Biology for MX

MSE – 212

Prof. Maartje M.C. Bastings
Programmable Biomaterials Laboratory

Course 4: ECM, adhesion and artificial matrices

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Course Content

BLOCK 1: Introduction and engineering with cellular components

Lecture 1.	Intro to biology and cells	(February 21)
Lecture 2.	Proteins and protein based materials	(February 28)
Lecture 3.	DNA and DNA-based materials	(March 6)
Exercise 1.	*Proteins, peptides and DNA*	*(March 13)*

BLOCK 2: Inter- and intracellular action

Lecture 4.	**ECM, adhesion and artificial matrices**	**(March 20)**
Lecture 5.	Virus, antibodies and immune engineering	(March 27)
Lecture 6.	Bacteria	(April 10)
Exercise 2.	*Nanoparticles and Scaffolds*	*(April 17)*

BLOCK 3: Physics of biological processes

Lecture 7.	Receptors and targeting	(April 24)
Lecture 8.	Endocytosis	(May 1)
Lecture 9.	Signaling and communication	(May 8)
Exercise 3.	*Engineering functionality*	*(May 15)*
Lecture 10.	Revision and conclusion	(May 22)
Open office.	*Questions, discussion, exam prep*	*(May 29)*

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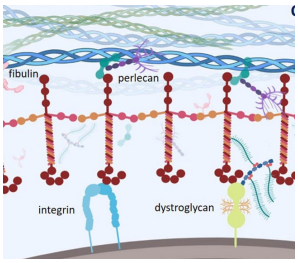
On Today's Menu:

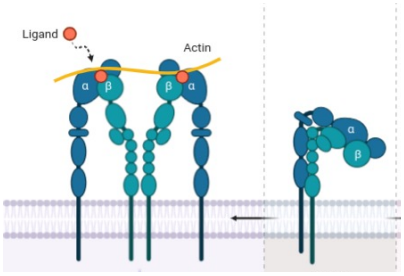
Part 1

Extracellular Matric (ECM)
- Structure
- Function
- Properties



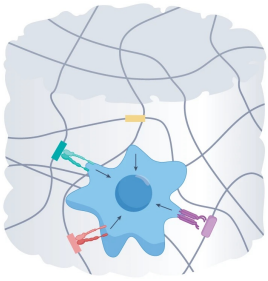
Part 2

Adhesion
- Cell-ECM
- Integrins



Part 3

Artifical ECMs
- Structure
- Function
- Challenges



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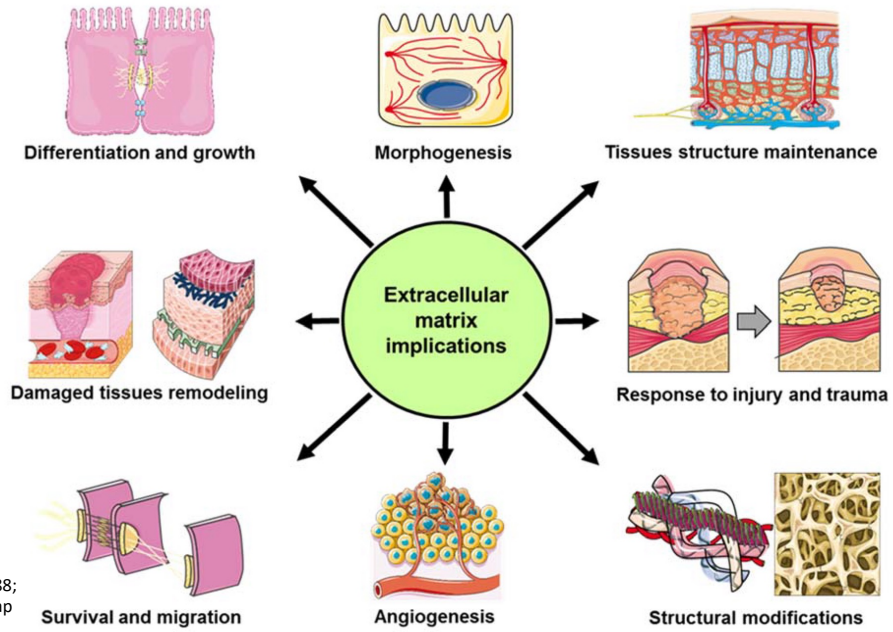
The extracellular matrix functions



Appl. Sci. 2020, 10(7), 2388; https://doi.org/10.3390/app10072388

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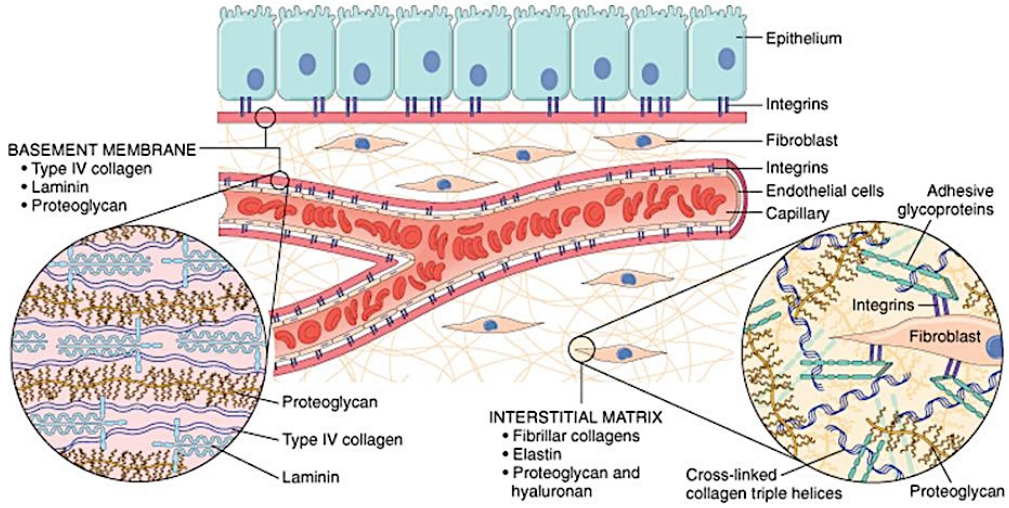
The Space Between Cells



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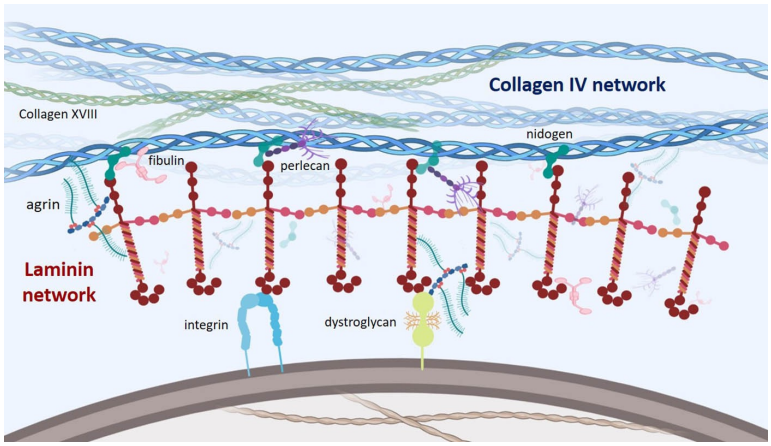
Basement Membrane

Network architecture of all epithelia



Type IV Collagen

Proteoglycans

Laminin

Illustration by Maryline Fresquet (University of Manchester, UK).

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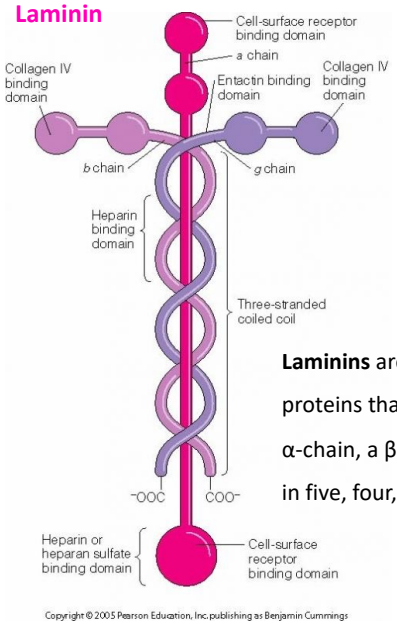
Laminin: Critical Organizer

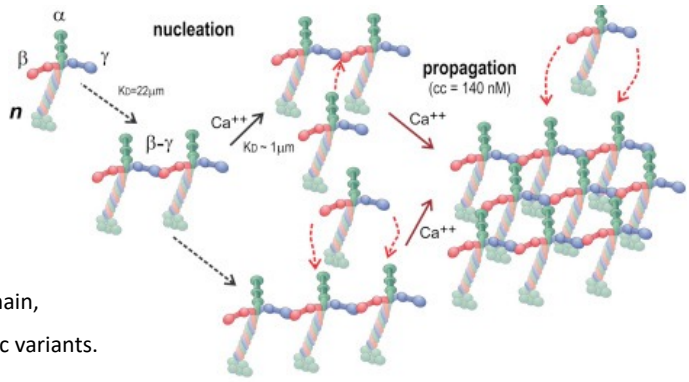
Laminin



High-molecular weight proteins, a major component of the basal lamina.

Important and biologically active part of the basal lamina,

Influencing cell differentiation, migration, and adhesion.



Laminins are heterotrimeric proteins that contain α-chain, a β-chain, and a γ-chain, in five, four, and three genetic variants.

https://doi.org/10.1016/bs.ctm.2015.05.001

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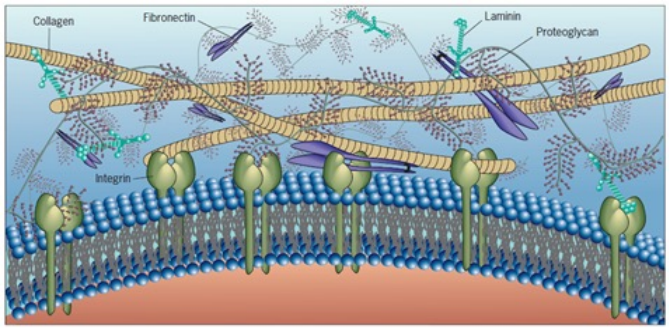
Extracellular Matrix (ECM)



- Is made and remodeled by cells that reside within it
- Is a well-defined composite of proteins and polysaccharides (sugars)
- Regulates cell function, including adhesion, survival, migration
- Contains unique chemical and physical features in each tissue

3 major components:

- Adhesive proteins: Connect cells to the ECM
  - **Integrins** / Cadherins
- Structural proteins: give tissues tensile and compressive strength
  - **Collagen** / **Elastin** / Keratin / **Fibronectin**
- Proteoglycans: Fill space in between, hydrate, cushion cells
  - Consist of a **protein** core with **sugar** side chains

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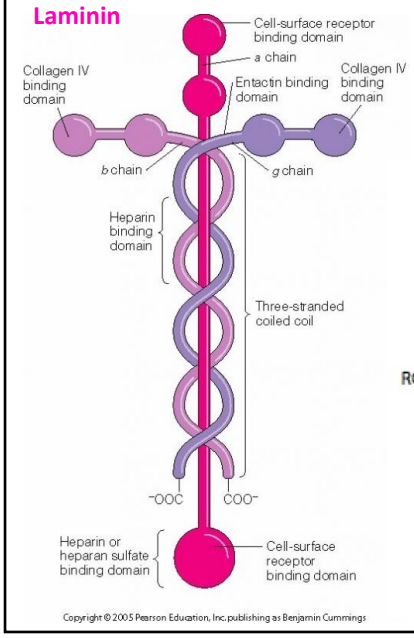
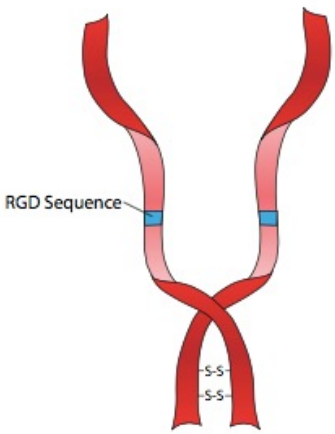
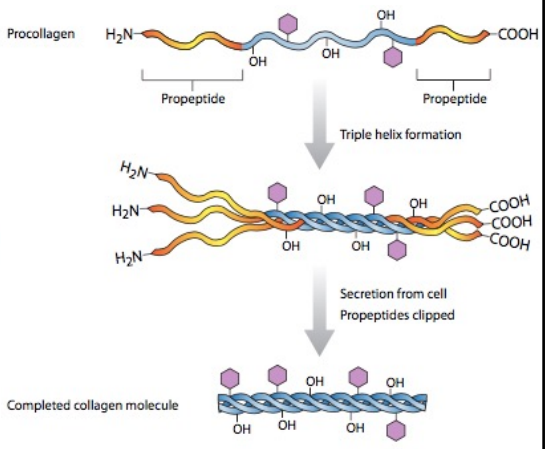
Structural Proteins

Laminin



Fibronectin





Collagen

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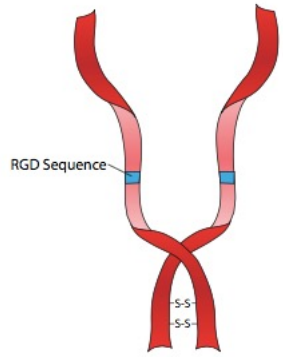
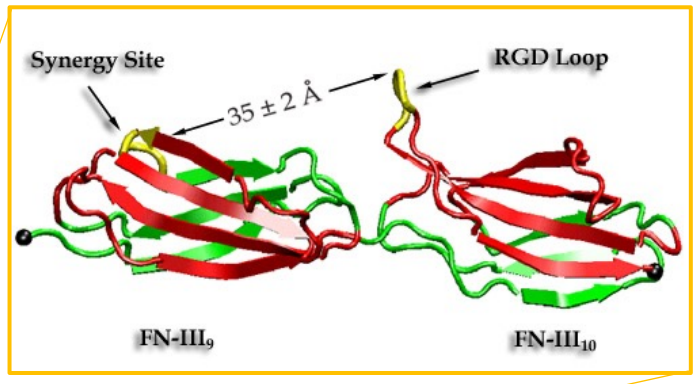
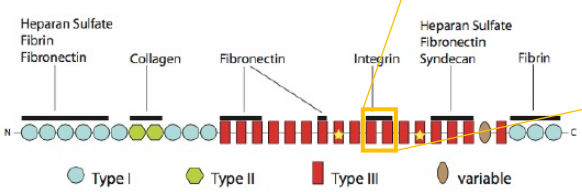
Fibronectin







Fibronectin is a high-molecular weight (~500 kDa) glycoprotein of the extracellular matrix that binds to membrane-spanning receptor proteins called integrins. **Fibronectin** also binds to other extracellular matrix proteins such as collagen, fibrin, and heparan sulfate proteoglycans (e.g. syndecans).

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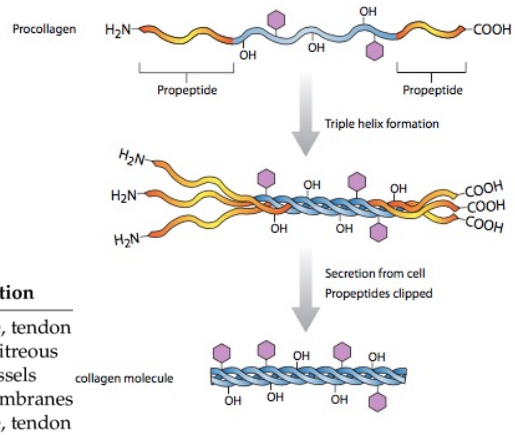
Collagen

collagen I is processed in the cell but not completely assembled :

the three pro-α-chains are assembled into a procollagen triple helix, which is secreted.

Extracellularly, they must then be cleaved at both termini to form the active collagen protein, which is fibrillar.



Type	Class	Distribution
I	Fibrillar	Dermis, bone, tendon
II	Fibrillar	Cartilage, vitreous
III	Fibrillar	Blood vessels
IV	Network	Basement membranes
V	Fibrillar	Dermis, bone, tendon
VI	Filaments, 100 nm	Dermis, bone, tendon
VII	Fibers with antiparallel dimers	Dermis, bladder
VIII	Hexagonal matrix	Membrane
IX	Fibril-associated collagens with interrupted triple helices	Cartilage, vitreous
X	Hexagonal matrix	Cartilage
XI	Fibrillar	Cartilage
XII	Fibril-associated collagens with interrupted triple helices	Tendon

Collagen

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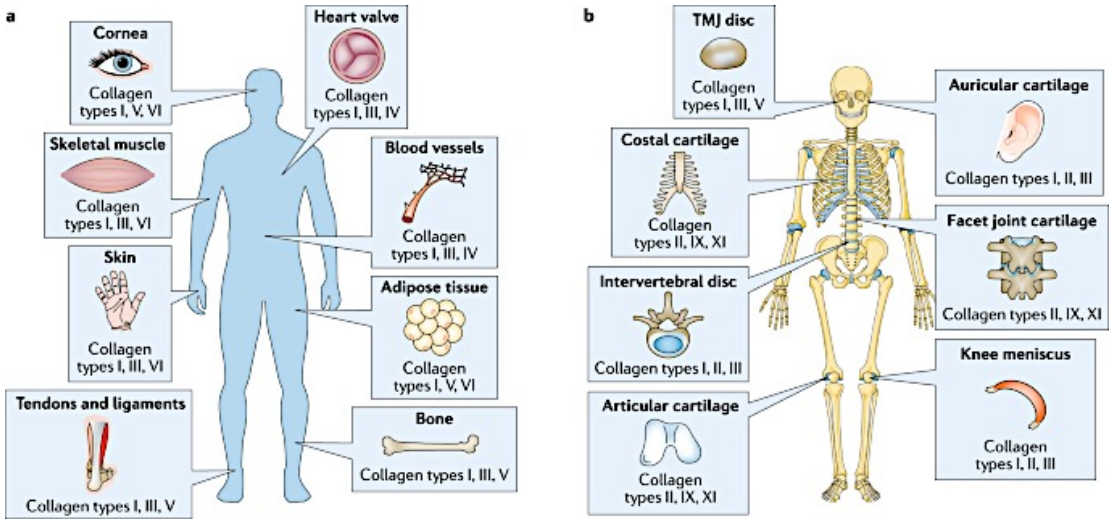
Collagen types in our body



Nature Reviews Materials, 5, 730–747(2020)

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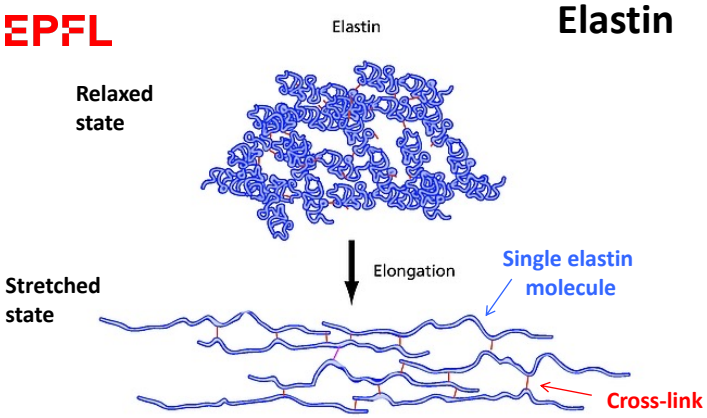
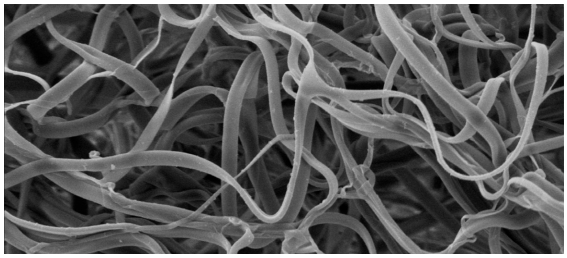
Elastin



Disordered spaghetti

Elastic properties due to cross-links between single elastin molecules

Resulting network is 5x more extensible than rubber!



Elastin is a key protein of the extracellular matrix. It is highly elastic and present in connective tissue allowing many tissues in the body to resume their shape after stretching or contracting. **Elastin** helps skin to return to its original position when it is poked or pinched.

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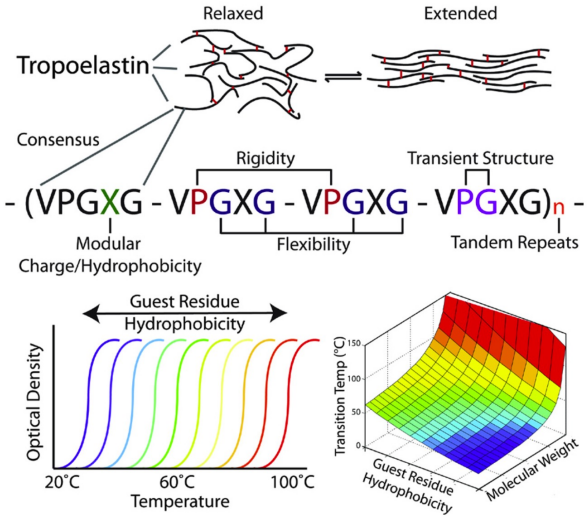
Elastin-like Polypeptides



Elastin-like-polypeptides (ELPs): Disorder encoded at the sequence level. ELPs are tandem repeat proteins derived from tropoelastin. The consensus repeat unit **VPGXG** promotes high conformational flexibility at low temperatures and a disordered molten globule aggregate at higher temperatures.

ELPs are water-soluble below their LCST, but coacervate and phase-separate above it in a reversible process that can take place in temperature intervals as narrow as 1–2 °C.

X can be any amino acid except proline and is usually referred to as the guest amino acid.

The behavior of ELPs has inspired many researchers to develop dynamic ELP-based stimuli-responsive materials.

S. Roberts et al. / FEBS Letters 589 (2015) 2477–2486
http://dx.doi.org/10.1016/j.febslet.2015.08.029

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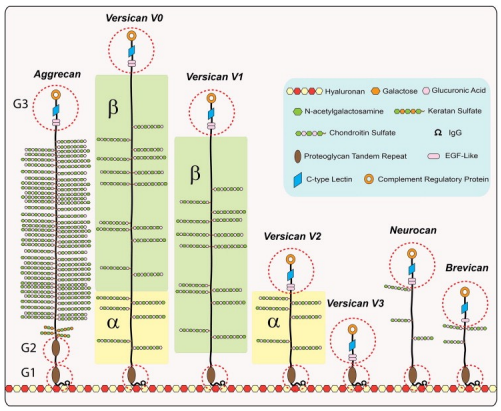
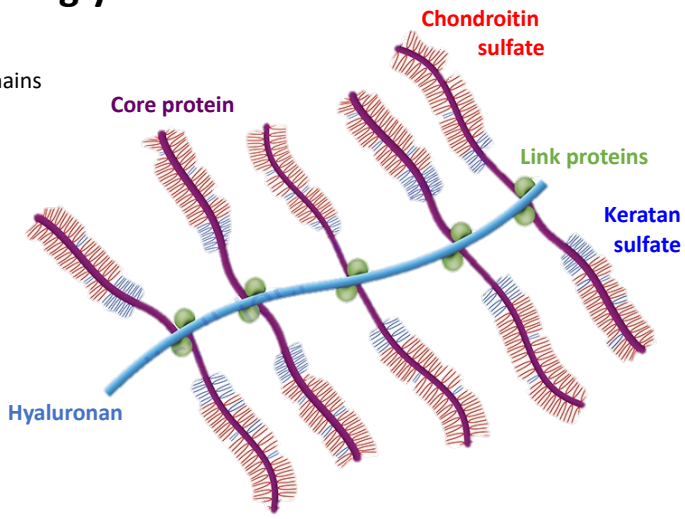
Proteoglycans

Consist of a sugar/protein core with sugar side chains

- Resist compressive forces
- Fill space in between, hydrate, cushion cells
- Connect to other ECM components
- Bottlebrush structure

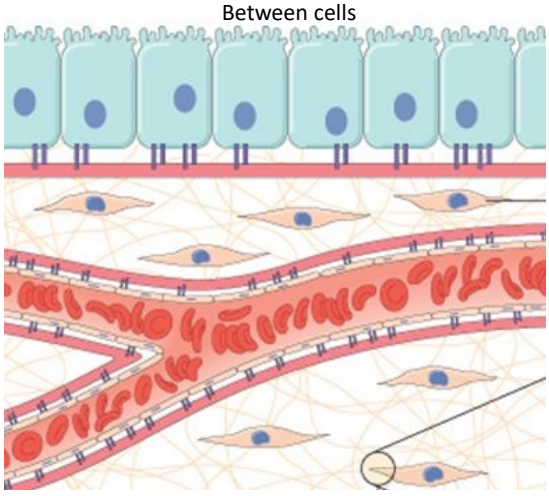




https://doi.org/10.1016/j.matbio.2015.02.003

Part 2: Adhesion

Between cells

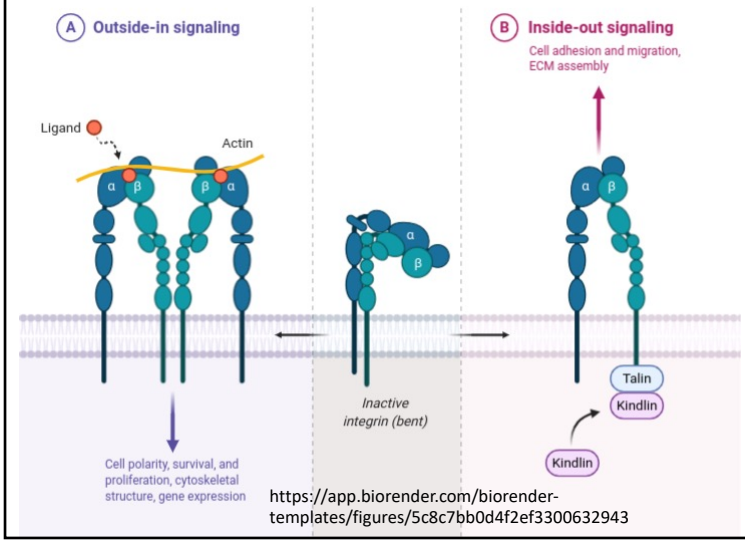


Between cells and the BM

Between cells and the ECM

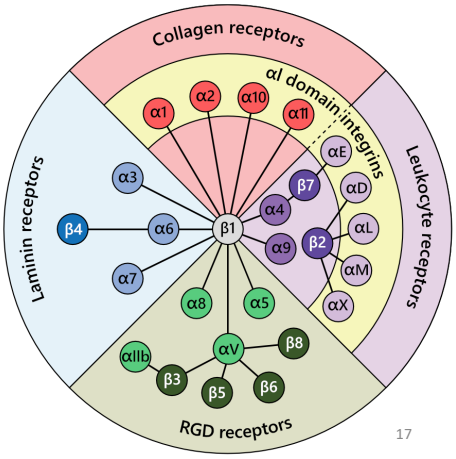
Integrin

Integrins are the principal receptors used by animal cells to bind to the extracellular matrix. They are heterodimers and function as transmembrane linkers between the extracellular matrix and the actin cytoskeleton. A cell can regulate the adhesive activity of its **integrins** from within.



https://app.biorender.com/biorender-templates/figures/5c8c7bb0d4f2ef3300632943

24 unique integrins by combinations of the α and β



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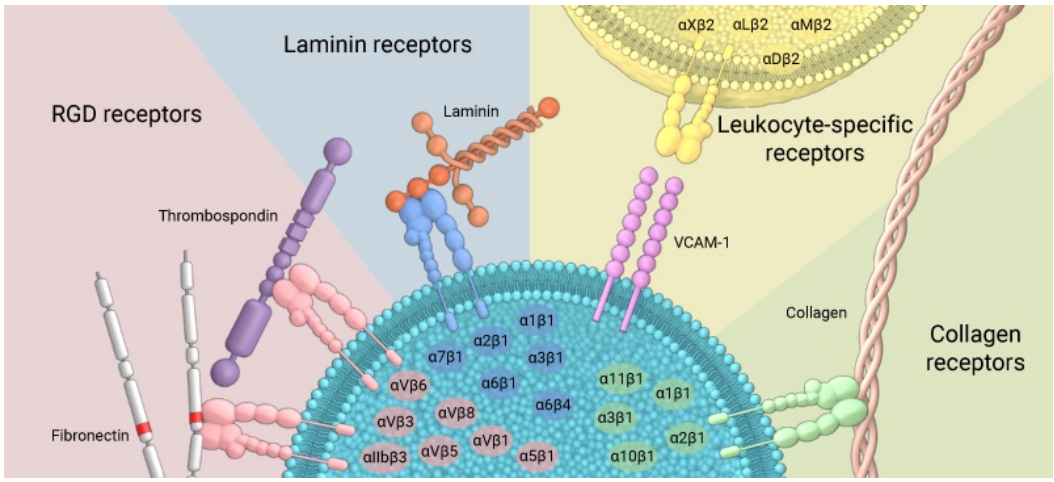
Integrin



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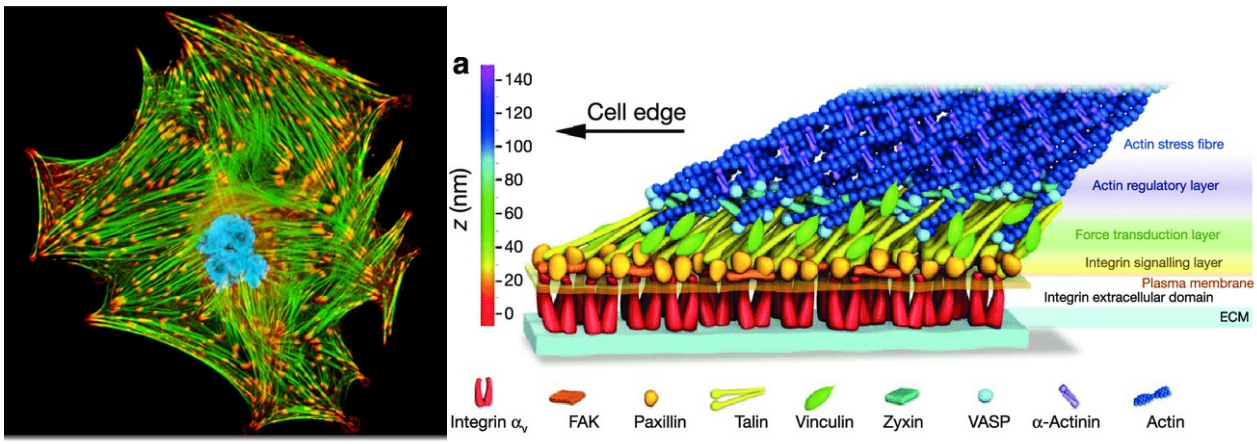
Binding to Integrins

Actin structures are linked to **focal adhesions** that provide the pathway of **force transmission** from inside the cell to the elastic matrix and associated with the focal-adhesion complexes are a number of well-known signaling molecules that are well-placed to act as **mechano-transducers**

At the molecular scale, cells to pull against the matrix and, secondly, a cellular mechano-transducer(s) **generates signals based on the force** that the cell must generate to deform the matrix.



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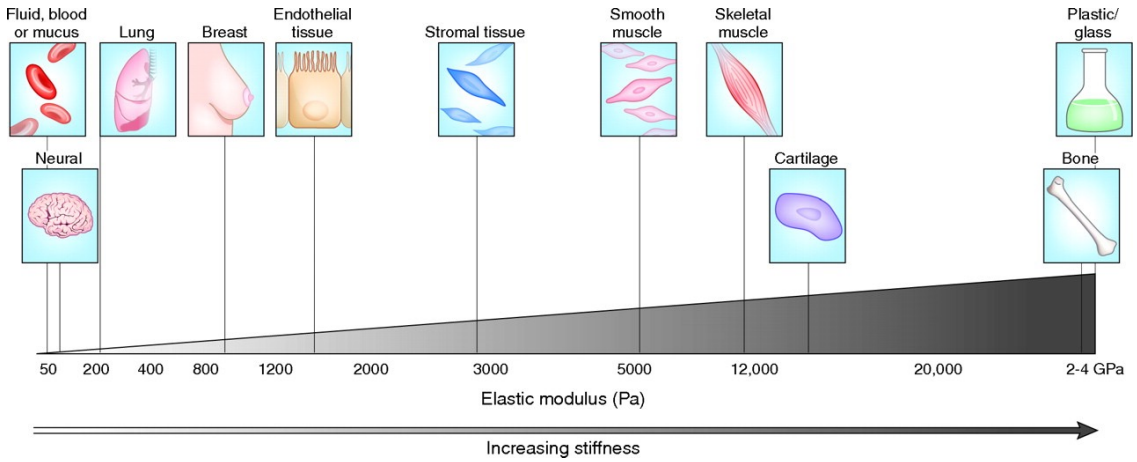
Mechanical properties



Disease Models & Mechanisms 2011 4: 165-178; doi: 10.1242/dmm.004077

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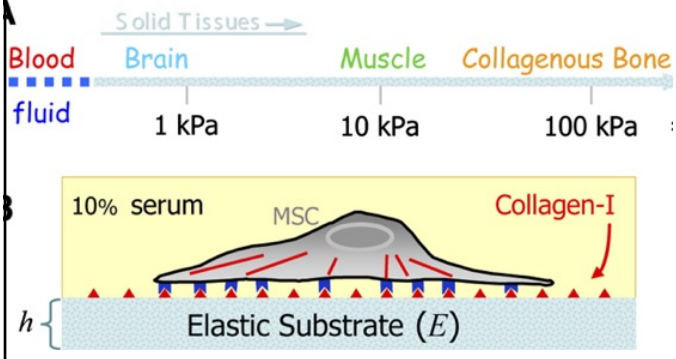
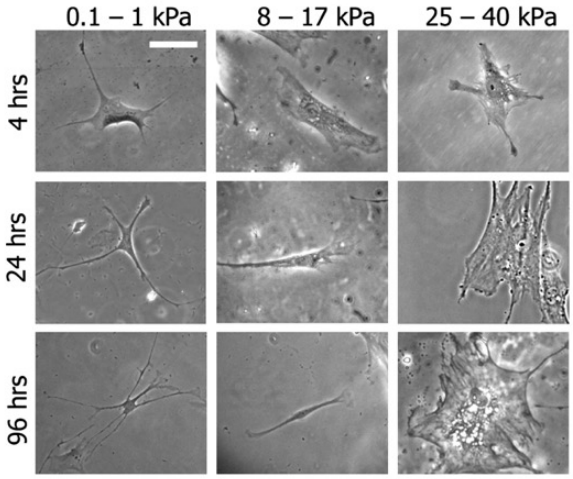
Effect of stiffness

Naive mesenchymal stem cells (MSCs) are shown to specify lineage and commit to phenotypes with extreme sensitivity to tissue-level elasticity.

Soft matrices that mimic brain are neurogenic,
stiffer matrices that mimic muscle are myogenic,
rigid matrices that mimic collagenous bone prove osteogenic.





Engler et al. Cell 2006

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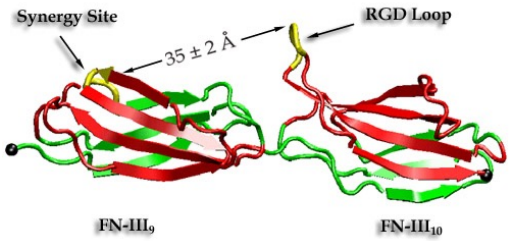
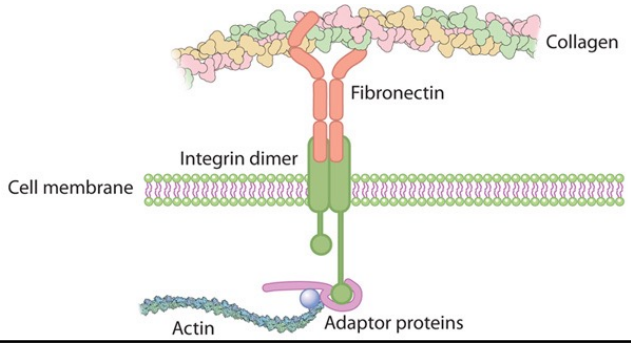
Synthetic Adhesion: RGD

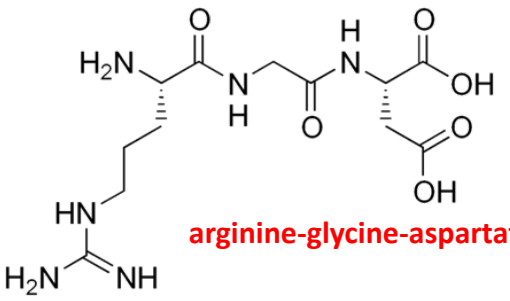
The most widely studied adhesive peptide in the biomaterials field is the tri-amino acid sequence, arginine-glycine-aspartate, or "**RGD**".

Principal integrin-binding domain present within ECM proteins

Synthesis is simple and inexpensive
Easy sterilization
Minimizes the risk of immune reactivity
Controlled densities and orientations.







arginine-glycine-aspartate

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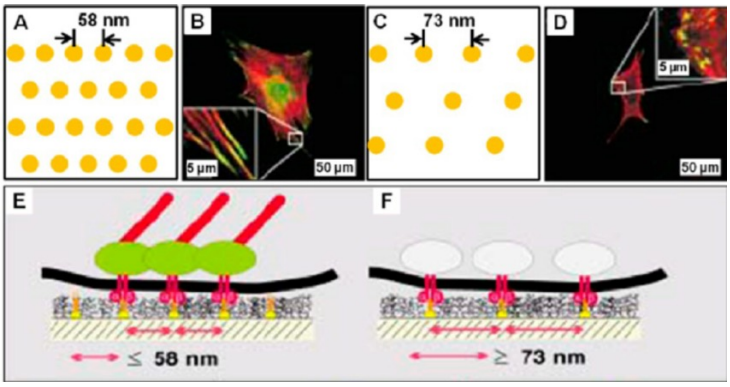
RGD Spacing

Thanks to progress in materials surface patterning, engineers have found that a spacing between **58-73nm** is required to enable integrin-signaling. Binding and clustering of integrins in FAs and cell spreading are strongly influences by nm changes.

Local ligand distribution is a key surface parameter for the assembly and stability of FA complexes.



https://doi.org/10.1529/biophysj.106.089730 ChemPhysChem 2004, 5, 383–388

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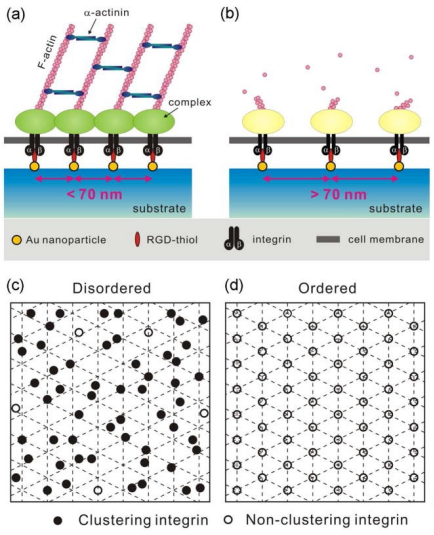
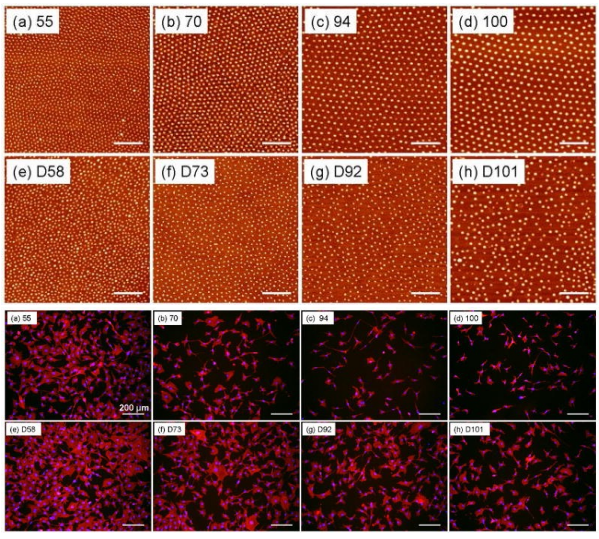
RGD order vs disorder

the disordered surface provided a much wider range of variation in local inter-ligand spacing for positive cell adhesion, which is due to the polydispersity of local inter-ligand spacings.





Nano Lett. 2009; 9(3): 1111–1116.
doi: 10.1021/nl803548b

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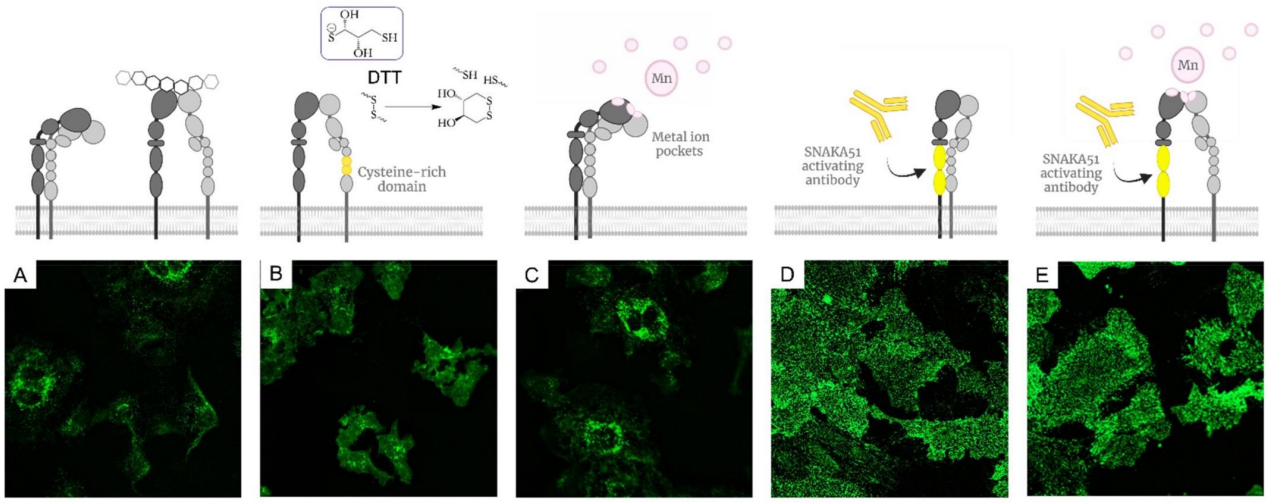
RGD patterns (PBL)



Molecules **2022**, *27*(15), 4968; https://doi.org/10.3390/molecules27154968

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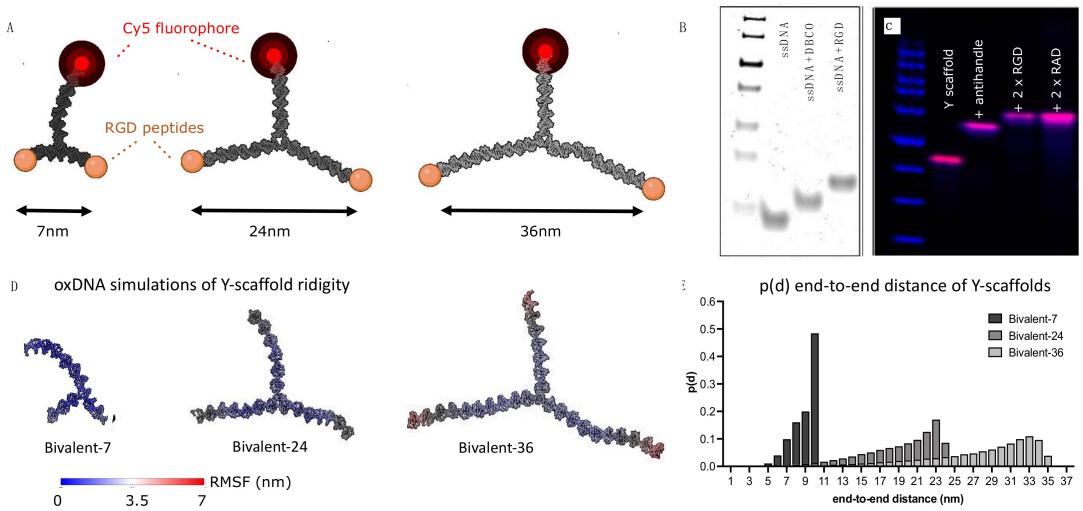
RGD patterns (PBL)



Molecules **2022**, *27*(15), 4968; https://doi.org/10.3390/molecules27154968

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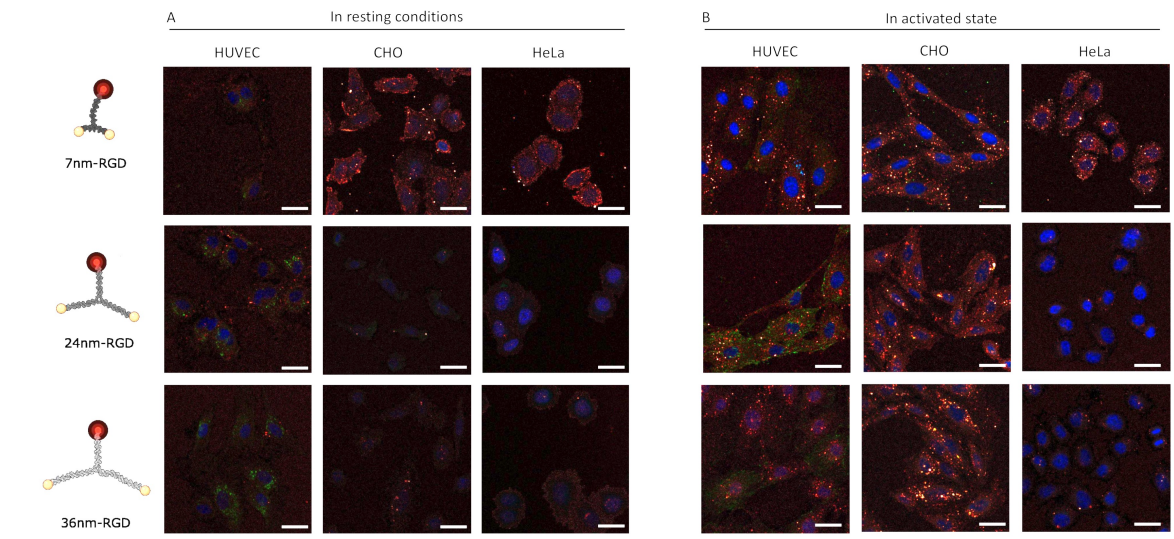
RGD patterns (PBL)



Molecules **2022**, *27*(15), 4968; https://doi.org/10.3390/molecules27154968

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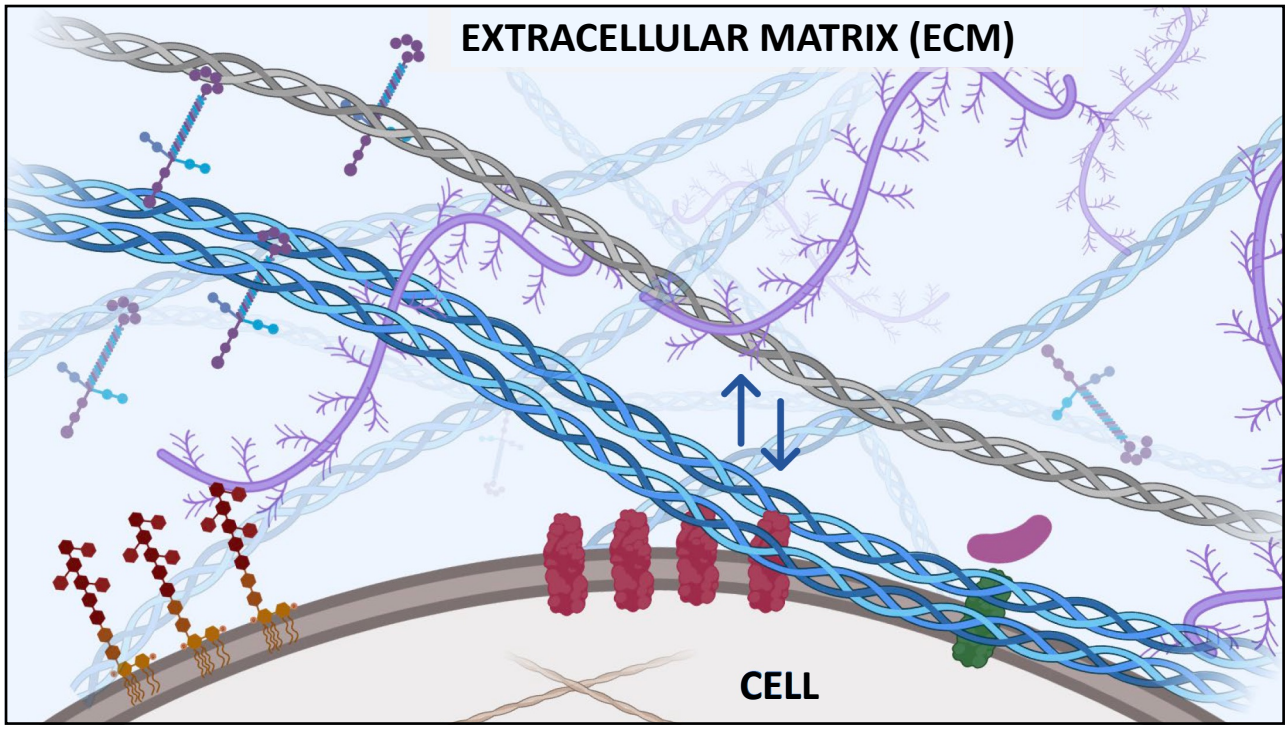
EXTRACELLULAR MATRIX (ECM)



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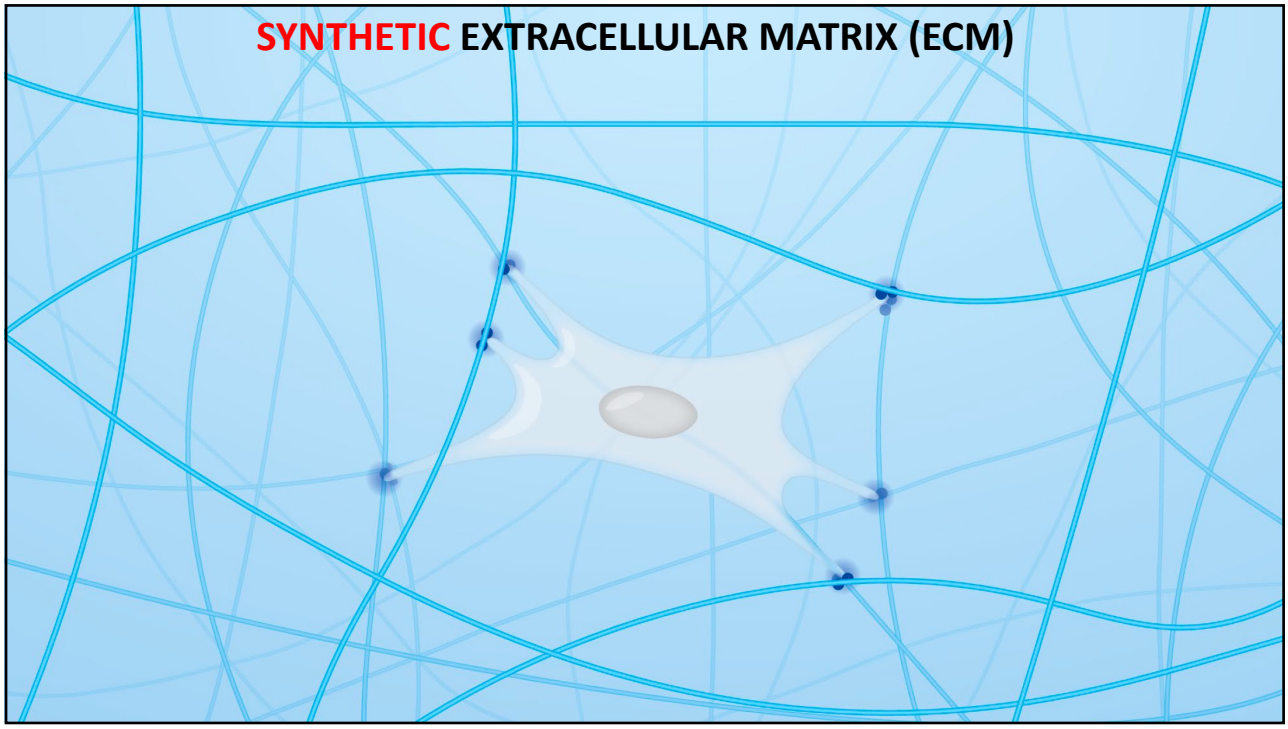
SYNTHETIC EXTRACELLULAR MATRIX (ECM)



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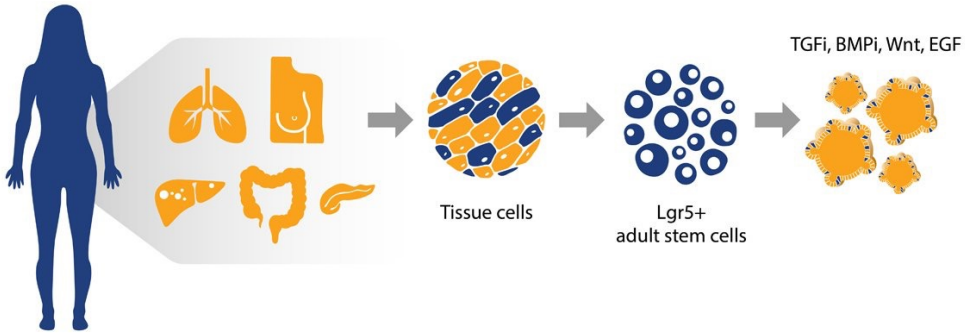
EPFL

Engineering of cellular organization: Organoids

An **organoid** is a **miniaturized** and **simplified** version of an organ produced *in vitro* in three dimensions that shows **realistic micro-anatomy**.

Production of Organoids



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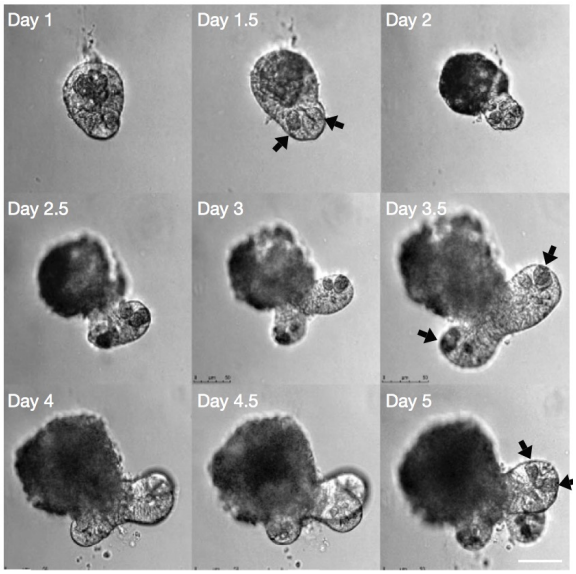
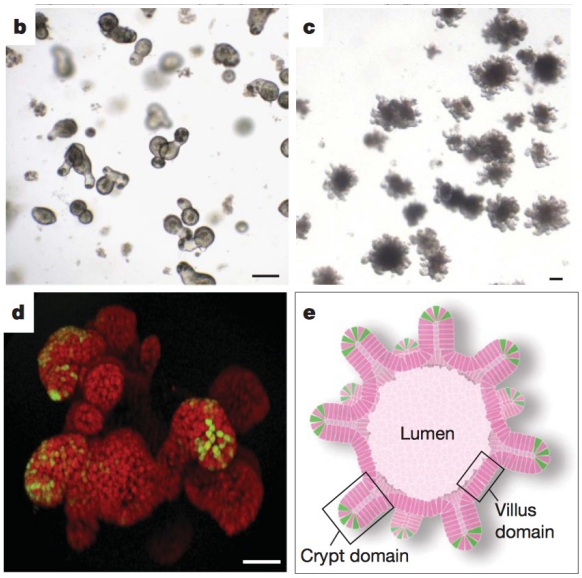
Intestinal Organoids





Vol 459|14 May 2009|doi:10.1038/nature07935

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Matrigel

Matrigel is the trade name for a gelatinous protein mixture secreted by Engelbreth-Holm-Swarm mouse sarcoma cells produced by Corning Life Sciences. Matrigel resembles the complex extracellular environment found in many tissues and is used by cell biologists as a substrate for culturing cells

Matrigel is **limited** in its applicability to cellular biology, therapeutic-cell manufacturing and drug discovery, owing to its complex, ill-defined and variable composition.

Variations in the mechanical and biochemical properties within a single batch of Matrigel —between batches — have led to uncertainty in experiments and a lack of reproducibility.

Moreover, Matrigel is **not conducive to physical or biochemical manipulation**, making it difficult to fine-tune the matrix to promote intended cell behaviours and achieve specific biological outcomes.

Composition:
laminin (~60%),
collagen IV (~30%),
entactin (~8%)
perlecan (~2–3%)

Aisenbrey, E.A., Murphy, W.L. Synthetic alternatives to Matrigel. *Nat Rev Mater* **5,** 539–551 (2020).

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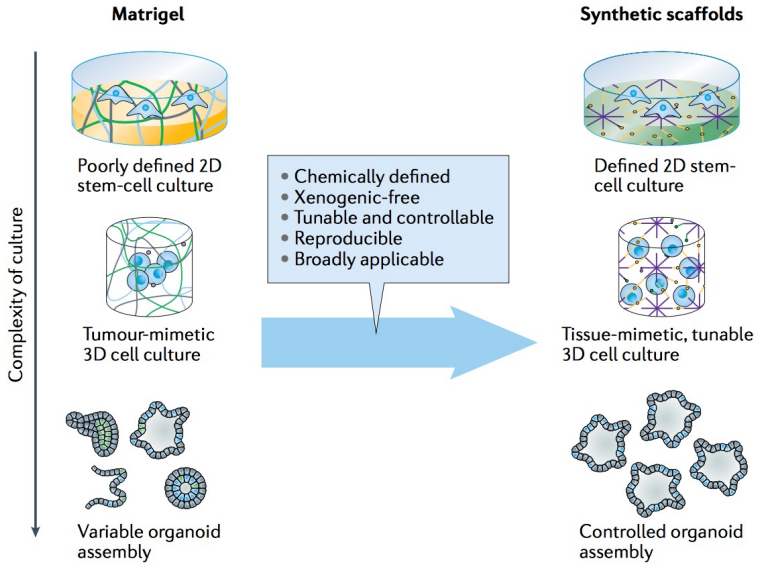
Replacing Matrigel



https://doi.org/10.1038/s41578-020-0199-8

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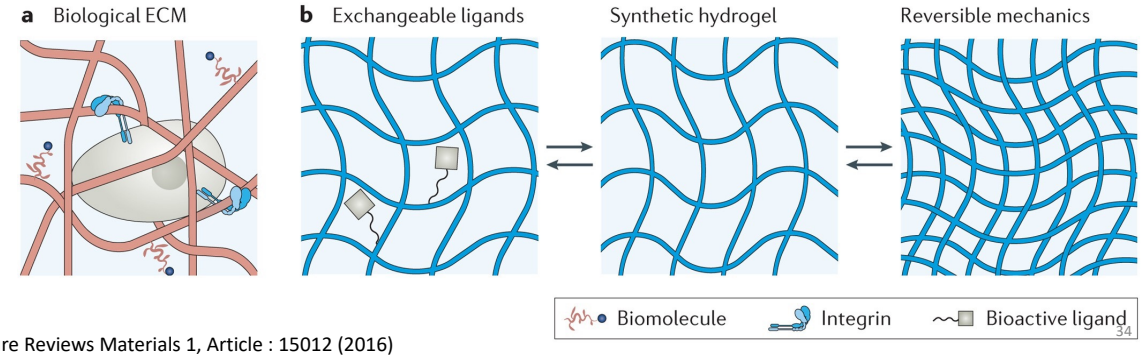
Artificial Matrices

The microenvironment can influence and dictate cellular behaviour despite the cell's genetic programming; This is a continual process with no defined end point.

Efforts in tissue engineering have focused on creating stem cell niches for medical therapies.

Made using structural, relatively static biomaterials with predefined properties.
Many applications — for example, cell differentiation — require more advanced materials, enabling chemical and mechanical adaptation over time.



Nature Reviews Materials 1, Article : 15012 (2016)

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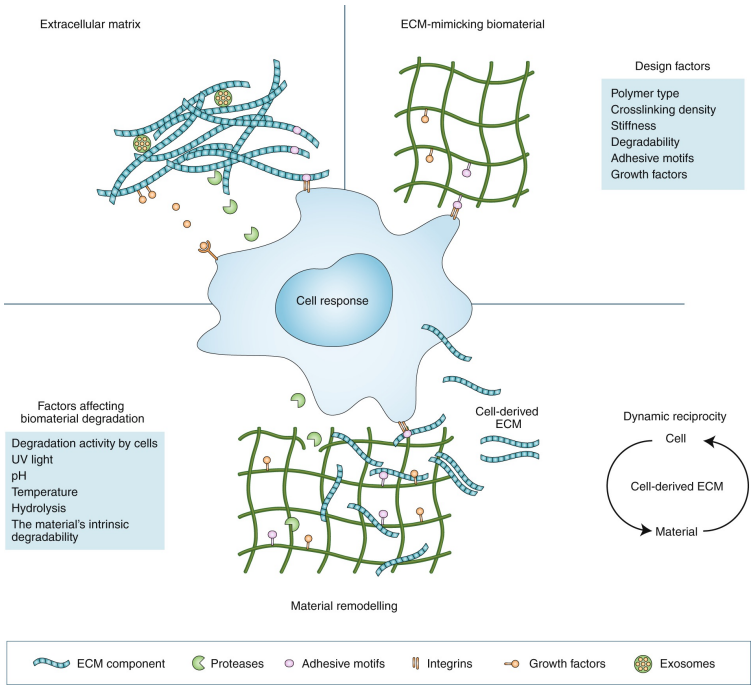
ECM-mimicking biomaterials can be rendered **cell-instructive** by incorporating protein-based or peptide-based motifs that recapitulate key features of the native ECM.

Cells encapsulated within hydrogels remodel their surroundings through a combination of matrix secretion and biomaterial degradation.

Over time, **the cell-secreted matrix can override cues** provided by the hydrogel.

This behaviour parallels the phenomenon of **dynamic reciprocity** observed in tissues, by which cells modulate their surroundings biochemically and mechanically, regulating intracellular signalling, gene expression and, ultimately, cell behaviour.



Blache, U., Stevens, M.M. & Gentleman, E. Harnessing the secreted extracellular matrix to engineer tissues. *Nat Biomed Eng* **4,** 357–363 (2020).

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EPFL

Artificial Intestinal Organoid Matrix

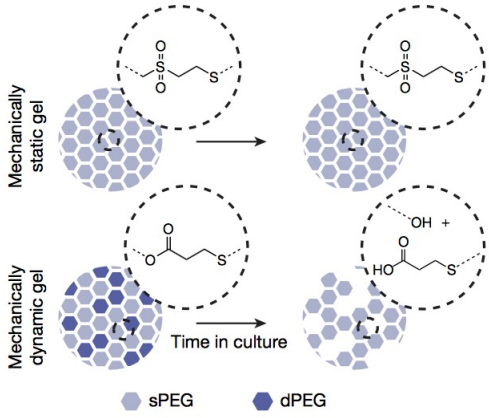
Modular synthetic hydrogel networks to define the key extracellular matrix (ECM) parameters that govern intestinal stem cell (ISC) expansion and organoid formation

Separate stages of the process **require different mechanical environments** and **ECM components**.

Fibronectin-based adhesion (RGD) was sufficient for ISC survival and proliferation.

High matrix stiffness significantly enhanced ISC expansion through a yes-associated protein 1 (YAP)-dependent mechanism.

ISC differentiation and organoid formation required a **soft matrix** and **laminin-based adhesion**.



Nature 539, 560–564(2016)

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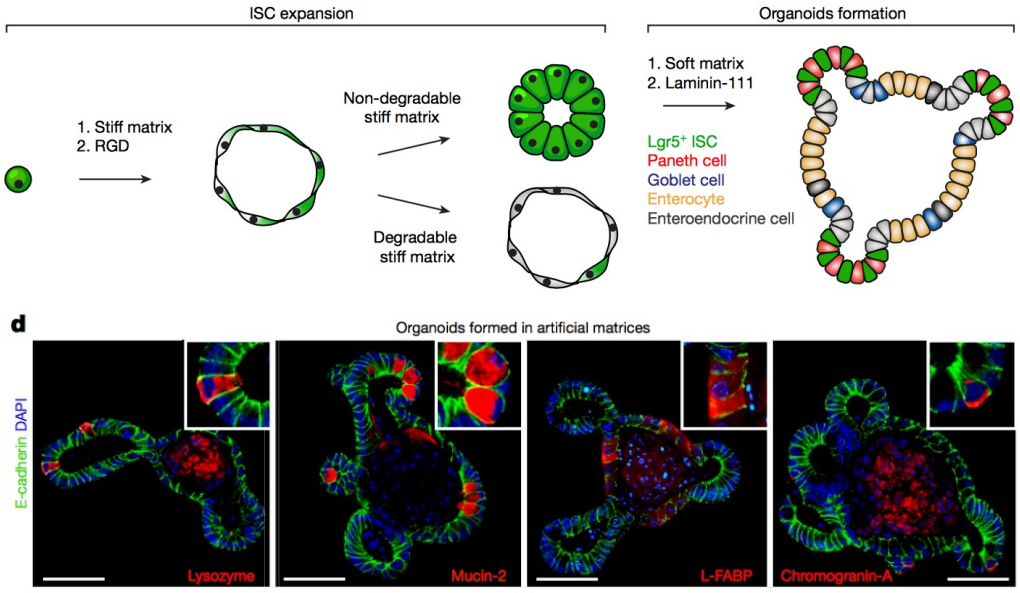
Artificial Intestinal Organoid Matrix



Nature 539, 560–564(2016)

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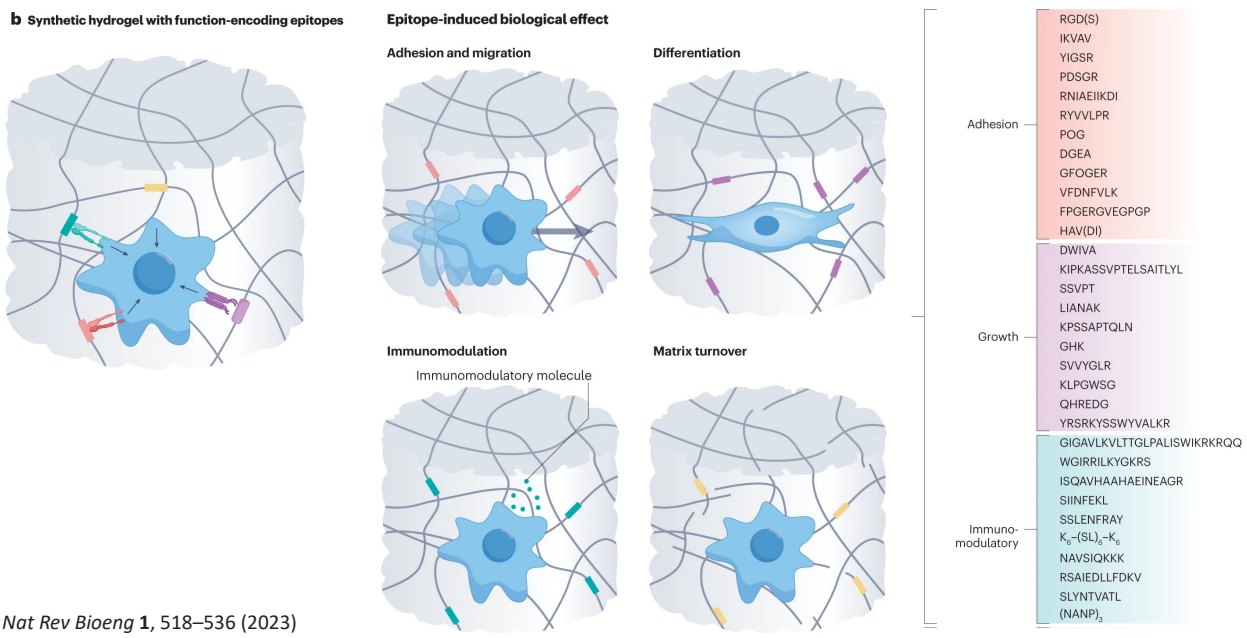
Peptides in Hydrogels for ECM Function



Nat Rev Bioeng **1**, 518–536 (2023)

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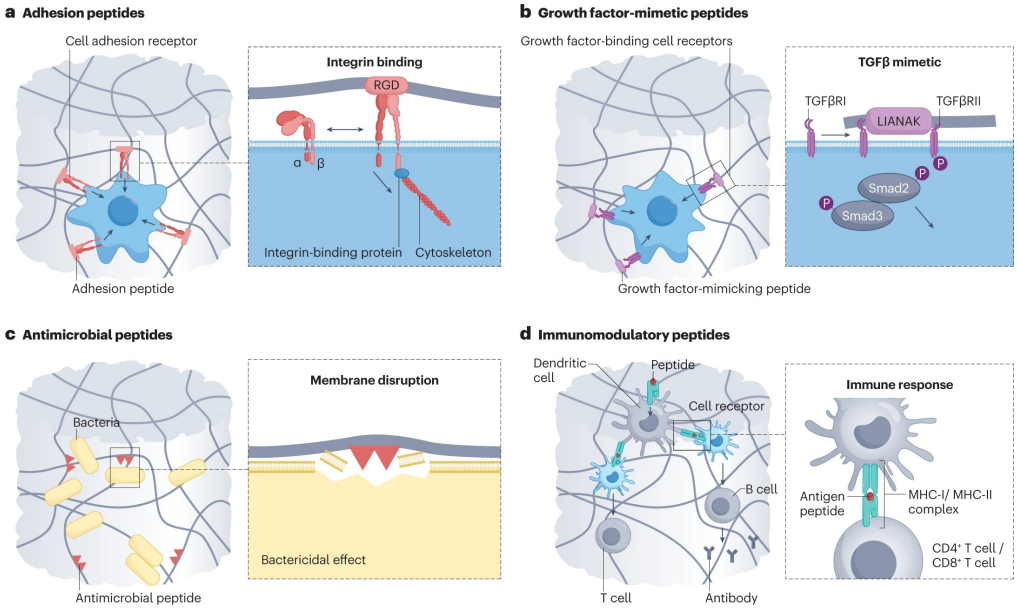
Direct signaling to the cells



Nat Rev Bioeng **1**, 518–536 (2023)

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Conclusion

The cellular surrounding is full of engineering masterpieces.

All molecules are organized in a spatio-temporal network that provides both mechanical support as well as biological signaling information.

Recreating such an architecture in the lab is proving to be very difficult.

It will take the efforts of many future materials engineers to solve the ECM puzzle.

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