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Epithelial machines of morphogenesis and their potential application in organ assembly and tissue engineering

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Abstract

Sheets of embryonic epithelial cells coordinate their efforts to create diverse tissue structures such as pits, grooves, tubes, and capsules that lead to organ formation. Such cells can use a number of cell behaviors including contractility, proliferation, and directed movement to create these structures. By contrast, tissue engineers and researchers in regenerative medicine seeking to produce organs for repair or replacement therapy can combine cells with synthetic polymeric scaffolds. Tissue engineers try to achieve these goals by shaping scaffold geometry in such a way that cells embedded within these scaffold self-assemble to form a tissue, for instance aligning to synthetic fibers, and assembling native extracellular matrix to form the desired tissue-like structure. Although self-assembly is a dominant process that guides tissue assembly both within the embryo and within artificial tissue constructs we know little about these critical processes. Here, we compare and contrast strategies of tissue assembly used by embryos to those used by engineers during epithelial morphogenesis and highlight opportunities for future applications of developmental biology in the field of tissue engineering.

Keywords

epithelia; apical contractility; origami; organogenesis; cell shape change; scaffold

Introduction

Embryos create tissues and assemble functioning organs using information from a range of sources including mechanical stimuli, genes and biochemical gradients, and the environment but how they integrate these sources of information to coordinate cell and tissue movements is largely unknown. Cell contraction generally and apical cell contraction specifically forms the initial step for numerous morphogenetic events (Sawyer et al. 2009). The formation of functioning organs such as liver, heart, and eyes demonstrate the remarkable capacity of embryonic cells to create complex three-dimensional (3D) structures. Heart development, as an example, involves orchestrated directional movement of two populations of cardiac progenitor cells from the right and left sides of the embryo toward the ventral midline. Multiple cell types join these progenitor cells and assemble the heart. Many of the steps of heart formation including cell specification, migration, joining of the two progenitor populations, heart tube formation and looping, and chamber formation (Warkman and Krieg 2007; Stainier 2001; Abu-Issa and Kirby 2007) have been described. Many of the critical steps in patterning cell identities that make up the heart are evolutionarily conserved, sharing common signaling pathways and gene regulatory networks (Gelb 2004; Nemer 2008),

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however, we know little about how gene expression and morphogenetic processes are integrated within these tissues and how cells interact with each other during heart formation. Thus, a more mechanistic understanding of how cells integrate information and coordinate movement can improve our understanding of organ assembly and morphogenesis and provide guidance to tissue engineers working to apply or co-opt endogenous programs of morphogenesis and self-organization for organ regeneration and the production of replacement tissues (Ingber et al. 2006).

Just as the developmental biologist asks the question “how does the *embryo* build tissues,” the tissue engineer asks “how can *we* build tissues?” By studying the principles that guide embryonic cells as they construct tissues in the early embryo we can learn to make use of these programs to engineer tissues. The programs of development revealed in comparative studies across species and morphogenetic movements can reveal principles of tissue assembly and help identify the molecular mechanisms used by cells and tissues (Simons and Mlodzik 2008; O’Farrell et al. 2004; Wang and Steinbeisser 2009; Keller 2002, 2006). Although, precise details of cell behaviors and the resulting morphogenetic movements differ from species to species, there appear to be universal physical principles that govern the morphogenetic process of tissue assembly (Grill 2011; Varner and Taber 2012; Beloussov 2012, 2008). In the sections below we discuss aspects of epithelial morphogenesis in this context.

Epithelial morphogenesis plays a critical role in many phases of embryonic development; understanding the mechanisms by which epithelial cells build three dimensional organs is an important step in understanding and using the principles of morphogenesis to direct tissue assembly. During the tissue formation phase, embryos routinely create structures such as columns, pits, grooves, capsules, and hollow tubes. At later stages these structures, often composed of multiple types of progenitor cells, serve as the foundation of functioning organs. For instance, the spinal cord and brain are formed as a flat epithelial sheet on the surface of the embryo rolls to form a tube (Lacalli 1994; Holland et al. 1996). In other examples, epithelial cell proliferation and coordinated changes in cell shape have been shown to drive branching morphogenesis of the salivary gland (Bernfield and Banerjee 1982; Spooner and Wessells 1972) and the mammary gland (Ewald et al. 2008). Coordinated epithelial cell shape change has also been shown to drive critical aspects of epithelial morphogenesis within *in vitro* cell cultures (Berdichevsky et al. 1994; Nogawa and Ito 1995; Wozniak et al. 2003; Gjorevski and Nelson 2010). Differential actomyosin contraction is widely considered one of the key molecular processes that coordinates cell shape change during tissue formation (Martin 2010; Sawyer et al. 2010; Blanchard et al. 2010). To understand how actomyosin-driven cell contractility mechanically shapes tissues requires detailed descriptions of cell shape change, how those changes depend on the state of the cytoskeleton, how cells adhere to their surroundings, and how these events relate to the physical constraints acting on the epithelial sheet (Davidson 2011). With the advent of four-dimensional (4D) imaging technologies there has been a resurgent interest in developing ways to visualize, map and quantify epithelial cell shape change during morphogenesis and to understand how these movements reflect operation of cellular and molecular machines (e.g. (Blanchard et al. 2009)).

To guide tissue engineers interested in applying the principles of morphogenesis we have divided our review into three sections: 1) a review of how embryos assemble epithelial structures from the large-scale folding to molecular-scale regulation of the cytoskeleton, 2) briefly review the current strategies for engineering tissues, and 3) propose for a new approach that uses principles evolved for use during morphogenesis to engineer novel tissue structures.

1. The embryo's strategy for building tissues

How do embryonic cells build a tissue? Within hours after fertilization, embryonic cells participate in dynamic morphogenetic programs by undergoing multiple rounds of cell division, signaling, and movement. These self-organizing processes shape the initial embryonic body, elaborate organs, and maintain anatomical structures as the embryo grows into the adult form. As an example, in a frog embryo, epithelial cells undergo distinct patterns of asymmetric cell division, cell shape change, and cell rearrangement, eventually providing material for epidermis and endoderm (Keller et al. 2003). Tissues assemble into anatomical structures by incorporating extracellular matrix (ECM). ECM can form a natural scaffold capable of directing 3D tissue movements. For instance, prior to gastrulation the sea urchin embryo assembles a stiff ECM on the outer, apical surface of the embryo (Davidson et al. 1999). The ECM provides mechanical support for the cellular processes that drive sea urchin invagination (Davidson and Keller 2007; Davidson et al. 1995; Lane et al. 1993). ECM is critical to epithelial folding in a range of animals during both early development (Marsden and DeSimone 2003; Pulina et al. 2011) and later during metamorphosis (Fu et al. 2007; Hasebe and Ishizuya-Oka 2007). Embryos thus serve as excellent examples of how cells interact with each other and with their ECM to remodel tissues during critical steps of tissue assembly and organogenesis.

Several examples of animal development demonstrate the capacity of embryonic cells to sculpt tissues. For instance, mediolateral cell intercalation, one of many forms of cell motility described during *Xenopus* morphogenesis, plays a critical role in driving the process of convergence-extension of the body axis (Keller et al. 2000). During this process cells differentiate and intercalate in a programmed way to move and assemble structures such as the notochord and somites (Shih and Keller 1992a, b). Cell contraction and shape change plays important role during bottle cell morphogenesis (Lee and Harland 2007). In general, *Xenopus* gastrulation is driven by cell motility and shape change (Keller et al. 2000). Programs of morphogenesis similar to those used during frog gastrulation can be seen in other organisms such as avians (Voiculescu et al. 2007), nematodes (Simske and Hardin 2001; Roh-Johnson et al. 2012), ascidians (Munro and Odell 2002; Sherrard et al. 2010), and insects (Lecuit et al. 2011; Martin et al. 2009; Harris and Peifer 2005). In these examples, embryonic cells play active, autonomous roles in shaping the embryonic body form. In addition, it has been suggested that cells can adopt behaviors or differentiation characteristics of their neighbors via a "community effect" (Gurdon 1988), for instance allowing them to be recruited into group of cells undergoing epithelial morphogenesis. Little is known about the molecular pathways that might allow cells to sense cues from their immediate neighbors. Such a community effect may represent yet another universal mechanism driving or coordinating morphogenesis within complex 3D tissues (Cossu et al. 1995; Kaneko et al. 2011; Gorfinkel et al. 2009). These studies suggest cells can act both as unitary agents and as groups that coordinate their behaviors during tissue self-assembly.

Cellular machines, epithelia, and cell shape change

How are cells coordinated during morphogenesis? In considering this question we will focus on the cellular machines that shape epithelial sheets. Epithelial sheets are constructed of cells that are tightly connected at their apices (Fristrom 1988), which form a tessellated structure capable of deforming itself by both cell shape change and cell rearrangement. Physical connections attach epithelial cells at their apical ends, lateral cell-cell boundaries, and basal surfaces. In principle, each of these connections is capable of transmitting force between adjacent cells to form a mechanically contiguous sheet. Epithelial morphogenesis can be driven exclusively by cell shape change, however, many cases of epithelial morphogenesis such as branching morphogenesis involve dynamic cell rearrangements (Ewald et al. 2008). During cell rearrangement, cells change shape according to rules that

appear to preserve geometric-order as they accommodate new cells or occupy space created cells that have undergone apoptosis (Gibson et al. 2006). Such rules are likely based on underlying mechanics of cell packing, contractility and adhesion like those that regulate geometric order in the fly retina (Hayashi and Carthew 2004; Hilgenfeldt et al. 2008; Kafer et al. 2007). Thus, cell shape change and cell rearrangement are coupled mechanically within the epithelial sheet and play central roles in driving epithelial morphogenesis.

Epithelial cells change shape in specific ways as they drive different types of epithelial morphogenesis, for instance, apically constricting to create grooves (Lee and Harland 2007; Rolo et al. 2009), to enable programs of epithelial-to-mesenchymal transitions (Mani et al. 2008; Nakaya and Sheng 2008), or to remove material after apoptosis (Toyama et al. 2008). In many cases, cell shape changes directly drive tissue movement, shaping two-dimensional (2D) cell sheets into 3D structures. To create 3D structures, groups of cells may undergo defined programs of apical contraction accompanied by basal expansion to produce a groove or a pit with the apically-contracted cells at the center (Figure 1A; adapted from (Davidson et al. 1995)). Furthermore, isotropic cell contractions and anisotropic mechanical tensions can result in invagination and groove formation (Martin et al. 2010). The resulting pit or invagination can be extended into a groove if the same program is expressed in multiple rows or stripes of cells. Apical contraction based mechanisms for shaping 3D tissues, illustrated by studies of sea urchin (Davidson et al. 1995), *Drosophila* (Brodland et al. 2010), and ascidian (Sherrard et al. 2010) gastrulation highlight a universal principle of epithelial morphogenesis that may operate to construct organs and to shape embryos of other species (Nakaya and Sheng 2008; Lecuit and Lenne 2007).

Cell contraction is a key physical process that regulates cell shape change in epithelial sheets. Cell contraction can be driven by a number of mechanisms such as 1) apical contraction 2) apico-basal contraction 3) circum-apical or circum-basal contraction 4) apical cortex contraction 5) basal expansion and apical contraction 6) whole cell contraction, which could modify the cell shape into cell-cylinders, wedges-like cells, etc by simple physical processes like thinning, thickening, bending and elongation (Sawyer et al. 2010; Davidson 2011) and commonly depend upon F-actin polymerization and remodeling (Pollard and Cooper 2009; Cortese et al. 1989; Kim and Davidson 2011; Lee and Harland 2007; Mermelstein et al. 2003). Autonomous programs of cell contractions facilitate cell movements and shape change during morphogenesis. Coordinated contraction in groups of cells drives epithelial morphogenesis in a range of embryonic model systems and cell culture studies (examples listed in Table 1). Studies of cell contractions in embryos (Rolo et al. 2009; Farber et al. 2011) parallel observations of cell contractions in culture (Alenghat et al. 2004) and can be recapitulated in computer models (Deshpande et al. 2006). Recent studies of contractile embryonic epithelial sheets in *Drosophila* suggest that mechanical stimuli may induce additional contractions (Fernandez-Gonzalez et al. 2009; Pouille et al. 2009), however, our group has not been able to confirm these studies in vertebrate *Xenopus* embryos either by direct indentation (Joshi et al. 2010) or by micro-aspiration (von Dassow et al. 2010). Future work with more diverse developmental models that exhibit robust responses to both physiological and non-physiological stimulation will be needed to decode the details of signal transduction pathways and evaluate the universality of mechanosensing during development.

Studies of both embryonic and cultured cells suggest that mechanical cues may play an important role in directing cell differentiation. Epithelia in early *Drosophila* embryos are sensitive to both internally generated and externally applied forces (Fernandez-Gonzalez et al. 2009; Desprat et al. 2008; Farge 2003; Pouille et al. 2009). Many types of cultured cells also respond to both internally generated and externally applied forces. Actomyosin driven contractility and cadherin mediated connectivity has been shown to drive cell growth on

micro-fabricated surfaces and that the regions of high cell density correspond to regions of high traction stress (Nelson et al. 2005). Several studies have shown that the cell fate can be modulated by geometric cues (Chen et al. 1997; Dike et al. 1999). Cells cultured on well defined bowtie-shaped substrates show cell proliferation can depend on the levels of cell-cell contact and reiterate the importance of geometric or structural cues in a multicellular tissue (Nelson and Chen 2003). The stiffness or elasticity of a cell's substrate can also direct differentiation of cultured stem cells down specific lineages and suggests the mechanical properties of the local microenvironment may play important inductive roles during development (Discher et al. 2005; Engler et al. 2006).

The actomyosin cytoskeleton

How is the operation of a cellular machine controlled and regulated? To address this question we will focus on the operation of the actomyosin cytoskeleton that controls many of the mechanical functions of cells. Cell culture studies have focused on the connection between biophysical principles of mechanotransduction to molecular regulation of contraction (e.g. (Chen 2008; Ingber 2005; Alenghat et al. 2004; Katsumi et al. 2005; Treppe et al. 2007; Sbrana et al. 2008; Cai et al. 2006; Jaalouk and Lammerding 2009)). Still, little is known about the complex regulation of the spatiotemporal dynamics of the F-actin cytoskeleton that drive cell contraction or whether feedback mechanisms control the extent of contraction and neighbor cell responses. For instance, many questions remain regarding F-actin feedback control in events such as hyper-restoration (Belousov et al. 1988; Taber 2007), the roles of feedback during cell shape change (Cuvelier et al. 2007; Reinhart-King et al. 2005), and in coordinating the actomyosin cytoskeleton with changes in cell adhesion (Balaban et al. 2001; Bershadsky et al. 2006; Bershadsky et al. 2003; Riveline et al. 2001). Actomyosin regulated cell contractions have been observed in a variety of species and developmental contexts (Gorfinkel and Blanchard 2011). Quantitative approaches and high-resolution imaging have significantly advanced our understanding of F-actin dynamics (Martin et al. 2008; Solon et al. 2009; Kim and Davidson 2011). Continuing advances in four-dimensional (3D time-lapse) imaging technologies are leading to new ways to visualize, map and quantify epithelial cell shape change and cytoskeletal dynamics and are already providing new insights into the operation of cellular and molecular machines during morphogenesis (Martin 2010; Sherrard et al. 2010; Blanchard et al. 2009).

2. Comparing mechanisms of morphogenesis with tissue engineering strategies

Engineers seeking to create organ replacements are free to invent novel approaches as they combine engineered cells with synthetic materials (Langer and Vacanti 1993). The field of tissue engineering has advanced our understanding of cell behaviors and their interaction with non-biological materials such as degradable or non-degradable synthetic scaffolds. In general, scaffolds are produced from synthetic polymer foams (Sachlos and Czernuszka 2003) and minimally aim to provide the structural support for cell attachment and tissue development (Chan and Leong 2008). Scaffold synthesis can be controlled to yield foams of defined geometry or porosity including polymer fiber diameters and pore sizes, surface chemistries, and biocompatibility (Hutmacher 2001; Hutmacher et al. 2001a; Hutmacher et al. 2001b).

Scaffolds in tissue engineering

Engineers have created tissue-like structures using cell-scaffold composites (Cao et al. 1998; Kamil et al. 2004; Saim et al. 2000). Following the pioneering work by Vacanti and coworkers (Cao et al. 1997), tissue engineers began combining various cell types with synthetic materials, investigating biocompatibility, cell-scaffold interactions, and exploring

the possibility of using these fully synthetic or composite constructs as initial steps in organ creation or regeneration. The intended goal of these engineered tissues have been to induce organ repair by supplying bio-active factors or to provide surgeons with engineered materials, containing scaffold or cells and scaffolds, to fill gaps in damaged tissues. One of the most common strategies has utilized cell-populated scaffold constructs for the repair of damaged tissues. Designing the optimal scaffold has been major focus of this strategy resulting in advanced applications of synthetic foams based on PLLA (Poly(L-lactic acid)) and PLGA (Poly(l-lactide-co-glycolide)) (Wright et al. 2010; Kavlock et al. 2012), PMMA (Polymethylmethacrylate) (Xing et al. 2012), PEG (Poly(ethylene glycol)) (Chiu et al. 2011) and PTFE (polytetrafluoroethylene) (Bax et al. 2011) and optimization of these polymers for cell viability and proliferation. Fabrication techniques such as freeze drying (Ohya et al. 2004), solvent evaporation (Devin et al. 1996) and electrospinning (Zeng et al. 2005) among others are routinely used to produce scaffolds with the necessary porosity and mechanical strength to allow degradation, cell attachment, and the binding of survival and growth factors. Cells attach, penetrate and populate the artificial polymer foam to produce a tissue-like construct. The goal is to produce artificial materials that can be implanted by surgeons or as raw material that can be used in constructing replacement organs (Figure 2) such as an artificial esophagus (Badylak et al. 2005; Matkowski et al. 2011).

Many studies have focused on testing the effects of externally applied mechanical loads on cell-scaffold constructs (Seliktar et al. 2000; Niklason et al. 1999) in order to mimic physiological conditions where cells are subjected to mechanical loading. Systematic approaches have illustrated that different loading parameters such as strain amplitude and frequency affect cell behaviors and mechanical strength of the scaffold (Joshi and Webb 2008) which indicates that the cell sense mechanical signals and translate them into their behaviors. These studies have advanced the field of regenerative medicine but have relied exclusively on fabricated synthetic scaffolds for imparting 3D shapes to tissues. The cell-material constructs have limitations such as risk of disease transfer and immune rejection for allografts (Burg et al. 2000), structural limitations (Boland et al. 2001) and lack of functional aspects in the synthetic scaffolds (Chen et al. 2002).

Engineers have used a variety of “smart” scaffolds in attempts to produce tissue replacements. Tissue-engineered bladder (Atala et al. 2006), skin (Metcalf and Ferguson 2007), bone (Burg et al. 2000), heart (Hecker and Birla 2007), auricle (Kamil et al. 2004), liver (Dvir-Ginzberg et al. 2003), and tendon (Liu et al. 2008) have been constructed from biocompatible materials. Once fabricated scaffolds and their cells can be cultured in bioreactors where cells proliferate and produce their own ECM. Mature scaffolds can be harvested and treated like any biopsied tissue where cell morphology, gene expression, or physiological function can be assessed. Whereas embryos do not use this strategy to assemble tissues, the majority of engineered tissues have relied on scaffolds to guide their assembly. In part, the use of scaffolds has allowed rapid testing of biocompatibility but has only produced limited products for organ implants (Lysaght and Hazlehurst 2004; Griffith and Naughton 2002; Badylak et al. 2011). To advance the field, biologists, clinicians, and engineers need to investigate the responses of progenitor cells to fixed scaffolds, how cellular self-assembly may be hampered or augmented by these artificial structures, and investigate alternative strategies for scaffold-free tissue assembly.

Artificial organ production using alternative strategies

To provide whole organs for replacement and research, several groups have focused on assembling more complex organ-like tissues and have developed technologies for organ manufacturing. Given the difficulties overcoming inflammation and immune rejection of constructs using exogenously sourced-cells or proteins there has been interest in using autografts, i.e. engineered tissue replacements constructs containing cells collected directly

from the patient (Basu and Ludlow 2010; Jayo et al. 2008). Following the lead of new manufacturing technologies there have been advances in the use of 2D and 3D printing platforms to ‘print’ organs as scaffold-free tissues (Boland et al. 2006; Pardo and Boland 2003; Xu et al. 2005; Norotte et al. 2009) and rely upon cell-specific programs of self-assembly to create physiologically functional organs (Jakab et al. 2006; Jakab et al. 2008).

Scaffold-free approaches

The principles of self-organization, whether during development, within organoids, or within scaffolds are poorly understood but assembly and remodeling of ECM is thought to play a critical role. Within the developing salivary gland the production of a fibronectin matrix is required during branching (Harunaga et al. 2011; Hsu and Yamada 2010; Sakai et al. 2003; Wei et al. 2007). Embryonic stem cells aggregated in embryoid bodies (EBs) are able to self-assemble, develop an anterior-posterior axis and successfully differentiate through Wnt signaling (ten Berge et al. 2008). EBs driven to differentiate as anterior neural tissues are able to self-assemble structures remarkably like the optic-cup in developing embryos (Eiraku et al. 2011). These self-organizing tissues demonstrate that tissue engineers may be able to direct organ formation without the use of artificial scaffolds. Detailed observation and elucidation of principles of self-organization in EBs could provide engineers with a tool-box for “forward-engineering” complex tissues.

3. Epithelial origami - the art of tissue folding

Origami inspired tissue engineering could be used to produce 3D tissues. We suggest engineers seek to create “smart” cell sheets capable of self-assembling into 3D forms. We have been inspired by the art of paper folding (popularly known as Origami) where a series of basic folding operations can create complex 3D structures. The paper sheet, with a single fold can be made into a 3D tent-like structure with two planes (Figure 3A) or a model with three planes using two folds (Figure 3B). With more folds, complex box and cylinder models can be made (Figure 3C and D). We can compare the simple paper sheet in origami to a multicellular 2D epithelial sheet (Figure 3E). If the tightly connected cells contract at their apical ends, a hollow tube can be generated (Figure 3F). If few cells (with red circles in Figure 3G) in a large epithelial sheet contract at their apical ends a groove or invagination can form (Figure 3H), if cells contract at their basal ends the tissue can bend outward or evaginate. More complex patterns of contraction may be used to produce even more complex architectural forms (Figure 3I). Grooves, pits, and tubes are common intermediate structures that serve as substrates for organogenesis during later stages of development (Ettensohn 1985). We suggest that such programs of contractility generating multiple folds might be used to construct cylinders, boxes, and other complex shapes. Engineered 3D cellular structures may be fabricated by series of folding steps where specific cell behaviors are induced to contract along a line or within a microdomain. Multiple rounds of induction could produce multiple folding steps capable of producing complex scaffold-free structures that are currently beyond the capability of scaffold-based approaches. Industrial scale manufacturing processes often combine complex folding, annealing, and shaping processes that have not yet been applied to produce living multicellular tissues.

Experimental control of cell contractility

In order to direct the self-assembly of tissues engineers will need to control similar cellular processes that the embryo uses to control morphogenesis. Our group has explored several approaches to control cell contractility and have applied these external stimulation techniques to trigger cell contraction in embryonic cells (Joshi et al. 2010). In that study, we discovered three simple triggers of epithelial contraction: 1) electrical stimulation, 2) laser activation, and 3) nano-perfusion of cell-extracts. Laser stimulation, activation, or ablation

has been used to induce cell lysis (Rau et al. 2006), formation of giant plasma membrane vesicle (GPMV) on cells, and single-cell and multicellular wounds (Sonnemann and Bement 2011). Interestingly, previous work in *Xenopus* highlighted unique programs of embryonic cell behaviors during wound closure (Davidson et al. 2002). Remarkably, all three triggers induced contractions with similar kinematic properties such as duration, time-to-peak and delay between stimulus and contraction. Contraction responses to all three triggers similarly correlate with rapid remodeling of the F-actin cytoskeleton. These common features suggest that the three stimulation methods share some or all aspects of the signaling pathway. Future studies will be needed to identify downstream signaling pathways and the specific ligands, receptors, and channels involved in transducing triggering stimuli.

Tissue engineering and development biology can together build organs

As we envision applications of organ origami, we recognize significant challenges must still be overcome. Key among these challenges are the development of experimental platforms where live tissues can be studied under controlled exposure to precisely controlled stimulus and where the status of cells and their signaling systems can be assessed directly. With these tools we expect engineers to develop techniques for creating both transient shape changes and for locking those shape changes into permanent 3D deformations in 2D cell sheets. With this level of control, engineers will face the challenge of stabilizing or growing origami-based organs. Current efforts at tissue engineering focus on providing implantable devices scaled for their final application in patients. By contrast, organogenesis proceeds by first assembling small organ rudiments that then grow as the organism grows. The size difference between embryonic organs and adult organs highlights one of the major challenges to adapting developmental processes for tissue engineering. Overcoming this limitation will necessitate the development of new bioreactors that can simulate the special environment of the growing organism. Lastly, we must not forget that as replacement organs become an integral part of a patient they will need to be able to grow or adapt to the physiological needs of the patient and be capable of regenerating if damaged.

Tissue engineering and developmental biology are becoming complementary fields on the one hand seeking to elaborate the principles of development and morphogenesis and on the other seeking methods to build 3D organs that are mechanically strong and function as well as native tissues. The processes that shape embryonic tissues are in many ways analogous to the processes that shape tissue-engineered constructs. Both tissue engineering and embryonic development share similar biological and biomechanical principles and fundamental constraints of biological systems. The mechanical principles revealed in morphogenetic studies will aid future tissue engineers and researchers in regenerative medicine to design efficient and long lasting replacement organs (Ghosh and Ingber 2007). Methods for triggering cell contractility and “printing” organs and other technologies to create scaffold-free cell sheets and reliably pattern cell shape change could be combined to engineer complex 3D tissues whose assembly can be guided through intermediate geometries of grooves, pits, tubes, and cavities.

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Figure 1. Apical contraction and epithelial morphogenesis

Epithelial bending shapes the vegetal plate during invagination in the Sea Urchin late mesenchyme blastula (A). *Lytechinus pictus* embryo stained with phalloidin. Finite element models demonstrate that the vegetal plate of the late mesenchymeblastula (B) can invaginate after gel swelling (C), apical-basal shortening (D), or apical contraction (E). F) The neural groove in *Xenopus laevis* displays apically localized F-actin and (G) β -catenin showing both structural proteins are apically localized in the superficial layer. H) Apically contracting cells (red apices) within an otherwise resting epithelium. (A–E) are modified from (Davidson et al. 1995; Davidson et al. 1999).

Figure 2. Cell attachment and penetration in porous scaffolds

Cells are added to porous biomaterial scaffolds (A) and allowed to attach to the biomaterial surface (B). With time, cells attach and penetrate the porous structure (C) and eventually populate the entire scaffold and deposit their own extracellular matrix (D). Cells attach and align to the porous fibres of the biomaterial scaffold (E) via integrins and move using their actin cytoskeleton (F).

Figure 3. Origami and engineering of 3D tissues

A sheet of paper, with a single fold along the red dotted line can be made into a structure with 2 planes (A) or other 3D shapes (B, C, D). A simple paper sheet in origami is compared with 1-dimensional epithelial sheet (E). If the cells (in E) contract at their apical ends, a 3D structure can be generated (F). If few cells (with red circles in G) in a large epithelial sheet contract at apical or basal ends (H), a trough or a crest could be created, which when replicated at multiple locations make intricate architectural forms(I).

Table 1

Examples of cell contraction, epithelial morphogenesis, and tissue assembly.

Contraction	Organism	Morphogenetic event	Phylum	Reference
Embryonic development				
Cell Autonomous	Drosophila	Dorsal closure (Apoptosis of supernumerary cells)	Arthropoda	(Toyama et al. 2008)
	Xenopus laevis, Hymenochirus	Wound healing	Chordata	(Davidson et al. 2002; Minsuk and Keller 1996)
	Mouse	Epithelial to mesenchymal transition	Chordata	(Williams et al. 2012)
Cell groups	Drosophila	Ventral furrow formation	Arthropoda	(Leptin and Grunewald 1990; Martin et al. 2008)
	Mouse	Neurulation	Chordata	(Smith et al. 1994)
	Ascidian	Invagination	Chordata	(Sherrard et al. 2010)
	Sea-urchin	Gastrulation	Echinodermata	(Nakajima and Burke 1996)
	Xenopus laevis	Neural tube formation	Chordata	(Haigo et al. 2003)
Cultured cell aggregates				
	Human	Branching mammary morphogenesis	Chordata	(Berdichevsky et al. 1994)
	Mouse	Optic-cup morphogenesis	Chordata	(Eiraku et al. 2011)
	Human	Airway branching morphogenesis	Chordata	(Franzdottir et al. 2010)
	Dog	Renal tube formation	Chordata	(Eisen et al. 2004; Shimazu et al. 2001)
	Rabbit	Branching networks and polarized vesicles	Chordata	(Mori and Miyazaki 2000)
	Rat	Testicular cord formation	Chordata	(Gassei et al. 2006)