Commentary

Untiring steps toward the maturation of human stem cell-engineered heart tissue

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Although the generation of a human heart *in vitro* would be an attempt somewhat beyond human wisdom, many researchers are tackling the subject with enthusiasm, taking advantage of technological progresses in stem cell biology and bioengineering in recent decades. The discovery of human induced pluripotent stem cells (hiPSCs) (1) was a paradigm shift on this research field which opened the door to employ abundant human heart cells (cardiomyocytes, vascular cells and so on) as a source of autologous or allogeneic engineered human heart constructs, which have never been possible with stem cell populations reported so far. Novel protocols to efficiently induce heart cells from hiPSCs (2-4), and bioengineered methods to reconstruct the 3-dimensional (3-D) heart based on porous biomaterials (5-11), decellularized heart (12) or scaffold-free approaches such as cell sheet technologies (3,13-15) are under investigation.

Generation of human heart tissues, a partial achievement of the whole heart reconstruction, is thought to give rise to valuable fundamentals for the new frontiers in the treatment of severe heart diseases resistant to conventional therapies from two standpoints. The first point is the establishment of transplantable heart tissues which may compensate the impaired cardiac function due to heart injury associated with myocardial infarction or idiopathic dilated cardiomyopathy which are representative cardiac disorders leading to severe heart failure requiring heart transplantation. The establishment of functioning heart tissue *in vitro* would serve as a new technological basis for cardiac regenerative therapy in which numerous attempts including intracoronary of myocardial injection of stem cell populations and their progenies possessing cardiogenic potential have been carried out with rather disappointing results. The second point is the substantiation of the cardiac biology, physiology and physics *in vitro* which can be used as a toolbox for drug discovery and the elucidation of the etiology of heart diseases which is restricted because of rather limited opportunities in obtaining diseased human heart samples from surgical resection or biopsy.

A crucial requirement for the better quality of the 3-D construct suitable for the abovementioned two applications is the maturation of the tissue. Cardiac cells differentiated from hiPSCs mostly used for recent studies in this research field are juvenile and immature, and have only acquired characteristics of terminally differentiated cell lineages for 1–2 months. These cells are equivalent to cells at the fetal stage at the first trimester of pregnancy (7-10). It has been reported that the electrophysiological properties of the vast majority of hiPSC-derived cardiomyocytes lack the I_{K1} current leading to immature repolarization capacity (16) which encourages researchers to drive cellular maturation, and also maturation as a tissue. During the early embryonic

development, the primitive heart tube composed of endocardium and myocardium layers derived from the lateral plate mesoderm starts to be compartmentalized along with looping, leading to the formation of the nascent heart organ (17). In this process, the volume and number of cardiomyocytes and other cardiac cells drastically increase to form a myocardial structure with enough thickness comparable to functional heart tissue. What kind of mechanisms are taking place in this early stage of heart development to drive the maturation of heart tissue? It would be a reasonable approach to apply this innate system during early embryonic heart development to drive engineered tissue maturation by putting the artificial tissue in culture conditions that recapitulate the dynamic physical microenvironment of early heart development.

Recently, Ruan *et al*. reported a unique method to achieve tissue maturation using the novel combination of physical stretch and electrical stimulation to a bioengineered heart tissue composed of hiPSC-derived cardiomyocytes and collagen I (11). The combination of physical stretch and electrical stimulation, possibly inspired by the bona fide processes of early heart development, is a new insight into this research field to pursue stem cell-derived 3-D tissue maturation. They indicated that static physical stretch enhanced contractile force, cellular and extracellular matrices (ECMs) alignment, tissue tensile stiffness, cellular size and SERCA2 expression which indicated maturation of the sarcoplasmic reticulum. They also reported that the addition of electrical stimulation further promoted tissue force production with unchanged cellular alignment and cell size, suggesting that the combination of physical stretch and electrical pacing promoted the maturation of excitation-contraction coupling. These intriguing findings imply that the closer recapitulation of innate developmental systems associates with higher effects on tissue maturation.

We may propose two perspectives of physical training which may further simulate or modify the innate developmental system and subsequently promote the tissue maturation. The first perspective is external cyclic stretching (not only static stretching by anchoring both edges of the tissue). A previous report of external cyclic stretching on bioengineered tissue showed positive results in increased gap junction formation and calcium handling (18). Considering another report that a pacing frequency higher than the intrinsic beating rate led to a more matured phenotype of cardiomyocytes among tissue structure including promoted calcium handling and I_{K1} current (8), non-physiological stimulation would accelerate tissue maturation, and it is worth attempting as long as it is guaranteed that the physical stress would not affect cellular viability, or promote undesirable cellular migration or other adverse effects. Another perspective is the supplementation of other cell lineages consisting native heart tissue such as vascular cells (vascular endothelial cells or vascular mural cells), or stromal cell lineages (e.g., cardiac fibroblasts) besides cardiomyocytes. Cell sheet experiments or 3-D engineered cardiac tissue experiments have shown that the co-existence of vascular cells with cardiomyocytes within the structure promotes reinforced secretion of humoral factors working on tissue repair, cellular alignment or sarcomeric maturation (7,19). A report from Tulloch *et al*. (same group as that of Ruan *et al*.) also indicates the additional effect of non-myocytes in which the supplementation with endothelial cells enhanced cellular alignment especially under conditions of cyclic stretching (20). These results indicate the importance of co-existence of various cardiac cell lineages among the 3-D construct, and that the addition of electrical stimulation potentially further enhances tissue maturation.

It still remains unclear what is indispensable to achieve tissue maturation comparable to the native adult heart tissue. What we learned from literatures and the recent report from Ruan *et al*. would be that a multidisciplinary approach is required to resolve this challenge considering the complex machinery of human heart development under various stage-specific physical and physiological conditions which may affect cellular proliferation and lineage specification as well as maturation. Untiring steps may pioneer the frontier.

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Footnote

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