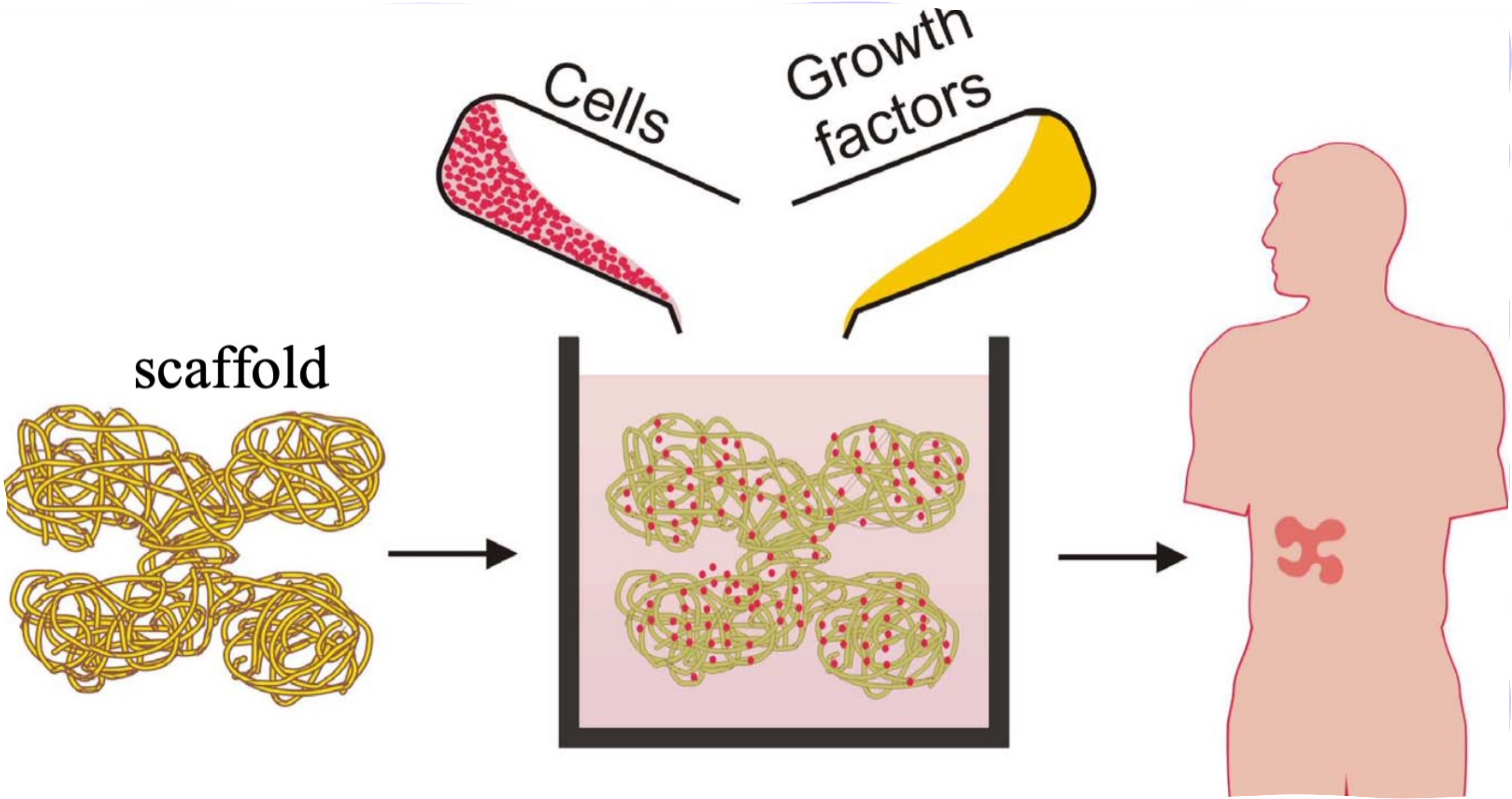
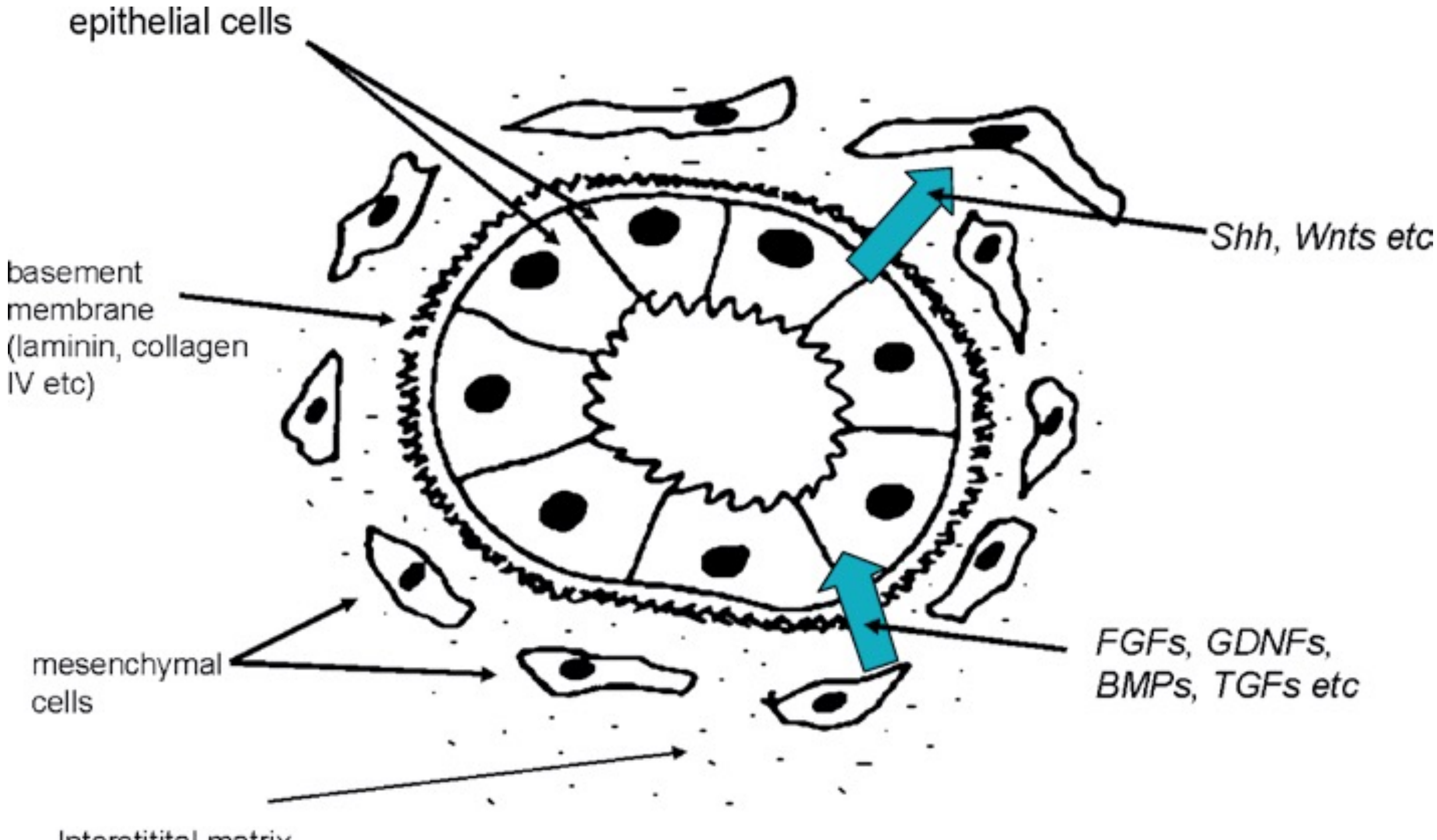

Cells
Growth factors
scaffold

An ideal scaffold must possess the requirements listed below

- Biocompatibility
- Structure = biomimicry ✓
- Fiber parameters ✓
- Mechanical properties ✓
- Biodegradability ✓
- Differentiation ✓
- Immunomodulation

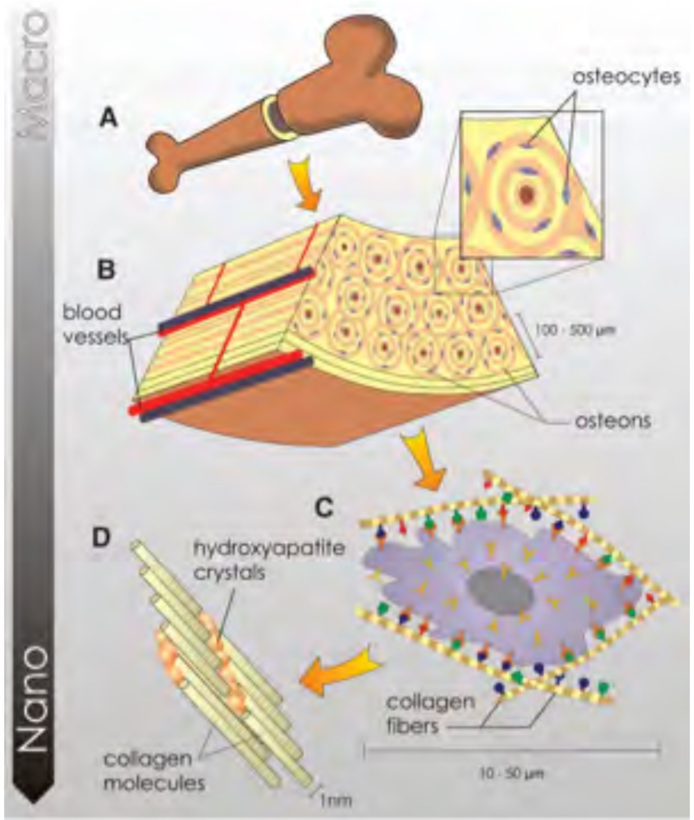
Structure = biomimicry

Control of Organogenesis: Towards Effective Tissue Engineering



Molecules that typically mediate communication between cell types during the development of typical organs

Can we design synthetic biomaterials that ***regenerate functional native-like tissues***?



Biological tissues are complex

tissue composition and organization leads to biological function

Biomimicry of the scaffolds

"How closely does a biomaterial scaffold have to approximate the normal tissue structure, mechanical function, and cell-matrix interaction to achieve desired tissue reconstruction?"

1. Characterize normal tissue structure, mechanical function, and cell-matrix interactions.

2. Engineer scaffolds with well-controlled structure, mechanical function, and cell matrix that can be quantitatively compared to normal tissue characteristics

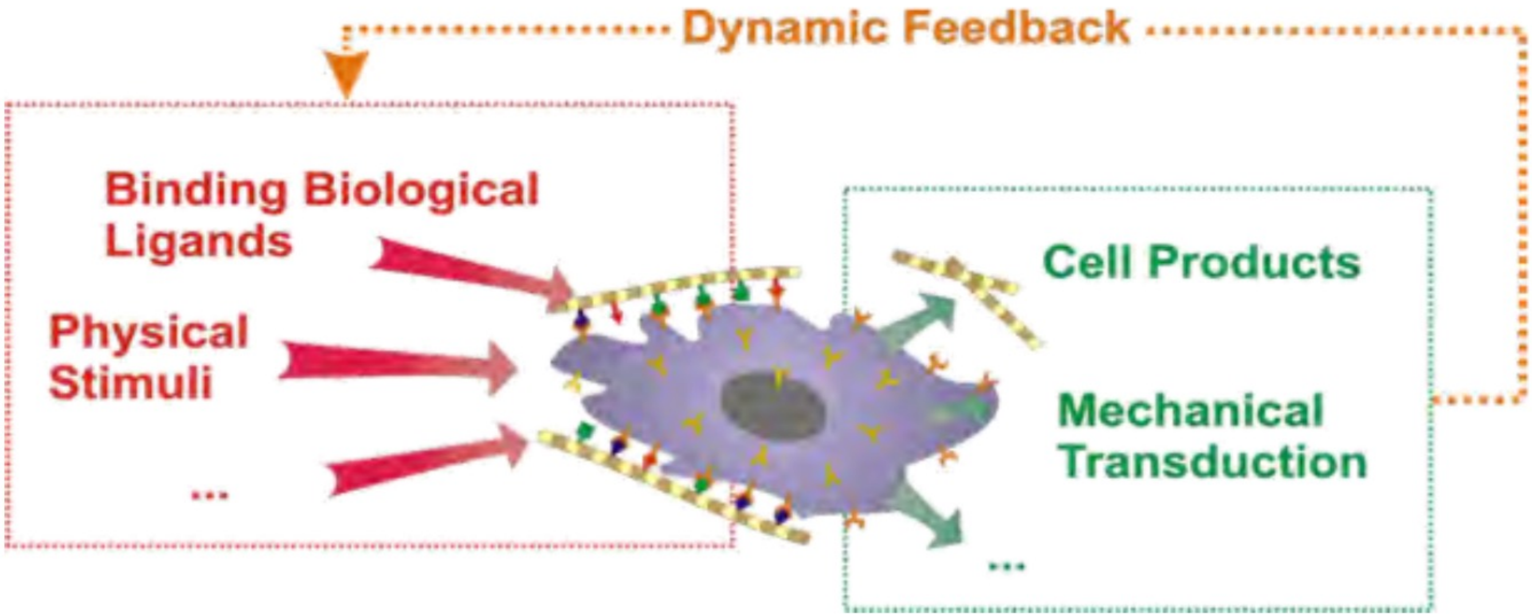
3. Perform *in vivo* experiments where tissue regeneration success is correlated to the engineered scaffold characteristics

What is the best design?

- Nature is the best designer for organ development. What should we do is just to follow Nature’s design---Mimetic.

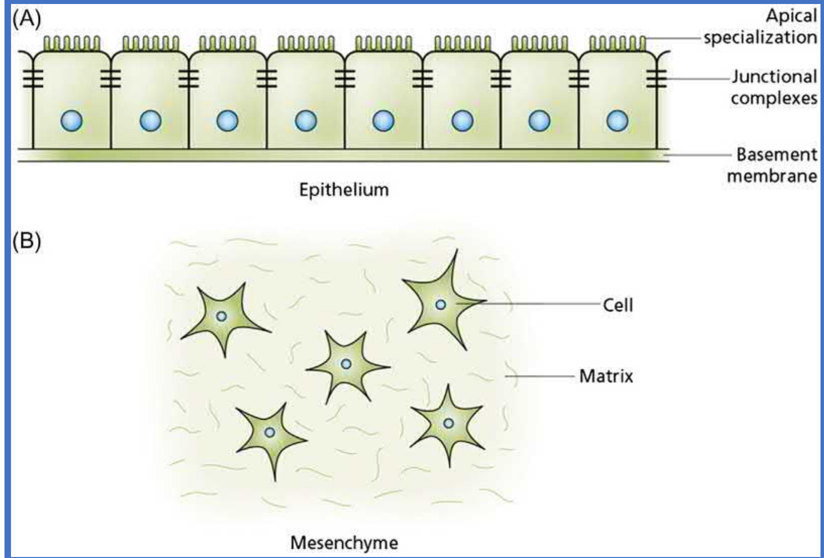
- Repair by tissue engineering In a specific tissue, the Natural ECM of this tissue with hierarchical structure and mechanical behavior should be ideal scaffold.

Designing materials to mimic ECM to regenerate tissues

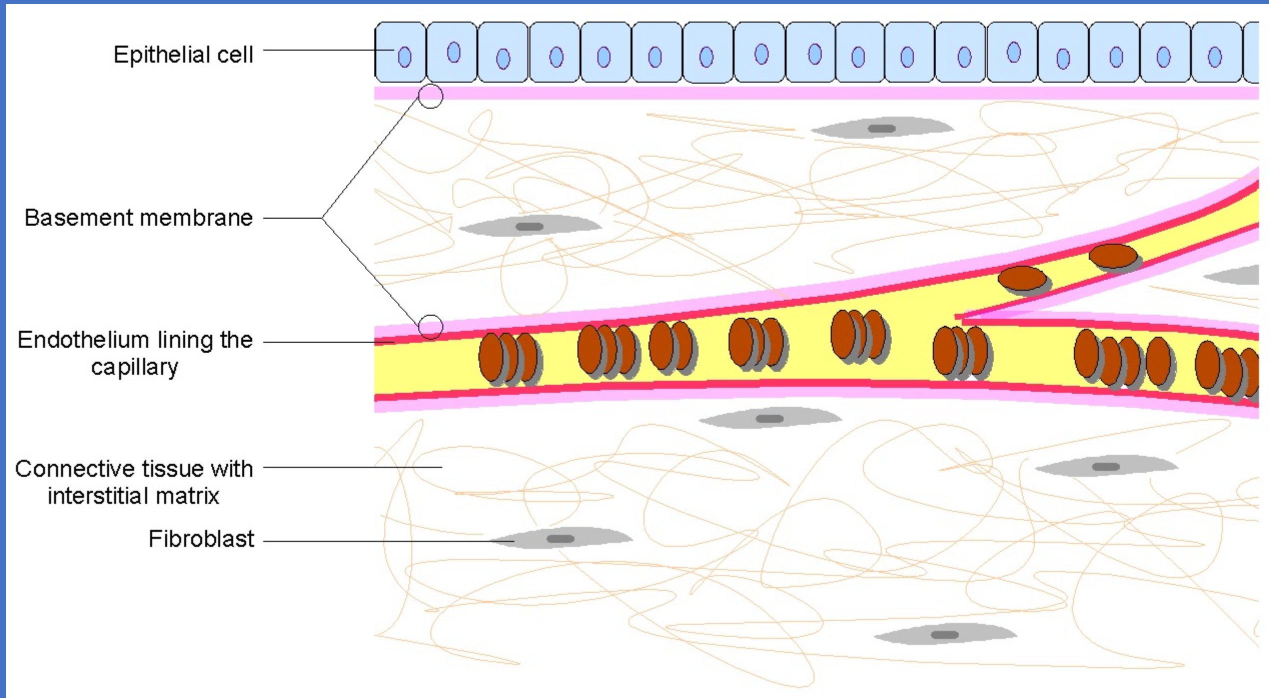


- apply principles and techniques from ***materials science and engineering*** to help understand biological processes and design systems
- take what we learn from nature to create ***biomimetic materials*** that can "jumpstart" regeneration

TISSUES

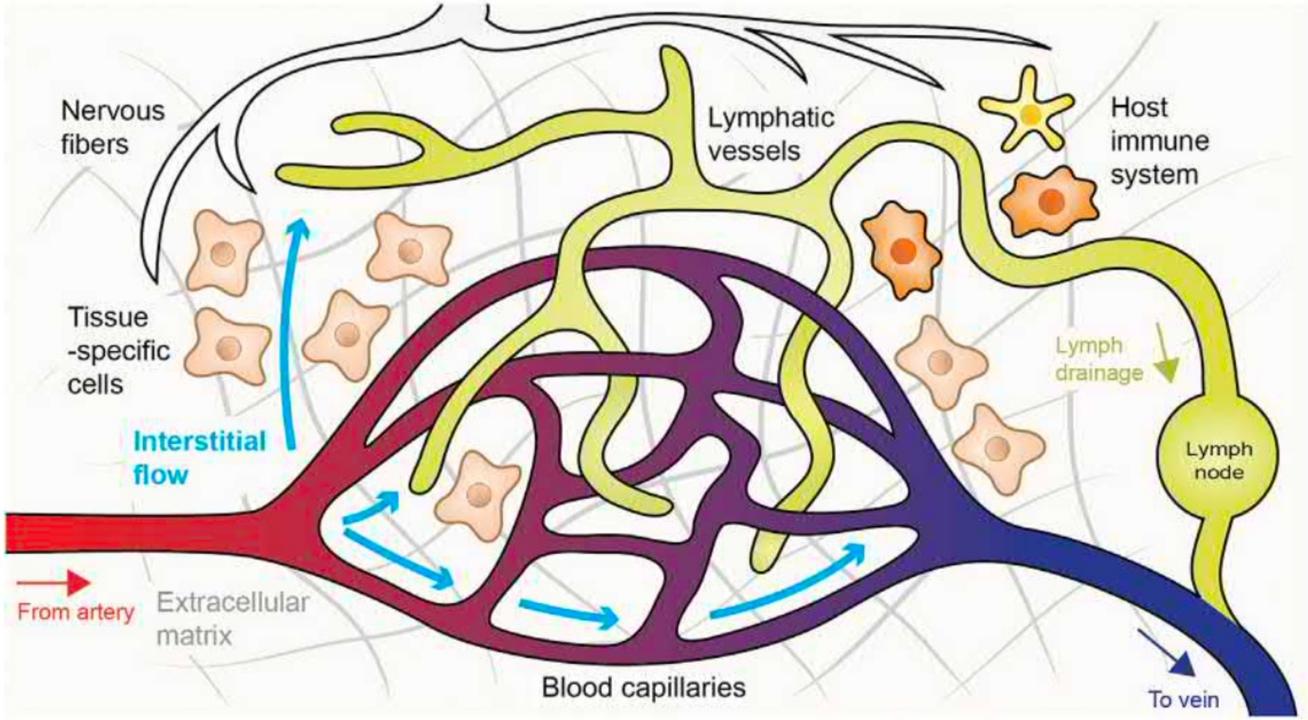


(A)
Apical specialization
Junctional complexes
Basement membrane
Epithelium
(B)
Cell
Matrix
Mesenchyme



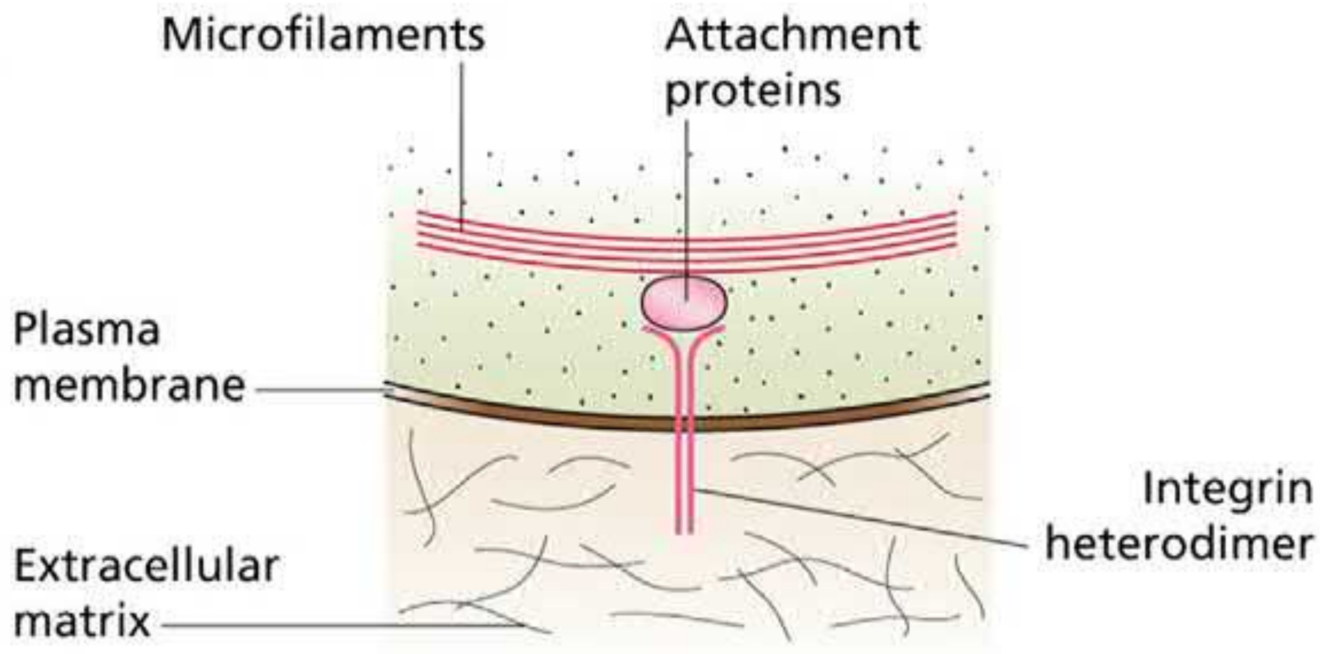
Epithelial cell
Basement membrane
Endothelium lining the capillary
Connective tissue with interstitial matrix
Fibroblast

Tissues as integrated systems in the body

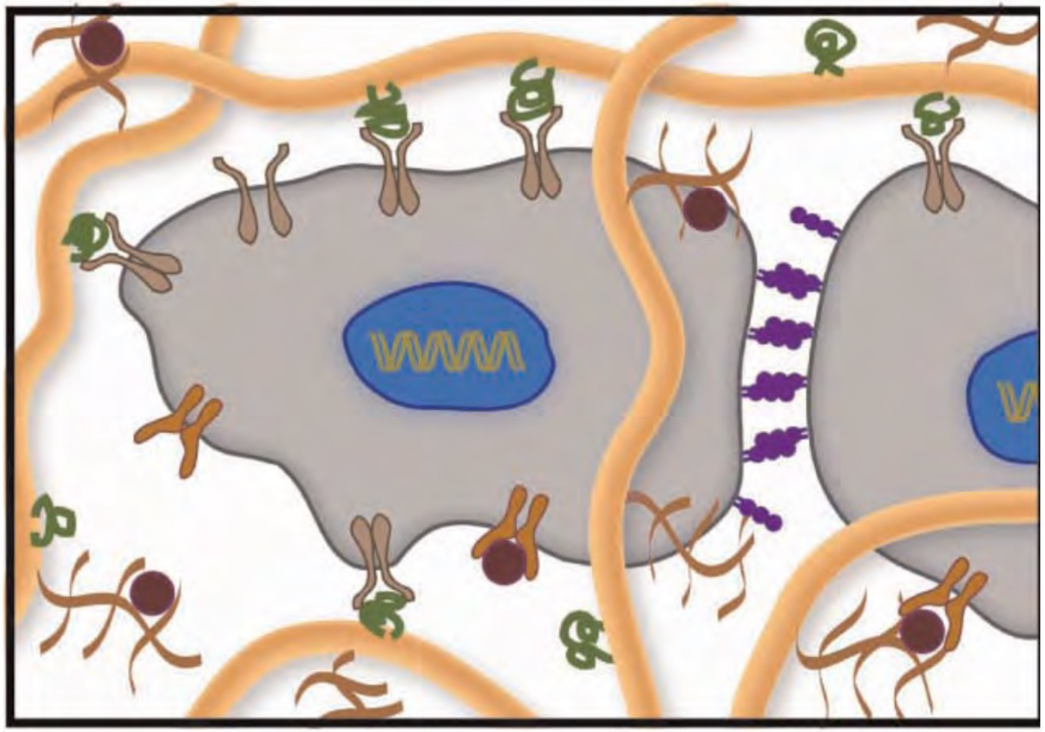


In addition to tissue-specific structures, most of tissues in the body are irrigated by blood capillaries that deliver oxygen, nutrients, and signaling molecules to tissue-specific cells. Into the tissue the interstitial flow increases the bio-availability of such molecules to distant cells, which is further regulated by specific interactions within the ECM. Tissue fluid and metabolic cell waste are collected by veins and lymphatic vessels and return back to the systemic circulation. The immune and nervous systems, also present in most of the body tissues, fulfill important roles in maintaining tissue homeostasis, modulating or instructing tissue functions, notably via systemic feedback loop signaling, and in repairing tissue upon injury.

The dynamics of cell-extracellular matrix interactions, with implications for tissue engineering



Extracellular matrix (ECM): home for cells



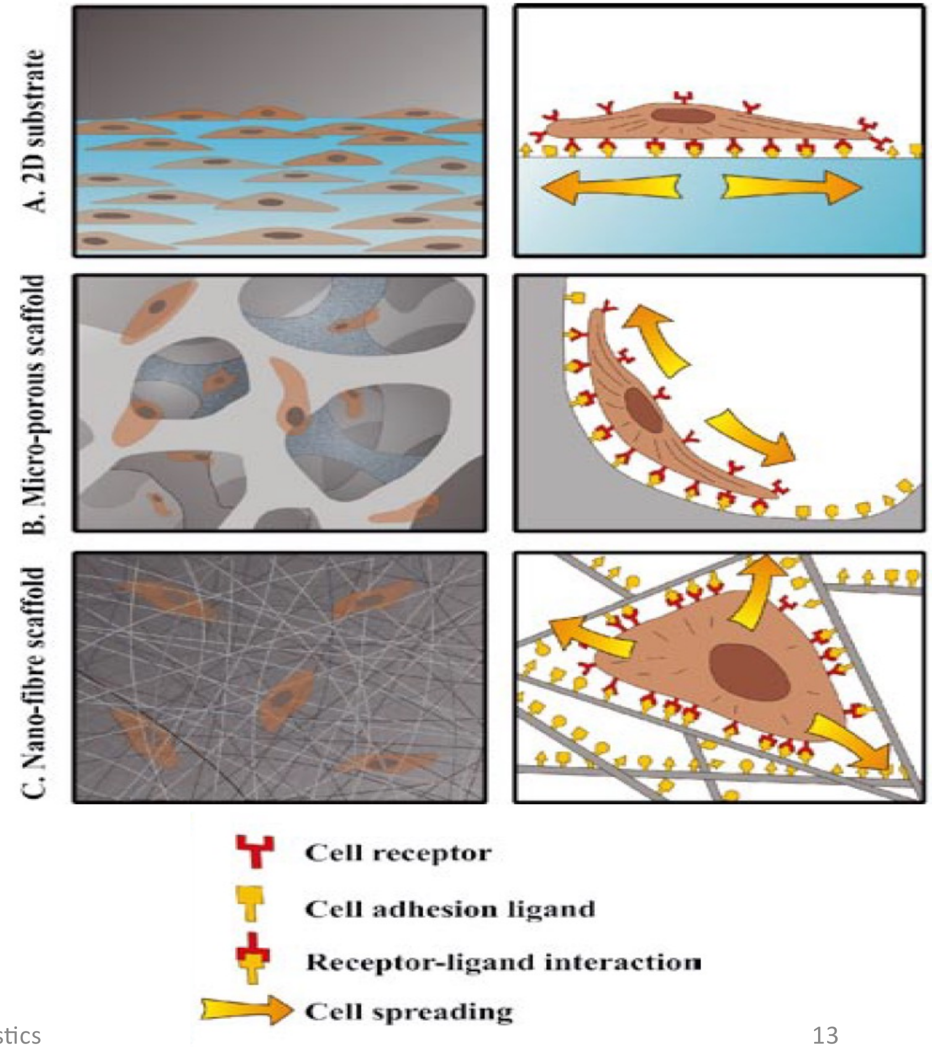
Tibbitt & Anseth, *Biotech & Bioeng* 2009

- composed of many cross-linked proteins and biopolymers
- provides mechanical support
- regulates biological functions such as cell adhesion, proliferation, migration, differentiation, etc.

- In vivo, cells are surrounded by a biological matrix comprising of tissue-specific combinations of insoluble proteins and inorganic crystals that are collectively referred to as the extracellular matrix (ECM).

- The varied composition of the ECM components not only provides the physical architecture and mechanical strength to the tissue, but also contains a reservoir of cell-signalling motifs (ligands) and growth factors that guide cellular anchorage and behaviour.

- The spatial distribution and concentration of ECM ligands, together with the tissue-specific topography and mechanical properties (in addition to signals from adjacent cells and from the surrounding fluid), provide signalling gradients that direct cell migration and cellular production of ECM constituents.



EXTRACELLULAR MATRIX (ECM)

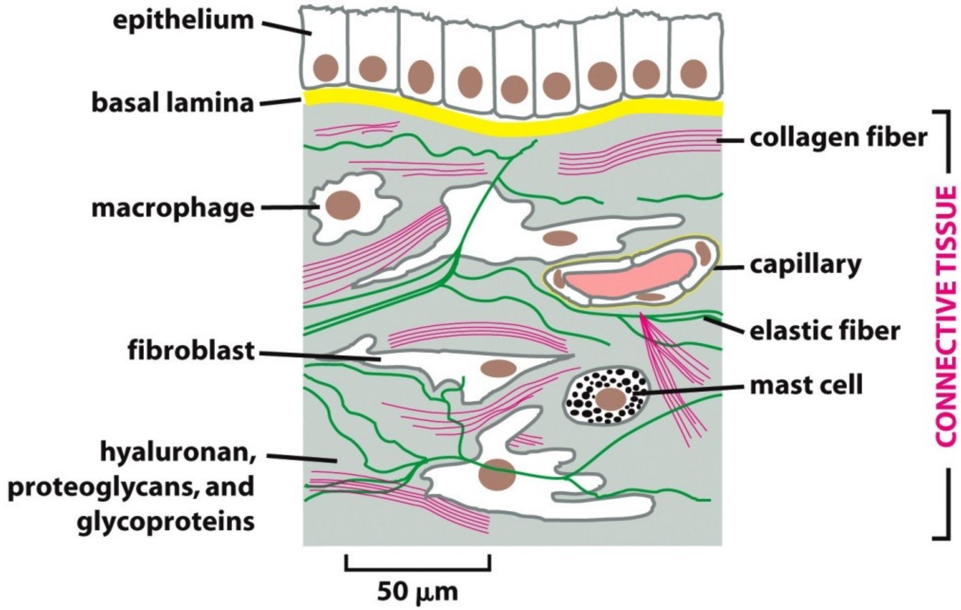
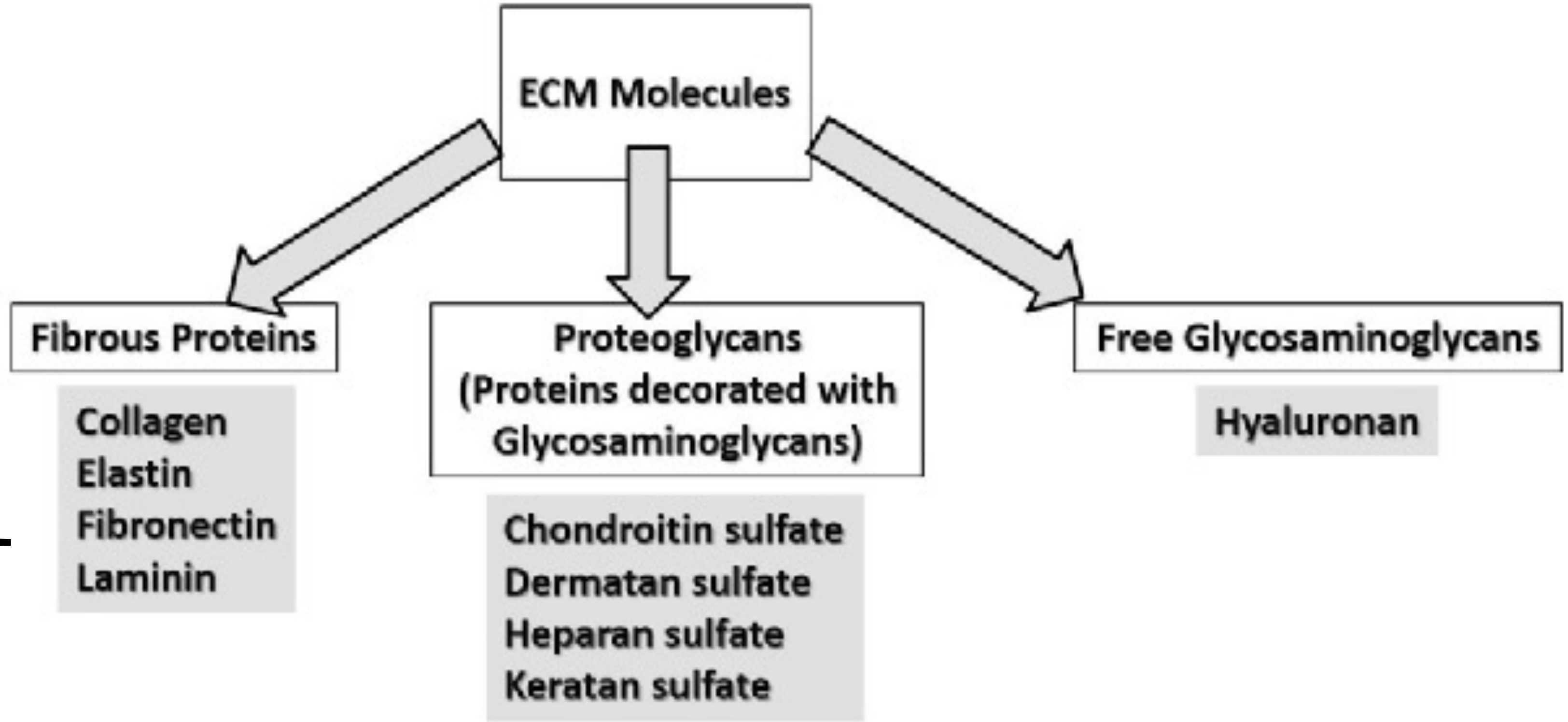


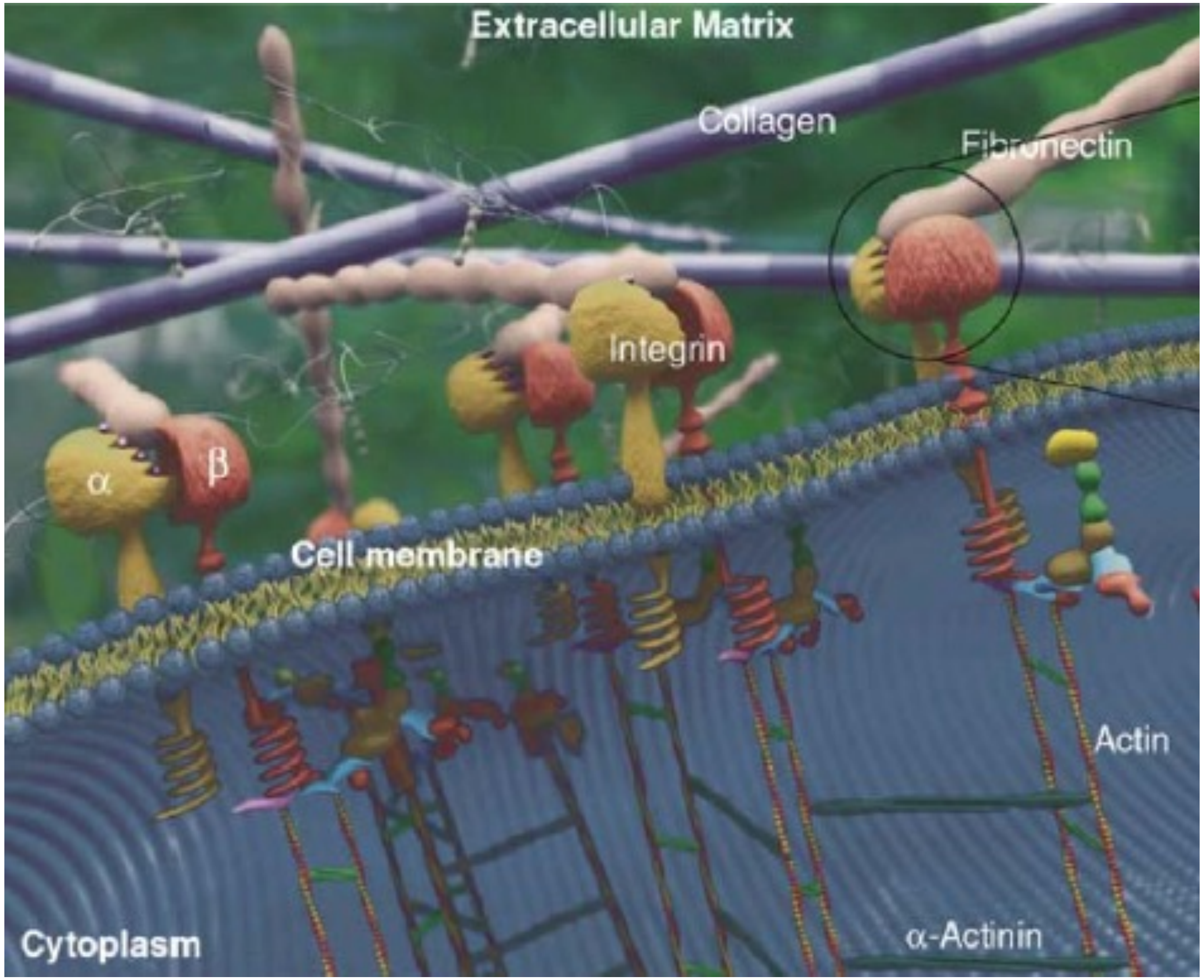
Figure 19-53 Molecular Biology of the Cell 5/e (© Garland Science 2008)

- Consists of a 3D array of protein fibers and filaments embedded in a hydrated gel of glycosaminoglycans
- ECM molecules
  - Glycosaminoglycans
  - Proteoglycans
  - Proteins

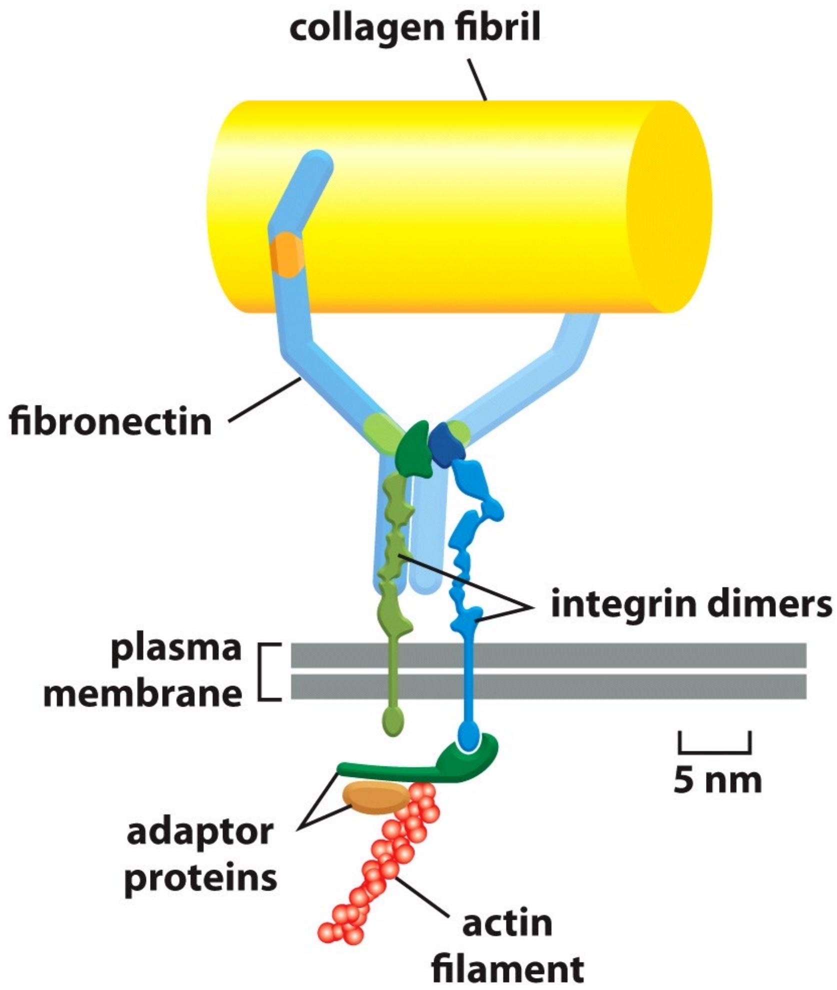
Structural proteins
- Collagen
- Elastin

Adhesion proteins
- Fibronectin
- Laminin

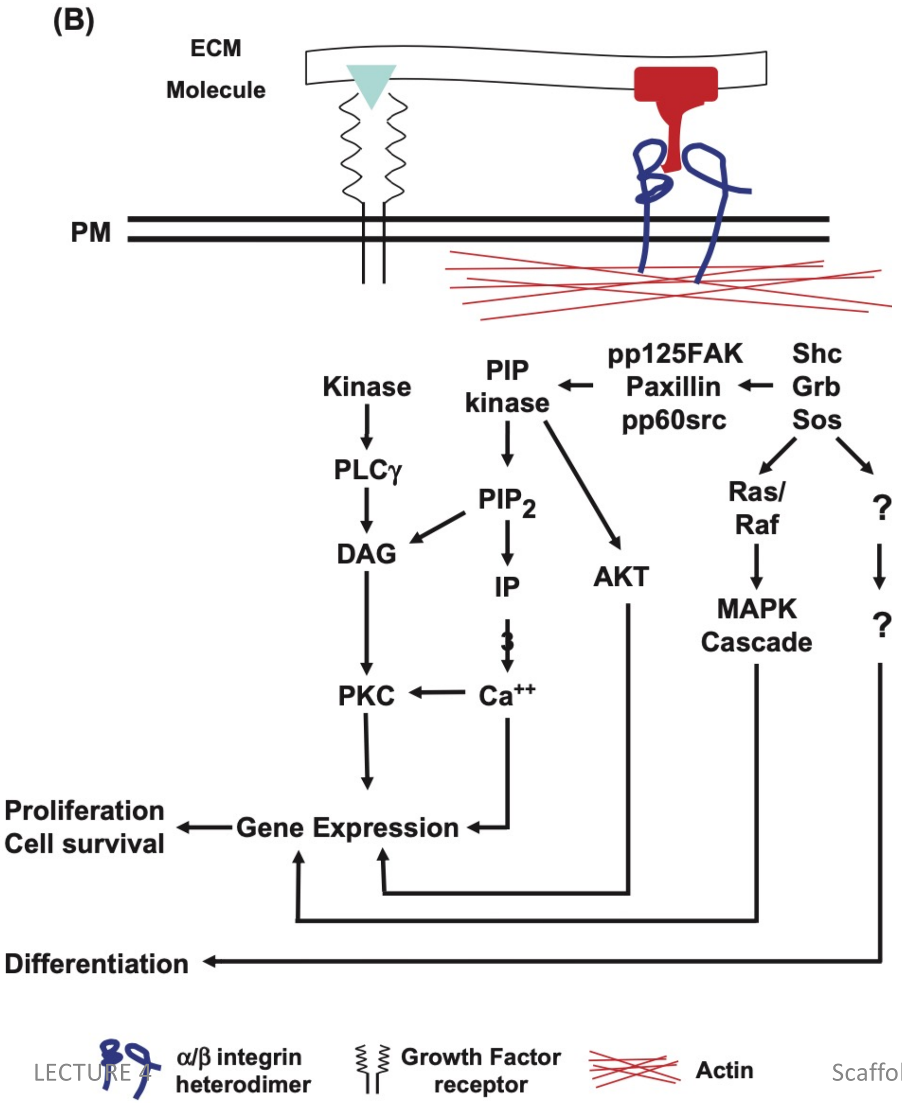


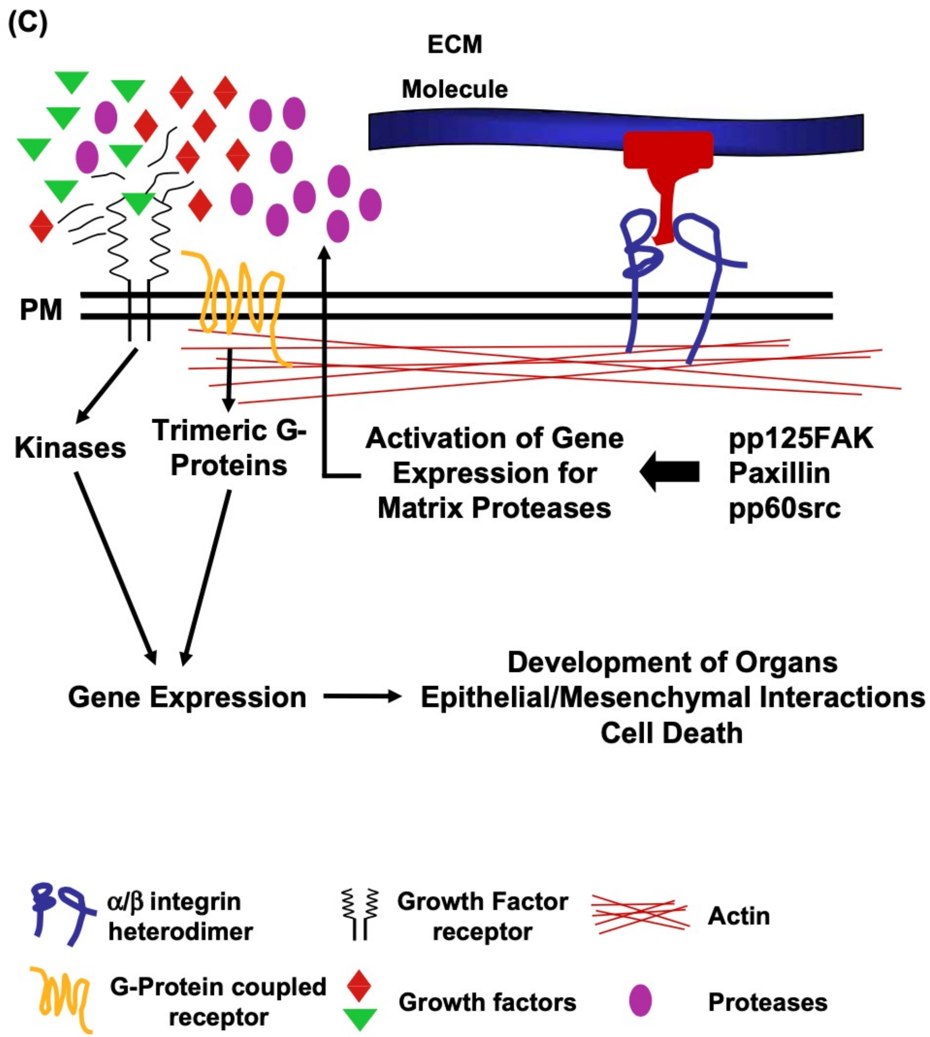

Extracellular Matrix
Collagen
Fibronectin
Integrin
α
β
Cell membrane
Actin
Cytoplasm
α-Actinin

Matrice extracellulare e adesione cellulare

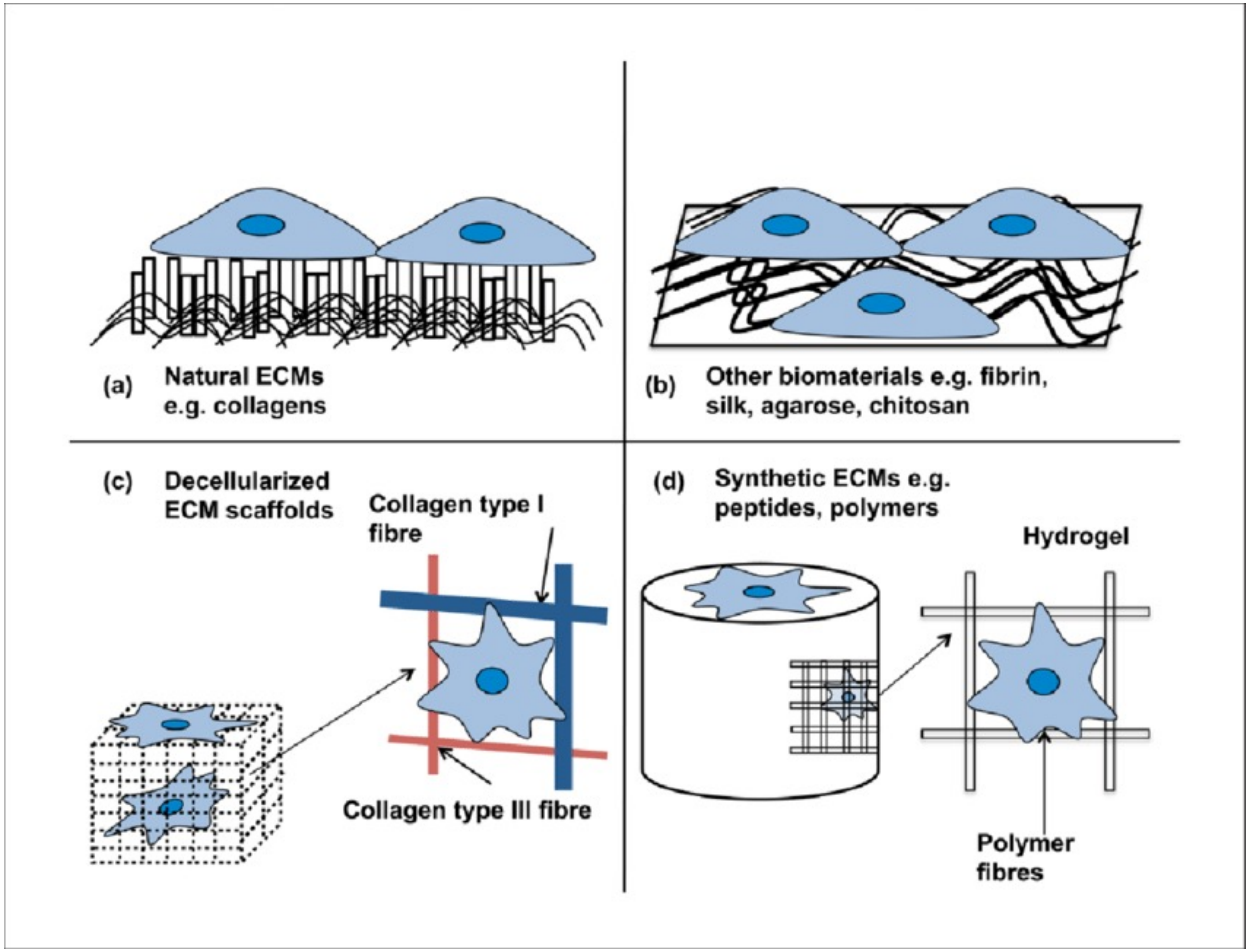


Cell-extracellular matrix interactions

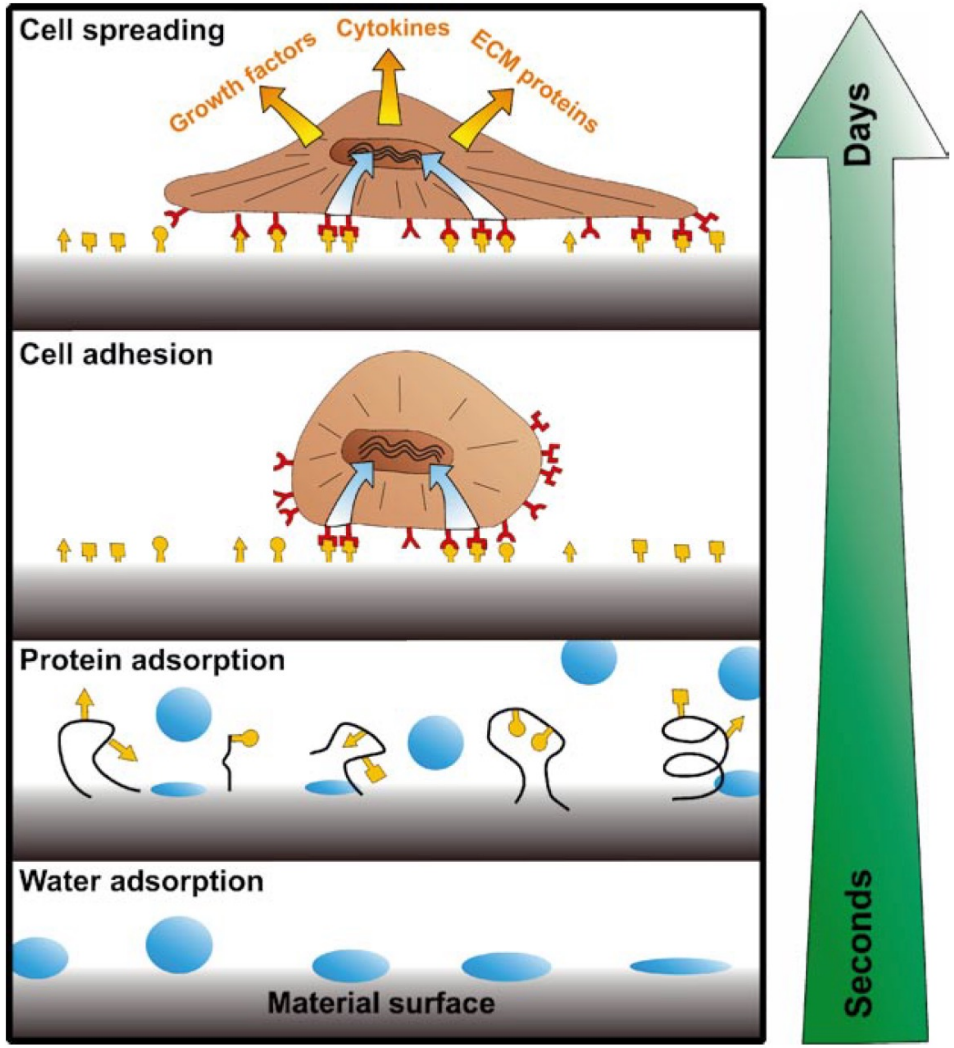




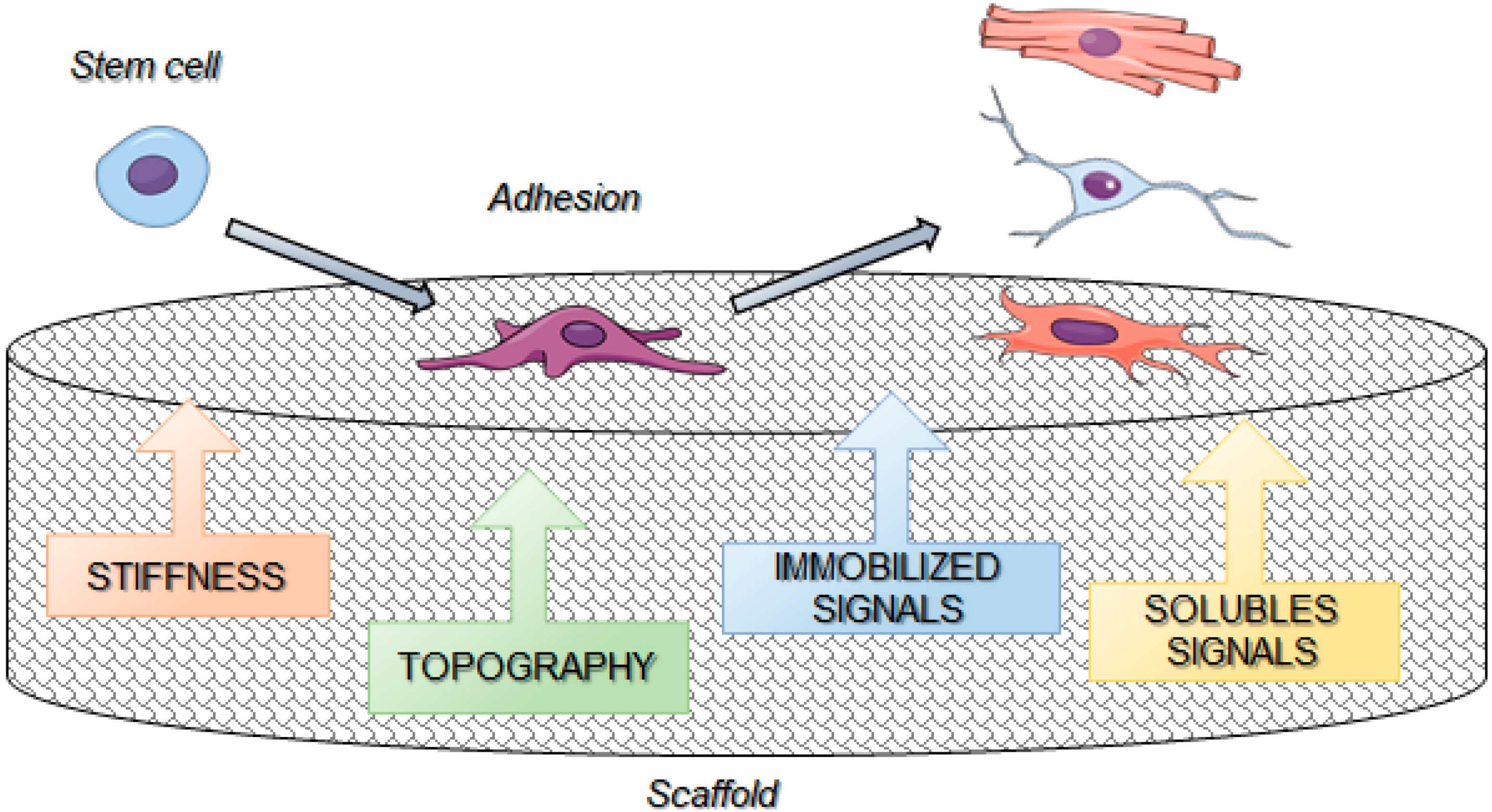
Creating the proper substrate for cell survival and differentiation



- The relative substrate activates various mechanotransduction and cellular pathways, which in turn trigger gene expression.
- In vivo micromechanical stimuli are important environmental cues that enable cell attachment, migration and organogenesis.

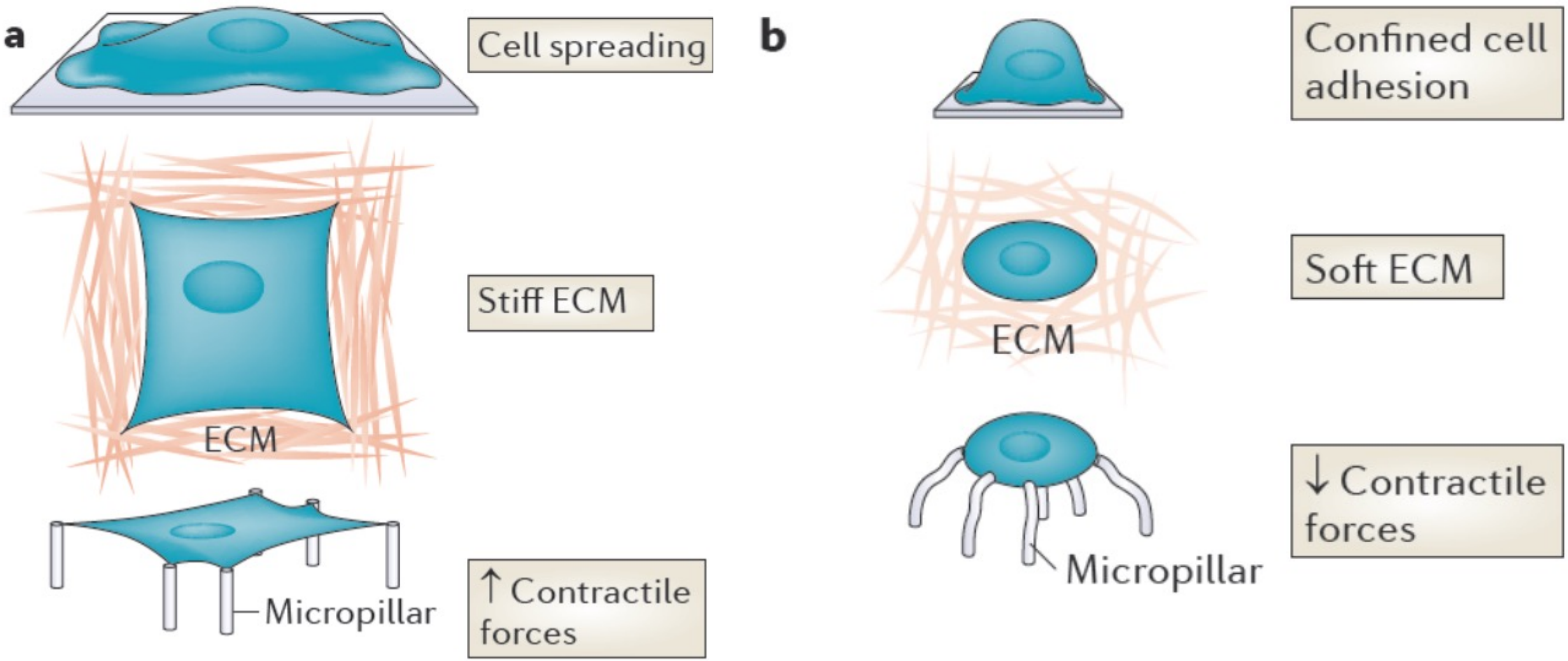


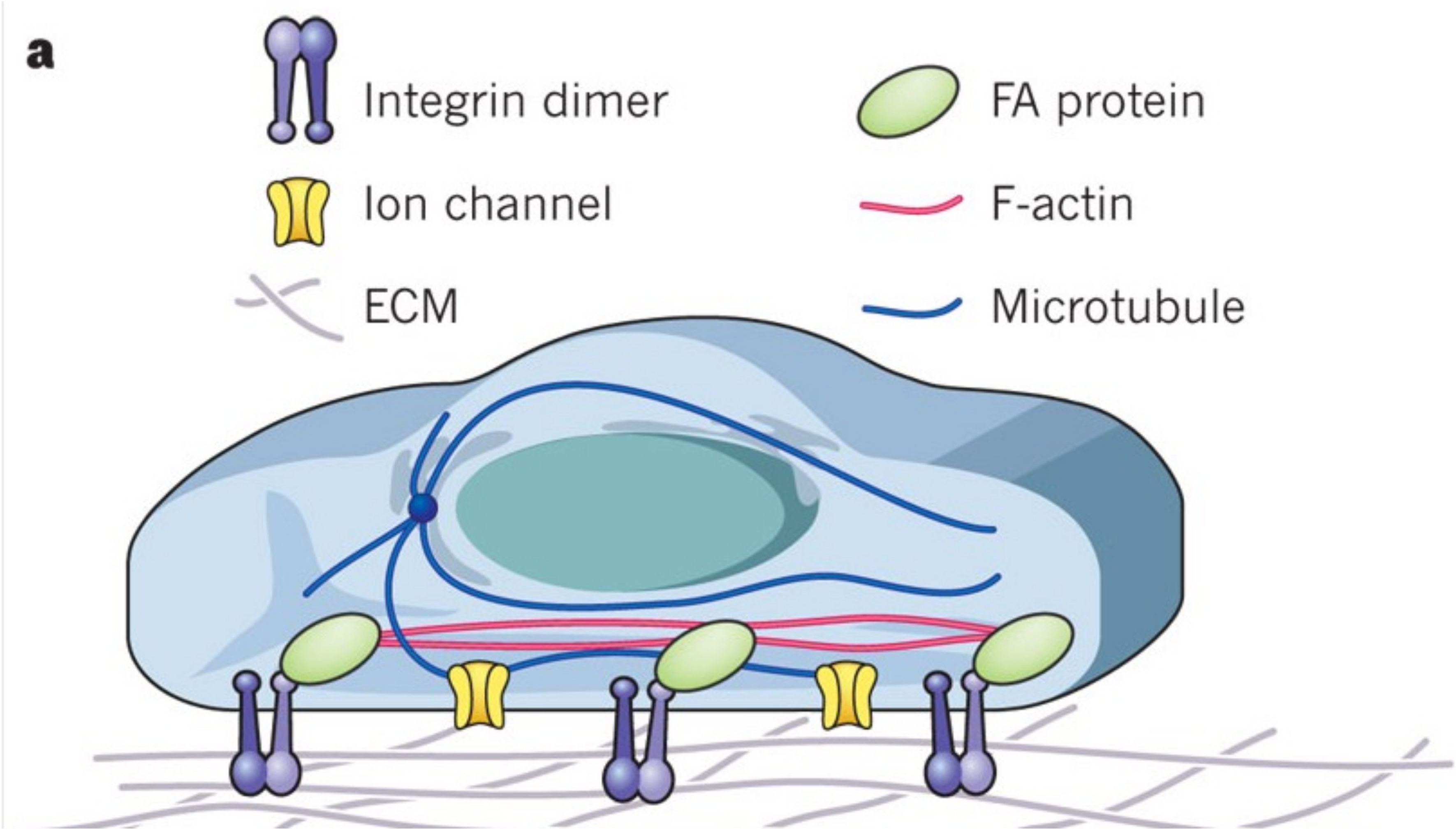
Mechanotransduction



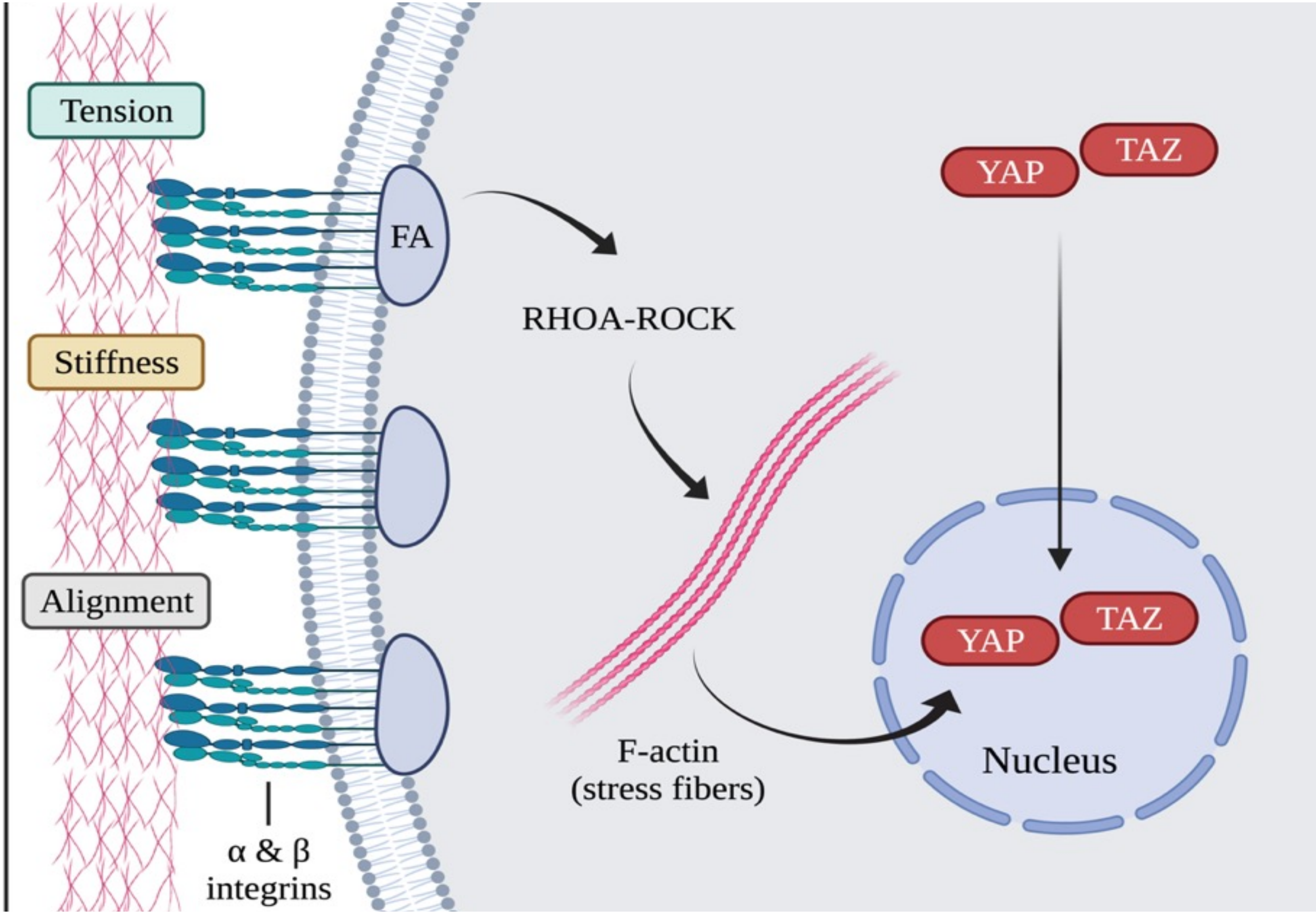
The mechanotransduction is the process by which cells sense mechanical stimuli of extracellular matrix or scaffold and translate them into biochemical signals.

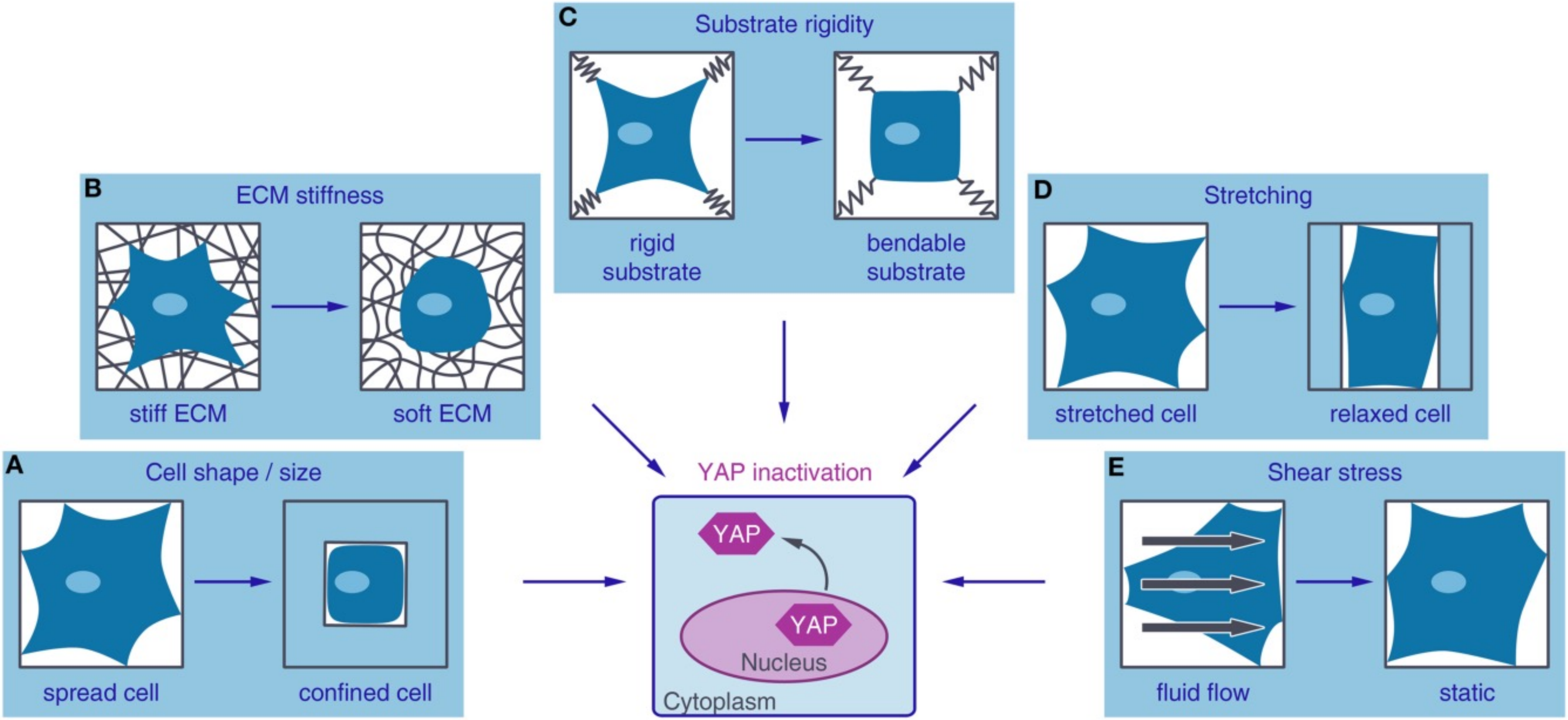
Mechanosensitivity and mechanotransduction

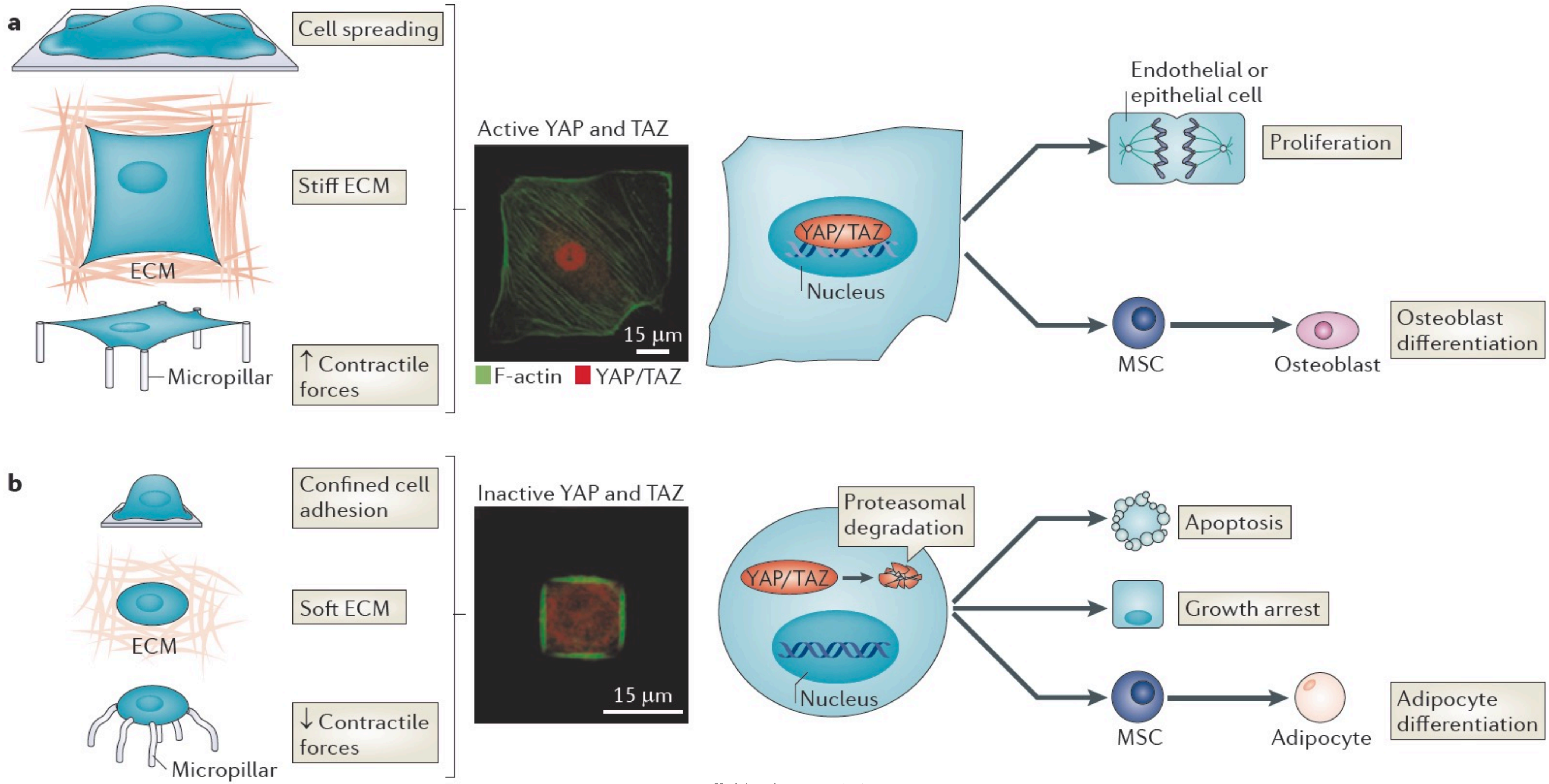



a
Integrin dimer
Ion channel
ECM
FA protein
F-actin
Microtubule

Electrospun scaffold fibers




C
Substrate rigidity
rigid substrate
bendable substrate
B
ECM stiffness
stiff ECM
soft ECM
D
Stretching
stretched cell
relaxed cell
A
Cell shape / size
spread cell
confined cell
YAP inactivation
YAP
YAP
Nucleus
Cytoplasm
E
Shear stress
fluid flow
static

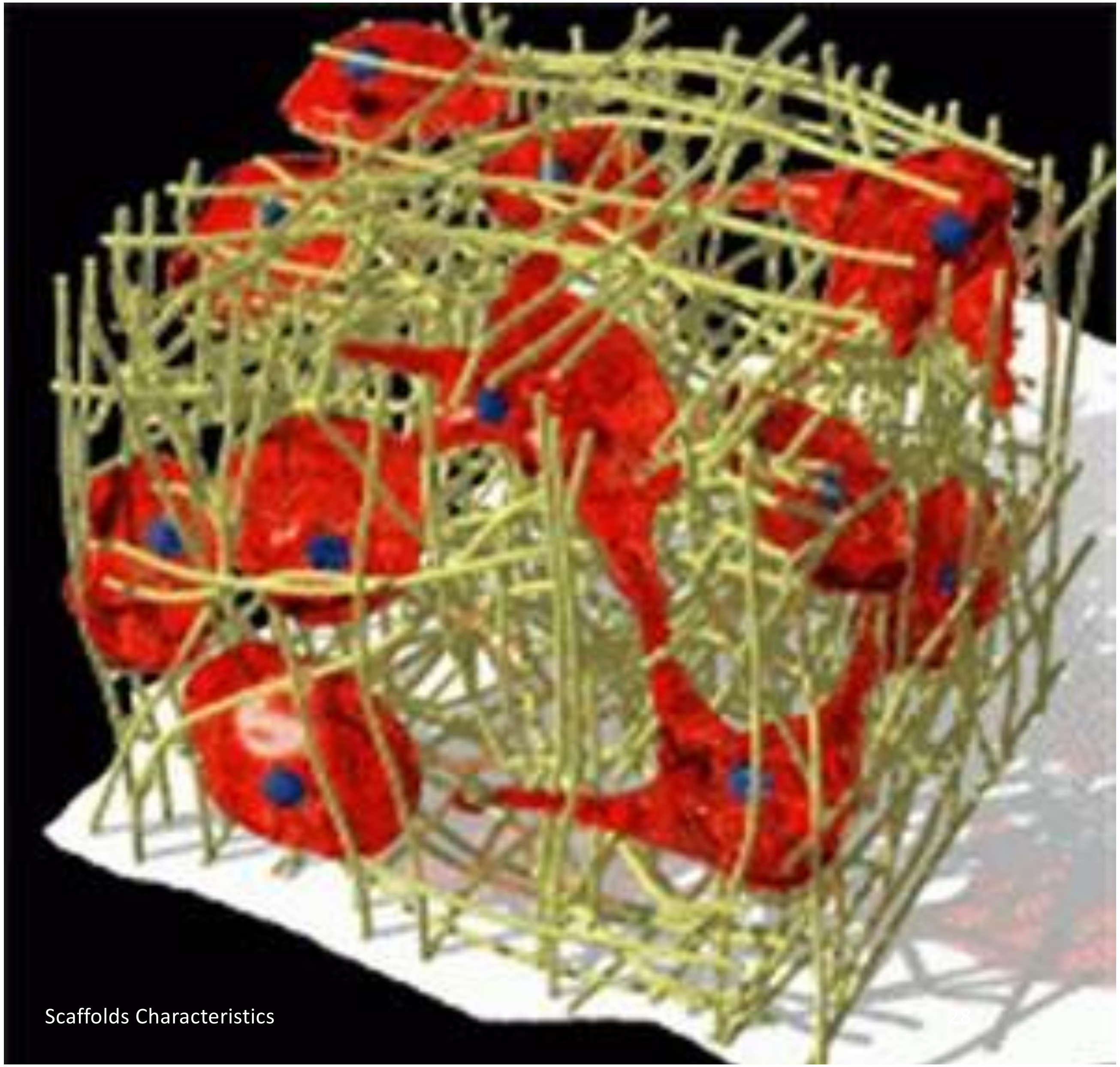

a
Cell spreading
Stiff ECM
ECM
Micropillar
↑ Contractile forces
Active YAP and TAZ
15 μm
F-actin
YAP/TAZ
YAP/TAZ
Nucleus
Endothelial or epithelial cell
Proliferation
MSC
Osteoblast
Osteoblast differentiation
b
Confined cell adhesion
Soft ECM
ECM
Micropillar
↓ Contractile forces
Inactive YAP and TAZ
15 μm
Proteasomal degradation
YAP/TAZ
Nucleus
Apoptosis
Growth arrest
MSC
Adipocyte
Adipocyte differentiation

Fiber parameters

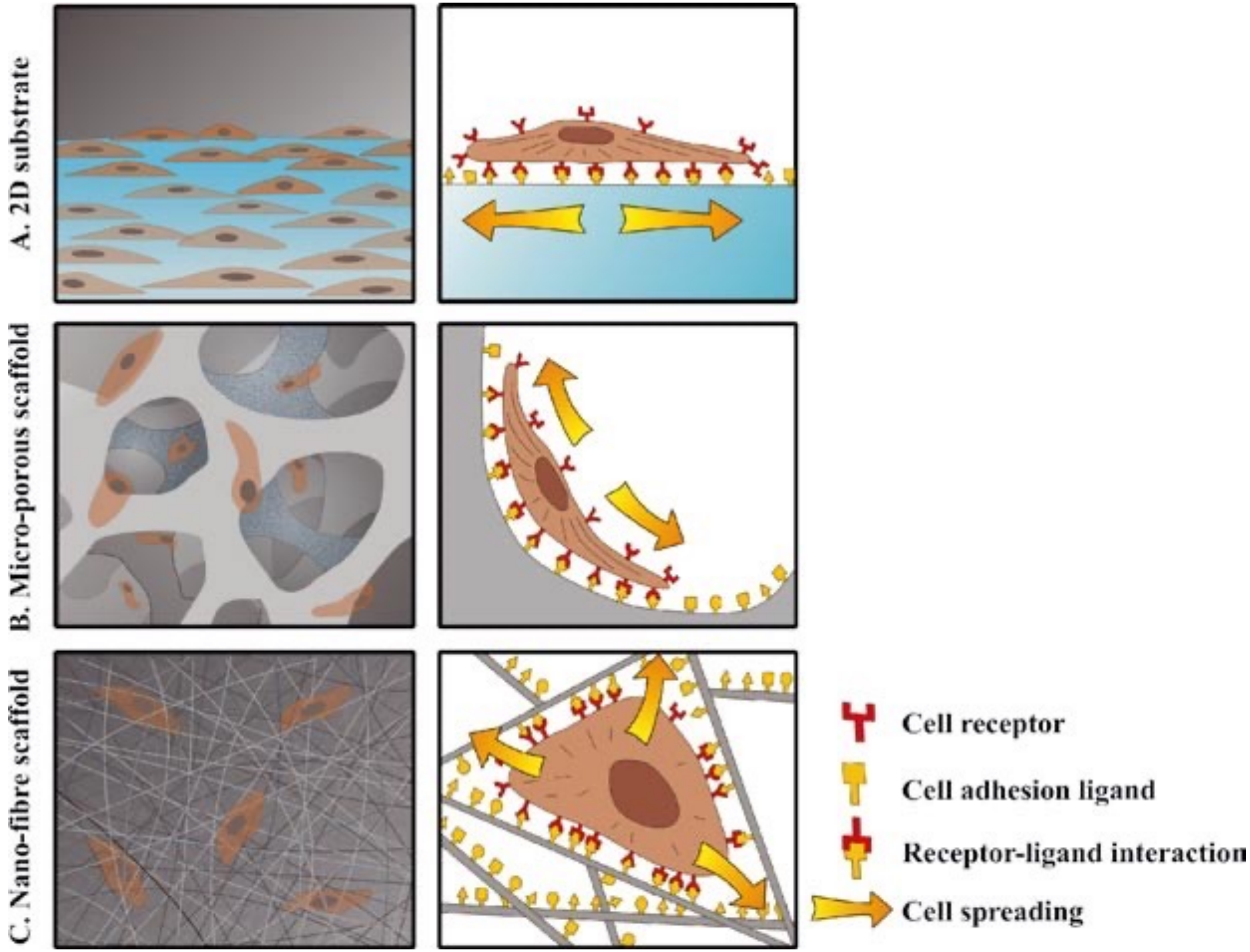
Differentiation

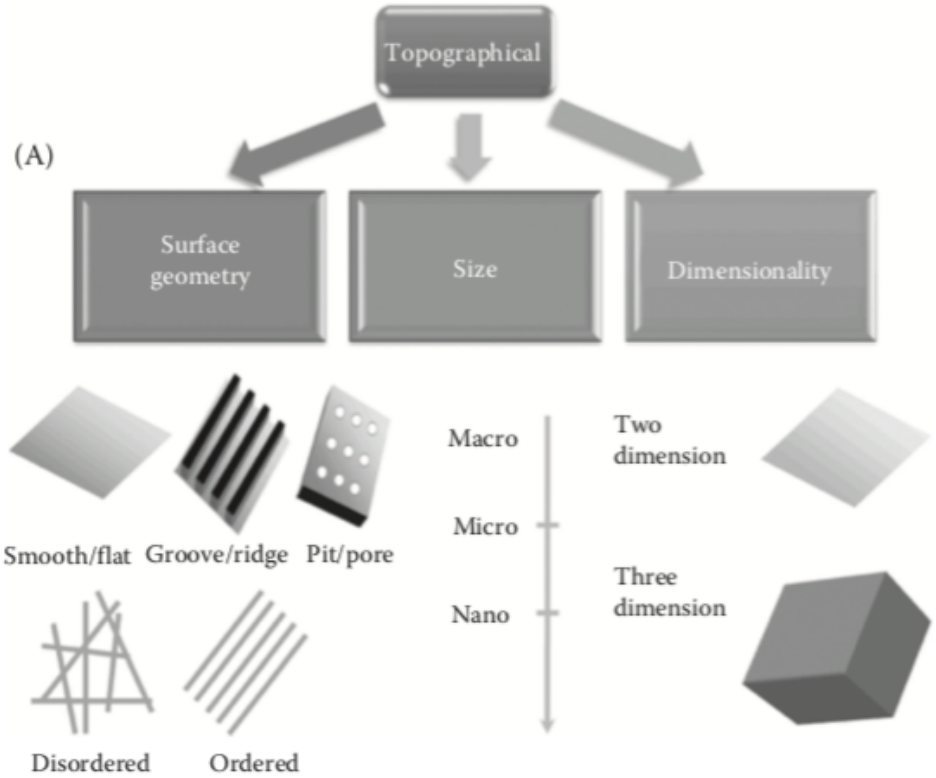
The surface topography significantly affects stem cell behaviors such as:

- **Morphology**
- **Orientation**
- **Differentiation**



Mimicking the Extracellular Matrix





- Recently, much attention has been paid to topographical cues as a useful tool for controlling stem cell fate and guiding stem cell differentiation.

- The use of topographically guided scaffolds for supporting stem cells has a great advantage over the use of chemical reagents due to allowing cells to grow and differentiate in the absence of potentially harmful inducing reagents.

- Surface geometries such as smooth/flat, groove/ridge, pit/pore, and disordered/ordered structure significantly influence cellular morphology, proliferation, and differentiation ability.

- Understanding influences of topographical cues on stem cell behaviors plays an important role in designing suitable scaffolds for tissue regeneration to expedite expansion and differentiation of stem cells without changing the plasticity nature of stem cells.

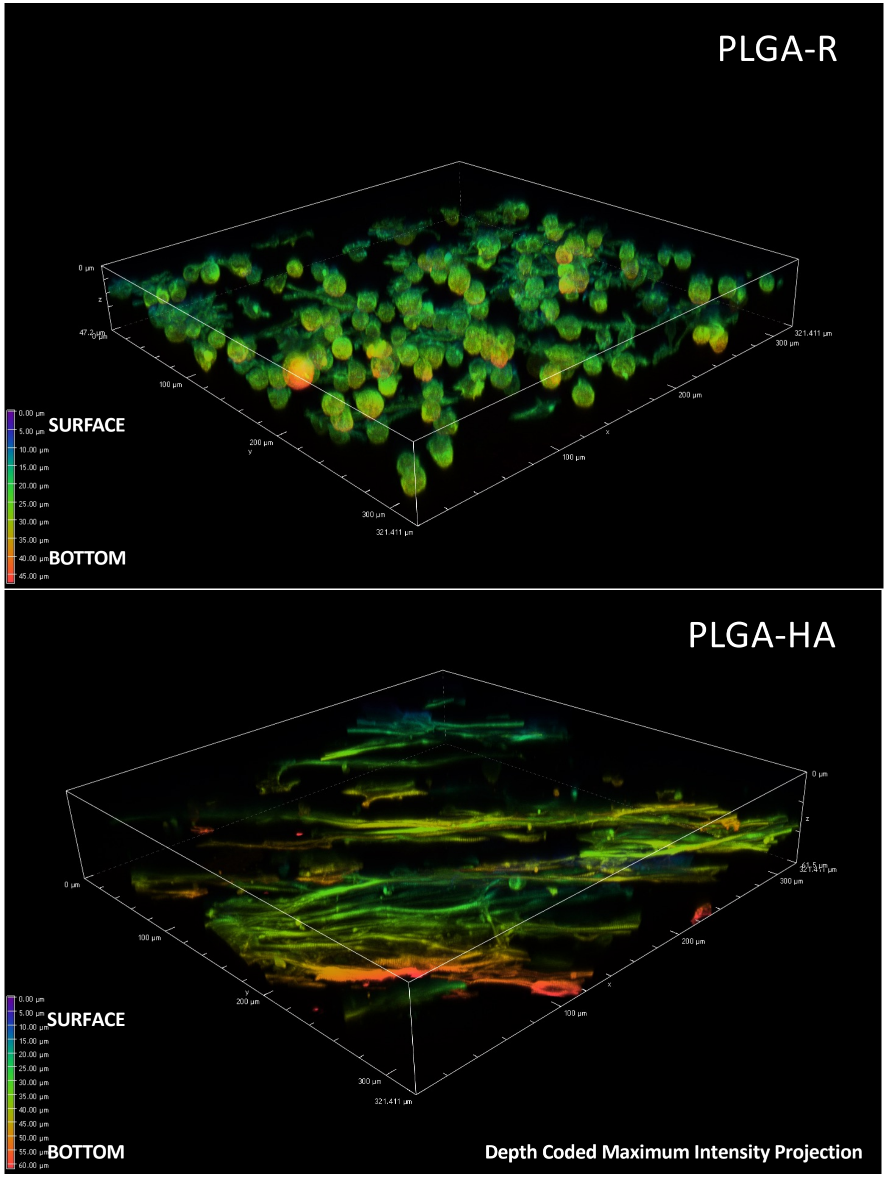
Table 3. General trends of cell response to fiber parameters

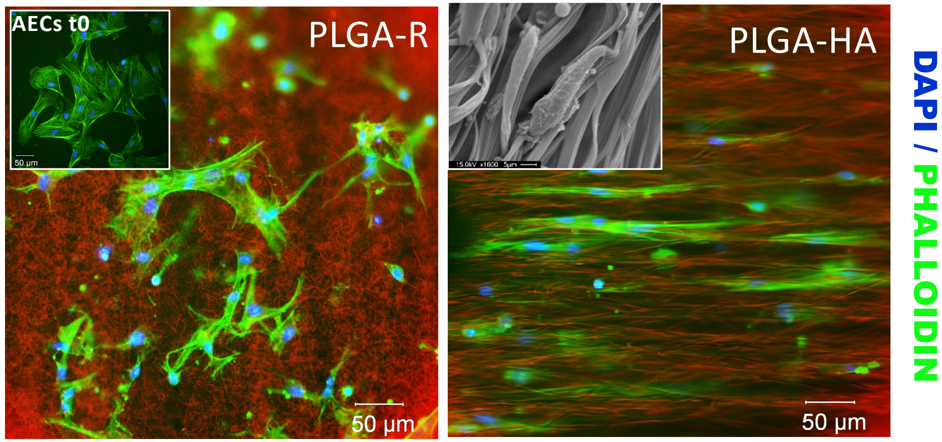
Fiber Parameters	Differentiation	Morphology	Migration
Diameter			
Nanofibers	Osteogenesis Chondrogenesis Tenogenesis Myogenesis Neurogenesis (Glial)	Rounded Larger focal adhesions	↑ Velocity
Microfibers	Adipogenesis Chondrogenesis Tenogenesis Neurogenesis (Neuronal)	↑ Elongation ↑ Aspect Ratio ↑ Alignment ↑ Area More focal adhesions	↑ Distance
Alignment			
Random	Neurogenesis (Glial)	Round Polygonal Random Orientation	
Aligned	Osteogenesis Tenogenesis Myogenesis Neurogenesis (Neuronal)	↑ Elongation ↑ Alignment Spindle Shape Cytoskeletal Alignment	↑ Velocity ↑ Distance Direction of Fibers

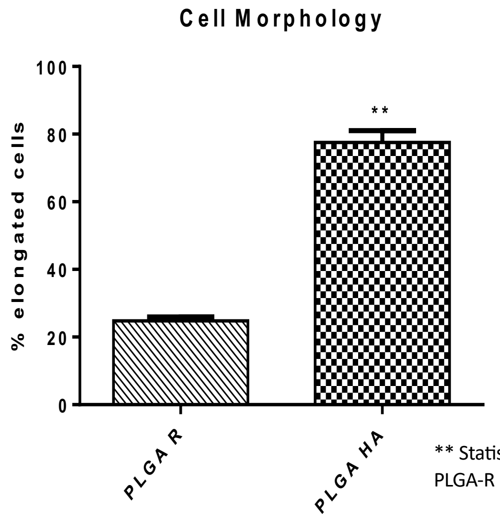
Table 3. General trends of cell response to fiber parameters

Fiber Parameters	Differentiation	Morphology	Migration
Porosity			
Low	Myogenesis	Rounded ↑ Spreading Attach to Multiple Fibers	
High	Neurogenesis (Glial)	↑ Elongation Larger Pseudopodia Attach to Single Fibers	↑ Velocity ↑ Distance

Influence of fibre topography on AECs



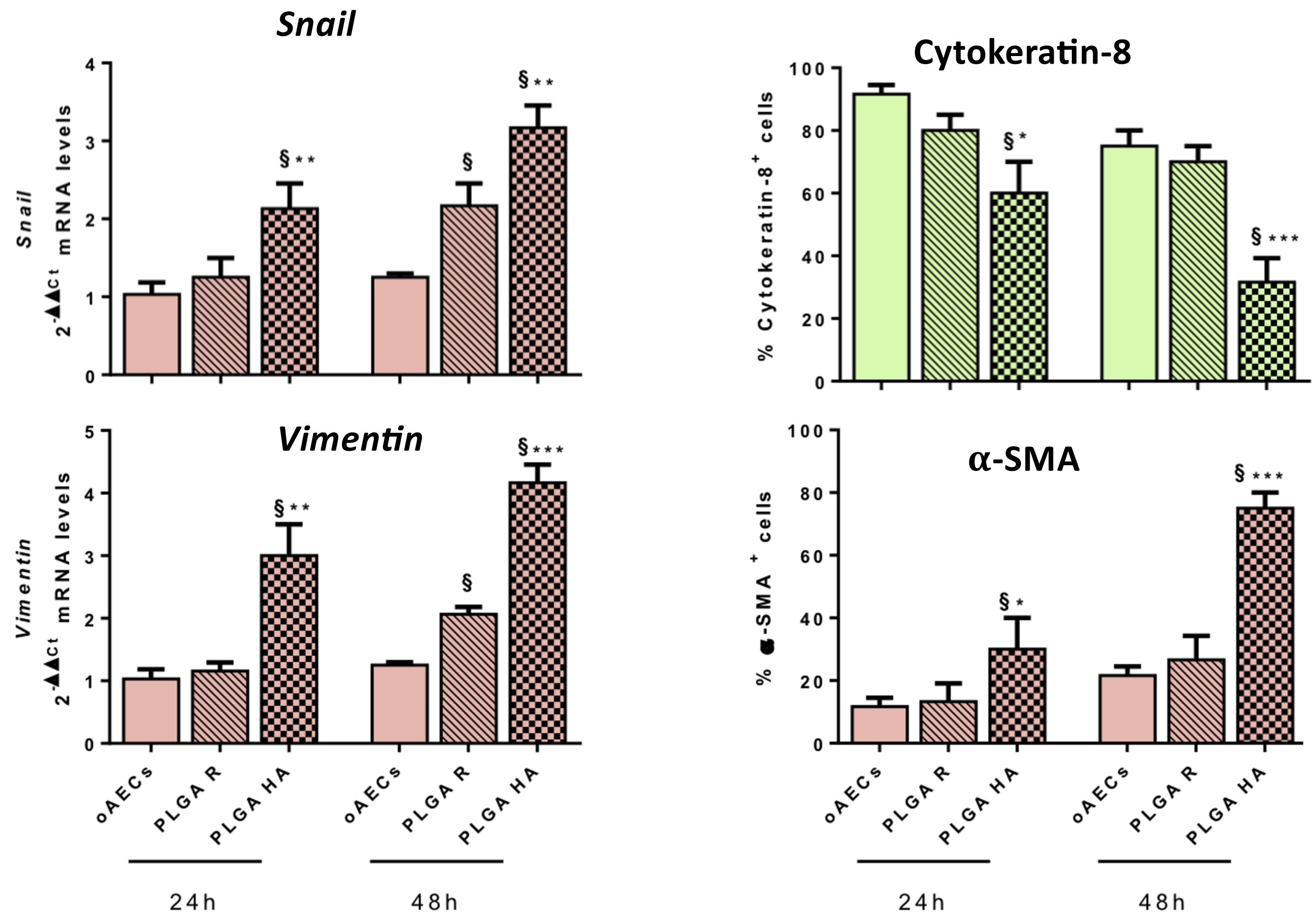


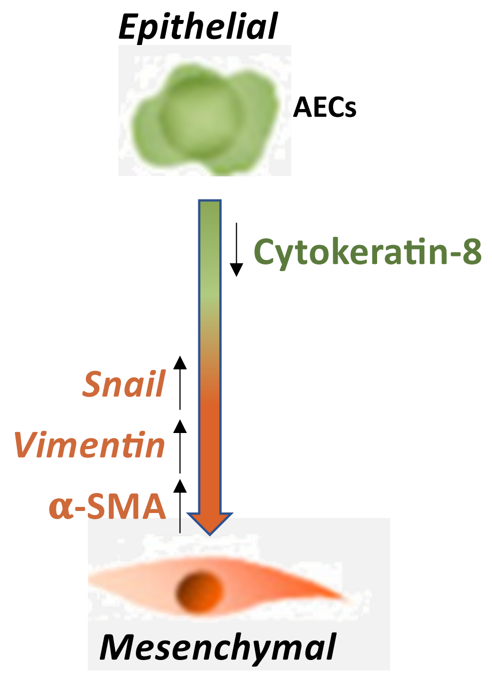


48h

** Statistically significant values between PLGA-R and PLAGA-HA scaffolds (p<0.01)

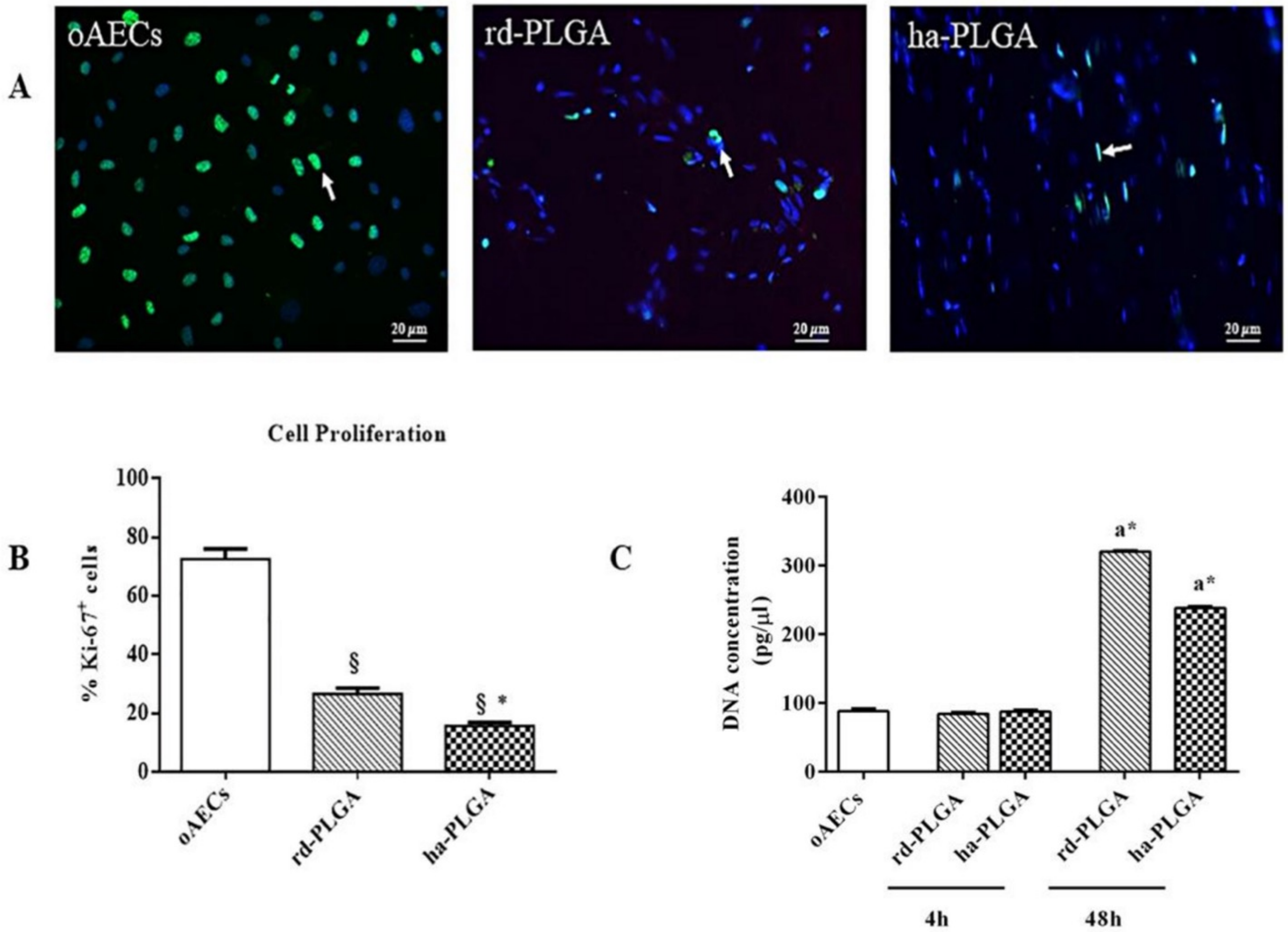
Fibre Topography induces AECs' Epithelial Mesenchymal Transition (EMT)



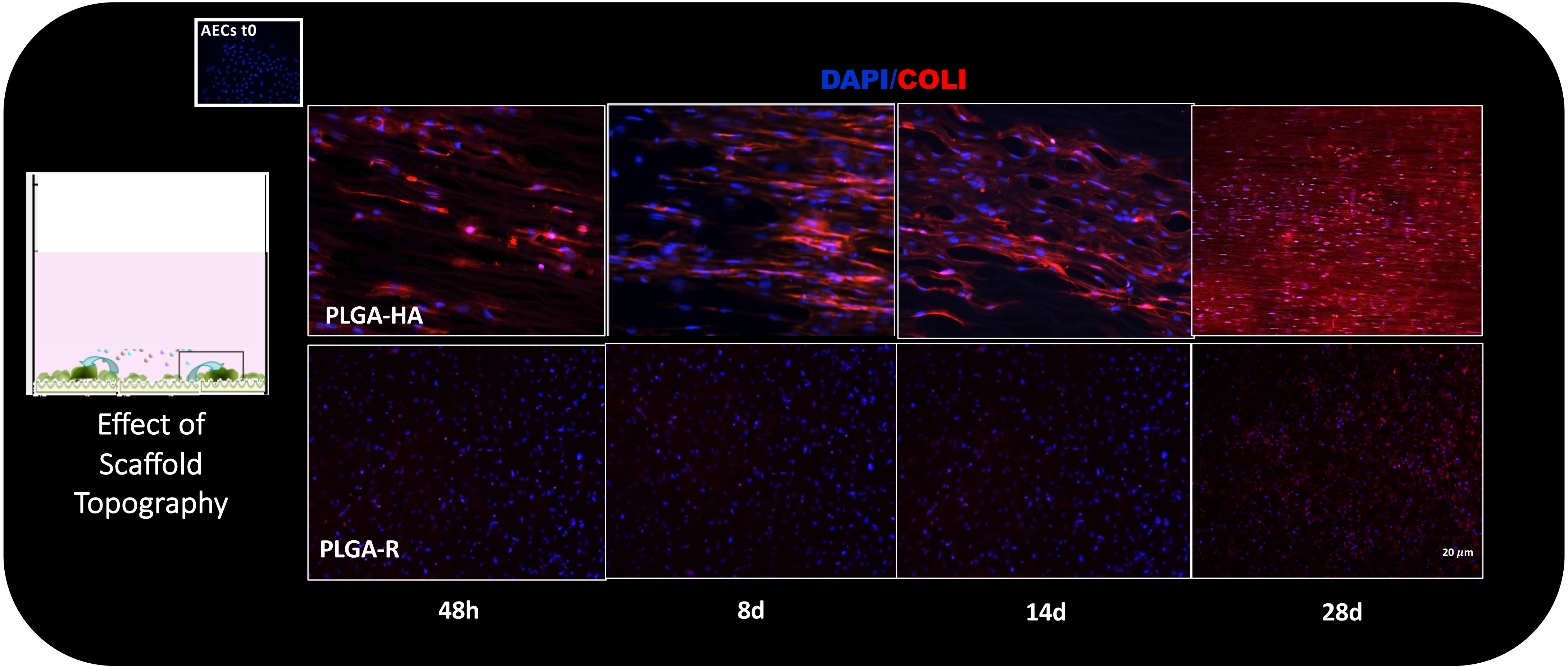


*Statistically significant between PLGA-HA and PLGA-R ($p<0.05$)
§ Statistically significant between PLGA-HA and AECs ($p<0.001$)

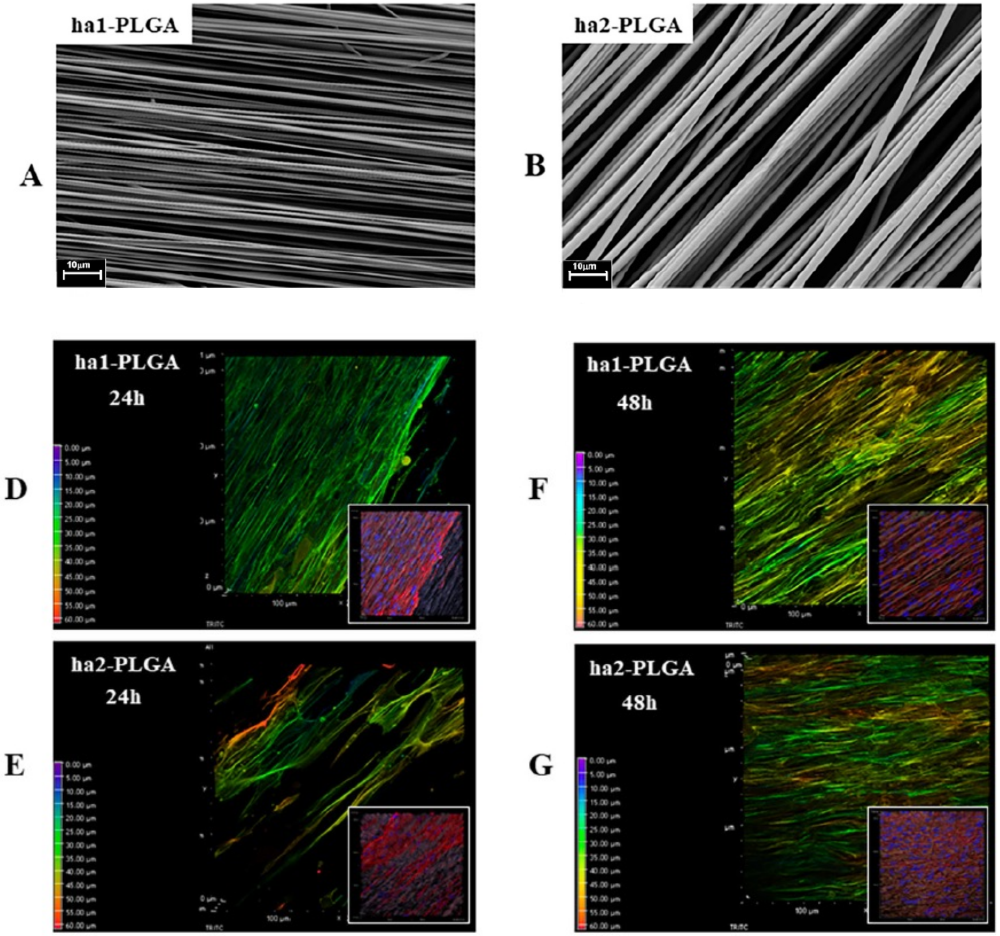
Effect of fiber alignment on oAEC proliferation.

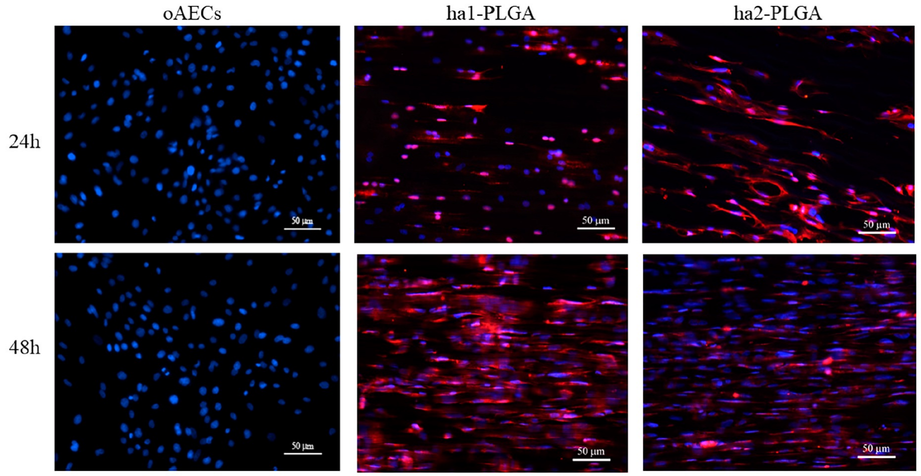


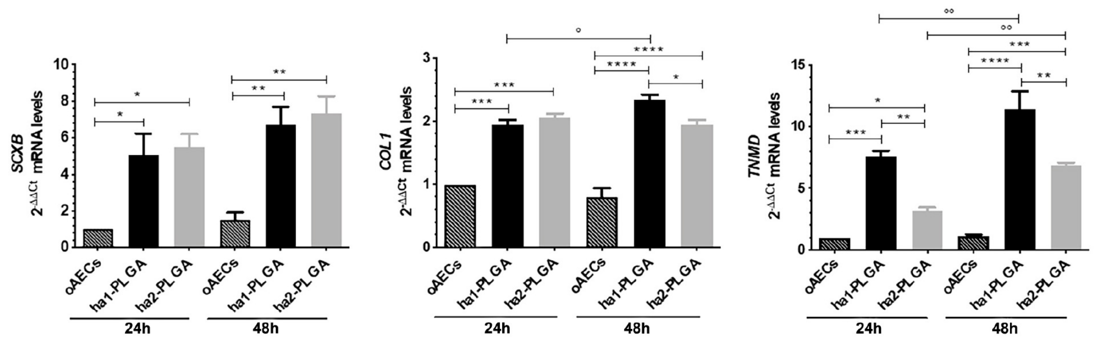
PLGA-HA scaffold teno-inductive properties on AECs



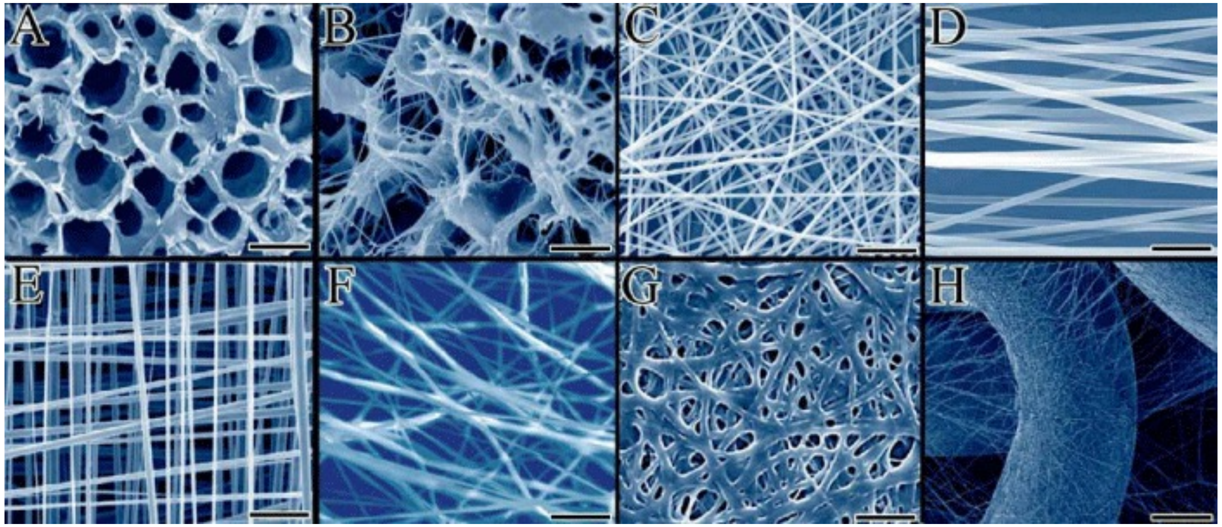
Effect of fiber diameter

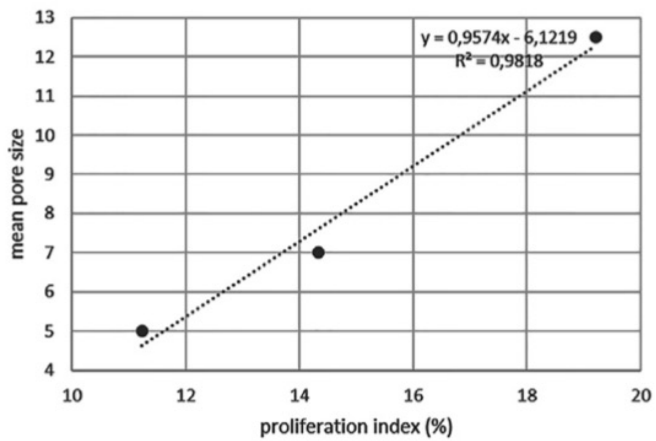






Porosity





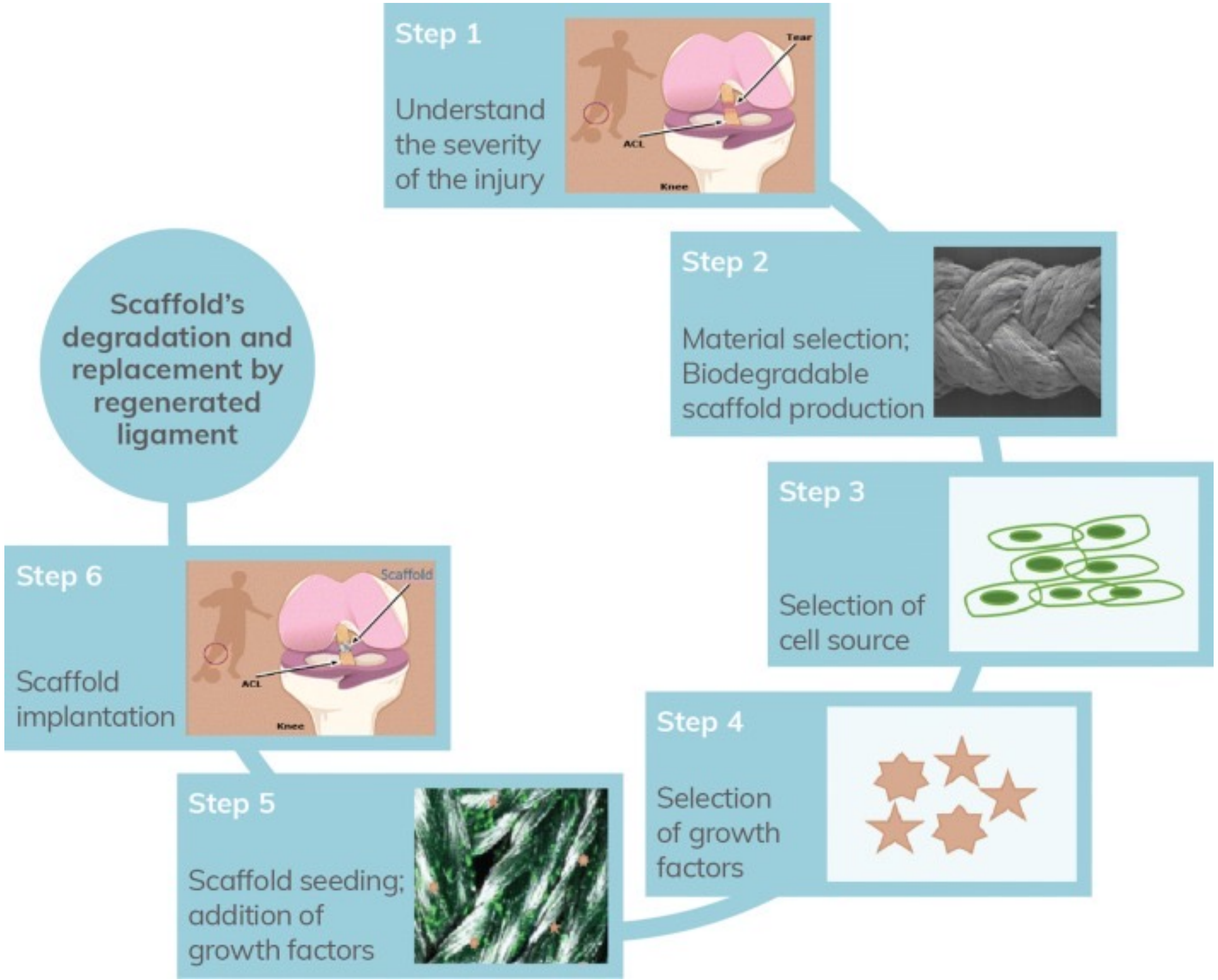
is a key parameter for providing nutrients for cells allowing their proliferation, penetration, and permitting waste exchange

Mechanical properties

Tissue (species)	*A* coefficient range in MPa	*B* coefficient range	Tangent modulus at 1% strain (MPa) = $ABe^{B\varepsilon}$	Tangent modulus at 10% strain (MPa) = $ABe^{B\varepsilon}$	Reference for data fit
Trabecular bone (human)	100.0–100,000.0	0.06–0.0152	6.0–1,520.0	6.0–1,520.0	21
Meniscus	1.6–3.2	27.5–31.9			48
Articular cartilage	0.3–2.1	1.3–5.0	0.4–11.0	0.444–17.3	48
Medial collateral ligament (human)	0.3	12	4.06	11.95	56
Intervertebral disc—fibrocartilage	0.05–0.07	4.95–11.9	0.26–0.94	0.41–2.74	34
Spinal cord grey matter (cow)	0.0066	9.06	0.066	0.148	28
Spinal cord white matter (cow)	0.0041	6.54	0.029	0.052	28
Bladder smooth muscle (rat)	0.0022	25.7	0.073	0.739	20
Fat (human)	0.002	9.64	0.002	0.005	45
Heart valve	0.00153	28.81	0.06	0.78	71
Myocardium	0.0013	6.0	0.008	0.014	62
Skin/subcutaneous tissue	0.000057	21.52	0.0015	0.011	76

Results show the tremendous variation in tissue mechanical properties ranging from stiff linear elastic (bone) to very compliant nonlinear elastic for smooth muscle, fat, cardiovascular, and skin tissues

Biodegradability



Step 1
Understand the severity of the injury
Tear
ACL
Knee
Step 2
Material selection; Biodegradable scaffold production
Step 3
Selection of cell source
Step 4
Selection of growth factors
Step 5
Scaffold seeding; addition of growth factors
Step 6
Scaffold implantation
Scaffold
ACL
Knee
Scaffold's degradation and replacement by regenerated ligament