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Forces of Change: Mechanics Underlying Formation of Functional 3D Organ Buds

Paul J. Wrighton[1] and Laura L. Kiessling[1,2,*]
[1]Department of Biochemistry, University of Wisconsin–Madison, Madison, WI 53792, USA
[2]Department of Chemistry, University of Wisconsin–Madison, Madison, WI 53792, USA
*Correspondence: kiessling@chem.wisc.edu
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3D organ buds that can recapitulate organ function have myriad applications for regenerative and personalized medicine. Here, Takebe et al. (2015) describe a generalized method for organ bud formation, demonstrating that mechanosensitive mesenchymal stem cells drive condensation of heterotypic cell mixtures to create buds from diverse organs.

A goal at the forefront of stem cell research is treating patients with degenerative diseases arising from lack of a specific cell type, tissue, or organ. A synergistic goal is to develop useful tools for drug screening and toxicity testing. Both of these objectives would benefit from a means of replicating the function of organs. Recapitulating such function requires complex organization of multiple functional and supporting cell types, which single purified sources of cells, for instance human pluripotent stem cell (hPSC)-derived cells, often fall short of providing. Budding research into organoid generation aims to overcome these limitations by relying on the historical principle of cellular self-organization. Through mechanisms whose molecular bases are largely unresolved, cells can self-sort and self-assemble to guide tissue formation. These principles can be applied in vitro by culturing hPSC-derived cell types under spatial constraints to generate organoids that resemble the gut, kidney, brain, and retina (reviewed in Lancaster and Knoblich, 2014). Limitations of organoid technology include size restriction due to insufficient in vitro nutrient diffusion, lack of vascularization upon transplantation, and the exiguous subset of mimicked organs. Now in *Cell Stem Cell*, Takebe et al. (2015) describe a generalizable method for generating functional, 3D organ buds from a variety of tissues that overcomes these issues.

Takebe and colleagues previously introduced their organ bud strategy to circumvent the limitations associated with organoid culture (Takebe et al., 2013). hPSC-derived hepatic endoderm was combined with mesenchymal stem cells (MSCs) and human umbilical cord endothelial vein cells (HUVECs), and the mixture aggregated and self-organized into 3D spheroid structures termed liver buds, or heterotypic cell collectives. Following transplantation into mice subjected to liver injury, the liver buds were vascularized, due to the inclusion of endothelial cells, and integrated with host tissues to increase host survival. The potential utility of such buds in pharmacological research was illustrated when human-specific drug metabolites were detected in the blood of the transplant-recipient mice (Takebe et al., 2013).

Now, Takebe et al. (2015) report optimized conditions for organ bud generation and demonstrate the general applicability of their technique to additional tissues. First, they observed liver bud formation using time-lapse microscopy and discovered that cells initially formed 2D circular colonies but quickly compacted to form 3D structures. Because the rate of cell movement exceeded that expected for cell migration, it was postulated that cytoskeletal contraction drove this cell condensation. Culturing the collectives with a small molecule inhibitor of myosin II ablated condensation, bolstering cytoskeletal contraction as the underlying mechanism. Furthermore, colony condensation failed if MSCs were not included. Thus, the authors concluded that MSC cytoskeletal contraction likely drives condensate formation.

Cytoskeletal contraction is influenced by the mechanical properties of the extracellular matrix (ECM). Synthetic hydrogels with tunable elasticity and substrate properties have revealed that mechanical forces alone can determine whether hPSCs undergo self-renewal or neuronal differentiation (Musah et al., 2014). MSCs are exquisitely sensitive to the mechanical properties of the ECM, and simply changing the stiffness of the substratum modulates MSC morphology and behavior (Engler et al., 2006). Takebe and colleagues hypothesized that the stiffness of the culture substratum could facilitate condensate formation. They cultured cell mixtures on hydrogels of tunable elasticity and found that hydrogels with intermediate stiffness (~10 kPa) promoted condensation (Figure 1). On softer surfaces, cells condensed more aggressively into small clusters rather than forming larger 3D organ buds. On stiffer surfaces, cells preferentially proliferated to confluence in two dimensions and failed to form the desired 3D assemblages. These findings highlight the importance of mechanical cues from the ECM in driving organ bud generation.

By analyzing the formation and function of liver buds, Takebe and colleagues concluded that the endothelial cells drive vascularization after transplantation and that ECM properties drive cell condensate formation via effects on MSCs. To assess the generality of their findings, the authors tested whether exchanging the hepatic endoderm cells with functional cell types of other tissues could produce cell condensates representative of a variety of organs. The authors mixed MSCs and HUVECs with either MIN6 murine β-cells or dissociated murine embryonic kidney cells and afforded pancreatic and kidney buds, respectively. After transplantation into murine hosts, both types of buds generated vascular networks and

integrated with the host circulatory system. When transplanted into a mouse model of type I diabetes, the pancreas buds restored body weight and glucose homeostasis. The transplanted kidney buds could filter blood and produce urine. The kidney results are especially intriguing because the organoids were derived from a heterogeneous collection of kidney-specific cell types rather than a single functional cell type. These kidney cells assembled into functional nephrons, demonstrating a sophisticated cell self-organizing behavior. The parameters optimized by Takebe and colleagues maximized this intrinsic self-organizing capacity. Because the mechanosensitive MSCs promote condensate formation regardless of the identity of co-cultured functional cells, these findings provide a robust platform to understand functions of diverse organs and to dissect how forces can drive tissue formation.

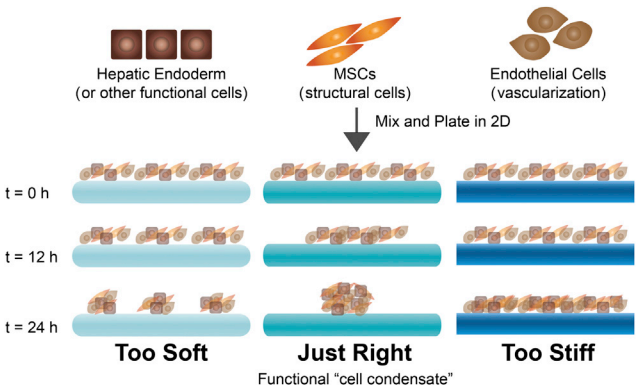

Figure 1. Mechanical Properties of the Substratum Drive Contraction of MSCs to Form Heterotypic Cell Condensates

HPSC-derived hepatic endoderm (or one or more functional cell type) is combined with MSCs and HUVECs, and the mixture is cultured in 2D on hydrogel substrata of varying stiffness. The MSCs respond to the mechanical properties of the substrate and condensation occurs on the surface with "Just Right" stiffness, forming a liver bud (or bud corresponding to the functional cell type).

Despite the rapid progress on heterotypic cell collectives and organoids, fundamental questions about cellular self-organization remain unanswered. It is unclear whether mechanical signals are transmitted through either or both cell-cell and cell-matrix interactions to facilitate condensate formation. The relative influences of cell adhesion receptor engagement versus bulk biophysical properties (i.e., mechanical or shear stress forces) on organoid generation are also unclear. Takebe and colleagues speculate that the balance of cell-cell and cell-matrix interactions governs condensate formation, but there is mounting evidence that the ECM alone can drive some cell-sorting behaviors. For example, in breast tissue, cell-matrix interactions can promote self-organization, while cell-cell contacts are mostly dispensable (Cerchiari et al., 2015). Generation of Cerebral and retina organoids (Nakano et al., 2012) relies on Matrigel, a murine source of ECM proteins, to induce self-organization, as do protocols for intestinal organoid generation (Sato et al., 2009). However, Matrigel can also provide signals inhibitory to differentiation (Wrighton et al., 2014), and the specific properties of this matrix responsible for organoid induction are unclear. Chemically defined matrix components would help elucidate the interplay between cell adhesive cues and mechanical forces in guiding condensate and organoid generation.

The results of this study highlight that changing the matrix can elicit major changes in how multiple cell types assemble. Incorporating additional supporting cell types in the condensates could further increase the utility of organ bud transplants. For instance, the inclusion of neural stem cells could allow innervation of the condensates, much like how endothelial cells allow vascularization. However, the limited sizes of the generated organ buds and organoids potentially impacts their utility. An alternative approach bypassing this issue utilizes ECM scaffolds from decellularized cadaveric organs. The decellularized scaffolds, once repopulated with cells, can drive cellular self-organization and recapitulate organ function (Song et al., 2013), thereby facilitating the generation of larger, more complicated geometries. The advantages of organoid and decellularization strategies might be combined to facilitate organ replacement.

The results from Takebe et al. highlight how controlling matrix properties to optimize the generation of organoids and heterotypic cell collectives can lead to enhanced functionality. The strategy can be used to elucidate the molecular underpinnings of basic cellular mechanisms like self-organization or transmission of mechanical cues from cell to cell throughout tissues. Applying the mechanistic insights that accrue will continue to fuel advances in regenerative medicine.

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