly synthesized proteins and samples were fixed at prescribed time points. Imaging was performed by fixing samples and 'clicking' AHA or HPG with a complementary fluorescently labeled alkyne or azide. For ExM, samples were immunostained, then fluorophores were linked to the expansion gel, which was generated by a thiol/acrylate mixed-mode photopolymerization. Unlinked proteins were then digested, the biomaterial was degraded, and the photopolymerized expansion gel was immersed in diH_2O for expansion. High resolution, post-expansion imaging (PostExM) was performed by conventional confocal microscopy.

Results: Intestinal organoid growth was achieved in both Matrigel and PEG-based hydrogels. ncAA tagging revealed extracellular nascent protein production as

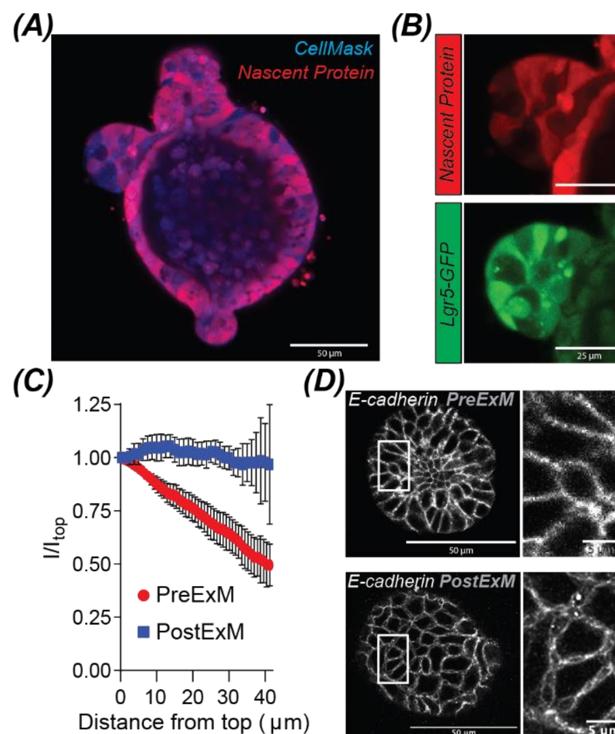

Figure 1. (A) Intestinal organoid nascent protein deposition. (B) Nascent protein deposition is dependent on cell type. (C) Signal attenuation is reduced following ExM. (D) Higher resolution imaging is possible with ExM.

organoids developed from spherical colonies to form physiologically relevant crypt structures (Fig. 1A). Interestingly, nascent protein production was dependent on stem cell fate, with less nascent protein accumulation adjacent to GFP+ intestinal stem cells, indicating cell type heterogeneity is not only critical for soluble biochemical signaling, but for inside-out cell-ECM signaling (Fig. 1B).

We next tested whether expansion microscopy could be used to obtain superresolution images of intestinal organoids, with a reduction in signal attenuation. Indeed, using cell-cell junctional E-cadherin as a model antibody, we saw significant attenuation reduction upon expansion (Fig. 1C), as well as increased resolution, as evidenced by visualization of heterogeneity in E-cadherin signal between cells, and patterns of E-cadherin at the single cell level that could not be observed without ExM (Fig. 1D).

Conclusions: Translation of novel imaging techniques enabled insight into ECM dynamics of intestinal organoids cultured in Matrigel and user-designed synthetic hydrogels. Moving forward, proof-of-concept studies using expansion microscopy will be co-opted to study cell-ECM interactions at the subcellular level to understand the interplay between cells and their local microenvironment.

References: [1]Sato, T. *Nature*, 2009. [2]Bonnans, C. *Nat Rev Mol Cell Biol*, 2014. [3]Loebel, C. *Nat Mater*, 2019. [4]Wassie, A.T. *Nat Methods*, 2018.

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