Environment for life (Tibbitt group) MSE 493

Prof. Tiffany Abitbol

2025

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).

- IF = 3.6
- 2009
- > 3400 citations
- About 200 citations/year over 16 years

- A perspective, not a research article
- Very clear title
- Very clear abstract
- This tackles the “materials” part – what kind of environment do we need to culture cells?

About the authors

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Translate

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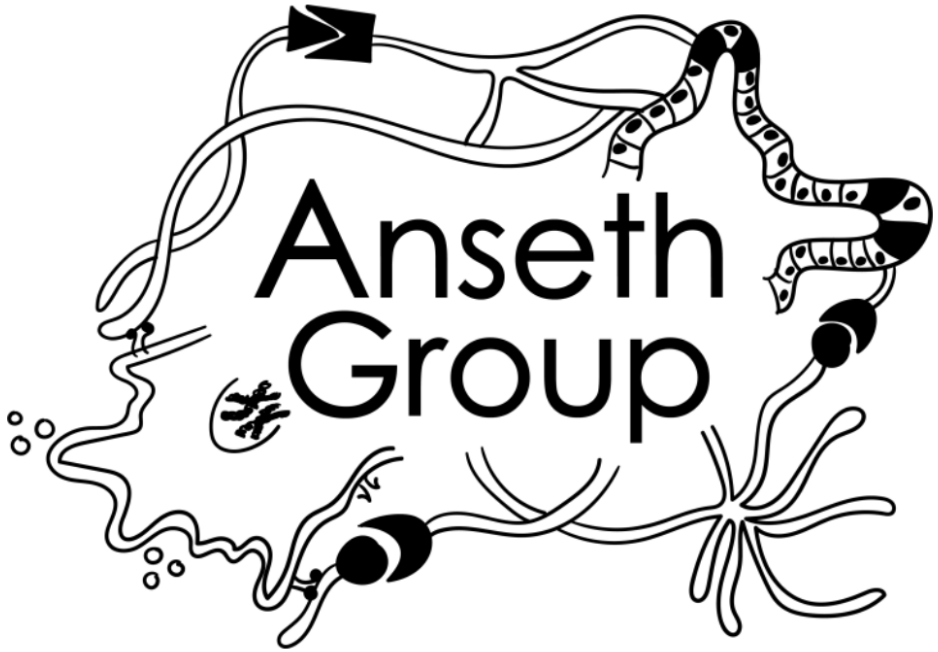

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TITLE	CITED BY	YEAR
Hydrogels as extracellular matrix mimics for 3D cell culture MW Tibbitt, KS Anseth Biotechnology and bioengineering 103 (4), 655-663	3479	2009
Photodegradable hydrogels for dynamic tuning of physical and chemical properties AM Kloxin, AM Kasko, CN Salinas, KS Anseth Science 324 (5923), 59-63	2039	2009
Mechanical properties of hydrogels and their experimental determination KS Anseth, CN Bowman, L Brannon-Peppas Biomaterials 17 (17), 1647-1657	1534	1996
Photoinitiated polymerization of PEG-diacrylate with lithium phenyl-2, 4, 6-	1498	2009

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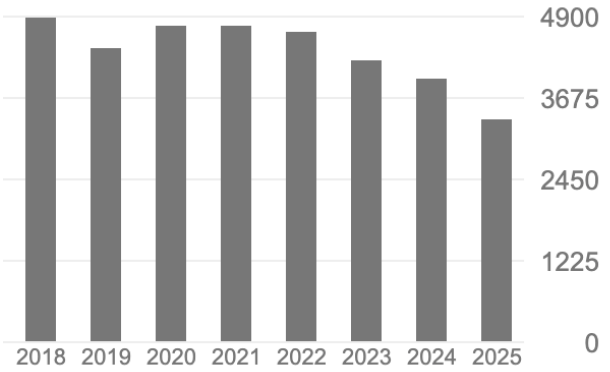

https://en.wikipedia.org/wiki/Kristi_Anseth

Welcome to the Macromolecular Engineering Lab!

These are the members of the Macromolecular Engineering Lab

Mark W. Tibbitt

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macromolecular engineering hydrogels biomaterials medical devices mechanobiology

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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	1293	2014
Emerging frontiers in drug delivery MW Tibbitt, JE Dahlman, R Langer Journal of the American Chemical Society 138 (3), 704-717	1066	2016
Self-assembled hydrogels utilizing polymer–nanoparticle interactions	601	2015

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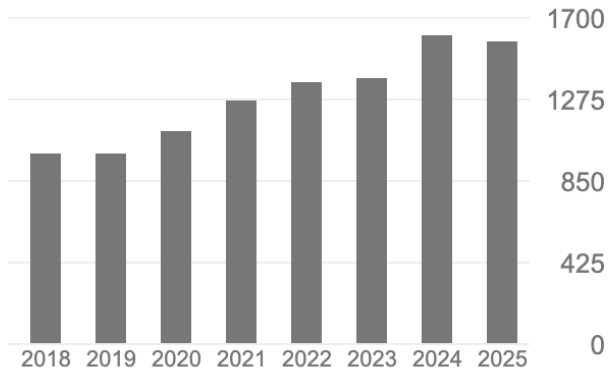

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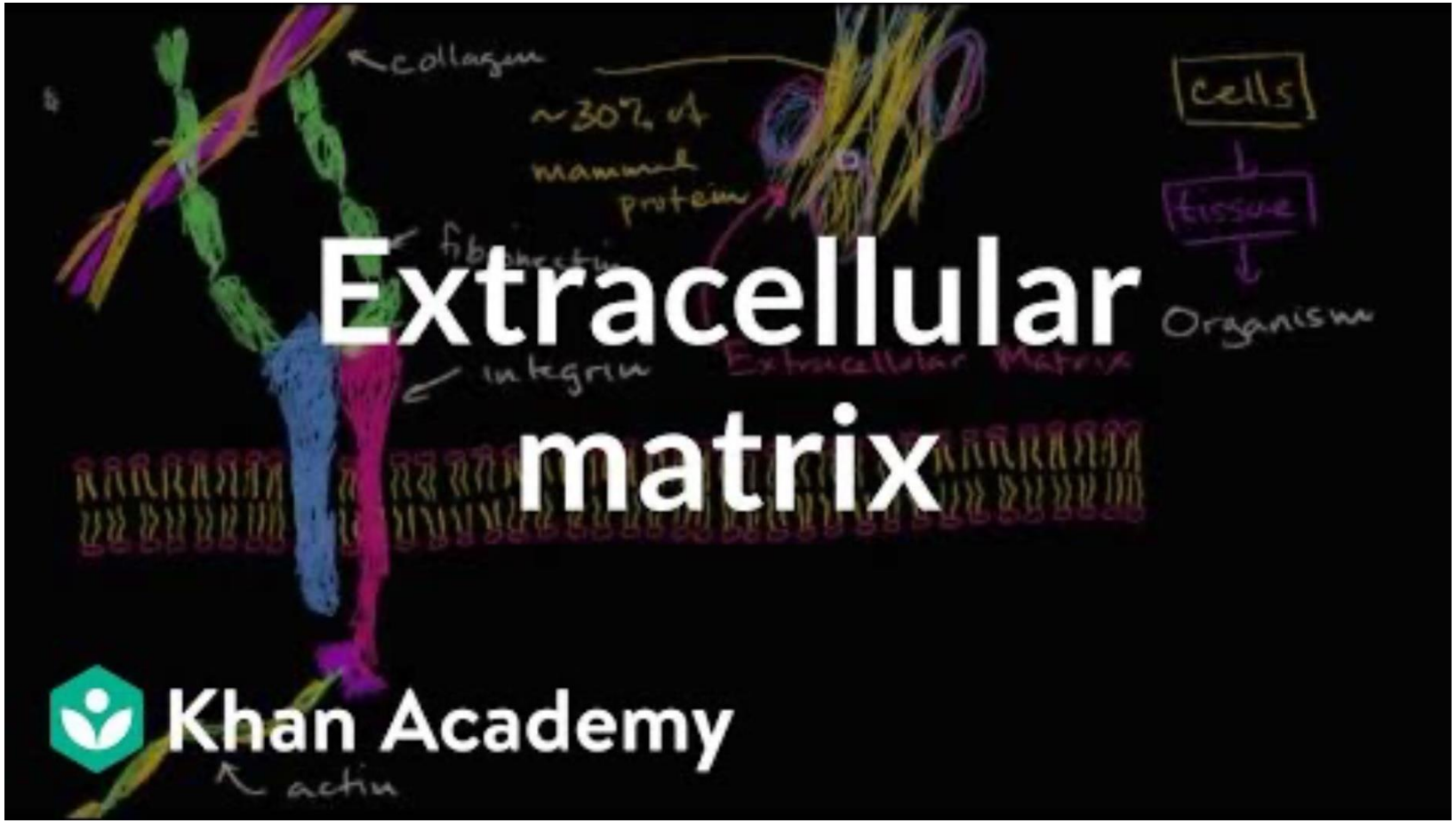
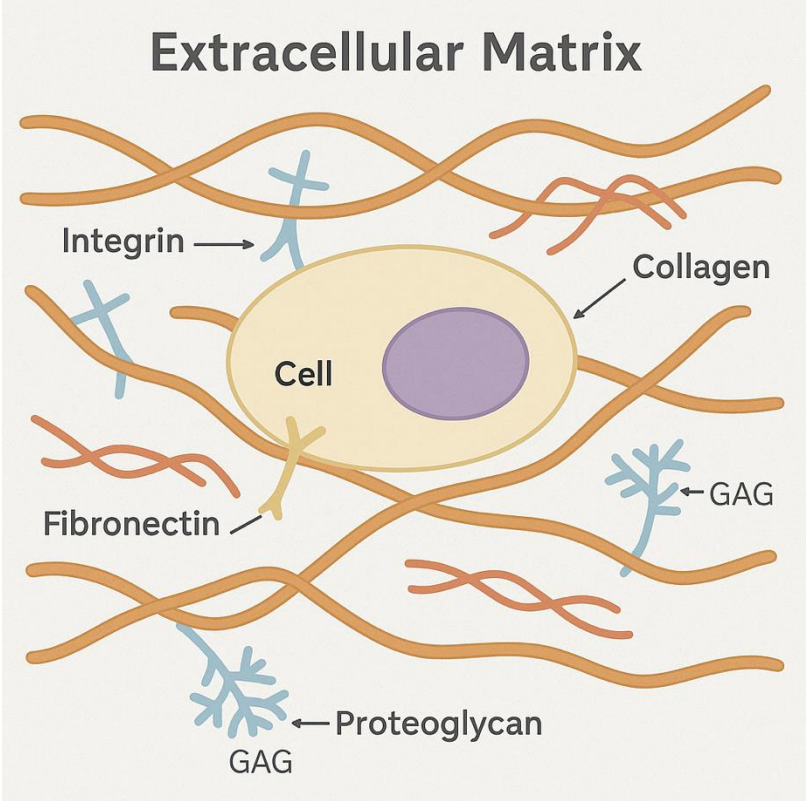


ECM video

What is the ECM?

- A network of macromolecules surrounding cells in tissues and organs, providing structural support (scaffold) and biochemical signaling to regulate cell behavior
- Made up of the proteins and polysaccharides secreted by cells: structural proteins (e.g., collagen & elastin), adhesive glycoproteins (fibronectin, laminin) to connect cells to the matrix (these are the proteins that bind to integrin – see next slide), & GAG that trap water, growth factors and ions
- Key functions: 3D scaffold, adhesive, signaling to regulate cell growth, migration, and differentiation, dynamic remodeling – constantly produced and degraded to adapt to tissue changes, barrier and filter
- E.g., Bone ECM = very stiff from mineralized collagen; brain ECM = rich in hyaluronic acid = soft and hydrated, etc.,



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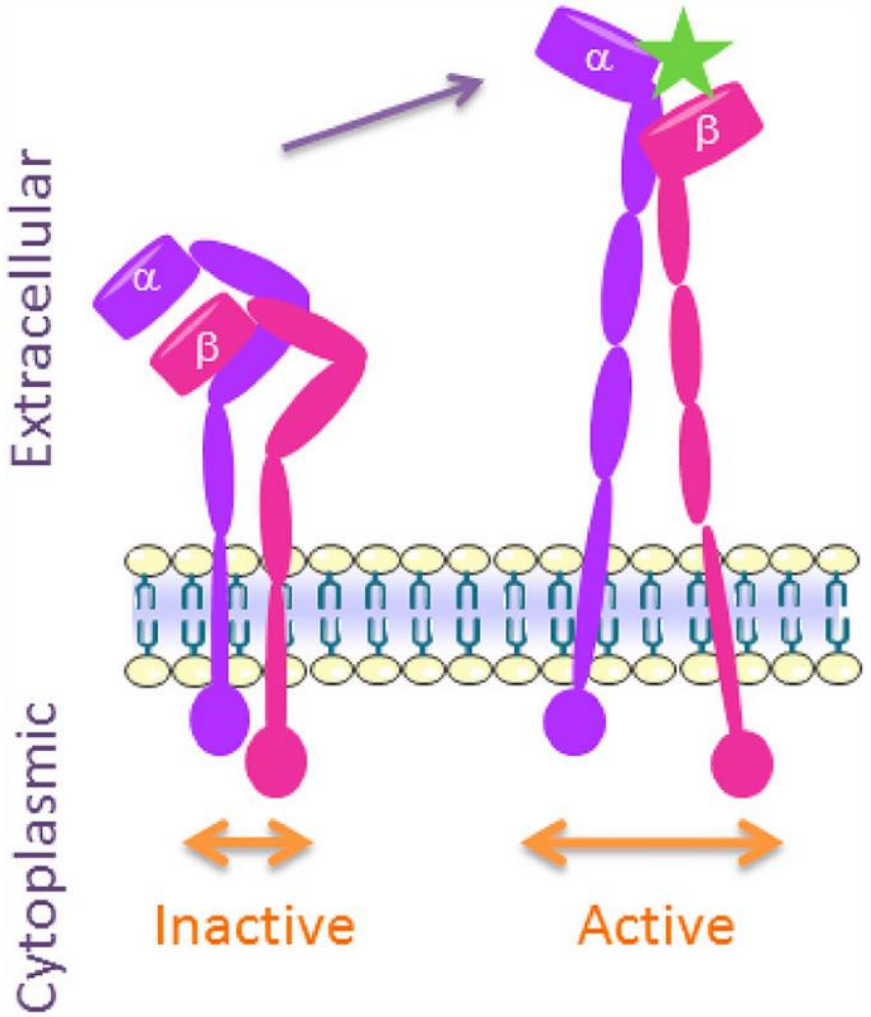
What is an integrin?

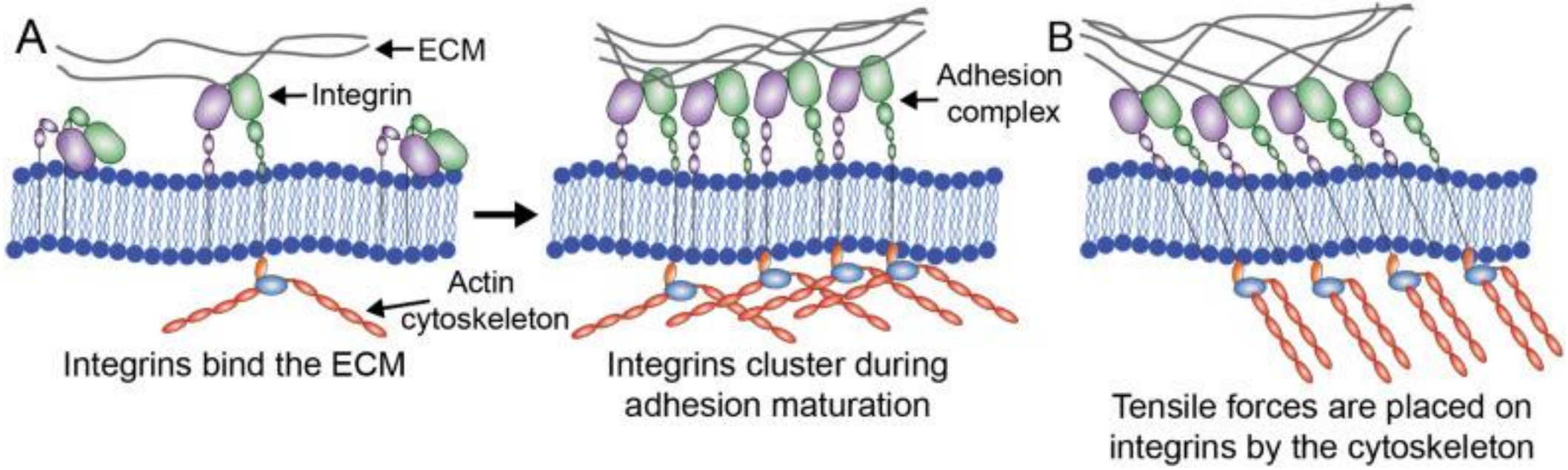
- Transmembrane receptor protein that **connects the cell's cytoskeleton (inside) to the extracellular matrix (outside)**
- Made up of a heterodimer with alpha and beta subunits, many combinations – each recognize a different ECM protein
- They enable **cell adhesion** to ECM proteins like fibronectin and collagen
- Upon binding, integrins cluster and trigger intracellular cascades that regulate cell shape, proliferation, migration, and differentiation: this process is called **mechanotransduction** – how cells sense and respond to their physical environment
- In synthetic hydrogels, cells need integrin-mediated anchoring points to attach, spread, and organize, OTHERWISE, they stay rounded and and inactive because they can't interact with their surrounding matrix



An example

doi.org/10.1016/j.actbio.2025.04.037

"Cells sense matrix properties through dynamic protein assemblies, such as focal adhesions, where integrins bind the local matrix and serve as mechanical links between the ECM and the cytoskeleton."



(a) Cells adhere to the ECM using integrins, which cluster to form focal adhesions

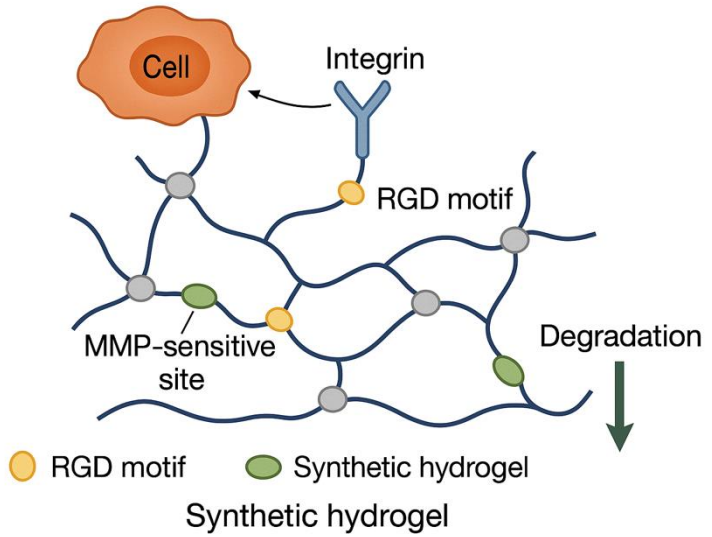
(b) Mature focal adhesions connect to the actin cytoskeleton, allowing cells to exert forces on the surrounding matrix

RGD & MMP sensitive sites

- **RGD** motif is a short peptide sequence (Arg-Gly-Asp) that is recognized by integrins
- Can be added to synthetic hydrogels to provide specific integrin binding sites
- Controls how cells adhere, spread, and form focal adhesions

- **MMP-sensitive peptides** are short sequences that can be cleaved by MMPs (matrix mettaloproteinases)
- MMPs are enzymes secreted by cells that locally degrade the matrix, allowing remodeling and invasion

- **In a hydrogel:**
- RGD provides attachment points of the cell to the polymer
- MMP-sensitive peptides provide scissors to cut and move through the polymer hydrogel



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Main points of paper:

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 – **2D vs. 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

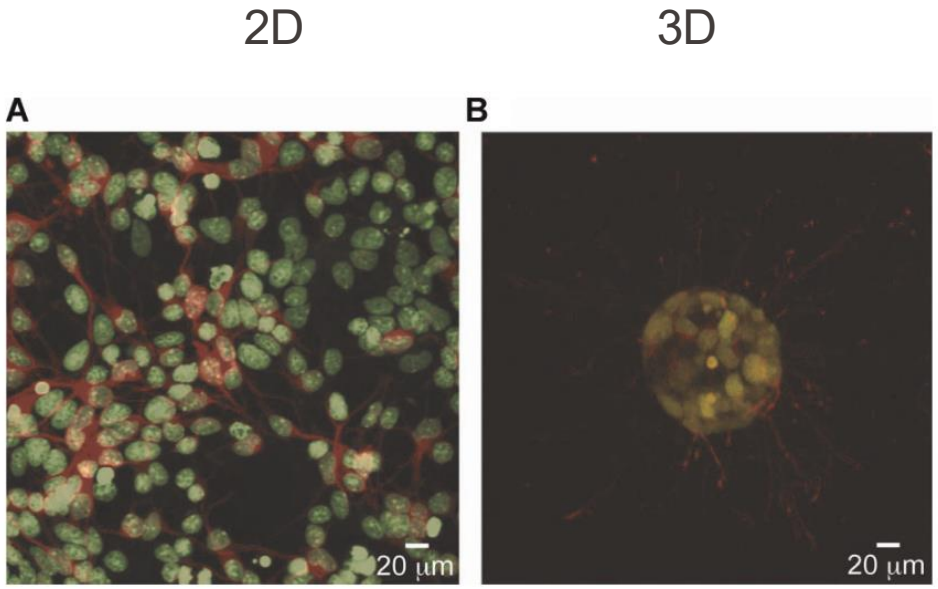
The context matters

In 2D culture

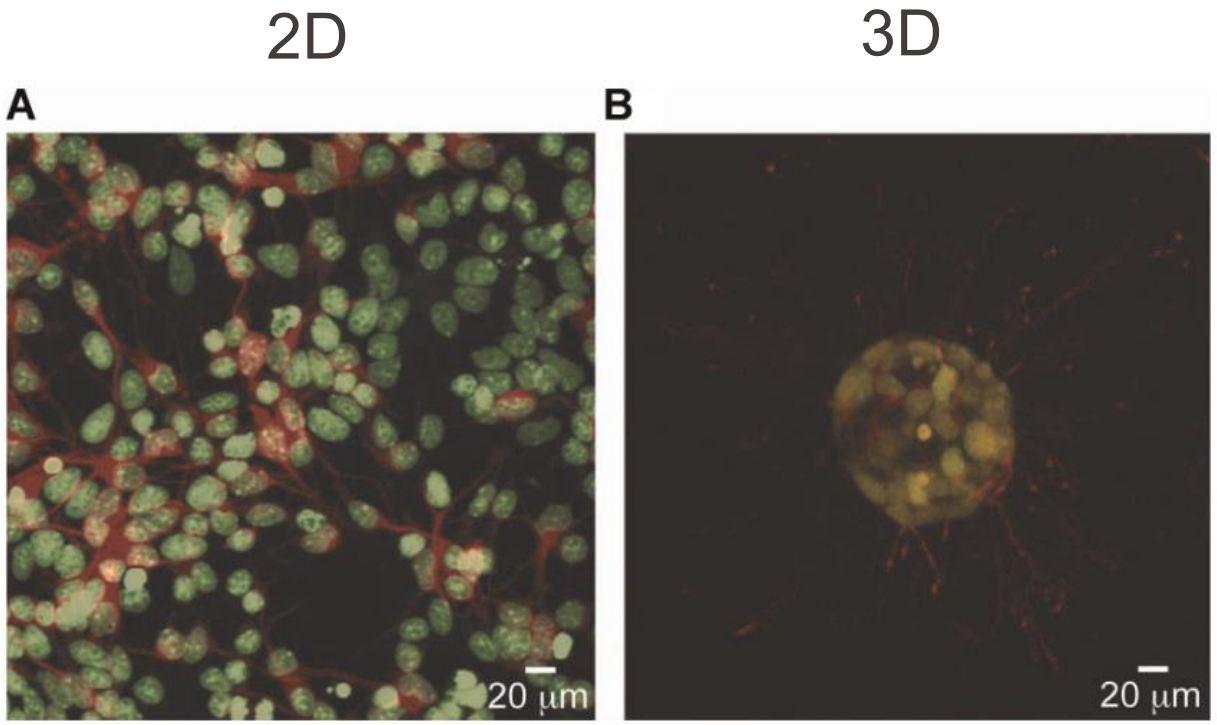
- Cell is confined to a planar environment
- Complex morphologies seen in vivo are not possible
- only a part of the cell is exposed to the ECM and neighboring cells, and the rest of the cell is exposed to the bulk culture media.

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



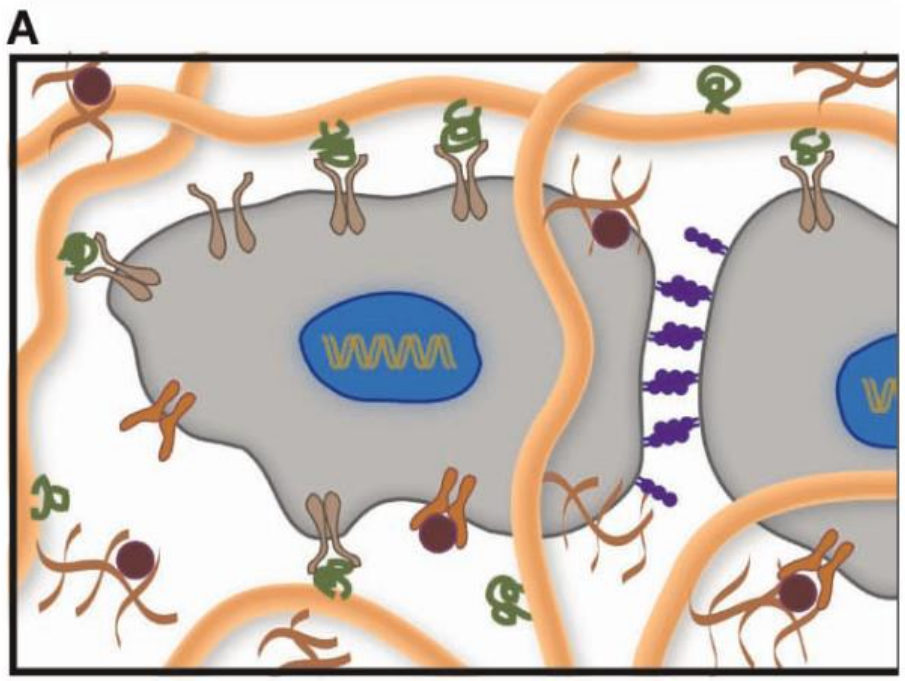
The context matters



- 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

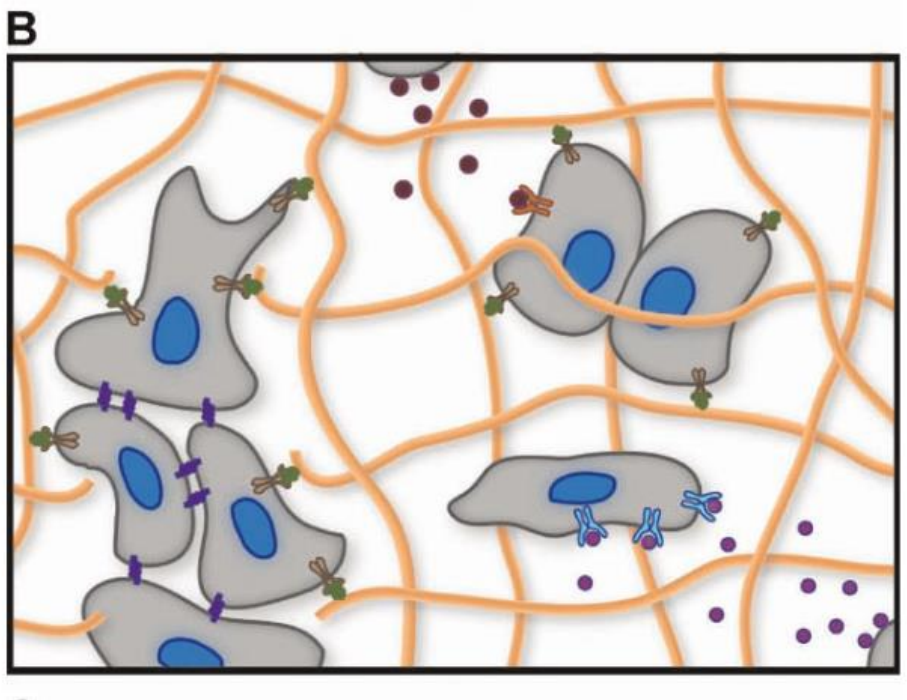
Figure 1. Cells experience a drastically different environment between 2D and 3D culture. For instance, neural cells cultured in monolayer (**A**) are constrained to extend processes in the plane. Cell bodies are stained green and β-tubulin in axonal extensions is stained red. When cultured within hydrolytically 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 native ECM



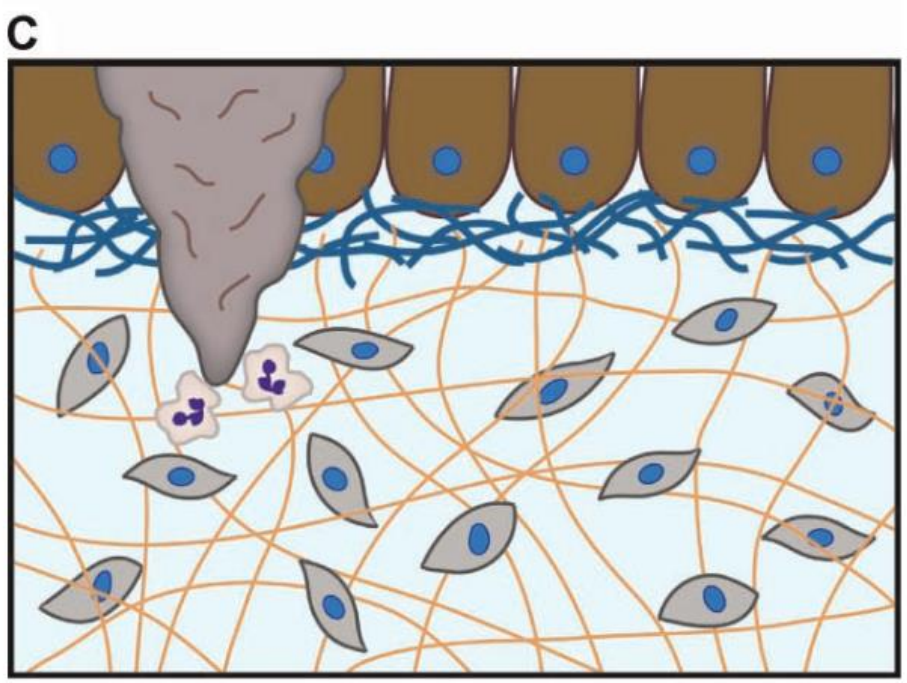
- Integrin-binding with ECM proteins
- Growth factor sequestration
- Cell-cell contacts

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?

How the paper discusses the natural ECM: "promoting, natural hydrogels"

- In vivo, the ECM provides a complex and bioactive scaffold that provides mechanical support while directing cell adhesion, proliferation, morphology, and gene expression; composed of 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 (e.g. RGD)**

- ECM composition varies significantly from tissue to tissue

- Cells **dynamically remodel** the microenvironment to allow different functions; critical for tissue homeostasis and becomes more pronounced in pathological and developing states

- **Engineered functional scaffolds for 3D cell culture should mimic the prototypical ECM!**

Permissive vs. promoting hydrogels (SOA)

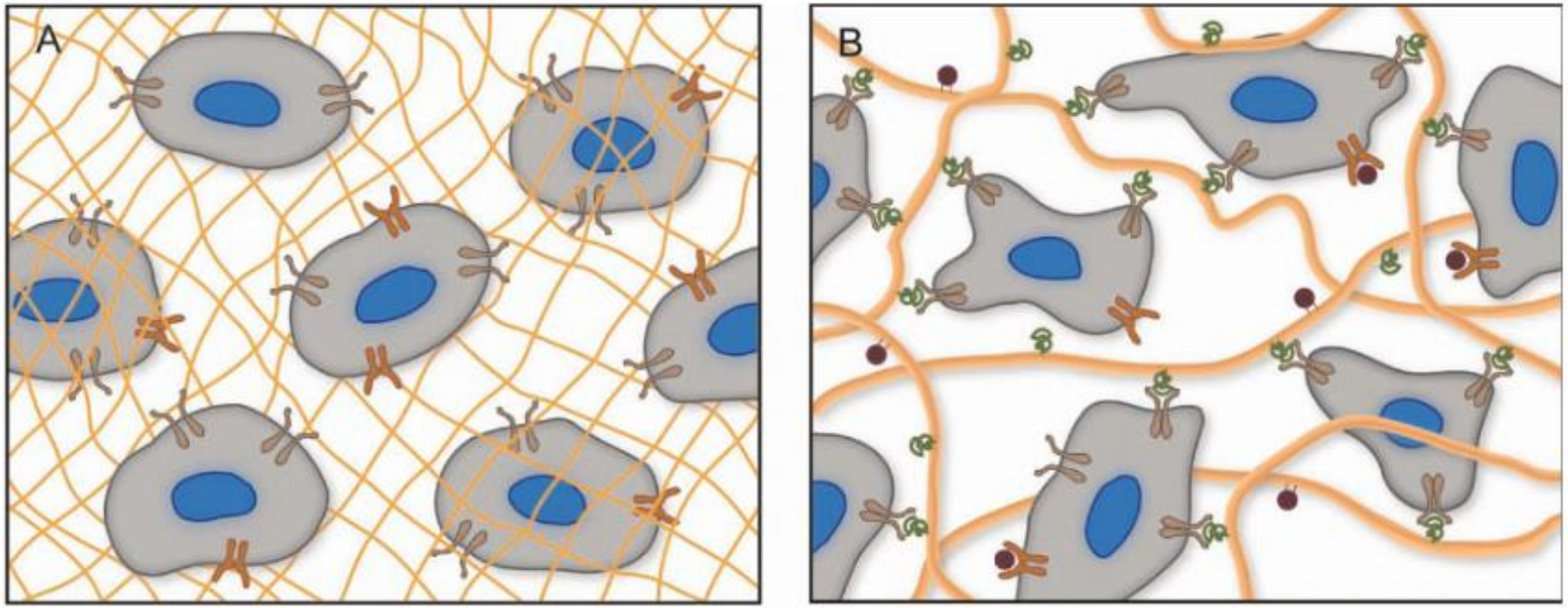
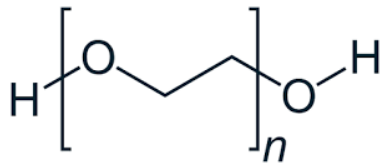


Figure 2. Permissive hydrogels (**A**) composed of synthetic polymers (yellow mesh) provide a 3D environment for culturing cells; however, they fail to activate integrins (brown) and other surface receptors (orange). The synthetic environment simply permits viability as cells remodel their surrounding 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.

Different types of ex-vivo ECMs – how can you best mimic a native ECM?

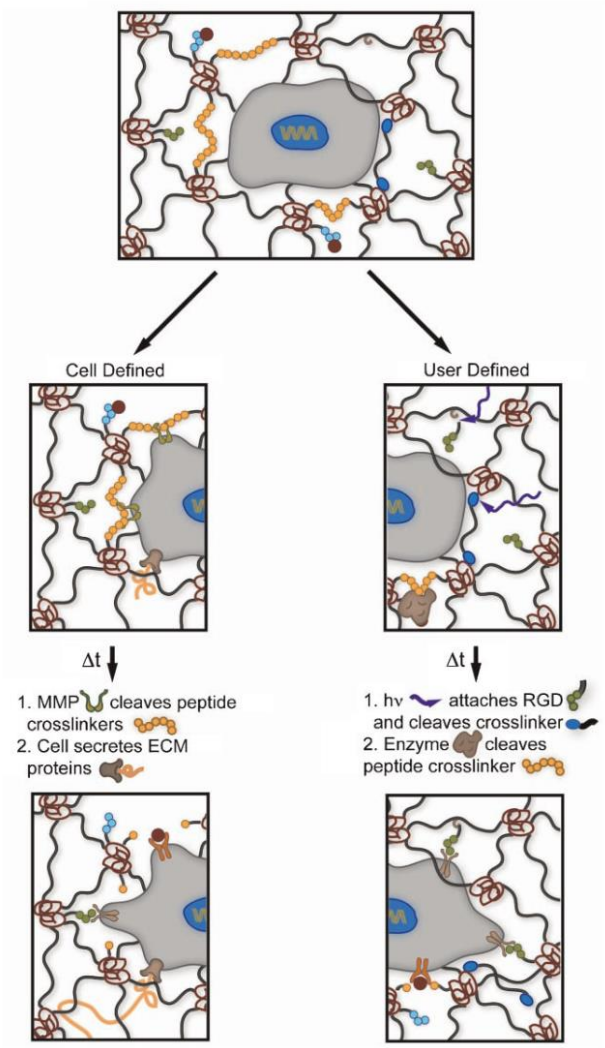
Bridging the GAP!

Type	**Composition**	**Bioactivity**	**Control**	**Example**
Permissive synthetic	Pure synthetic polymers (e.g., PEG)	None (inert)	Maximal chemical/mechanical control	PEGDA hydrogel
Synthetic–biologic (hybrid)	Synthetic + bioactive moieties	Tunable, modular	Intermediate	PEG–RGD–MMP hydrogel
Promoting natural	Natural ECM components	High (native cues)	Minimal tunability	Collagen or fibrin gel



Bioinert, biocompatible, non-toxic

Bridging the gap



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

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

Takeaways

- 3D is better
- Synthetic hydrogel to mimic native ECM requires understanding the cell's native environment: how cells 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