Developmental Biology 420 (2016) 221–229

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/locate/developmentalbiology

Review article

Reverse engineering liver buds through self-driven condensation and organization towards medical application

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ARTICLE INFO

Article history:
Received 26 March 2016
Received in revised form
24 May 2016
Accepted 25 June 2016
Available online 27 June 2016

Keywords:
Organ buds
Organoids
iPS cells
Self-condensation
Self-organization

ABSTRACT

The self-organizing tissue-based approach coupled with induced pluripotent stem (iPS) cell technology is evolving as a promising field for designing organoids in culture and is expected to achieve valuable practical outcomes in regenerative medicine and drug development. Organoids show properties of functional organs and represent an alternative to cell models in conventional two-dimensional differentiation platforms; moreover, organoids can be used to investigate mechanisms of development and disease, drug discovery and toxicity assessment. Towards a more complex and advanced organoid model, it is essential to incorporate multiple cell lineages including developing vessels. Using a self-condensation method, we recently demonstrated self-organizing "organ buds" of diverse systems together with human mesenchymal and endothelial progenitors, proposing a new reverse engineering method to generate a more complex organoid structure. In this section, we review characters of organ bud technology based on two important principles: self-condensation and self-organization focusing on liver bud as an example, and discuss their practicality in regenerative medicine and potential as research tools for developmental biology and drug discovery.

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1. Introduction

To treat patients with degenerative diseases arising from the lack of a specific cell type, tissue or organ, regenerative technology has been increasingly expected by applying stem cell wisdom, and these techniques serve as a novel research tool for investigating

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http://dx.doi.org/10.1016/j.ydbio.2016.06.036
0012-1606/

disease mechanisms, drug discovery and toxicity assessment. However, guiding a targeted cell fate from stem cells still remain challenging due to the failed or limited recapitulation of a natural developmental program. As a conventional strategy, two-dimensional (2-D) and unidirectional differentiation platforms have enabled dramatic advances in our ability to control the specific fate of pluripotent stem cells (Si-Tayeb et al., 2010b; Swistowski et al., 2010; Zhang et al., 2009), although it has been difficult to establish models with complex function in organs because of immaturity or limited functions.

Alternatively, a paradigm shift away from conventional strategies has begun to emerge through the use of more holistic models of human organ development with induced pluripotent stem (iPS) cell technology, namely an organoid model. The model produced by the organoid-based approach has shown immense potential for modeling development and disease with the use of organ-like tissues of various tissue types of health and disease (Huch and Koo, 2015; Lancaster and Knoblich, 2014). Our parallel efforts for engineering the additional tissue complexity, which is considered important to establish an advanced organoid model (Passier et al., 2016), enabled self-organizing "organ buds" that recapitulated aspects of early morphogenesis. In general, an organ develops from a condensed tissue mass prior to blood perfusion, and this mass is termed the organ bud, emerging at early stages of organogenesis through the process of self-condensation, by which neighboring mesenchyme condenses with organ progenitors to form the organ bud. Interestingly, self-organizing organ buds that incorporate multiple cell lineages follow aspects of the developmental program *in vitro* and *in vivo*, showing a therapeutic potential against lethal liver failure by iPS cell-derived organ bud transplantation (Takebe et al., 2014a, 2014b, 2015). Recent mechanistic follow-up study of the organ bud formation process suggests the importance of mesenchymal cells and surrounding matrix rigidity for self-organization into three-dimensional (3-D) liver bud like tissue (Takebe et al., 2014b). Here, we review state-of-art of organ bud technology on the basis of two important principles: self-condensation and self-organization, and discuss their potential application in regenerative medicine, developmental biology and drug discovery.

2. Reverse engineering organ bud via self-condensation and self-organization

Reverse engineering is an approach of extracting basic design information towards a new equivalent or improved technology, usually from a man-made system or device, but recently redefined in an organs-on-chips field (Ingber, 2016). Identifying the minimal set of design principles that are necessary to guide organogenesis using stem cells is also a key to advance organoid technology. As a method to reverse engineer organoids, we here propose two important principles with an organ bud example; 'self-condensation' and 'self-organization' (Fig. 1). A reverse engineered organ bud is a highly complex and organized organ rudiment comprised of a heterogeneous mixture of progenitor cells. Relative to numerous published reports about self-organization, there are few publications summarizing the self-condensation principle. Self-condensation provides a structural preparedness towards following self-organization, whereby a number of heterotypic (or optimal) progenitors undergo a large-scale morphogenetic change to generate a condensed tissue following a biophysical discipline. Here, we would like to discuss the mechanistic basis of this process from a biophysical perspective.

2.1. Biophysical mechanism of self-condensation

Self-condensation is the first key step for the generation of organ buds in our method and provides an extremely large-scale morphogenetic transition from 2-D mixtures of dissociated cells to 3-D cell collectives. In particular, we found that the presence of mesenchymal stem cells (MSCs) in the mixture is essential to induce self-condensation (Takebe et al., 2015). Such multicellular orchestration led by mesenchymal cells is also found in the early step of 3-D liver bud morphogenesis when drastic delamination

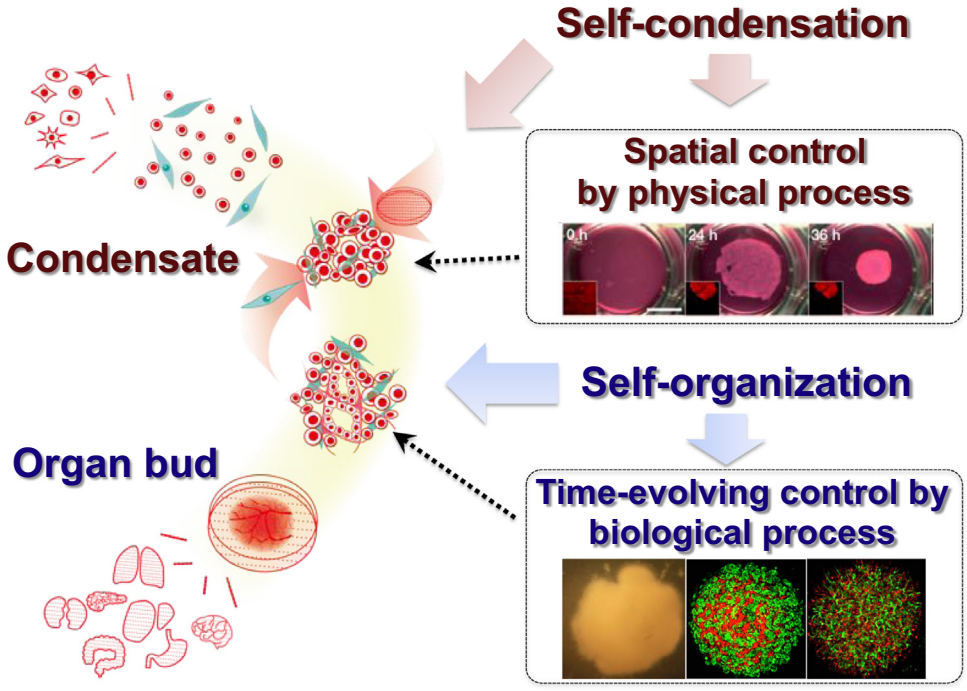

Fig. 1. Overview of organ bud technology Organ bud generation is initiated by a 'self-condensation' step, where the co-culture of heterotypic and optimal cell mixtures on petri dishes spontaneously. Then, the cell condensate undergoes a 'self-organization' step with time-evolving control by biological process and organ-like functional structures such as a vascular network are formed.