HHS Public Access

Author manuscript

Cell Stem Cell. Author manuscript; available in PMC 2019 January 16.

Published in final edited form as:

Cell Stem Cell. 2017 January 05; 20(1): 9–10. doi:10.1016/j.stem.2016.12.010.

Hacking the Matrix

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Abstract

Recently in *Nature*, Gjorevski et al. (2016) describe a fully defined synthetic hydrogel that mimics the extracellular matrix to support in vitro growth of intestinal stem cells and organoids. The hydrogel allows exquisite control over the chemical and physical in vitro niche and enables identification of regulatory properties of the matrix.

Derivation, expansion, and long-term culture of biopsy and pluripotent stem cell-derived tissues in three-dimensional (3D) culture (organoids) has transformed our ability to conduct in vitro research on primary tissues and holds vast promise across research and medical disciplines (Kretzschmar and Clevers, 2016; St Johnston, 2015). Organoid systems have already yielded an impressive wealth of knowledge about development, homeostasis, and disease. Given the appropriate culture environment, which includes culture within an extracellular matrix (ECM), many organoids will self-assemble into units that recapitulate some aspects of the complex tissue architecture and cellular heterogeneity found in vivo (Turner et al., 2016). However, defining the correct in vitro growth environment for diverse tissue-specific organoids has not been trivial, given the complex chemical and mechanical cues that are present in vivo.

Current approaches to grow organoids in vitro have focused heavily on the signaling cues that maintain stem cell self-renewal, which is required for long-term growth of organoids. With few exceptions, these approaches have relied heavily on animal-derived ECM, such as that harvested from Engelbreth-Holm-Swarm (EHS) mouse sarcoma cells (marketed commercially as Matrigel). However, these ECMs are poorly defined, contain numerous unknown growth factors, vary from batch to batch, and introduce significant variability, making experimental precision and reproducibility difficult. Moreover, it is unclear how much these tumor-derived ECMs mimic the actual in vivo conditions of healthy tissues. The continued reliance on such an uncontrollable and potentially non-physiological physical environment severely limits the power of organoid systems to further our understanding of biology and disease, and it greatly limits clinical and translational applications of in vitro cultured organoids.

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Exciting new research describes a fully synthetic hydrogel for growing primary mouse and human epithelial intestinal organoids in vitro (Gjorevski et al., 2016). By deconstructing the 3D physical environment and rebuilding it from the minimal essential component pieces, Gjorevski et al. created an ECM-mimetic hydrogel with controlled adhesive ligands in addition to defined and tunable mechanical and proteolytic degradation properties. Similar systems have been implemented successfully for growing transformed cell lines and for iPSC reprogramming (Caiazzo et al., 2016; Enemchukwu et al., 2016); however, the current work marks a milestone for long-term growth of primary tissue (Gjorevski et al., 2016). The researchers used Polyethylene glycol (PEG) as the core component of the hydrogels in this study and were able to empirically test individual or combinations of variables within the synthetic matrix to identify mechanical and adhesive properties critical for proper intestinal stem cell (ISC) expansion and differentiation.

While pure PEG hydrogels could produce a physical 3D support similar to that of Matrigel, Gjorevski et al. quickly found that these inert gels, completely lacking in biological matrix components, failed to support ISC expansion. Previous research had identified many of the ECM components, such as fibronectin, laminin-111, collagen IV, hyaluronic acid, and perlecan, that exist within the ISC niche at the base of intestinal crypts. Leveraging this information, the authors modified soft PEG gels, which mimicked the basic physical properties of Matrigel, with individual ECM adhesive peptides to determine those that were essential. Surprisingly, experiments showed that inclusion of any one of these ECM components in PEG hydrogels leads to the formation of spherical epithelial colonies from ISCs cultured in these gels.

To further define the absolute minimal essential chemically defined environment that supported ISC expansion and survival in PEG gels, the researchers generated gels that included only the RGD sequence (Arg-Gly-Asp) of fibronectin. This RGD sequence is the domain within the fibronectin molecule that serves as the site of cell attachment via integrins. PEG gels containing the RGD sequence peptides were found to support ISC culture in a dose-dependent manner. Further characterization of ISC colonies grown long-term in PEG RGD gels showed that Lgr5, a well-established marker of ISCs (Barker et al., 2007), was maintained even after many passages. However, the generation of differentiated cell types, such as Paneth, goblet, and enteroendocrine cells, was lacking in RGD-PEG gels compared to those grown in Matrigel.

Gjorevski et al. next set out to precisely define the mechanical properties, including hydrogel stiffness, necessary for optimal ISC growth and differentiation. Previous research has identified YAP, an effector of the Hippo signaling pathway, as a critical factor in the sensation of substrate rigidity (Aragona et al., 2013). YAP has also been identified as an essential component in organoid formation from cells originating from a wide variety of tissues/organs, and it is important for intestinal organoid formation in vitro (Panciera et al., 2016; Gregorieff et al., 2015). Using nuclear localization of YAP as a readout for proper ISC culture conditions, Gjorevski et al. identified the optimal hydrogel rigidity for ISC expansion and demonstrated that stiff gels supported Lgr5+ ISCs; however, RGD-PEG gels that optimally support ISCs did not allow for multicellular differentiation, which required a softer gel environment. In an effort to produce a dynamic hydrogel that could function to

promote ISC colony formation followed by differentiation, the team initially generated RGD-PEG gels that were degradable by matrix metalo-proteases, which failed to support long-term ISC maintenance. After testing a number of conditions, the authors produced an engineered dynamic gel that exhibited the desired properties of supporting ISCs but also allowed for differentiation of different intestinal epithelial cell types.

By combining stable PEG (sPEG) with a hydrolytically degradable PEG polymer (dPEG), a hydrogel was formed with a high initial stiffness that could degrade and soften over time at a defined rate. While this dynamic gel did allow for the physical properties necessary for organoid differentiation, the addition of laminin-111 was required in addition to the RGD peptide. With this final formulation of RGD-laminin-111-sPEG:dPEG gel, ISCs expand in a rigid stem cell promoting environment to form spherical colonies that, upon gel softening, could differentiate into organoids with a full complement of mature epithelial intestinal cells.

The combined results of this study define a functional method for the establishment and long-term culture of ISCs and generation of intestinal organoids in a fully defined chemical and physical environment. This overcomes several challenges, including the use of animal products, which is a significant clinical challenge due to potential zoonotic contamination, and it opens the door to organoid research directed specifically for clinical applications. The ability to both define the chemical environment and fully control and tune stiffness and degradation will both minimize experimental variables and increase the precision with which experimental perturbations can be carried out. This study also marks a major step forward by providing experimental tools to cleanly study the influence of physical properties on a complex biological system derived from primary human or mouse tissue, and it provides a solid framework for innumerable research and clinical applications in the future.

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