SYNTHETIC HYDROGELS

Defined matrices bring IBD to 3D

An immune cell population enriched in inflamed gut tissue is shown to play a role in driving CD44⁺ intestinal organoid proliferation, while also regulating extracellular matrix deposition and remodelling in a synthetic hydrogel platform.

Bauer L. LeSavage and Sarah C. Heilshorn

Three-dimensional (3D) organoid models of intestinal tissue are seeding a new perspective on gut development and disease. Organoids are defined as 3D assemblies of tissue-specific cell types that self-organize into physiologically relevant morphologies. These multicellular structures are currently being used to study developmental processes and disease progression in a dish, enabling unprecedented possibilities for complex genetic and environmental manipulation. For example, intestinal organoids have recently been fabricated into microfluidic-based cocultures with parasites to model infection, or with other non-epithelial cell types including myofibroblasts and macrophages[1]. Intestinal organoids have also been used to model viral infections, including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[2]. Additionally, intestinal organoids with 'flipped' apical-out polarity have been developed by regulating matrix cues, enabling the unique study of host–pathogen interactions without the need for a complex experimental set-up[3]. Despite these advancements, mechanistic understanding of the roles that these supporting cells and pathogens play in intestinal development and disease are not well understood, primarily owing to limited accessibility of rare (particularly human) cell types as well as limited alternatives to animal-derived matrices that preclude mechanistic studies of how cocultured cell types influence the extracellular matrix (ECM). Overcoming these limitations, Geraldine Jowett and colleagues illustrated the influence of type-1 innate lymphoid cells (ILC1) on intestinal organoids in an in vitro model of inflammatory bowel disease (IBD) and used a custom-designed matrix to demonstrate ILC1-mediated ECM remodelling[4].

ILC1 are a rare immune cell population, enriched in IBD, known to coordinate rapid responses to tissue damage; however, the explicit role these cells play in regulating intestinal epithelial phenotype and matrix reorganization is largely unknown. In this work, the authors discovered an unforeseen relationship between ILC1 and gut organoids — ILC1-specific secretion of transforming growth factor beta-1 (TGFβ1) drives CD44 expression and proliferation of intestinal epithelium. Interestingly, CD44 upregulation was found to be variant-specific (CD44v6), although the overall impact of this variant on downstream signalling remains unclear. Clinical relevance of their platform was realized upon generation of human induced pluripotent stem cell (hiPSC)-derived intestinal organoids and subsequent coculture with meticulously purified human ILC1. Excitingly, their human

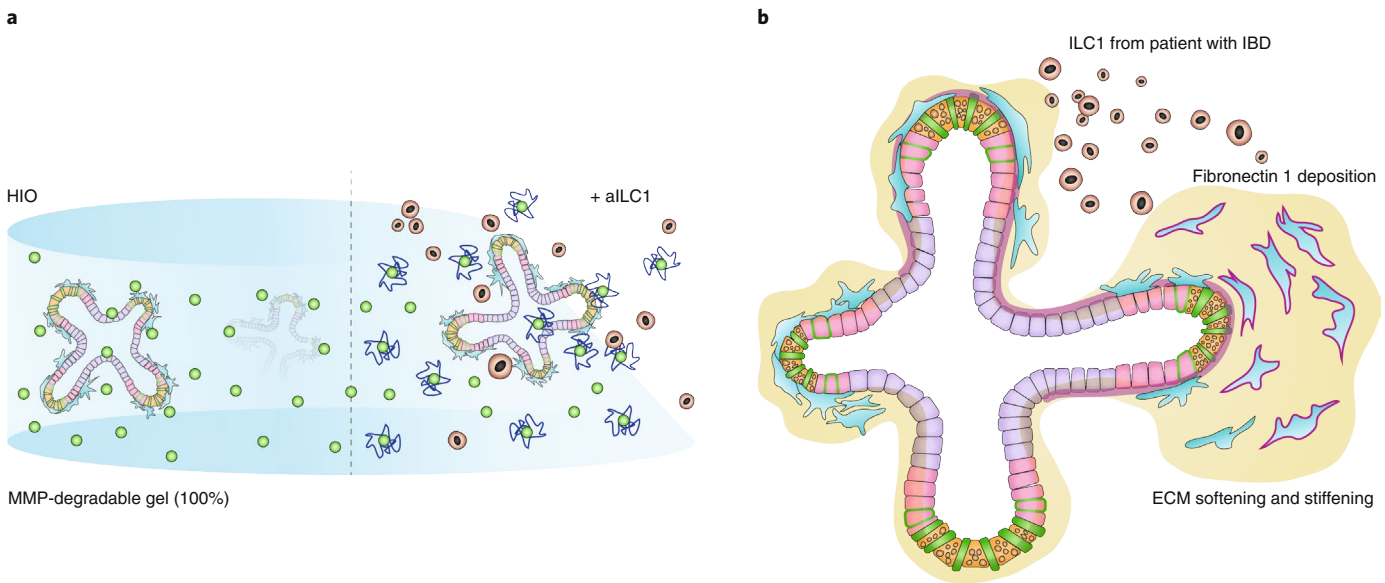

Fig. 1 | ILC1 support human intestinal organoid matrix remodelling within synthetic hydrogels. a, Schematic of human intestinal organoids (HIOs) cultured within a synthetic hydrogel indicating matrix degradation in the presence of ancillary ILC1 (aILC1). Upon matrix degradation, gel-encapsulated microparticles (green in schematic) transition from a trapped, immobile state (left) toward Brownian motion (right), which was quantified using particle tracking microrheology. **b**, Schematic of an HIO cultured with ILC1 that stimulate the enhanced deposition of fibronectin around the organoid and also lead to MMP-mediated matrix degradation with resultant ECM softening and stiffening. Credit: Adapted with permission from ref. [4] Springer Nature Ltd.

model corroborated their initial findings in murine samples and was validated through analysis of IBD patient-derived intestinal tissue biopsies that also showed enriched CD44v6 expression in both intestinal epithelium and neighbouring mesenchymal cells. As researchers move toward modelling complex diseases using organoid cocultures, the use of primary human tissue sources is a necessary criterion for reproducing patient-specific biology with meaningful clinical impact. As highlighted in this work, this step forward will often require the careful purification of exceedingly rare cell types, further accelerating the need for robust tools and platforms to characterize and culture human cell types of interest.

Beyond the influence of ILC1 on the intestinal organoids, the authors also noticed ILC1-mediated ECM remodelling; however, animal-derived matrices, such as the Matrigel hydrogels initially used in this study, have limited tunability and contain ill-defined matrix components. To better model ILC1–matrix interactions, the authors generated a family of fully-synthetic polyethylene glycol (PEG)-based 3D matrices with tunable matrix degradation. External mechanical measurements suggested that ILC1 could simultaneously induce matrix softening and stiffening via matrix metalloproteinase (MMP)-mediated remodelling (Fig. 1). Interestingly, ILC1 were also found to drive fibronectin deposition and subsequent matrix stiffening by peri-organoid fibroblasts, which was also observed in patient biopsies of IBD.

This study is a compelling example of exploiting synthetic materials and organoids to answer a defined and disease-relevant biological hypothesis. Previous seminal studies have developed similar synthetic matrix platforms to identify key parameters that support human intestinal organoid culture, including matrix degradation and remodelling. For example, PEG-based synthetic matrices were developed to support the growth and transplantation of hiPSC-derived intestinal organoids in a regenerative medicine model[5]. Synthetic PEG-based materials have also recently been designed to support the first primary human intestinal organoid cultures, which may enable future patient-specific disease modelling[6]. These recent studies have reaffirmed the importance of using engineered matrices and human cell types to make reproducible, clinically relevant organoid models.

Over the last decade, matrix remodelling, which includes matrix degradation, matrix reorganization and nascent matrix deposition, has become increasingly recognized as a key parameter in regulating cellular biology. To gain mechanistic understanding of these interactions, new strategies to design synthetic materials with exquisite control over matrix degradation will be essential. Common biomaterial strategies to control matrix degradability include use of enzyme-degradable peptides within the hydrogel, which is the method employed here, or the incorporation of hydrolytically degradable chemical moieties into the polymer network[7]. Alternatively, control of matrix remodelling has also been achieved through manipulation of the hydrogel network connectivity and through the use of reversible, dynamic crosslinks[8].

Characterization techniques that can accurately and non-destructively measure dynamic matrix remodelling are essential for decoding temporal changes that cells impart on their surrounding matrix. Current techniques, including macrorheology and atomic force microscopy, often only probe bulk or surface matrix properties and therefore are not able to report on local, cellular-level heterogeneity within the 3D material. Additionally, these methods are often destructive, preventing multi-day analysis of dynamic processes. To address these limitations, microrheological techniques, including video particle tracking used in this work, have facilitated the non-destructive study of local matrix remodelling with improved spatiotemporal accuracy[9]. As an alternative approach, recent progress suggests that future innovations in materials design will enable the development of synthetic matrices with embedded sensors for real-time analysis of local biomechanical properties[10]. The importance of nascent, cell-produced matrices within synthetic hydrogels is also becoming increasingly apparent, and new techniques were recently reported that enable the in situ characterization of this dynamic aspect of matrix remodelling[11]. Overall, the parallel application of both novel materials design and robust characterization methods will enable future insights into how dynamic cell–matrix remodelling plays a critical role in tissue development and disease.

The results presented here underscore the multifaceted roles that rare cell types can play in driving intestinal cell phenotype and ECM remodelling in gut development and disorder. Importantly, the experimental framework of combining reproducible, synthetic matrices with human-sourced cells can be applied to many different tissue types for human-specific disease modelling, enabling identification of new therapeutic targets with clinical impact. ❐

Bauer L. LeSavage[1]✉ and Sarah C. Heilshorn[2]✉

[1]Department of Bioengineering, Stanford University, Stanford, CA, USA. [2]Department of Materials Science & Engineering, Stanford University, Stanford, CA, USA.

✉*e-mail: lesavage@stanford.edu; heilshorn@stanford.edu*

Published online: 27 January 2021
https://doi.org/10.1038/s41563-020-00904-1

References

1. Nikolaev, M. et al. *Nature* **585**, 574–578 (2020).
2. Lamers, M. M. et al. *Science* **369**, 50–54 (2020).
3. Co, J. Y. et al. *Cell Rep.* **26**, 2509–2520.e4 (2019).
4. Jowett, G. M. et al. *Nat. Mater.* https://doi.org/10.1038/s41563-020-0783-8 (2020).
5. Cruz-Acuña, R. et al. *Nat. Cell Biol.* **19**, 1326–1335 (2017).
6. Hernandez-Gordillo, V. et al. *Biomaterials* **254**, 120125 (2020).
7. Gjorevski, N. et al. *Nature* **539**, 560–564 (2016).
8. Madl, C. M. et al. *Nat. Mater.* **16**, 1233–1242 (2017).
9. Schultz, K. M., Kyburz, K. A. & Anseth, K. S. *Proc. Natl Acad. Sci. USA* **112**, E3757–E3764 (2015).
10. Mok, S. et al. *Nat. Commun.* **11**, 4757 (2020).
11. Loebel, C., Mauck, R. L. & Burdick, J. A. *Nat. Mater.* **18**, 883–891 (2019).

Competing interests

The authors declare no competing interests.