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PERSPECTIVE

BIOTECHNOLOGY and BIOENGINEERING

Hydrogels as Extracellular Matrix Mimics for 3D Cell Culture

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ABSTRACT: Methods for culturing mammalian cells ex vivo are increasingly needed to study cell and tissue physiology and to grow replacement tissue for regenerative medicine. Two-dimensional culture has been the paradigm for typical in vitro cell culture; however, it has been demonstrated that cells behave more natively when cultured in three-dimensional environments. Permissive, synthetic hydrogels and promoting, natural hydrogels have become popular as three-dimensional cell culture platforms; yet, both of these systems possess limitations. In this perspective, we discuss the use of both synthetic and natural hydrogels as scaffolds for three-dimensional cell culture as well as synthetic hydrogels that incorporate sophisticated biochemical and mechanical cues as mimics of the native extracellular matrix. Ultimately, advances in synthetic–biologic hydrogel hybrids are needed to provide robust platforms for investigating cell physiology and fabricating tissue outside of the organism.

Biotechnol. Bioeng. 2009;103: 655–663.

KEYWORDS: hydrogels; tissue engineering; 3D cell culture; biomaterials

et al., 1993). Furthermore, 2D experiments have given rise to seminal findings in the dynamic relationship between cell function and interactions with the cellular microenvironment. Discher and coworkers demonstrated that the differentiation of human mesenchymal stem cells (hMSCs) is dependent on the mechanical stiffness of the 2D culture platform (Engler et al., 2007, 2006). Further, Ingber and coworkers have shown that the degree to which a cell is mechanically distended on a 2D scaffold dictates relative growth and apoptotic rates (Chen et al., 1997; Singhvi et al., 1994). Thus, in vitro cell constructs can be used to examine how epigenetic factors affect physiological phenomena; however, recent work has shown that cells often exhibit unnatural behavior when they are excised from native three-dimensional (3D) tissues and confined to a monolayer.

In their groundbreaking work, Bissell and coworkers demonstrated that human breast epithelial cells develop like tumor cells when cultured in two dimensions, but revert to normal growth behavior when cultured in 3D analogs of their native microenvironment (Petersen et al., 1992). Also, enhanced chondrogenesis of embryonic stem cells has been observed when cells are cultured in 3D embryoid bodies as compared to monolayer culture (Tanaka et al., 2004).

- 2023 IF = 3.5
- 2022 citations (Web of Science, > 3200 Google Scholar)
- Very(!) highly cited

- A perspective, not a research article
- Very clear title
- Very clear abstract

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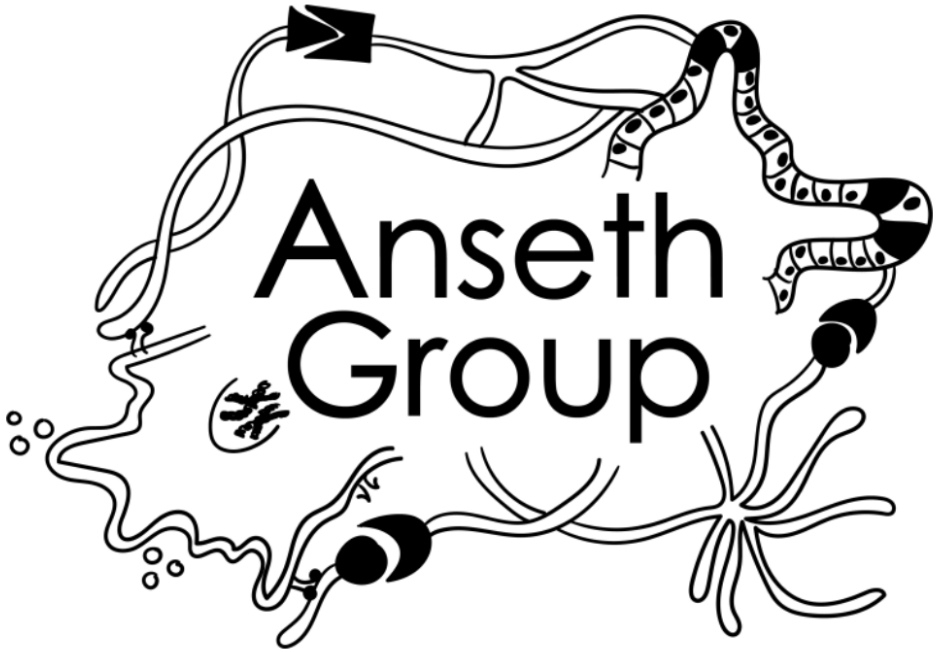

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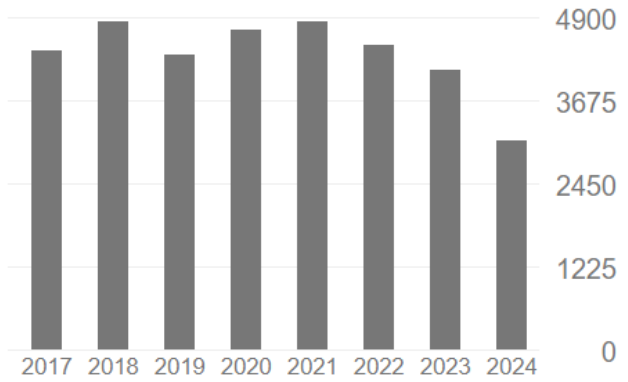

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The research in our lab is highly multidisciplinary, as our group members come from diverse backgrounds ranging from biology to engineering. We are involved in the design of biomaterial scaffolds with controlled biochemical and mechanical properties for 3D cell culture, the production of nanoparticles for drug delivery applications, 3D printed scaffolds for tissue regeneration and the development of hydrogels for thermal stabilization of complex biomolecules.

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Mechanical memory and dosing influence stem cell fate C Yang*, MW Tibbitt*, L Basta, KS Anseth Nature materials 13 (6), 645-652	1174	2014

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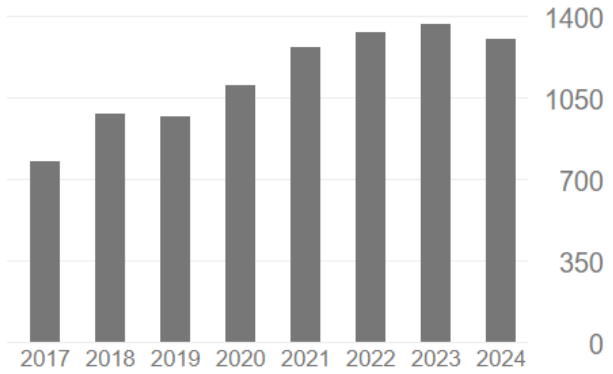

ABSTRACT: Methods for culturing mammalian cells ex vivo are increasingly needed to study cell and tissue physiology and to grow replacement tissue for regenerative medicine. Two-dimensional culture has been the paradigm for typical in vitro cell culture; however, it has been demonstrated that cells behave more natively when cultured in three-dimensional environments. Permissive, synthetic hydrogels and promoting, natural hydrogels have become popular as three-dimensional cell culture platforms; yet, both of these systems possess limitations. In this perspective, we discuss the use of both synthetic and natural hydrogels as scaffolds for three-dimensional cell culture as well as synthetic hydrogels that incorporate sophisticated biochemical and mechanical cues as mimics of the native extracellular matrix. Ultimately, advances in synthetic–biologic hydrogel hybrids are needed to provide robust platforms for investigating cell physiology and fabricating tissue outside of the organism.

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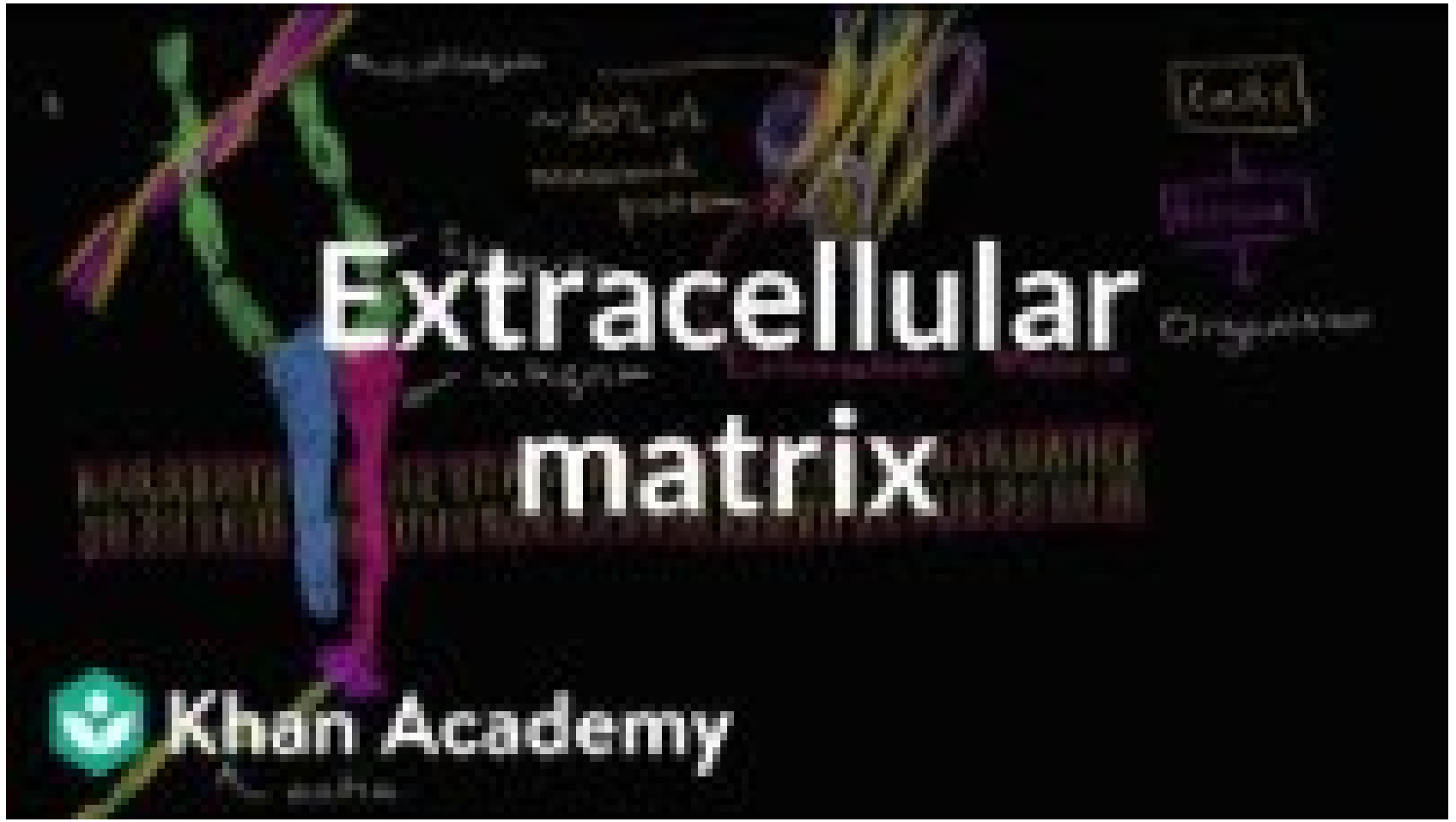
Main points:

1. We need methods to culture cells ex-vivo
2. 2D culture has been the paradigm for in vitro cell culture but cells behave more natively in 3D
3. “Permissive, synthetic hydrogels” and “Promoting, natural hydrogels” are becoming popular but they have limitations
4. Perspective will discuss both types of hydrogels AND synthetic hydrogels that incorporate biochemical and mechanical cues to better mimic native ECM (synthetic-biologic hybrids)
5. Perspective: advances in synthetic-biologic hybrids are needed to study cell physiology and tissue fabrication outside of the organism


Extracellular
matrix
Khan Academy

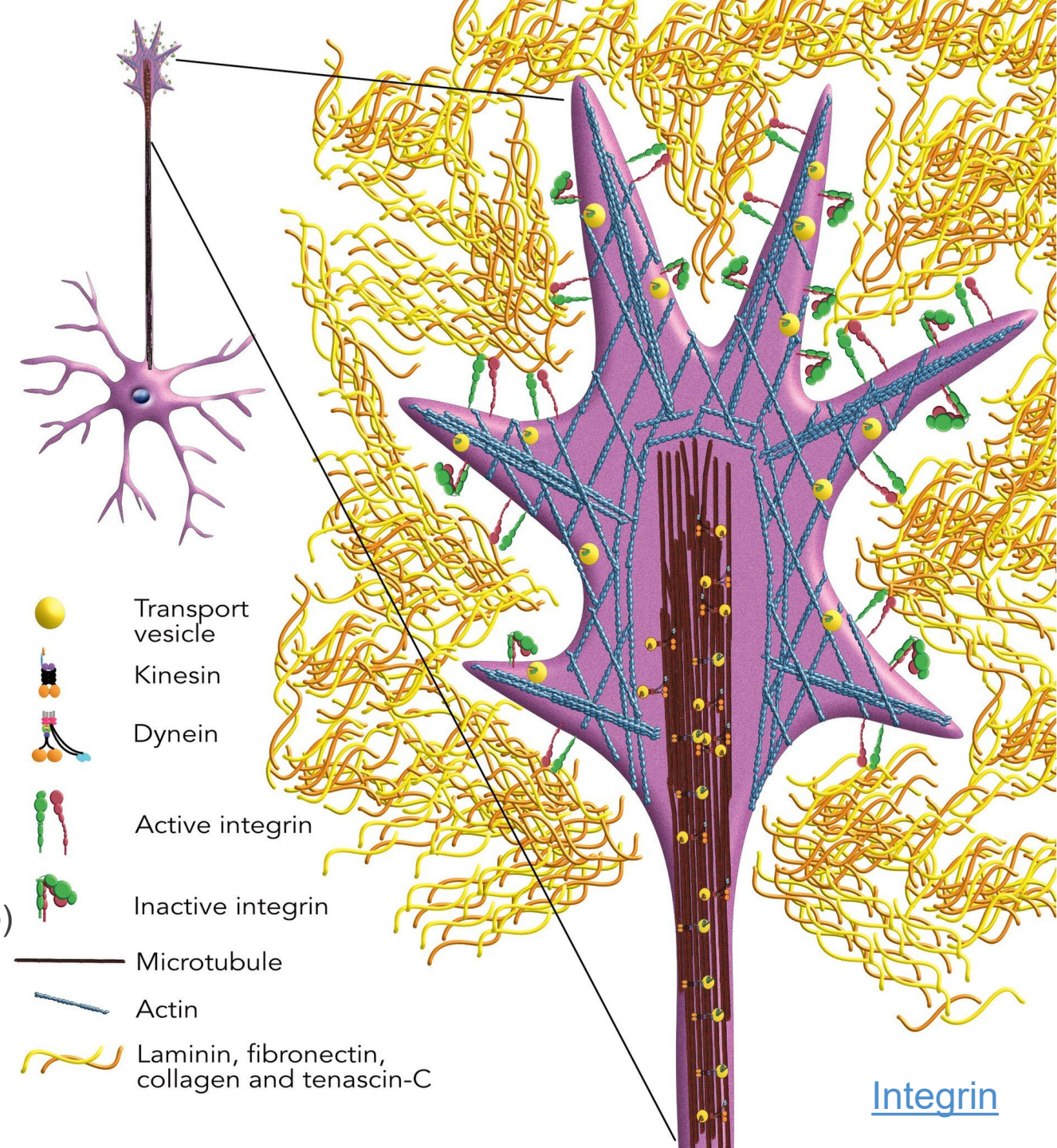
What is an integrin?

Transmembrane receptor protein, with roles in:

- Cell adhesion: helps cells stick to ECM or to other cells
- Signal transduction: transmit signals from ECM to cells
- Mechanotransduction: transmit mechanical stimuli into biochemical signals

Binding (important for a lot of things):

- Integrins bind to specific ligands
- Inactive ad active states
- Active state changes conformation to better bind ligands
- Binding often based on recognition of a specific amino acid sequence (like RGD motif – Arg-Gly-Asp)

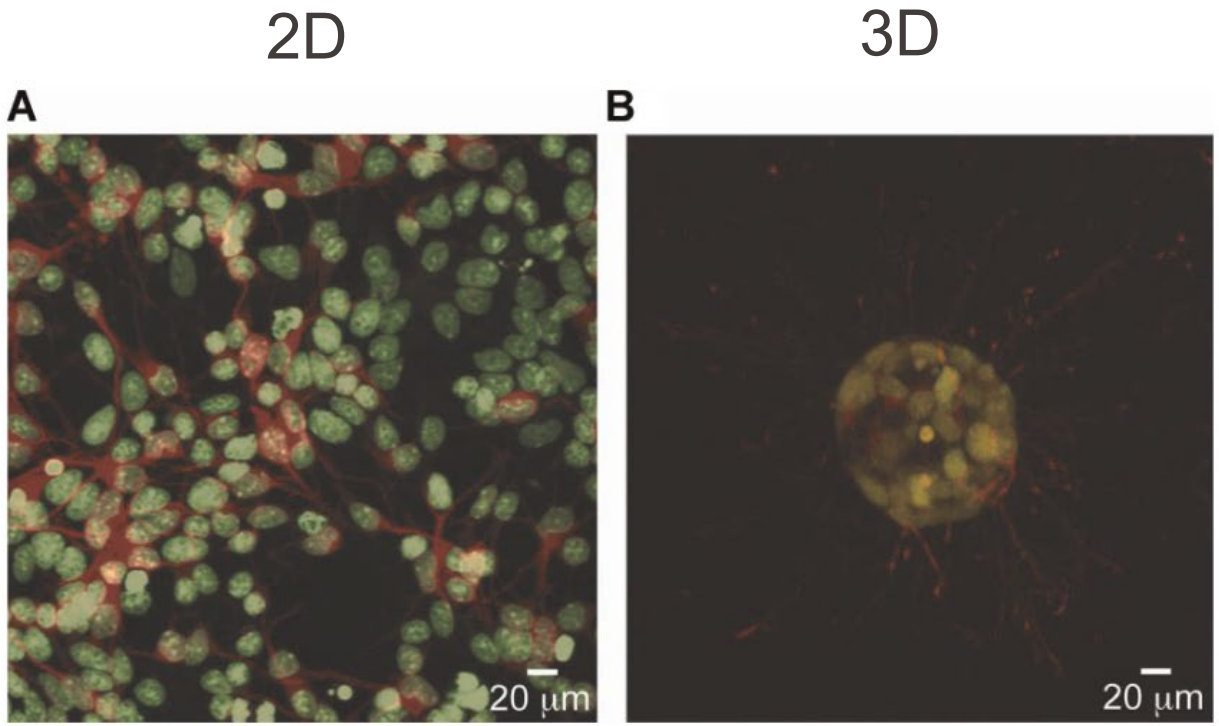


Integrin

The native ECM (from the paper)

- In vivo, the ECM provides a complex and bioactive scaffold that provides mechanical support while directing cell adhesion, proliferation, morphology, and gene expression
- Functional scaffolds for 3D cell culture should mimic the prototypical ECM
- The ECM backbone is a complex architecture of fibrous proteins, like collagen, provides mechanical properties
- Cells sense these mechanics through binding events between integrins on the cell surface and binding motifs of ECM proteins
- Hydrated polyglycans fill the interstitial voids of the backbone, sequestering soluble growth factors, small integrin-binding glycoproteins and matricellular proteins
- Cells **dynamically remodel** the microenvironment to allow different functions
- Remodeling is needed is needed for tissue homeostasis and becomes more pronounced in pathological and developing states
- ECM composition varies significantly from tissue to tissue, but **understanding how remodeling functions is an important design criteria for 3D cell culture platforms**

The context matters



- It is outdated to think of cellular scaffolds as passive vehicles
- Cell microenvironment contributes to the cell phenotype
- The differences in cell behavior due to 2D and 3D cultures come from perturbations in gene expression related to how the cell experiences its microenvironment
- Morphology influences cellular processes

Figure 1. Cells experience a drastically different environment between 2D and 3D culture. For instance, neural cells cultured in monolayer (**A**) are constrained to extend processes in the plane. Cell bodies are stained green and β-tubulin in axonal extensions is stained red. When cultured within hydrolytically degradable poly(ethylene glycol) based hydrogels (**B**) the same cells form neurospheres and extend processes isotropically in three dimensions. Images taken by M.J. Mahoney.

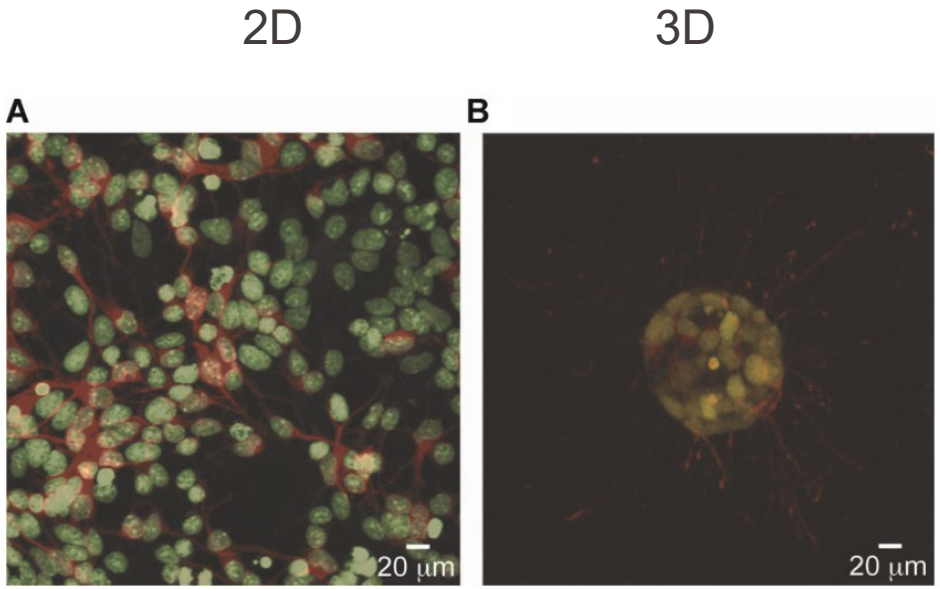
The context matters

In 2D culture

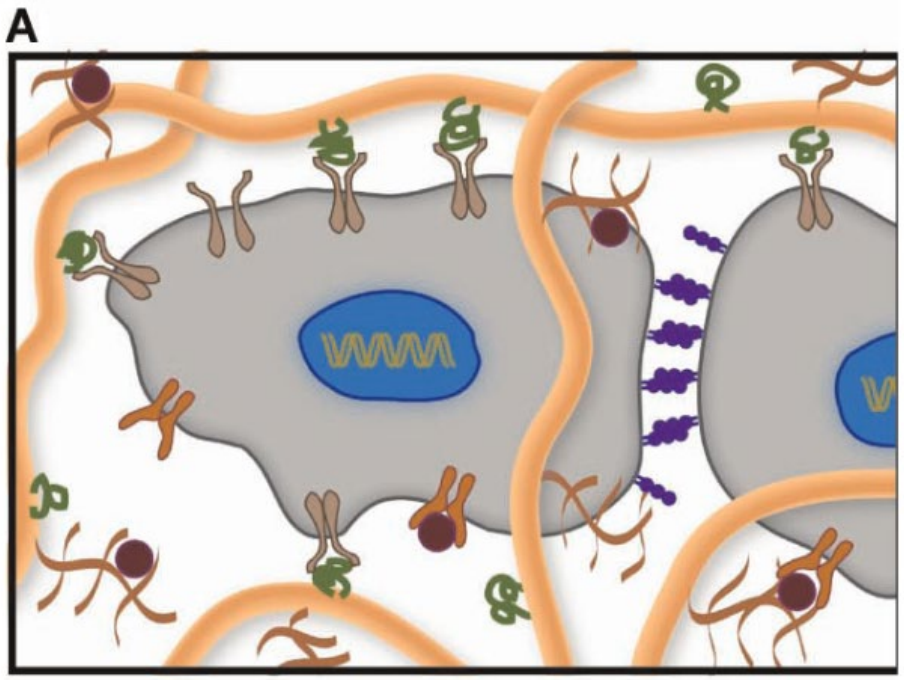
- Cell is confined to a planar environment
- Complex morphologies seen in vivo are not possible
- only a part of the cell is exposed to the ECM and neighboring cells, and the rest of the cell is exposed to the bulk culture media.

This can lead to:

- *Unnatural*, polarized integrin binding and mechanotransduction, both of which affect intracellular signaling and phenotype
- Little to no resistance to migration from the surrounding ECM

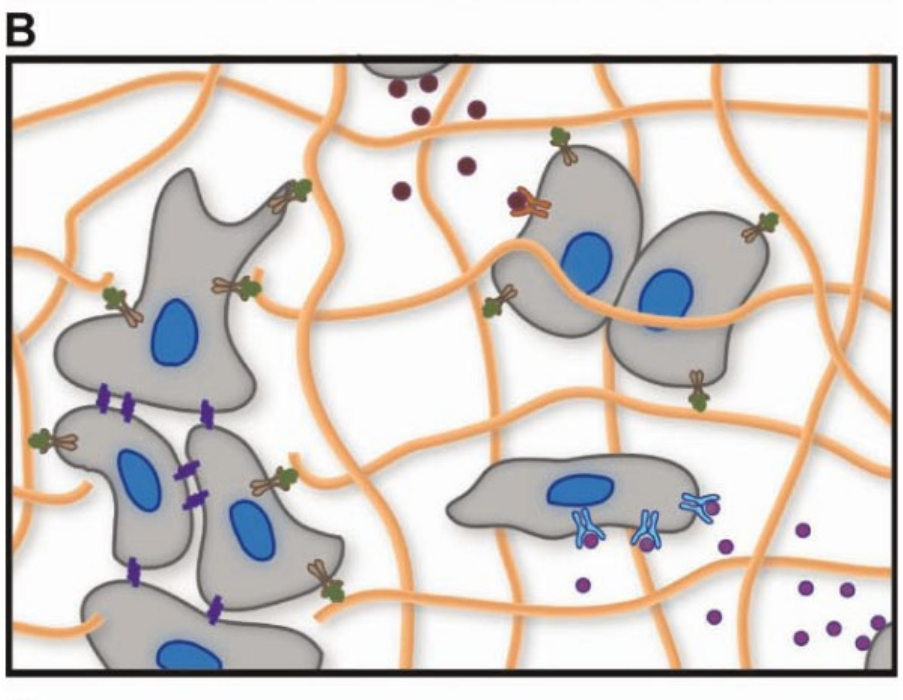


The native ECM



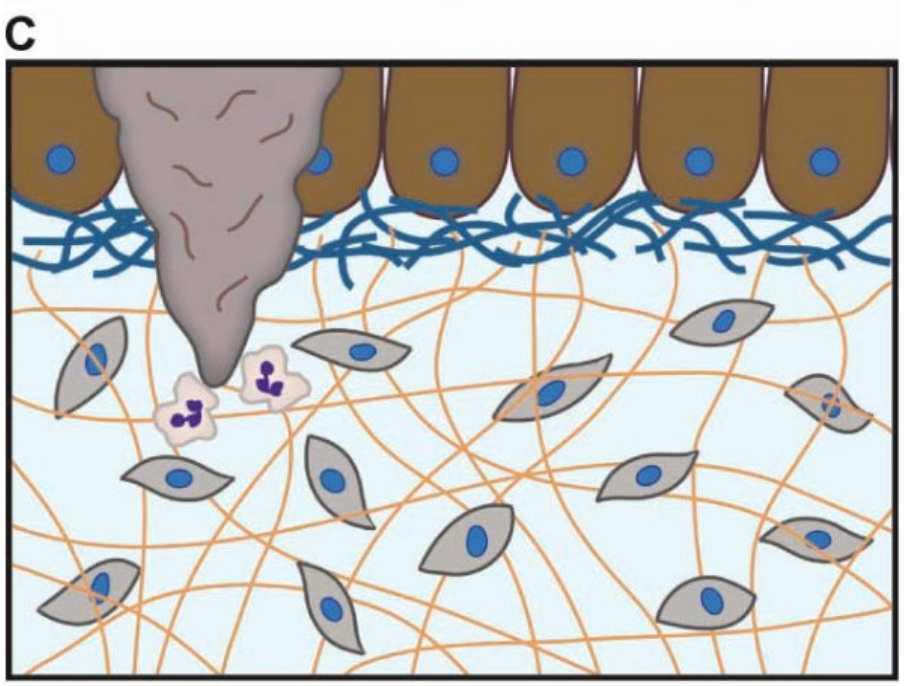
- Integrin-binding with ECM proteins
- Growth factor sequestration
- Cell-cell contacts

The native ECM



- Migration (critical to wound healing, tissue regeneration, and cancer metastasis)
- 10s to 100s of microns

The native ECM



- Tissue homeostasis, development, and wound healing
- Microns to centimeters

How to get all these features in a synthetic ECM?

Permissive vs. promoting hydrogels (SOA)

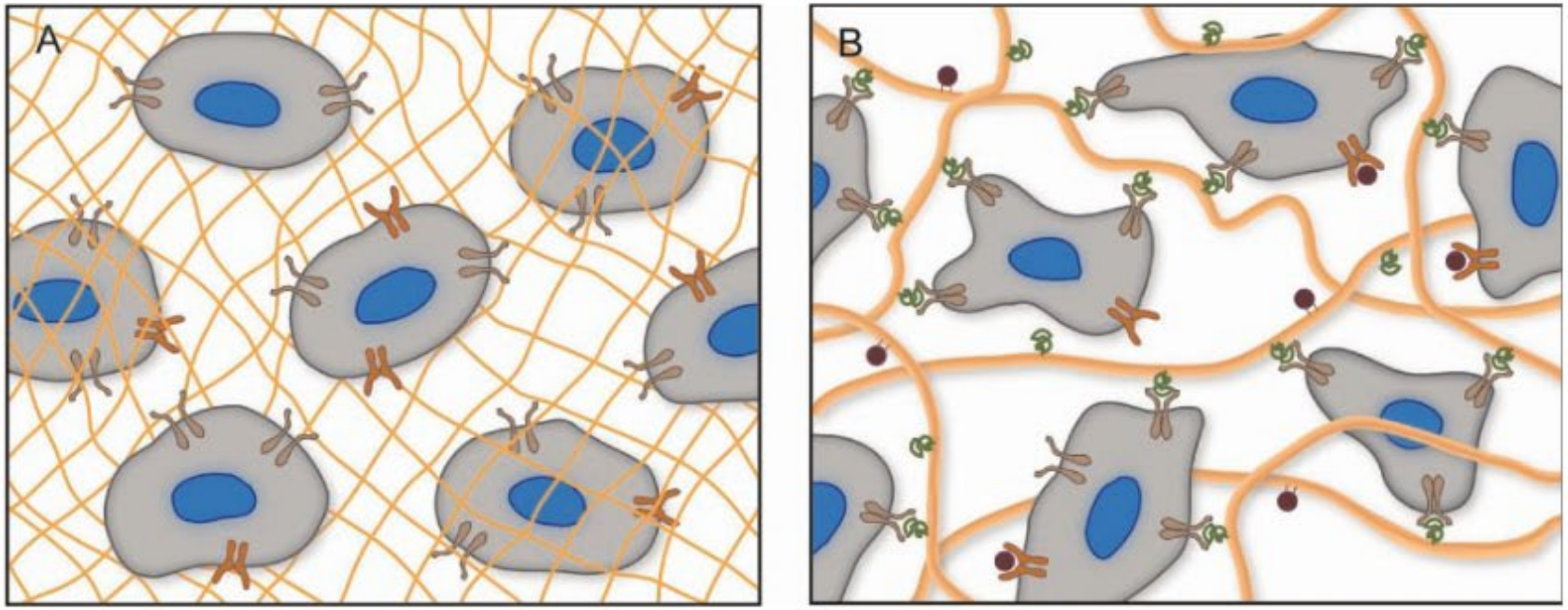
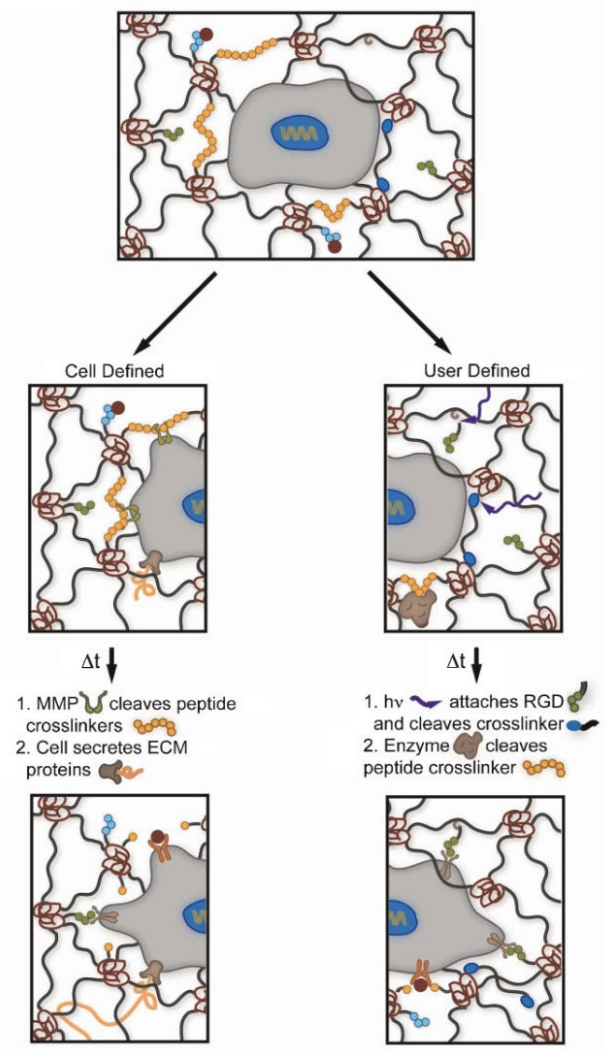


Figure 2. Permissive hydrogels (**A**) composed of synthetic polymers (yellow mesh) provide a 3D environment for culturing cells; however, they fail to activate integrins (brown) and other surface receptors (orange). The synthetic environment simply permits viability as cells remodel their surrounding microenvironment. On the other hand, promoting hydrogels (**B**) formed from naturally derived polymers present a myriad of integrin-binding sites (green) and growth factors (red) coordinated to the ECM (yellow fibers), which direct cell behavior through signaling cascades that are initiated by binding events with cell surface receptors.

Bridging the gap



Bridging the gap with **synthetic-biologic hydrogels**:

- Well-defined, orthogonal chemistries
- Cell or user-defined regulation of material properties to emulate the dynamic native ECM environment
- Both cell and user-defined may be needed

Conclusions

- 3D is better
- Synthetic hydrogel to mimic native ECM requires understanding the cell's native environment: how cell's interact with, remodel and migrate through the ECM
- To get biologically relevant conclusions from in vitro cell culture, critical matrix factors need to be incorporated into the 3D environment
- For tissue development, it might be advantageous to allow cells to dictate the changes in their own environment as they do in vivo
- To study specific cell-ECM interactions, ***user defined control of mechanical and biochemical properties*** can be useful to test complex hypotheses
- Designing an ECM mimic will likely rely on multiple orthogonal chemistries