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Tooling up against mechanobiological orthodoxy

Increasingly physiological in vitro models and techniques for measuring forces at multiple scales are enabling unanticipated findings in tissue mechanopathology.

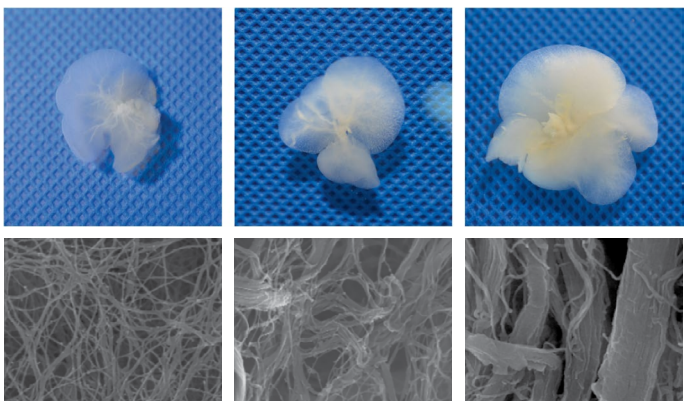

Fig. 1 | Decellularized liver extracellular matrices obtained from mice with increasing extents of liver fibrosis. Top: bright-field images; bottom: electron micrographs. Figure reproduced with permission from the Article by Du and colleagues, Springer Nature Ltd.

The big toolbox of molecular biology makes it routine to explore the biochemistry underlying disease, especially in vitro: a molecular pathway can be severed, a receptor can be blocked, and a gene can be knocked in or knocked out. However, when studying how physical forces impact disease, fewer tools are available, and they are less refined. Microenvironmental forces acting on a cell's surface cannot be precisely blocked by antibodies, the effects of shear flow on tissue may not easily be disentangled from those of compression, and there is no general recipe to trace the mechanics of the cellular microenvironment to phenotypic outcomes. This is perhaps unsurprising; after all, mechanical forces act across scales, and are unspecific from a biochemical viewpoint.

Yet advances in biomaterials chemistry, in physiological modelling of disease factors in vitro and computationally, and in force-measurement techniques are nevertheless facilitating the discovery of disease-relevant phenomenology. This issue of *Nature Biomedical Engineering* highlights five examples of largely unexpected findings prompted by clinical observations, and enabled by the rational design of microphysiological models and the clever use — through combination or adaptation — of biochemical and biophysical measurement techniques.

Biomineralization — the mineral-producing process involved in the formation of new bone — may regulate the formation of bone metastases of breast tumours. Indeed, observations of patients with breast cancer indicated that bone-mineral density affects the risk of developing bone metastases. The assumption has been that mineralization-induced rigidity drives metastatic progression via increased cell-adhesion forces. Yet, this process has been understudied because it is difficult to physiologically adjust the mineral content of bone tissue in vitro without affecting the phenotypes of the tumour cells on it. Claudia Fischbach, Lara Estroff and colleagues now report the use of collagen-based matrices with adjustable intrafibrillar mineralization (alongside traction force microscopy and biochemical assays) to study how bone-matrix mineralization affects the phenotype of breast cancer cells seeded on them. They found that, contrary to expectations, mineralization induces a more quiescent phenotype in the cancer cells (a finding that they validated in mice and with computational analyses of patient data). As highlighted by Cheyenne Ernst and Hai Wang in an accompanying News & Views article, further development of the model matrices to incorporate osteoclasts and immune cells and to capture effects from age-related factors, osteoporosis and vitamin D deficiencies may allow for new insights.

In another study, David Mooney and colleagues leveraged click chemistry to design collagen matrices for which stiffness and viscoelasticity could be independently adjusted, and used the matrices to generate functionally distinct T cell populations. Specifically, they showed that tissue viscoelasticity can be used to direct T cell function (via the activator-protein-1 signalling pathway, a regulator of the activation and fate of T cells), which confirmed observations that the phenotypes of T cells are linked to the properties of their surrounding tissue microenvironment. This association is often unappreciated.

The collagen matrix in fibrotically scarred liver tissue is aberrantly crosslinked, altering its stiffness and viscoelasticity and exacerbating fibrosis. Yet drugs targeting the enzymatic formation of crosslinks between collagen molecules have been ineffective in patients with cirrhosis. By using liquid chromatography–tandem mass spectrometry, Yanan Du and colleagues quantified the degree of crosslinking of the matrix of decellularized cirrhotic liver samples from patients and from mouse models of liver fibrosis (Fig. 1). They found that advanced glycation end-products (products of the glycation of proteins, lipids or nucleic acids resulting from their exposure to sugars) mediate the non-enzymatic crosslinking of collagen fibrils. By reconstructing such pathologically crosslinked collagen matrices in vitro to recapitulate the in vivo characteristics of the matrices of livers with late-stage fibrosis, the researchers uncovered that collagen fibrils crosslinked by advanced glycation end-products resist macrophage-mediated remodelling. In a News & Views article, Orit Kollet and Irit Sagi suggest that the matrices could be used to further study the responses of other immune cells to crosslinking by

advanced glycation end-products, and to assess the efficacy of drugs for the inhibition of extensive crosslinking.

It has long been assumed that the foreign-body response to implants is a reaction of the body to the chemical and mechanical properties of the implanted material. Geoffrey Gurtner and co-authors now show that forces scaling with body size can also drive the development of such pathological responses (and that they are mediated by *RAC2*, a signal-transduction molecule involved in the recruitment and activation of immune cells). This discovery was enabled by the authors' finite-element modelling of a vibrating silicone implant in mice designed to impart human-like levels of mechanical forces independently of implant size and chemistry. By showing that fibroblast activity can also be modulated by higher tissue-scale forces via mechanoresponsive immune cells, the authors' findings challenge the prevailing view of fibroblasts as the primary drivers of fibrosis. Moreover, as pointed out by Georgios Theocharidis and Aristidis Veves in a News & Views article, finite-element modelling "could be harnessed clinically for each individual's morphological characteristics as input to gauge the predicted amount of stress, and to facilitate the choice of potential therapeutic strategies for preventing the development of excessive foreign-body responses."

Solid stresses — which arise from forces generated and transmitted by a tissue's solid components — are a physical biomarker of tumours. The stresses contribute to a tumour's hypoxic status, impede the infiltration of drugs into tumour tissue (partly because the stresses compress blood vessels and lymphatic vessels) and promote cancer cell invasiveness. But the cellular responses underlying the generation of solid stresses are hard to investigate. Hadi Nia and colleagues used intravital measurements (via confocal microscopy, two-photon microscopy and optical coherence microscopy) of deformable and fluorescently labelled beads injected into breast tumours in vitro, in post-mortem tissue and in mice to compare solid stresses at the single-cell and tissue scales in primary and metastatic tumours. They found that the transmission of solid stresses is scale-dependent: the stresses experienced by individual tumour cells were notably lower than the stresses measured at the tissue scale. This may explain how cancer cells can survive at high solid stresses. The authors also found that the solid stresses in metastases of breast cancer in the lungs of mice were substantially higher than those in primary tumours, and that the stresses were higher in the peritumoural regions than within and outside the tumours. In a News & Views article, Bashar Emon and M. Taher Saif note that "integrating the technique with biomechanical sensors that can measure cellular forces and matrix remodelling may reveal whether there is a synergy between solid stresses, cell contractility and stromal stiffening."

Collectively, these research studies highlight that the exploration of the complex interplay between biophysical and biochemical processes in pathophysiological processes benefits from the rational development of experimental and computational models that leverage knowledge and techniques in microphysiological cell-culture systems, biomaterials, tissue engineering and mechanobiology. Such tooling may even force the remodelling of accepted knowledge.

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