Introduction to the Breadth of the Extracellular Matrix of Animal Tissues

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The ECM (and water within it) is a major component of tissue

- Variable amounts (bone to brain)
- Highly heterogeneous between and within tissues, and different states
- Highly dynamic
- Critical determinant of tissue properties
  - Skin, vessels, lungs: elasticity
  - Bone: strength
  - Cornea: transparency
Discrete ECM structures: normal

- Basement membrane
- Submucosal space
- Interstitial space
- Cornea
- Apical glycocalyx
- Cartilage
The ECM in pathology

Cancer microenvironment

Fibrosis
To think about

• Scale: size of different molecules and fibers relative to the size of a cell or vessel

• What do cells actually sense in the matrix (topography, mechanics)

• Appropriateness of the culture system and matrix proteins (i.e. relevance of matrix protein organization and topography)

• Mechanical import of the matrix proteins in a system: how do the proteins and their conformation affect the mechanics of the system, and vice versa (e.g. FN on plastic, collagen organization and stiffness....)

Fig. 2. Nanoscale topography and structure of basement membranes of anterior corneal epithelium (adapted with permission from Abrams et al., 2000), small intestine, and Matrigel (adapted with permission from Abrams et al., 2000).

3. Patterned cell culture substrates: fabrication methods & materials

Various methods and materials have been utilized to create 3D cell culture substrates and tissue culture scaffolds. Depending on desired 3D features as well as chemical and mechanical properties of the scaffold, a specific fabrication strategy can be selected. There are four main categories of methods reported in the literature for fabrication of a 3D cell culture substrate or scaffold: (1) methods resulting in precisely designed regular surface topographies or 3D features; (2) methods resulting in irregular topographies, such as 3D fibrils, pores, or simple increased surface nano-scale roughness; (3) methods aiming for exact replication of 3D feature of native tissue; (4) methods based on naturally derived biopolymer gels or decellularized ECM.

Micro- and nanofabrication methods, such as photolithography, electron-beam lithography, two-photon polymerization, microcontact printing and etching, have often been employed to produce surface features with controlled dimensions and specific shapes (reviewed by Bettinger et al., 2009)). Among these techniques, photolithography is the most popular approach and is often used to generate regular surface features, such as grooves, posts, and pits. Photolithography, and other micro-nanofabrication techniques are typically fine-tuned for silicon, silicon oxide, polycrystalline silicon, and other inorganic systems such as titanium. Therefore, either these inorganic materials, such as silicon or titanium, or organic polymer replicas of inorganic master molds have been utilized as cell culture substrates to study the effect of topography on cell behavior (Reviewed by Bettinger et al., 2009)).

Organic polymers used in this manner include poly (dimethylsiloxane), polystyrene, poly(methyl methacrylate), polycarbonate, and poly(ethylene glycol), as well as biodegradable polymers such as poly (H-caprolactone), poly(L-lactic acid), poly(glycolic acid), and poly(L-lactic-co-glycolic acid). Some more recently developed techniques, such as multiphoton lithography, are capable of fabricating much more complex 3D topographies than simple groove, post or pit arrays. For example, it was reported that layer-by-layer stereolithography was able to create free-form complicated 3D constructs: a layer of 400 mg/ml bovine serum albumin (BSA) was deposited and photocrosslinked by exposed to patterned UV light, and repeated many times to incrementally build a 3D structure. The
Functional and structural complexity

• **Architecture:**
  - Strength, resilience, resistance to compression
  - Volume (space filling, water retention and buffering)
  - Organizational complexity/topography at multiple levels
  - Regulates diffusion
  - Migration substrate and barrier
  - Adhesion scaffold and anchor

• **Signaling:**
  - Ligands for integrins and other receptors
  - Receptors and co-receptors
  - Sequester, target, and activate growth factors
The Matrisome

- Combined proteomics/bioinformatics approach
- Highly variable between tissues, normal vs. tumor
- Roughly divided in to basement membrane and interstitial matrix

Naba, Matrix Biology 2016
General scale of matrix proteins

- Major structural proteins: collagens and elastins
- Adhesive glycoproteins
- Glycosaminoglycans
Collagens

- 28 known collagens
- Gly-X-Y repeats (Pro, OH-Pro)
- 3 chains form a rigid triple helix (tightly packed due to glycine, stabilized by OH-Pro and OH-Lys)
- Significant post-translational modification
- Multiple families, depending on collagenous/non-collagenous domains
- Most abundant fibrous protein in the ECM
Collagen families

- Fibrillar (interstitial stroma)
- Network forming (basement membrane)
- FACITS (Fibril-Associated with Interrupted Triple Helices)
- Multiplexins (Multiple Triple-Helical Domains and Interruptions)
Fibrillar Collagens

- I, II, III, V, XI, XXIV, XXVII
- Most heterotrimers
- Cleavage of N and C termini leads to fibril self assembly (staggered=bands); diameter is key to fxn
- Fibrils organize into fibers
- Fibers are cross-linked by lysyl oxidase family enzymes (oxidative deamination of lysines), altering mechanics
Fibrous networks of collagen

• Required for long-range force transmission between cells

Wang H et al, Biophys J 2014;107:2592
Network-forming Collagens

- **IV, VI, VIII, X**
- IV has 6 α chains, forms “chicken wire” 3-D structure; **basement membrane**
- VI forms tetramers, then large aggregates; help anchor cells
- VIII: tetrahedrons, hexagonal lattices, associated with elastic fibers and basement membrane
**FACIT Collagens**

- VII, IX, XII, XIV, XX, XVI, XIX, XXI, XXII

- Interrupted triple helices, so flexible; retain N and C termini, so don’t form fibrils

- Associate in periodic way with surface of fibrillar collagen; determine matrix organization

- VII links basement membrane of skin to underlying matrix in dermis

Gordon and Hahn, Cell Tissue Res 2010;339: 247
Collagen IX binds to fibrillar collagens
Elastin: a major structural protein

- Provides resilience
- Major component of arterial vasculature
- 5x more extensible than rubber band; intertwined with collagen fibrils
- Binds to collagens, PGs
- Relatively protease resistant
Elastin fibrils provide resilience

- Rich in proline and glycine (some OH-Pro, no OH-Lys)
- Highly cross-linked by lysyl oxidase family members
- Chains have hydrophobic (extensible) and α-helical (cross-linked) parts
- Covered by sheaths of microfibrils (10 nm) of fibrillin, stabilize elastin
- Fibrillin involved in TGF-β activation
Adhesive glycoproteins

- **Laminins**: Self-assembling heterotrimer of basement membrane; binds to multiple other matrix proteins including nidogen, perlecan; receptors include integrins and dystroglycan

- **Tenascins**: Multiple domains, some similar to FN; binds multiple other matrix proteins
Fibronectin

- Adhesive glycoprotein with multiple domains, including cell binding, heparin binding, and collagen binding domains
- Organized by cells after deposition
- Is required for collagen organization, and this interaction is mechanically regulated
- Highly extensible, becomes extremely rigid
- Two forms (alternative splicing at C-terminus):
  - Plasma FN: soluble, disulfide-linked dimers produced by hepatocytes
  - Cellular FN: highly insoluble, organized by cells (integrins) into complex cell-associated arrays; produced by most cells
Fibronectins

White et al, J. Pathology 2008
Alternatively spliced cellular FN

White et al, J. Pathology 2008
Glycosaminoglycans

• Unbranched polysaccharides
• Repeating disaccharide units (amino sugar, often sulfated, alternating with iduronic or glucuronic acid); highly variable
• Highly negatively charged
• Form gels at low concentrations
• Occupy large area
• Hydrophilic, stiff, so contain large amounts of water; resist compression
Hyaluronan

- Non-sulfated
- Non-protein attached
- Enormous, especially important in driving shape changes
Proteoglycans

- Interstitial PGs
  - SLRPs (lumican, fibromodulin, decorin, biglycan)
  - Aggrecan family (aggrecan, versican, brevican)
- Basement membrane PGs (perlecan, agrin, col XVIII)
- Membrane-bound PGs (glipican, CD44, syndecans)
SLRPs and mechanics

• Regulate collagen organization and fibril size
• Regulation of collagen mechanical properties?
Basement membrane organization
### ECM breakdown

- Specialized enzymes to break down and remodel ECM
- Main enzymes are MMPs, secreted as zymogens
- ADAMs, ADAM-TSs (sheddases)
- TIMPs
- Highly regulated

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**Table: Enzyme ECM substrates**

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<th>Enzyme</th>
<th>ECM substrates</th>
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<td>MMP1</td>
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**Diagram:**

- *Propeptide* Catalytic domain
- *Ast-like Cat* GPI anchoring sequence
- *MAM* Signal peptide domain
- *TRAF* Fibrinopeptide A domain
- *EGF* GPI anchoring sequence
- *TM* Signal peptide domain
- *Cyto* Fibrinopeptide A domain
- *Zn* Activase-like catalytic domain
- *HPX* GPI anchoring sequence
- *GPI* Signal peptide domain
- *5KIPalRGRtKFGFoOaKP* Fibrinopeptide A domain
- *TM* GPI anchoring sequence
- *Cys* Signal peptide domain
- *Ig* Fibrinopeptide A domain
- *Dis* Activase-like catalytic domain
- *Cys* GPI anchoring sequence
- *6bs* Signal peptide domain
- *n* Fibrinopeptide A domain
- *Furin-like cleavage*
- *β* Activase-like catalytic domain
- *α* GPI anchoring sequence
- *ADAM9* Signal peptide domain
- *ADAM12* Fibrinopeptide A domain
- *ADAM15* GPI anchoring sequence
- *ADAM10* Signal peptide domain
- *ADAM15* GPI anchoring sequence
- *Meprin A5 protein tyr phospatase* Signal peptide domain
- *μ* GPI anchoring sequence
- *Dis* GPI anchoring sequence
- *&KsKPtGIrKPFoOaKP* GPI anchoring sequence
- *Meprin A5 protein tyr phospatase* Signal peptide domain
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Mechanics of individual ECM molecules

“Rather than maximizing toughness, as needed for silk and muscle titin fibers to withstand external impact, the much softer extracellular matrix fibers made from fibronectin (Fn) can be stretched by cell generated forces and display extraordinary extensibility. We show that Fn fibers can be extended more than 8-fold (>700% strain) before 50% of the fibers break. The Young's modulus of single fibers, given by the highly nonlinear slope of the stress-strain curve, changes orders of magnitude, up to Mpa.”

(This is NOT reflected in the bulk mechanics of tissues)
Take home messages

- Cannot think of cells and tissues without thinking of the ECM
- Extraordinary heterogeneity across ECM types, individual tissues, specific states
- Multifunctional: many architectural and signaling roles
- Scale is important when considering individual ECM molecules and ECM organization and cell sensing
- ECM-ECM interactions (like cell-cell and cell-ECM) are important
- Relevance for cell and tissue mechanics