Advanced Drug Delivery Reviews 96 (2016) 103–109

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

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

Three-dimensional cardiac tissue fabrication based on cell sheet technology☆

Shinako Masuda, Tatsuya Shimizu *

Institute of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University, 8-1 Kawada-cho, Shinjuku, Tokyo 162-8666, Japan

ARTICLE INFO

Available online 14 May 2015

Keywords:
Cell sheet engineering
Perfusable 3D cardiac tissues
Vascularization in 3D cardiac tissues
Cardiac tubes
Pluripotent stem cell-derived cardiac cells
Large-scale suspension culture systems

ABSTRACT

Cardiac tissue engineering is a promising therapeutic strategy for severe heart failure. However, conventional tissue engineering methods by seeding cells into biodegradable scaffolds have intrinsic limitations such as inflammatory responses and fibrosis arising from the degradation of scaffolds. On the other hand, we have developed cell sheet engineering as a scaffold-free approach for cardiac tissue engineering. Confluent cultured cells are harvested as an intact cell sheet using a temperature-responsive culture surface. By layering cardiac cell sheets, it is possible to form electrically communicative three-dimensional cardiac constructs. Cell sheet transplantation onto damaged hearts in several animal models has revealed improvements in heart functions. Because of the lack of vasculature, the thickness of viable cardiac cell sheet-layered tissues is limited to three layers. Pre-vascularized structure formation within cardiac tissue and multi-step transplantation methods has enabled the formation of thick vascularized tissues in vivo. Furthermore, development of original bioreactor systems with vascular beds has allowed reconstruction of three-dimensional cardiac tissues with a functional vascular structure in vitro. Large-scale culture systems to generate pluripotent stem cell-derived cardiac cells can create large numbers of cardiac cell sheets. Three-dimensional cardiac tissues fabricated by cell sheet engineering may be applied to treat heart disease and tissue model construction.

Contents

1. Introduction

Cell-based therapies are expected to be an alternative strategy to repair damaged cardiac tissue, because heart transplantation is restricted by a shortage of donors [1]. One of the promising approaches to repair damaged cardiac tissue is transplantation of a cell suspension, but it is difficult to apply a cell suspension for treatment of myocardial tissue

Abbreviations: ECM, extracellular matrix; 3D, three-dimensional; 2D, two-dimensional; PIPAAm, poly-(*N*-isopropylacrylamide); iPS cells, induced pluripotent stem cells; ES cells, embryonic stem cells.

☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Tissue engineering of the heart: from in vitro models to regenerative solutions".

* Corresponding author. Tel.: +81 3 5367 9945; fax: +81 3 3359 6046.
E-mail address: shimizu.tatsuya@twmu.ac.jp (T. Shimizu).

http://dx.doi.org/10.1016/j.addr.2015.05.002
0169-409X/

defects. Therefore, an aim of tissue engineering is to generate functional bioengineered cardiac tissue. Tissue engineering has emerged as field in regenerative medicine and tissue modeling. In general, the main components for tissue engineering include isolated cells or cell alternatives, appropriate signaling molecules such as cytokines and growth factors, and an extracellular matrix (ECM) [2]. When three-dimensional (3D) biodegradable scaffolds are used as an ECM alternative, the spaces occupied by biodegradable polymers are often replaced with large amounts of ECM, resulting in the formation of tissue with a low cell density. In addition, biodegradation of scaffolds causes an inflammatory response and pathological fibrous tissue formation. To overcome these issues, we have developed cell sheet engineering methods to fabricate 3D functional tissues by layering confluent two-dimensional (2D) cell sheets.

2. Methods for 3D cardiac tissue fabrication

There are several tissue engineering methods to fabricate 3D cardiac tissues. Eschenhagen and Zimmermann reported reconstitution of cardiac tissues by mixing cells with a solution of collagen I for gelling [3]. They developed effective hydrogels for cardiac tissue engineering by application of mechanical stretch, fabricated highly differentiated cardiac tissue exhibiting contractile and electrophysiological properties, and demonstrated improvement of heart functions in infarcted rats by implantation experiments [4–8]. Another cardiac tissue engineering approach is seeding cardiac cells into prefabricated scaffolds. Compared with other techniques, it is easy to fabricate the desired 3D form with prefabricated porous matrices. This technique has also been applied to generate various types of tissues. In addition to the abovementioned biomaterials, a decellularized ECM of heart tissue has been used as a scaffold to seed cells and fabricate cardiac tissues [9]. Decellularization is usually performed by treatment with detergents, hypo/hypertonic solutions, nucleases, and other enzymes. Decellularized heart tissue preserves the 3D anatomical architecture and vasculature [10,11], and spontaneous contractions and normal response to drug in decellularized mouse heart, which repopulated with human induced pluripotent stem (iPS) cell-derived multipotential cardiovascular progenitor cells, were observed [12]. However, it is insufficient for circulating blood by mechanical force generated from the engineered heart tissue, and electrical conduction in the construct is slower than in vivo heart tissue. It might be necessary for further studies to improve the heart functions.

Indeed, various biomaterials such as polyglycolic acid [13,14], gelatin [15], alginate [16], collagen [17], and fibrin [18] are used as biodegradable scaffolds to substitute for the ECM in tissue engineering. As mentioned above, biodegradable polymer-based techniques, which result in the production of cell-sparse tissue, are inadequate to regenerate cell-dense tissues such as the heart. To circumvent problems arising from biodegradable polymers, scaffold-less cell sheet engineering has been applied to fabricate 3D cardiac tissues by layering 2D cardiac cell sheets.

Cell sheets are harvested from intelligent cell culture surfaces that are covalently bonded with a temperature-responsive polymer, poly(*N*-isopropylacrylamide) (PIPAAm) (Fig. 1) [19]. The surface is slightly hydrophobic under normal cell culture conditions at 37 °C to allow adherence and proliferation of cells. By lowering the temperature to <32 °C, the surface becomes highly hydrophilic and nonadhesive for cells because of rapid hydration and swelling of the PIPAAm. This change in the surface properties leads to the cells detaching from the PIPAAm-coated surface by simply lowering the culture temperature. Cells are also attached to each other through cell adhesion proteins and deposited ECM in confluent cultures. Popular cell harvesting techniques by enzymatic digestion usually disrupt the cell adhesive components and membrane proteins. In contrast, confluent cells are harvested from temperature-responsive culture surfaces as intact cell sheets with the deposited ECM [20]. Cell sheets harvested from temperature-responsive culture surfaces can be directly attached to host tissue without fibrin glue or a suture because of the deposited ECM underneath the cell sheet.

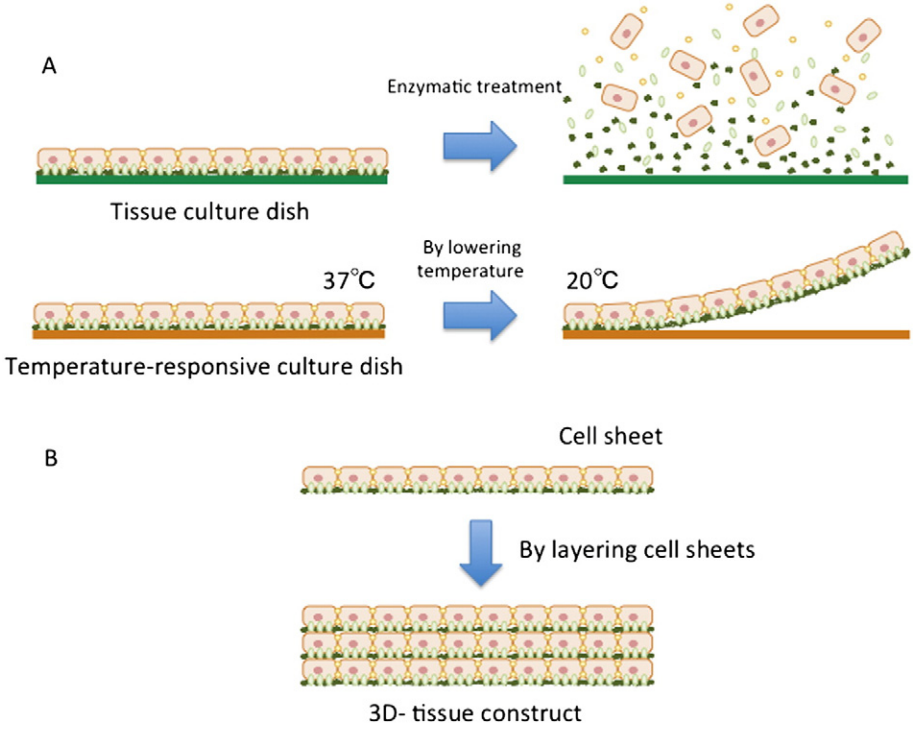

Fig. 1. A. Confluent cells connected to each other via cell-to-cell junction proteins and the extracellular matrix. Cell harvesting by enzymatic treatment results in the disruption of membrane proteins and the extracellular matrix. Using temperature-responsive culture dishes, confluent cells are released as a contiguous cell sheet with cell-to-cell connections and the deposited extracellular matrix. B. 3D tissue can be reconstructed by layering cell sheets harvested from temperature-responsive culture surfaces.