Lecture #3
Tissue Morphogenesis
Morphogenesis

- Morphogenesis describes the evolution and development of form.
- It refers to the structural and functional changes that we observe during development and the elucidation of the underlying mechanisms.
- In tissue engineering, can be broader: the formation and differentiation of tissues and organs
Morphogenetic Spatio-temporal Scales

• Space constraints
  - Not less than 10 and not more than 1000 cells participate in morphological processes
  - A few hundred microns, < 1 mm in size
  - Corresponds closely to size of most functional subunits of tissues, possibly due to communicative or diffusion limitations

• Time constraints
  - Few hours → few days
  - Influenced by speed of cellular fate processes (migration, differentiation, etc.)
Embryogenesis

Fertilization and the events of the first 6 days of development.
Implantation of the Blastocyst

(a) Blastocyst that has just adhered to the wall of the uterus (day 6).

(b) Implanting embryo (day 7). The embryos in this figure are in cross-sectional view.
Embryo is almost completely implanted in the wall of the uterus. It is now a bilaminar embryonic disc of epiblast and hypoblast situated between an amniotic sac and a yolk sac.
The primitive streak stage appears on the dorsal surface of the embryonic disc on about day 15. Shown is the relation of the primitive streak and the embryonic disc to the body that forms later. Ultimately, the primitive streak is reduced to a strip of skin epidermis over the tailbone (coccyx) in the cleft between the buttocks.
Origins of Tissues-
Developmental Hierarchy

- Blastocyst
  - Inner Cell Mass
    - Epiblast
      - Embryonic Epiblast
        - Embryonic ectoderm
          - Neural ectoderm
            - Neural crest
          - Epidermis
        - Embryonic endoderm
    - Hypoblast
      - Amniotic ectoderm
      - Extraembryonic endoderm
      - Primitive streak
        - Extraembryonic Mesoderm
        - Notochordal process
        - Embryonic Mesoderm
Endoderm - digestive tube, internal organs
Mesoderm - muscle, skeleton
Ectoderm - skin, brain, spinal cord
Week 4- Tube within a tube
Morphogenesis and Tissue Engineering

Hypothesis → Normal tissue morphogenesis can be effectively achieved by recreating *in vivo* developmental conditions.

Goal → Understand normal growth and development of different tissue types and organs and mechanisms of morphogenic control. Recapitulate some or all of the *controlling mechanisms of development* in an *in vitro* system.
Self-organization is a dynamic and lineage-intrinsic property of mammary epithelial cells

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Loss of organization is a principle feature of cancers; therefore it is important to understand how normal adult multilineage tissues, such as bilayered secretory epithelia, establish and maintain their architectures. The self-organization process that drives heterogeneous mixtures of cells to form organized tissues is well studied in embryology and with mammalian cell lines that were abnormal or engineered. Here we used a micropatterning approach that confined cells to a cylindrical geometry combined with an algorithm to quantify changes of cellular distribution over time to measure the with primary materials and a paucity of tractable culture systems for maintaining cell types from normal adult tissues. To facilitate a quantitative understanding of those processes in an adult epithelial tissue, we used a robust cell culture system that enables culture of pre-stasis normal HMEC obtained from reduction mammoplasties for 40–60 population doublings while maintaining both the LEP and MEP lineages (12). Flow cytometry-enriched cells from both lineages were placed in arrays of micropatterned microwells, where their distributions were
Mammary Gland Development: LEPs and MEPs

Gjorevski and Nelson, Nat Rev Mol Cell Biol 2011
Figure 1
Figure 3
Figure 4

A. E-cadherin expression

B. E-cadherin binding

C. Self-organizing dynamics

C’. Mean green and red fluorescence intensity over time.
Tissue Morphogenesis
Continued
Types of Induction

• Competence - responder cells must be capable of responding (change with t, animal)

• Instructive - Changes cell type of responder cells by instructing cell fate

• Permissive - Responder cells contain all potential needed and only require environment that permits expression (e.g. ECM)

• Reciprocal - Tissues signal each other

• Negative - one cell population restricts the potential of the other
Three of the Modes of Induction

- Diffusion of inducers from one cell to another
- Contact of matrix from one cell to another
- Contact between the inducing and responding cells

> mechanical forces, geometry
Gradient Model for Positional Information
 Ability of presumptive lung epithelium to differentiate with respect to the source of the mesenchyme:

(1) Lung epithelium does not differentiate when cultured in the absence of mesenchymal cells.

(2-6) Mesenchyme-specific differentiation of epithelium.
When cells of the dermis (mesoderm) are recombined with the epidermis (ectoderm) in the chick, the type of cutaneous structure made by the ectoderm is determined by the original location of the mesoderm.
Salivary-gland differentiation:
(1) Salivary-glands can be dissected
(2) treated to remove the basal lamina
(3) If mixed immediately with mesenchymal cells, there is no growth or differentiation.
(4) If cultured, they regenerate the basal lamina, but do not differentiate further (5) unless they interact with mesenchymal cells (6).
Epithelio-Mesenchymal Transitions

**Table 1. Genes That Promote Transformation**

<table>
<thead>
<tr>
<th>PROCESS</th>
<th>ACTION</th>
<th>MORPHOLOGY</th>
<th>EXAMPLE</th>
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<tbody>
<tr>
<td><strong>MESENCHYMAL CELLS</strong></td>
<td></td>
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<tr>
<td>Condensation</td>
<td>Mesenchyme becomes epithelium</td>
<td>Cartilage mesenchyme</td>
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<tr>
<td><strong>EPITHELIAL CELLS</strong></td>
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<tr>
<td>Dispersal</td>
<td>Epithelium → Mesenchyme (entire structure)</td>
<td>Müllerian duct degeneration</td>
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<table>
<thead>
<tr>
<th>Epithelium to Mesenchyme</th>
<th>Mesenchyme to Epithelium</th>
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</thead>
<tbody>
<tr>
<td>1. Cell surface programs</td>
<td>1. L-CAM, E-cadherin</td>
</tr>
<tr>
<td>α5/β1 Integrin</td>
<td>α6 Integrin</td>
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<tr>
<td>Oncogenes</td>
<td>Syndecan 1°</td>
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<tr>
<td>v-src</td>
<td>Laminin/ nidogen</td>
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<tr>
<td>c-fos</td>
<td>2. c-met</td>
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<tr>
<td>v-ras, v-mos</td>
<td>3. HGF/SF</td>
</tr>
<tr>
<td>Growth factors</td>
<td>4. Other genes</td>
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<tr>
<td>TGFβ1, TGFβ2</td>
<td>wnt-1°</td>
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<td>MIF</td>
<td>wnt-4β</td>
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<td>TGFα100</td>
<td>Pax 5a</td>
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<td>aFGF101</td>
<td>E1a102</td>
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Developmental Control by Apoptosis

(A) Chick Leg Primordium
Extensive cell Death

(B) Duck Leg Primordium
Minimal cell Death
Example of Genetic Control-Differentiation

- Sonic hedgehog (Shh) gene provides positional information
- Developmental character depends on distance from the Shh protein

Alberts, 1998