

MINI-REVIEW

Regeneration of a heart cell

Yibing Qyang* and Grant Senyei

Section of Cardiovascular Medicine, Department of Internal Medicine and Yale Stem Cell Center, Yale University School of Medicine, New Haven, Connecticut

Cardiomyocytes, the beating cells in all mammalian hearts, generally are believed to be terminally differentiated cells that do not proliferate after birth. Instead, the growth of a heart from the neonatal to adult stage results primarily from a phenomenon called hypertrophy, during which cardiomyocytes increase their cell size without undergoing cell division. This dogma was recently challenged by two outstanding publications from the Lee [1] and Frisen [2] groups. Using a mouse genetic fate-mapping strategy, the Lee group showed strong evidence that stem or progenitor cells refreshed murine cardiomyocytes after heart injury, but not during up to one year of normal aging [1]. Additionally, taking advantage of the incorporation of carbon-14 produced during the Cold War into DNA, the Frisen group established a way to determine the age of cardiomyocytes. Using this method, they demonstrated that human cardiomyocytes renew themselves, although at a low frequency [2]. These studies have opened a new and exciting avenue for research into cardiac repair mechanisms, such as develop-

ing pharmacological agents to stimulate the low level of cardiac repair function of mammalian hearts. However, the development of such agents is hindered by the identities of the cardiac stem or progenitor cells able to refresh the injured or aged cardiomyocytes remaining unknown. As an alternative strategy, one could isolate these cardiac stem/progenitor cells from heart tissues or embryonic stem (ES⁺) cells and use them for cell-based therapies for cardiomyocyte replacement. But before a fruitful clinical application of cardiac stem cell/progenitor cells can be established, there are a few roadblocks we need to clear up.

IDENTIFICATION OF THE OPTIMAL CELL TYPE AND NUMBER OF CELLS FOR POSTNATAL HEART REPAIR AND REGENERATION

The major barriers to the development of pharmacological agents or cell-based therapies for cardiac repair include obtaining not only an understanding of the identity of the resident cardiac stem/progenitor cells,

*To whom all correspondence should be addressed: Yibing Qyang, Section of Cardiovascular Medicine, Department of Internal Medicine and Yale Stem Cell Center, Yale University School of Medicine, New Haven, CT 06511; Tele: 203-737-6354; E-mail: yibing.qyang@yale.edu.

†Abbreviations: ES, embryonic stem; CPC, cardiovascular progenitor cells; PIPAAm, poly (N-isopropylacrylamide); ECM, extracellular matrix.

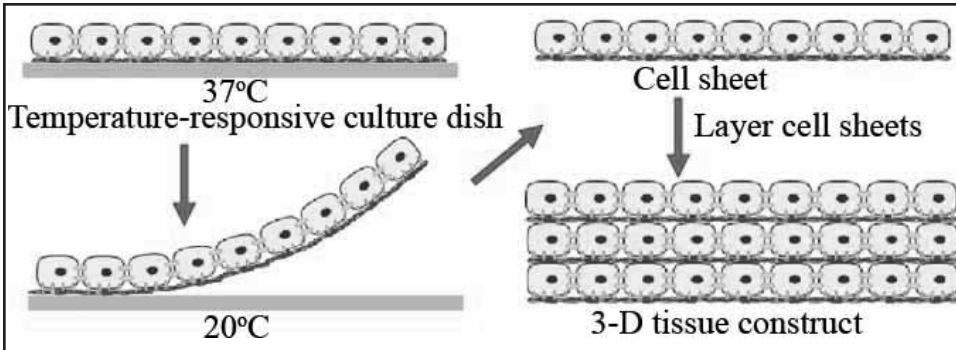


Figure 1. 3-D Engineered Heart Tissue (EHT) Construction with Cell Sheet Engineering. Cardiomyocyte cell sheets can be harvested by lowering culture temperature from 37°C to 20°C without enzymatic digestion. 3-D heart tissue can be established by layering the cell sheets (with permission from Masuda et al., 2007 [11]).

but also the ability to generate a sufficient number of cells for therapeutic use. Subsequent challenges involve the optimization of methods for the isolation and delivery of these cells, particularly concerning the timing of cell delivery. The recent discovery of various cardiovascular progenitor cells (CPC) in adult hearts has provided exciting candidates that could replace the injured or aged cardiomyocytes. In some cases, these cardiovascular progenitor cells can improve cardiac function after implantation into injured hearts in animal models. However, the developmental origins and long-term benefits of such cardiovascular progenitor cells remain largely unknown or unproven.

Our laboratory recently isolated and characterized a novel population of cardiovascular progenitor cells from rodent and human hearts, as well as from murine ES cells [3-5]. These cells are capable of making nearly an entire heart during embryonic and postnatal heart formation and are marked by expression of *ISL1* — a LIM-Homeo domain transcription factor [3,6]. *ISL1*⁺ cardiovascular progenitor cells are highly proliferative, multi-potent cells that have the capacity to differentiate into cardiomyocytes, smooth muscle cells, and endothelial cells [3,5], all of which are required for cardiac repair. Thus, these cells are an ideal source for cell-based cardiac repair therapies in postnatal hearts. Since we can derive unlimited amounts of functional *ISL1*⁺ CPC from ES cells, we will be able to

determine the optimal number of cells required for significant cell engraftment following implantation into injured hearts. Moreover, the discovery of *ISL1*⁺ CPC allows for high-throughput small molecule screening for the isolation of pharmacological agents that can stimulate the self renewal of these CPCs, which may play a critical role in the repair of mammalian hearts.

GENERATION OF HUMAN INDUCED PLURIPOTENT STEM (IPS) CELLS FOR THE ISOLATION OF CARDIOVASCULAR PROGENITOR CELLS AND CARDIOMYOCYTES

Another major challenge facing cell-based therapies for heart failure is the immune rejection of the ectopic cells transplanted into the host. Recently, several groups have reprogrammed terminally differentiated human cells into iPS cells by introducing a combination of genes, including *OCT4*, *SOX2*, *NANOG* and *LIN28*, using lentiviral as well as non-integrating episomal vectors [7-9]. These human iPS cell lines mimic human ES cells and have the potential to produce derivatives of all three germ layers, including cardiac cells [7-9]. This advancement provides an ideal means of isolating patient-specific, autologous heart cells, such as cardiovascular progenitor cells and functional cardiomyocytes for cardiac repair.

ESTABLISHMENT OF OPTIMIZED METHODS FOR DELIVERING CELLS INTO THE INJURED HEART

Current methods of cell delivery, such as cell suspension injection, often result in poor engraftment. These techniques and routes of delivery must be improved in order to increase the efficacy of cardiac cell-based therapies. Engineered heart tissues, generated by using biodegradable scaffolds, improved the efficiency of cell retention compared with cell suspension injection. However, this approach showed marginal benefit in improving cardiac function, possibly due to their limited attachment to the myocardium and the inflammatory and fibrotic responses caused by the scaffold's degradation [10].

A recently developed "cell sheet engineering" technology (Figure 1) has greatly improved the efficiency and efficacy of cell engraftment. This system of delivery has resulted in the production of functional engineered heart tissue as well as a marked improvement in cardiac function following implantation [11,12]. In order to prepare these cell sheets, tissue culture dishes are covalently coated with the temperature sensitive polymer, poly (N-isopropylacrylamide) (PI-PAAm). At 37°C, the surface is hydrophobic and allows cells to adhere and proliferate. However, at 20°C, the surface becomes hydrophilic, leading to cell detachment because of rapid hydration and swelling of the grafted PIPAAm. The 3-D heart tissue can be established by layering these cell sheets. As there is no enzyme digestion involved, the cell surface, extracellular matrix (ECM), and cell-cell interactions remain intact in the detached cell sheet. Our laboratory will use this technology to develop engineered heart tissues with human ES and iPS-derived cardiomyocytes or cardiovascular progenitor cells. The intact adhesive molecules and ECM of the engineered heart tissues will enable them to readily attach to the injured myocardium without any suture. Following implantation, these engineered heart tissues will engraft efficiently to the injured hearts, reducing scar formation and increasing cardiac function.

In summary, the discovery of several types of cardiovascular progenitor cells within the heart provides a unique opportunity to establish pharmacological agents capable of stimulating an increase in the low level of cardiac repair in mammalian hearts. Furthermore, the development of engineered cardiac tissues, using human ES and iPS cell-derived cardiomyocytes or cardiovascular progenitor cells, will offer exciting possibilities for the establishment of high-throughput, patient-specific evaluation of cardiac medicines, and the discovery of cell-based therapies to treat heart failure.

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