Photoinduced hydrogel network reorganization facilitates *in situ* modulation of intestinal organoid epithelial shape

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Statement of Purpose: Intestinal organoids are 3D cellular aggregates that self-organize from stem cells to recapitulate the structure and function of the intestines.[1] When encapsulated within a supportive matrix, crypts housing intestinal stem cells project off of a central, enclosed lumen, mimicking the crypt-villus architecture that is characteristic of the intestine. While these self-organizational principles endow organoid models with unparalleled structural and functional sophistication, they also create heterogeneous organoid morphologies that limit reproducibility.[2] Stochastic crypt branching produces architectures that differ from the native organ in many respects, including the size and spacing of crypts, and their cellular compositions. Such heterogeneity limits the translation of organoids towards clinical applications, including the interpretation of results from drug screens.

Organoid heterogeneity can be addressed by better understanding basic principles that influence organoid self-organization and development. For example, changes in cell shape and local epithelial curvature are observed prior to crypt budding.[3] While cell morphology is known to influence stem cell fate specification[4], the role of cell shape in directing fate specification of intestinal organoids is unknown. This question has been particularly difficult to address, as methods to control local cell shape in 3D are limited, resulting from deficiencies in programmable and adaptable materials. Here, we apply photopatterned light to allyl sulfide hydrogels[5], which undergo photoinduced network reorganization to spatially relax the hydrogel matrix surrounding encapsulated organoids. Relaxation of stress causes a disruption of the mechanical equilibrium between the expanding organoid and the compressive hydrogel, enabling a bud to protrude into the photopatterned region which locally alters the epithelial curvature. Control over pattern dimensions allows for precise and dynamic control over local epithelial curvature and cell shape, enabling investigations into the effect of epithelial curvature on crypt development *in vitro.*

Methods: Hydrogels were formed by the reaction of dibenzylcyclooctyne functionalized poly(ethylene glycol) macromers with allyl sulfide containing, diazide functionalized crosslinkers. Hydrogels were formed into 8 mm diameter, 500 μm thick disks and equilibrated with a solution containing the photoinitiator, lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP). A rheometer (TA Instruments DHR-3) with a parallel plate and light curing attachment was used to measure hydrogel stress relaxation and storage modulus as a function of irradiation time. Subsequently, intestinal organoids derived from Lgr5$^{\text{CreER-IRES-eGFP}}$ mice were dissociated into single cells, encapsulated into the allyl sulfide crosslinked hydrogels, and cultured in growth media. Cell laden hydrogels were equilibrated with LAP, and a 405 nm light from a confocal microscope (Zeiss LSM 710) was used to create shape-specific patterns proximal to the growing organoids. Fiji (ImageJ) was used to measure the organoid diameter and cell shape from immunostained images, while the Fiji plugin, Kappa, was used to measure changes in local epithelial curvature.

Results: Exposure of cell-laden hydrogels to 405 nm light initiated local bond rearrangement in the allyl sulfide crosslinks, enabling stress relaxation and bond rearrangement with minimal degradation (Figure 1a). A 405 nm laser was used to apply discrete and predictable changes in the local hydrogel properties, and ultimately the organoid shape (Figure 1b). This technique was used to pattern up to 8 buds within a single organoid (Figure 1c). Modulation of the photopatterning dimensions controlled the resulting epithelial curvature (Figure 1d). By culturing budded organoids in differentiation media, the relationship between epithelial curvature and crypt morphology can be quantified and correlated.

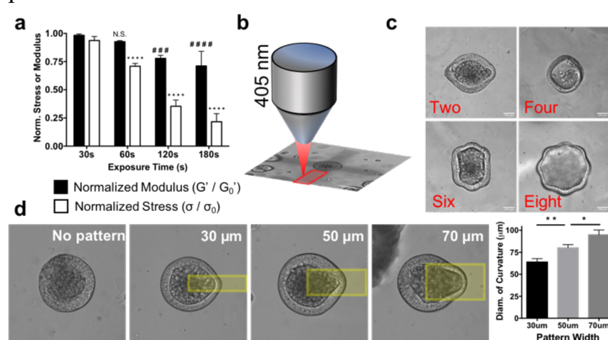

Figure 1. a Normalized stress relaxation (white) and normalized storage modulus (black) of allyl sulfide hydrogels irradiated with 405 nm light **b** Raster patterned light is applied to areas of the hydrogel adjacent to encapsulated organoids. **c** The location and number of buds can be controlled by varying the photopattern configuration. **d** Curvature of buds is dependent on photopattern dimensions.

Conclusions: Changes in local cell shape are thought to direct the morphology of developing intestinal organoid crypts. However, hydrogels used for organoid culture are incapable of modulating organoid shape *in situ*. We report the development of a photoadaptable hydrogel platform that enables such spatial control. Photopatterned light from a laser scanning confocal microscope enabled precise control over epithelial cell shape, including the location and amplitude of the epithelial cell deformation. Current experiments are focused on investigating the relationship between epithelial shape and fate specification to better understand and control processes that will lead to more homogenous intestinal organoid cultures.

References: [1](Sato T. *Nature.* 2009;459:262-265), [2](Brandenberg N. *Nat Biomed. Eng.* 2020;4:863-874), [3](Hartl L. *Dev Biol.* 2019;450:76-81), [4](McBeath R. *Dev Cell*. 2004;6:483-495), [5](Yavitt FM. *Adv Mater.* 2020; 32:1905366).

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