recorded electrocardiogram (ECG) and intraventricular pressure. The synchronized tracings of ECG and pressure were shown in Fig. 4B. Microscopic observation revealed that seeded cells adhered inhomogeneously to the decellularized ECM (Fig. 4C). We further examined whether cardiac proteins existed in recellularized hearts by western blot analysis (Fig 4D). As a result, cardiac contractile proteins, including sarcomeric alpha actinin, MYH and cardiac troponin I (TnI), were abundantly observed, while VE-cadherin and Cx43 were only faintly detected in engineered hearts compared to an adult rat heart. Among the seeded cells, sarcomeric alpha actinin positive cells were detected in the ventricles; these cells were surrounded by laminin-positive ECM (Fig. 5A). Although regions of the alpha actinin-positive cells were accompanied by Cx43, the expression was faint, which indicated that intercellular conduction might be immature as compared to adult hearts (Fig. 5B). An immunofluorescence study revealed a randomly-mixed alignment of cardiomyocytes, endothelial cells, and smooth muscle cells stained with alpha actinin, CD31, and smooth muscle (sm)-actin, respectively (Fig. 5C, D). CD31-positive cells and sm-actin-positive cells were not necessarily localized to decellularized vessel-like structures. Transmission Electron Microscope observations showed that the decellularized hearts preserved collagen fibers well in the ECM (Fig. 6A) and the recellularized hearts demonstrated the presence of sarcomeric structures surrounded by ECM (Fig. 6B).

Supplementary video related to this article can be found at http://dx.doi.org/10.1016/j.biomaterials.2014.05.080.

3.4. Excitation-propagation of recellularized hearts

Engineered hearts seeded with GCaMP2-expressing cardiac cells showed spontaneous beating within 2–3 days after recellularization, at which point they were subjected to optical imaging experiments. We observed that spontaneous excitations were generally well aligned and stably propagated in the engineered heart tissues (Fig. 7A and Supplementary Movie 3). Fig. 7A shows the representative images of propagation sequences in an engineered heart. Excitation seemed to emerge in the lateral wall of left ventricle (LV) and propagated through the free LV wall. Isochrone map of the propagating CaT suggested inhomogeneous conduction in substantial areas (Fig. 7C). Conduction velocity (CV) was an order of magnitude or slower (approximately 0.5–5 cm/s) compared to normal adult rat hearts.

Supplementary video related to this article can be found at http://dx.doi.org/10.1016/j.biomaterials.2014.05.080.

3.5. Arrhythmogenicity of recellularized heart tissues

To examine the underlying mechanisms of arrhythmogenesis in the engineered heart tissues and the time-course of maturation in electrical properties, live tissue fluorescence video imaging was employed. We used FFT analysis to examine the synchronicity. Spontaneous excitation of CaT recorded at the three discrete points in the engineered heart tissues were subjected to FFT analysis. In the sample of the first group, during spontaneous beating, the

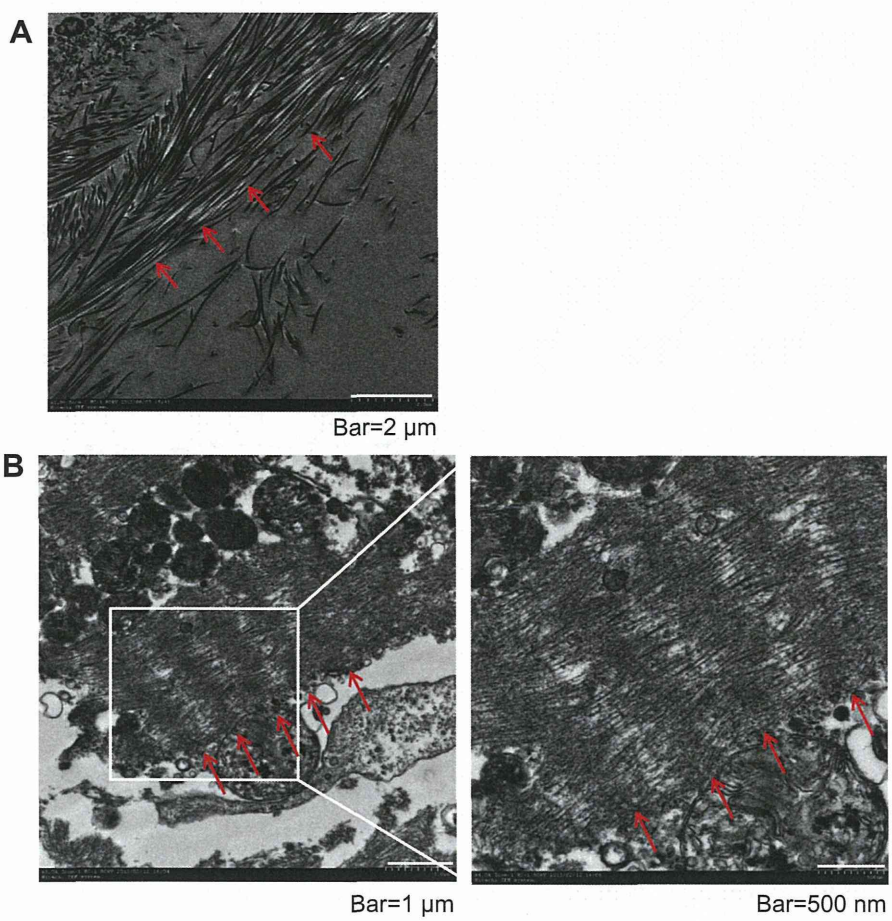

Fig. 6. Ultrastructure of decellularized and recellularized rat hearts by transmission electron microscopy. (A) Ultrastructure of a decellularized heart. Collagen fibers (arrows) in the ECM were well preserved after decellularization procedure. (B) Ultrastructure of a recellularized heart. Arrows show cells with well-organized stria. Cardiac cells seemed to be surrounded by ECM.

entire heart tissues were well-synchronized (Fig. 8A–E and Supplementary Movie 4). Isochrone map showed that excitation propagated rapidly through the left ventricle (Fig. 8D). Frequency analysis showed similar frequency profile pattern at each point (Fig. 8E). On the other hand, in the sample of the second group, although substantial parts of the tissues showed well-organized propagation of excitation, unsynchronized beatings were also observed around ROI-2 (Fig. 9A–E and Supplementary Movie 5). Isochrone map of the propagating CaT showed that initial activation generated from two distinct sites at basal and apical parts of the heart (Fig. 9D). The frequency profiles were accordingly similar at ROI-1 and ROI-3, but not at ROI-2 (Fig. 9E). These observations suggested that there were multiple re-entry like circuits. The sample of the third group exhibited markedly arrhythmogenic characteristics. The heart tissues showed spontaneous contraction, but each region did not show synchronization (Fig. 10A–E and Supplementary Movie 6). Conduction between left and right sides of the heart was hardly observed (Fig. 10D).

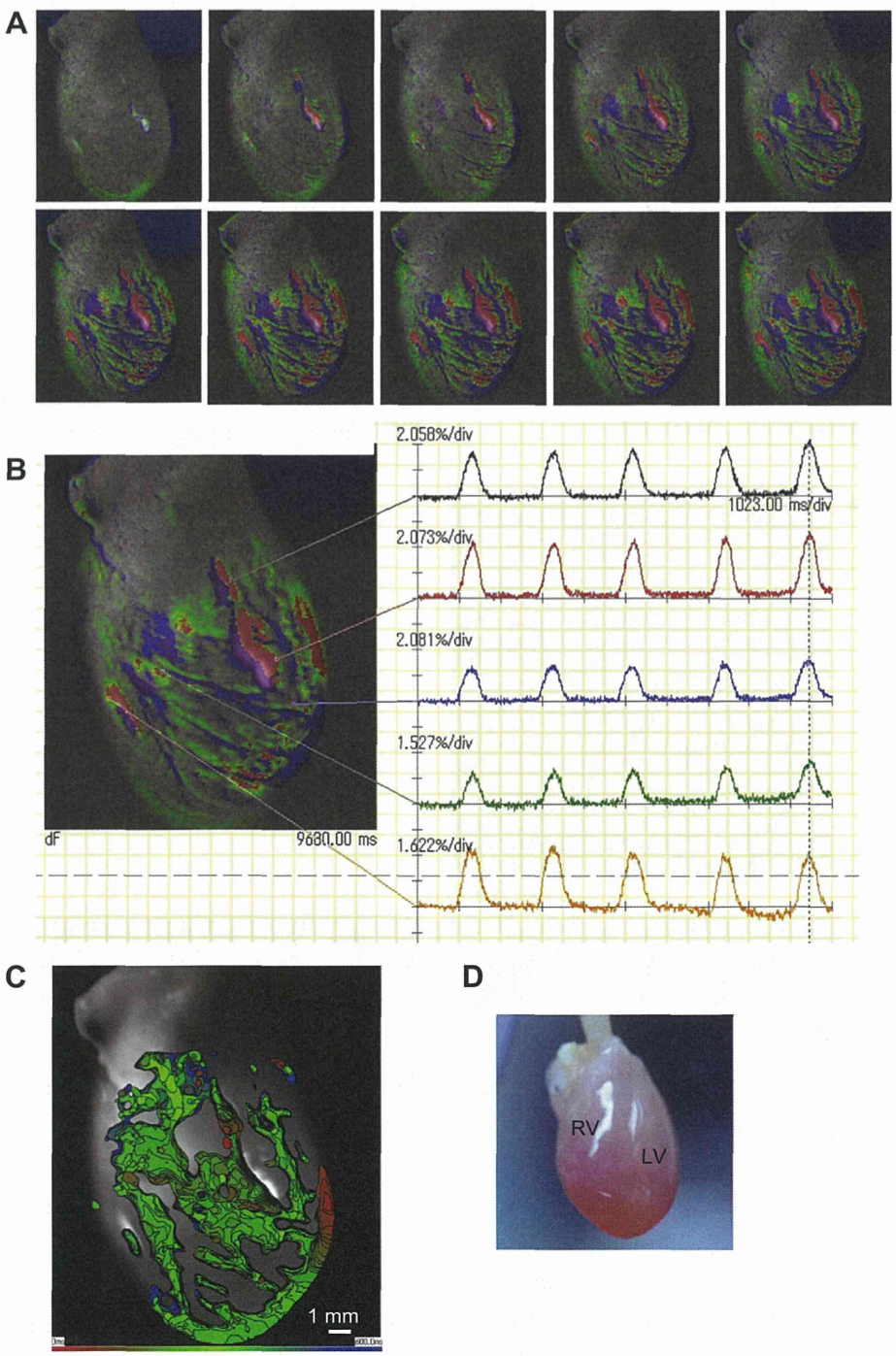

Fig. 7. Propagation of the intracellular calcium transient (CaT) in a recellularized heart. (A) Sequential images in spontaneous beating (every 40 ms). Optical images showed excitation-propagation throughout epicardial surface of the recellularized heart. (B) Detection of the intracellular CaT. (C) Isochrone map (in 10 ms color-coded intervals) of the propagating CaT. Isochrone map suggested inhomogeneous propagation in substantial areas. (D) Anatomical features of the recellularized heart from a frame of video file.

Frequency analysis showed that ROI-1, -2, and -3 beat independently (Fig. 10E). These observations suggested each region had automaticity although cardiac cells were continuously present among ROI-1, -2, and -3.

Supplementary video related to this article can be found at http://dx.doi.org/10.1016/j.biomaterials.2014.05.080.

4. Discussion

In the present study, we successfully constructed 3D engineered hearts by recellularizing adult rat decellularized hearts with neonatal rat cardiac cells, and demonstrated the excitation and propagation properties of 3D engineered heart tissues as an "organ".

For myocardial regeneration therapy of severe heart failure, sufficient wall thickness is required for engrafted cardiac tissues to assist pumping function of the failing hearts. To elucidate the point, several attempts have been made such as "3D cardiac patch" with promoted maturation from ES cell-derived cardiomyocytes [12] or "multi-layered cardiac cell sheets" based on temperature-responsive cell culture dishes [13]. Despite of these efforts, there still remains a limitation in oxygen delivery to thick myocardial tissues, and thus the thickness of the engineered cardiac tissues is confined to approximately 200 μm or less [14,15]. Recent techniques to engineer cardiac tissues with perfusable blood vessels have made thicker vascularized cardiac tissues feasible, which showed spontaneous beating and were transplanted with blood vessel anastomoses [16]. Eschenhagen and Zimmermann

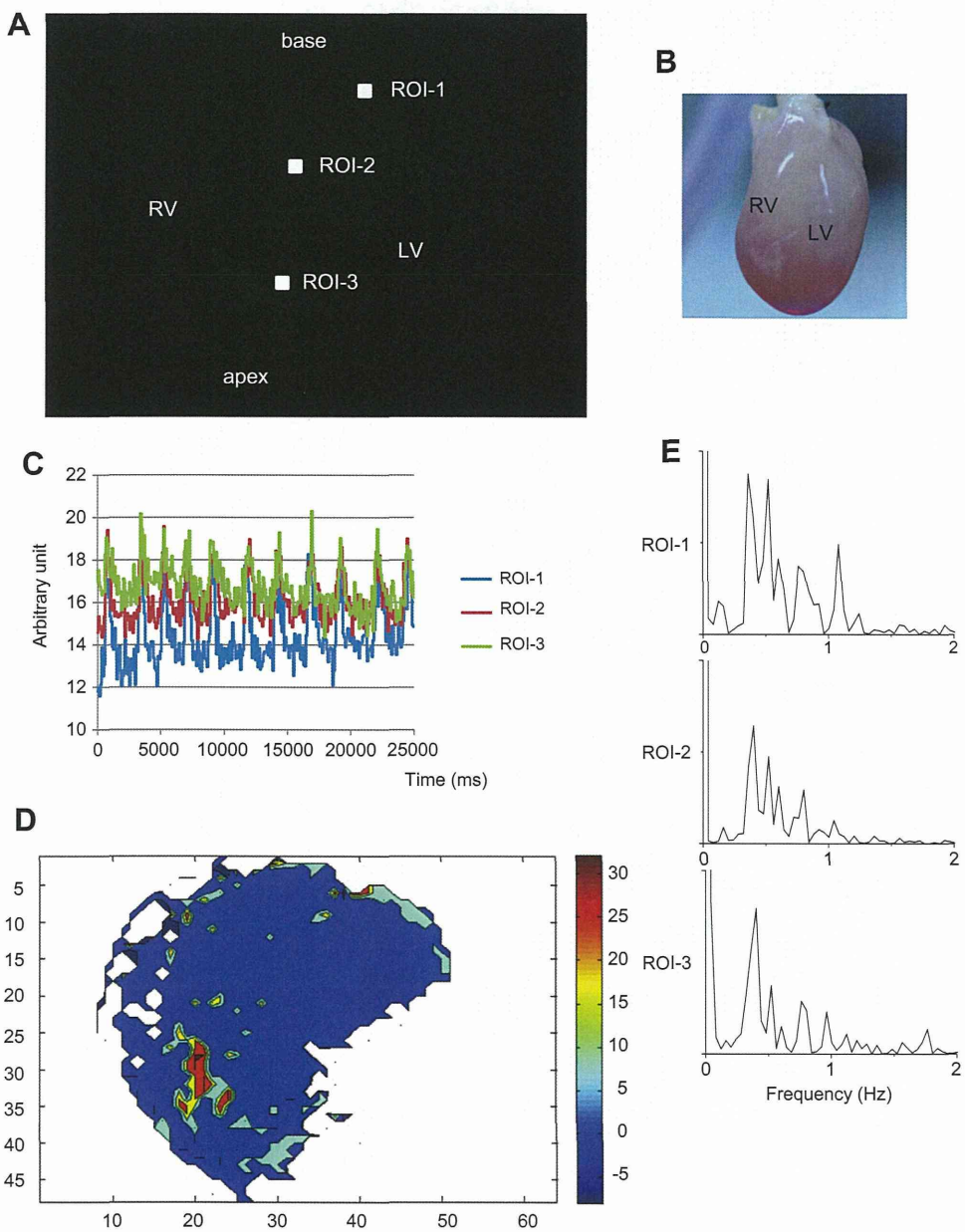

Fig. 8. Propagation of recellularized heart tissues ('well-synchronized' group). (A) Detection of the intracellular CaT driven by local propagation of electrical activity (ROI-1, -2, and -3) in the sample 1. (B) Anatomical features of the recellularized heart from a frame of video file. (C) Intracellular CaT at each site. We observed well-organized conduction of stable excitation in substantial areas of the engineered heart tissues. (D) Isochrone map of the propagating CaT. Note that excitation rapidly propagated throughout the left ventricle. (E) Fast Fourier Transform (FFT) analysis. Note that each site showed similar frequency pattern.

engineered cardiac tissues using a mixture of collagen I, ECM proteins (Matrigel®) and neonatal rat cardiomyocytes into molds; their constructs were intensively interconnected [17]. Their 1–4-mm-thick engineered heart tissue grafts improved the systolic and diastolic functions on implantation in infarcted rat hearts [18].

However, they could not preserve the large-scaled 3D architectures and natural matrix components of the heart. In this context, decellularized whole hearts may act as niches for repopulated cells and vessels for supplying the nutrients to the engineered myocardial constructs. It was indicated that ECM components and preserved mechanical properties of the decellularized heart had directed differentiation of the stem/progenitor cells into the cardiac lineage [19]. This observation also suggests that decellularized heart matrix may therefore be a promising alternative to synthetic scaffolds and a foundation for regenerative efforts for 3D tissue engineering [20].

Previous attempts have revealed that engineered myocardial tissues are not fully synchronized in the small regions of the whole hearts [11,21]. We achieved partial synchronization of significant regions of the heart, but also observed regions which beat independently. Synchronicity is an important factor for the construction of a transplantable artificial organ, since asynchronous regions impair the contractility, as well as potentially increase the risk of lethal cardiac arrhythmias. The calculated CV in our engineered heart tissues was markedly decreased (approximately 5 cm/s) as compared to that reported previously in an adult rat heart [22,23]

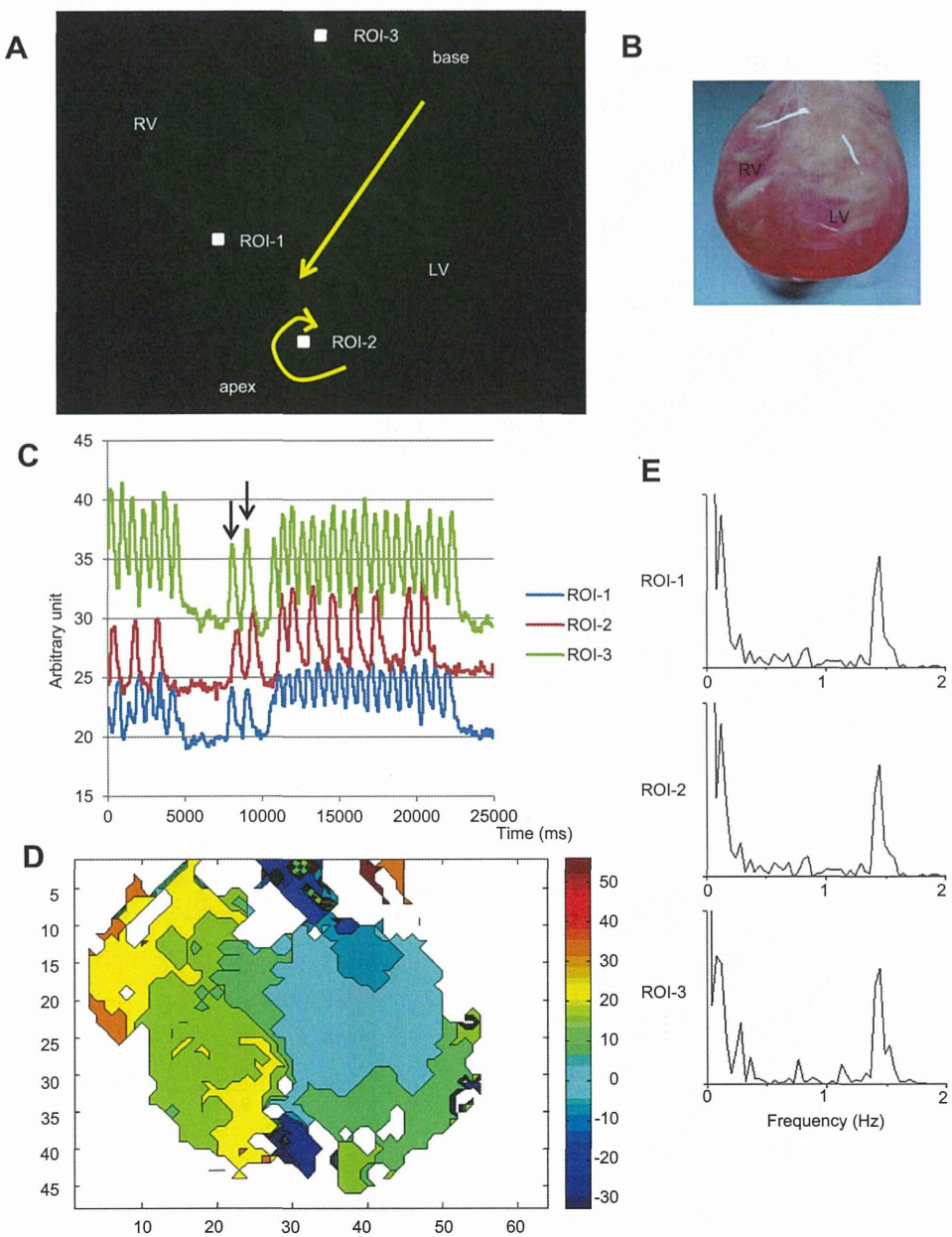

Fig. 9. Propagation of recellularized heart tissues ('unsynchronized' group). (A) Detection of the intracellular CaT driven by local propagation of electrical activity (ROI-1, -2, and -3) in the sample 2. (B) Anatomical features of the recellularized heart from a frame of video file. (C) Intracellular CaT at each site. Propagation of most parts showed the unsynchronized pattern. After 2 beats (arrows), there was a pause, and another wave emerged from the bottom of the heart and collided with the preceding wave. (D) Isochrone map of the propagating CaT. Note that initial activation generated from two distinct sites at basal and apical parts of the heart. (E) FFT analysis. The frequency profiles were similar at ROI-1 and ROI-3, but not at ROI-2. This suggested that at least two excitation-propagation existed.

or in an *in vitro* cultured neonatal rat cardiomyocyte (NRCM), whereas it was similar to that of NRCM with Cx43 mutation [24]. Slow conduction and electrical instability were more apparent in the whole heart. The mixture of automaticity from multiple sites and multiple re-entry leads to arrhythmogenic propensity and obstructs adequate contraction. To create a whole heart with sufficient contraction, electrophysiological evaluation of the whole heart is essential.

Our engineered heart showed a marked decrease in expression of Cx43, which is one of the reasons why our engineered constructs showed slow conduction and electrical instability as well as sparse adhesion of the repopulated cardiomyocytes. This might be improved by overexpressing Cx43 in the engineered cells. Immunofluorescence study revealed randomly mixed alignment of cardiomyocytes, endothelial cells, and smooth muscle cells stained with alpha-actinin, CD31, and sm-actin, respectively; these cells were located close to one another. The mixture of different cells may inhibit smooth electrical conduction. Although these cells were present simultaneously, it is possible that previously resident cadiomyocytes attracted other endothelial cells and attempted to

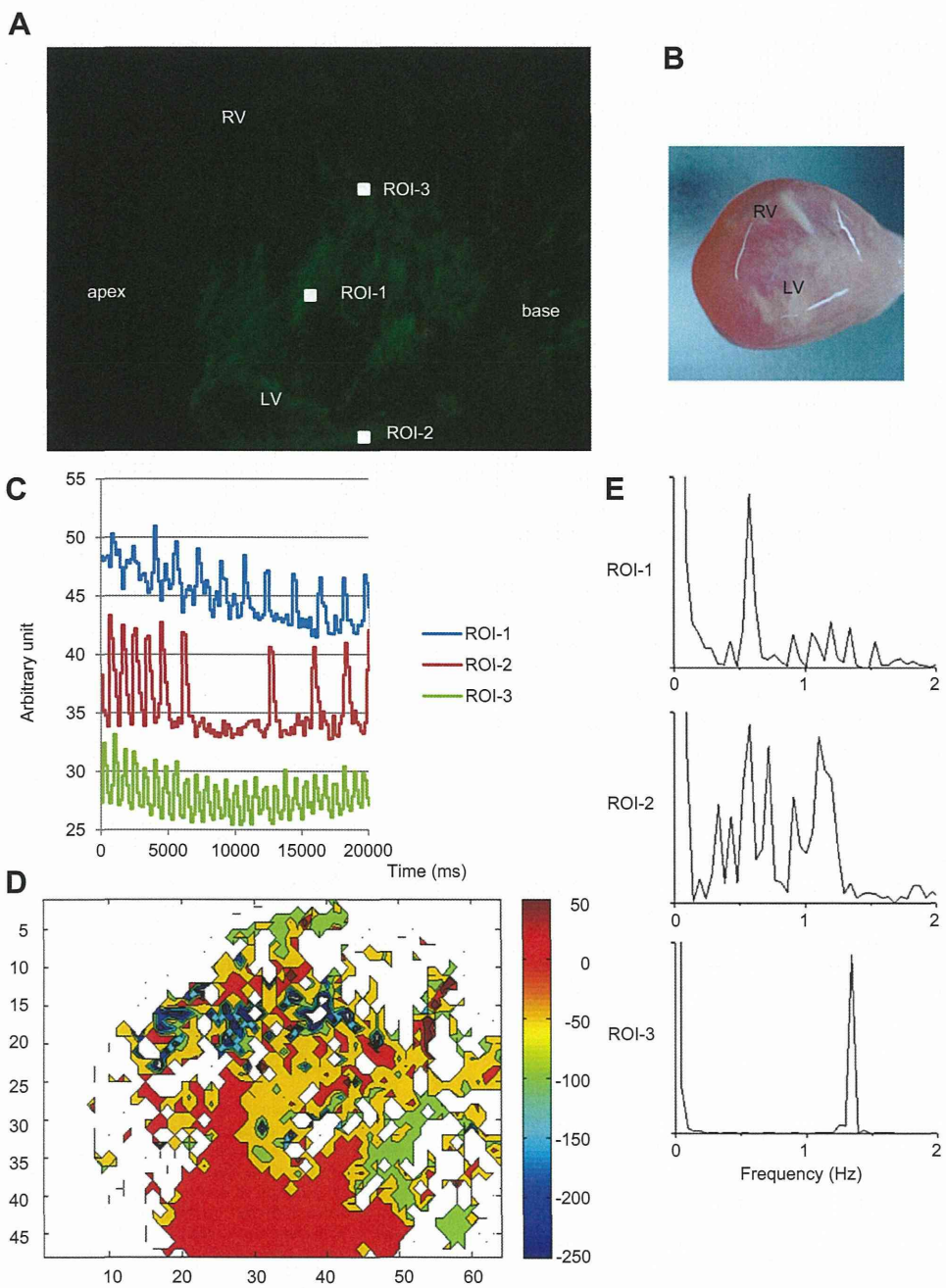

Fig. 10. Propagation of recellularized heart tissues ('disorganized' group). (A) Detection of the intracellular CaT driven by local propagation of electrical activity (ROI-1, -2, and -3) in the sample 3. (B) Anatomical features of the recellularized heart from a frame of video file. The heart sample is shown in the same direction with panels A and D. (C) Intracellular CaT at each site. We recorded disorganized propagation of asynchronous excitation with multiple origins, indicating automaticity. (D) Isochrone map of the propagating CaT. Note that left and right sides of the heart showed distinct automaticity and that conduction between these distinct sites was hardly observed. (E) FFT analysis. Note that each site showed different frequency pattern.

form capillary blood vessels. Cross-talk between endothelial cells and cardiomyocytes regulates not only early cardiac development but also adult cardiomyocyte contraction [25]. Also, it has been reported that the inductive signaling from endothelial cells plays an important role in cardiac conduction system development [26]. The co-existence of endothelial cells and cardiomyocytes may be compatible with this endothelial–cardiomyocyte interaction in the native heart. The density of laminin was high around the resident cardiomyocytes. The finding suggests that cardiomyocytes may produce laminin autonomously in addition to the natural matrix and adapt themselves at the resided space in the matrix. Supplementation with additional extracellular matrices could be possible experiments to attempt.

Extraction of the cadaveric heart *per se* could be potential controversies due to the notion on the death. For example, fabrication of scaffolds analogous to biological tissues using advanced technology such as 3D bioprinting technique may provide clues to solve the issue [27]. The technique has already been applied in various biological tissues including heart valves [28,29] and cartilage [30]. In this regard, 3D bioprinting could be a possible arsenal for heart regeneration in the future, however, arrhythmogenic evaluation as a whole organ will be still essential to create a sufficiently functional heart.

5. Conclusion

We engineered 3D hearts by recellularizing adult rat decellularized hearts with neonatal rat cardiac cells and observed excitation-propagation of spontaneous beatings of the recellularized heart tissues as an "organ". We observed disorganized asynchronous excitation arising from multiple origins while the engineered 3D heart tissues generally showed well-organized stable conduction. Although previous studies have shown the electrophysiological characteristics of 3D engineered heart tissues, those were only limited to the small area of the heart. In the present study, we clearly presented excitation and propagation properties of the entire heart tissues as an "organ". To construct functionally comparable and transplantable artificial heart, our strategy may be beneficial in the evaluation of the arrhythmogenic propensity, and it is necessary to establish the technique to engineer 3D heart tissues with stable excitation-propagation propensity as an organ.

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References

[1] Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012;380:2095–128.
[2] Pfister O, Della Verde G, Liao R, Kuster GM. Regenerative therapy for cardiovascular disease. Transl Res 2014;163:307–20.
[3] Ott HC, Matthiesen TS, Goh S-K, Black LD, Kren SM, Netoff TI, et al. Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart. Nat Med 2008;14:213–21.
[4] Ott HC, Clippinger B, Conrad C, Schuetz C, Pomerantseva I, Ikonomou L, et al. Regeneration and orthotopic transplantation of a bioartificial lung. Nat Med 2010;16:927–33.
[5] Petersen TH, Calle EA, Zhao L, Lee EJ, Gui L, Raredon MB, et al. Tissue-engineered lungs for in vivo implantation. Science 2010;329:538–41.
[6] Song JJ, Guyette JP, Gilpin SE, Gonzalez G, Vacanti JP, Ott HC. Regeneration and experimental orthotopic transplantation of a bioengineered kidney. Nat Med 2013;19:646–51.
[7] Nakayama KH, Batchelder CA, Lee CI, Tarantal AF. Decellularized rhesus monkey kidney as a three-dimensional scaffold for renal tissue engineering. Tissue Eng Part A 2010;16:2207–16.
[8] Uygun BE, Soto-Gutierrez A, Yagi H, Izamis M-L, Guzzardi M a, Shulman C, et al. Organ reengineering through development of a transplantable recellularized liver graft using decellularized liver matrix. Nat Med 2010;16:814–20.
[9] Macchiarini P, Jungebluth P, Go T, Asnaghi MA, Rees LE, Cogan TA, et al. Clinical transplantation of a tissue-engineered airway. Lancet 2008;372:2023–30.
[10] Cebotari S, Tudorache I, Ciubotaru A, Boethig D, Sarikouch S, Goerler A, et al. Use of fresh decellularized allografts for pulmonary valve replacement may reduce the reoperation rate in children and young adults: early report. Circulation 2011;124:S115–23.
[11] Lu T-Y, Lin B, Kim J, Sullivan M, Tobita K, Salama G, et al. Repopulation of decellularized mouse heart with human induced pluripotent stem cell-derived cardiovascular progenitor cells. Nat Commun 2013;4:2307.
[12] Zhang D, Shadrin IY, Lam J, Xian H-Q, Snodgrass HR, Bursac N. Tissue-engineered cardiac patch for advanced functional maturation of human ESC-derived cardiomyocytes. Biomaterials 2013;34:5813–20.
[13] Shimizu T, Yamato M, Isoi Y, Akutsu T, Setomaru T, Abe K, et al. Fabrication of pulsatile cardiac tissue grafts using a novel 3-dimensional cell sheet manipulation technique and temperature-responsive cell culture surfaces. Circ Res 2002;90:e40.
[14] Radisic M, Malda J, Epping E, Geng W, Langer R, Vunjak-Novakovic G. Oxygen gradients correlate with cell density and cell viability in engineered cardiac tissue. Biotechnol Bioeng 2006;93:332–43.
[15] Radisic M, Euloth M, Yang L, Langer R, Freed LE, Vunjak-Novakovic G. High-density seeding of myocyte cells for cardiac tissue engineering. Biotechnol Bioeng 2003;82:403–14.
[16] Sekine H, Shimizu T, Sakaguchi K, Dobashi I, Wada M, Yamato M, et al. In vitro fabrication of functional three-dimensional tissues with perfusable blood vessels. Nat Commun 2013;4:1399.
[17] Zimmermann W-H, Schneiderbanger K, Schubert P, Didie M, Munzel F, Heubach JF, et al. Tissue engineering of a differentiated cardiac muscle construct. Circ Res 2002;90:223–30.
[18] Zimmermann W-H, Melnychenko I, Wasmeier G, Didié M, Naito H, Nixdorff U, et al. Engineered heart tissue grafts improve systolic and diastolic function in infarcted rat hearts. Nat Med 2006;12:452–8.
[19] Ng SLJ, Narayanan K, Gao S, Wan ACA. Lineage restricted progenitors for the repopulation of decellularized heart. Biomaterials 2011;32:7571–80.
[20] Song JJ, Ott HC. Organ engineering based on decellularized matrix scaffolds. Trends Mol Med 2011;17:424–32.
[21] Kadota S, Minami I, Morone N, Heuser JE, Agladze K, Nakatsuji N. Development of a reentrant arrhythmia model in human pluripotent stem cell-derived cardiac cell sheets. Eur Heart J 2013;34:1147–56.
[22] Parikh A, Patel D, McTiernan CF, Xiang W, Haney J, Yang L, et al. Relaxin suppresses atrial fibrillation by reversing fibrosis and myocyte hypertrophy and increasing conduction velocity and sodium current in spontaneously hypertensive rat hearts. Circ Res 2013;113:313–21.
[23] Hardziyenka M, Campian ME, Verkerk AO, Surie S, van Ginneken ACG, Hakim S, et al. Electrophysiologic remodeling of the left ventricle in pressure overload-induced right ventricular failure. J Am Coll Cardiol 2012;59:2193–202.
[24] Kizana E, Chang CY, Cingolani E, Ramirez-Correa G a, Sekar RB, Abraham MR, et al. Gene transfer of connexin43 mutants attenuates coupling in cardiomyocytes: novel basis for modulation of cardiac conduction by gene therapy. Circ Res 2007;100:1597–604.
[25] Hsieh PCH, Davis ME, Lisowski LK, Lee RT. Endothelial-cardiomyocyte interactions in cardiac development and repair. Annu Rev Physiol 2006;68:51–66.
[26] Tian Y, Morrisey EE. Importance of myocyte-nonmyocyte interactions in cardiac development and disease. Circ Res 2012;110:1023–34.
[27] Derby B. Printing and prototyping of tissues and scaffolds. Science 2012;338:921–6.
[28] Duan B, Kapetanovic E, Hockaday L a, Butcher JT. Three-dimensional printed trileaflet valve conduits using biological hydrogels and human valve interstitial cells. Acta Biomater 2014;10:1836–46.
[29] Hockaday L a, Kang KH, Colangelo NW, Cheung PYC, Duan B, Malone E, et al. Rapid 3D printing of anatomically accurate and mechanically heterogeneous aortic valve hydrogel scaffolds. Biofabrication 2012;4:035005.
[30] Cui X, Breitenkamp K, Finn MG, Lotz M, D'Lima DD. Direct human cartilage repair using three-dimensional bioprinting technology. Tissue Eng Part A 2012;18:1304–12.
[31] Tallini YN, Ohkura M, Choi BR, Ji G, Imoto K, Doran R, et al. Imaging cellular signals in the heart in vivo: cardiac expression of the high-signal Ca^{2+} indicator GCaMP2. Proc Natl Acad Sci U S A 2006;103(12):4753–8.

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From bench to bedside, work in cell-based myocardial regeneration therapy

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ABSTRACT

In clinical cellular cardiomyoplasty, bone marrow cells and myoblasts are introduced mainly to ischemic cardiomyopathy tissue via several cell delivery systems, such as needle injection or catheter. These clinical studies have demonstrated the safety and feasibility of this technique, but its effectiveness for treating heart failure, especially in the long term, is still under discussion. Neither of these cell types can differentiate into cardiomyocytes; rather, they improve the failing heart mainly by the paracrine effects of some cytokines, such as Hepatocyte growth factor (HGF) and Vascular endothelial growth factor (VEGF). Thus, many researchers have a great interest in stem cells, which exist in bone marrow, circulating blood, atrium, and adipose tissue, and can differentiate into cardiomyocytes. Although several stem cells with the potential to differentiate into various cell types have been reported, few can differentiate into cardiomyocytes. Moreover, beating cells that can demonstrate synchronized contraction with native cardiomyocytes are critical for the complete repair of severe heart failure. Therefore, stem cells with a high differentiation capacity should be explored for the goal of completely repairing severely damaged myocardium. In this review, we summarize the clinical protocols and basic experiments for cellular cardiomyoplasty using bone marrow cells, myoblasts, and other stem cells.

KEYWORDS

Stem Cells; Heart Failure; Cell Therapy

1. INTRODUCTION

To overcome heart failure many basical studies have been done and some technologies have been introduced to the treatment of heart failure clinically based on the experimental data.

Although cell therapy was recently introduced to clinical situation in heart failure, tremendous experimental studies (Bench work) have been done before clinical trials (Bed side work). In this review we present and analyze recent achievements in the laboratory and clinic in cellular cardiomyoplasty.

2. CELLULAR CARDIOMYOPLASTY

The adult heart has no regenerative ability to repair damaged myocardium by itself. Cellular cardiomyoplasty was introduced to compensate for this lack of regenerative ability by delivering viable cells to distressed myocardium that has almost no functional cells (**Figure 1**). To develop this attractive method, many kinds of cells were implanted into small animal or large animal models through various routes. Based on the results of these experiments, several clinical trials have already been performed, and revealed the feasibility and efficacy of this technique. Although the mechanisms for the functional improvement after cellular cardiomyoplasty have not been completely elucidated, most researchers believe that its efficacy mainly depends on the paracrine effect of cytokines, because the incidence of transplanted cell differentiation to cardiomyocytes is low, there is no contractile function in most of the implanted cells [1], and the implanted cells represent only a small fraction of the LV mass [2].

2.1. Skeletal Myoblasts

2.1.1. Experimental Achievements

Unlike heart muscle, skeletal muscle has its own regenerative system. As soon as skeletal fibers are injured, skeletal myoblasts under the basal membrane of the skeletal fibers are mobilized and fuse with neighboring

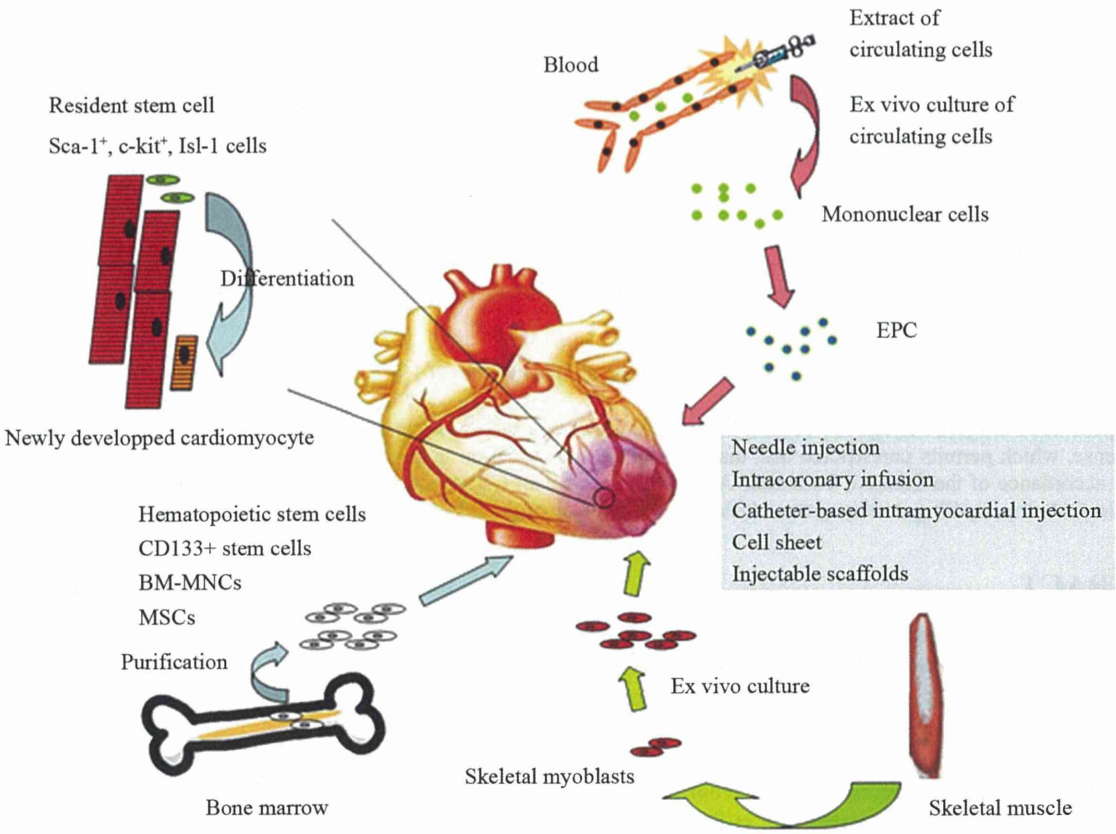

Figure 1. Cell sources for myocardial regeneration. Current clinical trials include the transplantation of skeletal myoblasts, bone marrow mononuclear cells, mesenchymal stem cells, or circulating progenitor cells. Cells used in experimental studies are resident stem cells and hematopoietic stem cells. There are various kinds of cell delivery routes, such as needle injection, intracoronary infusion, catheter-based intramyocardial injection, and cell sheet.

myoblasts, leading to regenerated, functional skeletal muscle. To exploit their self-regenerative capacity, myoblasts were implanted into the distressed myocardium, which has no regenerative system. The viability of the transferred myoblasts and their affinity for the myocardium were studied, and many experiments on the cell survival, differentiation to cardiomyocytes, and electrical coupling with recipient myocytes were performed to examine the effectiveness of myoblast implantation.

Implanted myoblasts engrafted to cryoinjured dog myocardium [3,4] prevented LV remodeling and improved cardiac performance [5,6]. The implanted myoblasts did not transdifferentiate into cardiomyocytes, showing a mature skeletal muscle phenotype [7]. Mature skeletal muscle grafts in the distressed myocardium had no connexin43 or N-cadherin, indicating they did not undergo electrical coupling with the host myocardium *in vivo* [8]. However, a low incidence of myoblast fusion with cardiomyocytes was observed [9], and a small number of these fused cells showed connexin43 expression [10]. Suzuki *et al.* reported that connexin43-overexpressing myoblasts formed functional gap junctions, suggesting the potential for synchronous contraction with host myocytes [11]. However, implanted myoblasts isolated from the recipient myocardium could not contract synchronously with host cardiomyocytes [12]. Myoblasts are thought to be the best candidate for cardiomyogenesis in the clinical setting, because cardiomyocytes cannot be cultured for clinical use, and only myoblasts can differentiate to muscle. However, implanted myoblasts can be isolated from the host myocardium electrically *in vivo*, indicating that they do not differentiate, and the cardiomyogenesis in the failing heart is quite incomplete.

2.1.2. Clinical Achievements

Despite the lack of affinity of implanted myoblasts for the host myocardium, many papers have reported functional improvement in small animal and large animal models following myoblast transplantation, leading to clinical applications. This technique is attractive because of its high resistance to ischemia and use of autologous cells [13], even though the mechanisms underlying its effects are still unclear.

Several groups reported the clinical efficacy of myoblast transplantation through needle injection [14-18] or catheter [19] (**Table 1**). These clinical studies indicated

Table 1. Clinical trials of myoblast transplantation. HD, high dose; LD, low dose; OMI, old myocardial infarction; TR, treatment; CABG, coronary artery bypass grafting; LVAD, left ventricular assist device; F.U. (mo), follow up (months); IM, intramuscular; TE, transendocardial; EF, ejection fraction; ESV, end systolic volume; EDV, end diastolic volume; PET, positron emission tomography; VT, ventricular tachycardia.

Study	Study design	Concomitant TR	Number of cells	F.U.(mo)	Route	Results	Adverse effects
Menasche, 2003	No control	CABG	8.7×10^8	10.9	IM	Symptom↓, EF↑(8%)	VT(4/10)
n = 10	OMI		(86% myoblast)			Systolic thickening↑(14/22)	
Herreros, 2003	No control	CABG	2.2×10^8	3	IM	EF↑(18%), Regional contractility↑	No
n = 12	OMI		(65.6% myoblast)			Regional Viability↑(PET),	
Smits, 2003	No control	~	2.0×10^8	6	TE	EF↑(9%)	VT(1/5)
n = 5			(55% myoblast)			Wall thickening ↑at target area	
Pagani, 2003	No control	LVAD	3.0×10^8	68 - 191 days	IM	Detection of matured myofiber	VT(3/5)
n = 5	OMI		(43% - 97% myoblast)			small vessel formation↑	
Ince, 2004	Controlled, n = 6	~	2.1×10^8	12	TE	EF↑(7.9%), Regional contractility↑	No
n = 6	OMI		(70% myoblast)			Symptom↓, EF↑(8%)	
Siminiak, 2004	No control	CABG	$0.04 - 5.0 \times 10^7$	12	IM	EF↑(6.8%)	VT(2/10)
n = 10	OMI		(65.4% myoblast)				
Dib, 2005	No control	CABG(n = 24)	$0.1 - 3.0 \times 10^8$	11 - 45	IM	EF↑(8%, 2 years), EDV, ESV↓	VT(3/30)
n = 30	Non randamised	LVAD(n = 6)	(79% myoblast)			Detection of skeletal myofiber	
	multicenter, OMI						
Siminiak, 2005	No control	~	$0.17 - 1.1 \times 10^8$	6	Transvenous	EF↑(6/9)	No
n = 10	OMI				approach		
Gavira, 2006	Controlled, n = 14	CABG	2.2×10^8	12	IM	EF↑(19.5%), Regional contactility↑	No
n = 12	OMI		(65.6% myoblast)			Myocardial viability, perfusion↑(PET)	
Hagege, 2006	No control	CABG	8.7×10^8	49.4	IM	EF↑(1mo, 6.7%), Symptom↓	VT(5/9)
n = 9	OMI		(86.6% myoblast)			EF→(long term follow up)	
Menasche, 2008	Randmised	CABG	HD; 8×10^8	6	IM	EF→, Regional wall motion→	VT(12%; LD, 17%; HD)
n = 33(HD)	Placebo-Controlled, n = 30		LD; 4×10^8			ESV↓(HD)	
n = 34(LD)	Double blind						
	Multicenter						

its feasibility by establishing human myoblast cell culture methods in Good Manufacturing Practice-approved facilities and procedures for injecting cells into the human myocardium [13]. Two papers successfully identified mature myofibers in the human myocardium after transplantation [17,20], but its efficacy needs to be further investigated because of the absence of control groups and concomitant coronary bypass surgery in many studies. Although the incidence of ventricular tachycardia after engrafting was high, occurring in 4 out of 10 patients [15] and 1 out of 5 patients [19], a causal relationship between the myoblasts and ventricular tachycardia remains unknown. To evaluate its efficacy and arrhythmogenicity, Menasche *et al.* performed a multicenter, randomized, placebo-controlled, double-blind study and revealed a high incidence of early postoperative arrhythmic events and prevention of LV remodeling with a high-dose cell injection, although no improvement in global systolic function was seen [21].

Recently myoblast sheet implantation technique in human DCM and ICM has already been introduced to clinical application. However, to prove its therapeutic effectiveness, a multicenter, randomized, placebo-controlled, double-blind study should be performed.

2.2. Bone Marrow Cells

2.2.1. Experimental Achievements

Progenitor cells within bone marrow cells (BMCs) play a large role in the regeneration of damaged skeletal muscle [22], and this exciting finding encouraged researchers to explore the regeneration ability of BMCs in the failing myocardium. The potential ability of BMCs to differentiate to cardiomyocytes *in vivo* has been shown in several reports. Orlic *et al.* sorted lineage-negative (Lin-) and c-kit+ BMCs by fluorescence-activated cell sorting from mice constitutively expressing enhanced green fluorescent protein (EGFP), and transplanted these cells into an infarcted area. Surprisingly, newly formed cardiomyocyte tissue was regenerated in approximately 68% of the infarcted area, and these transdifferentiated EGFP-positive cardiomyocytes expressed cardiomyocyte-specific markers, such as cardiac myosin and several transcription factors, and improved cardiac performance [23,24]. This discovery supported the idea of myocardial regeneration by multipotential BM stem cells. The work of Jackson *et al.* suggested that hematopoietic stem cells (HS cells; a component of BM stem cells that is CD34+, the so-called side population cells) can differentiate into cardiomyocytes. These researchers implanted HS cells from Rosa26 mice that constitutively expressed beta-galactosidase into lethally irradiated mice and made an infarction model of these mice. The transplanted beta-galactosidase-positive HS cells migrated to the periinfarcted region, and were identified as newly differentiated cardiomyocytes at a prevalence of approximately 0.02%, and as endothelial cells at 3.3%, of all myocytes [25]. Although this report is exciting, the incidence of differentiation to cardiomyocytes was revealed to be quite low, unlike the above-mentioned study by Orlic *et al.* Another exciting study appeared to show that BMCs can differentiate into cardiomyocytes in human samples. Deb *et al.* examined female uninjured hearts from patients who had undergone male bone marrow transplantation, and detected approximately 0.23% Y chromosome-positive cardiomyocytes by the FISH method. This study also indicated that the transdifferentiation to cardiomyocytes was very rare. Several groups have since examined the reproducibility of BMC differentiation to cardiomyocytes, motivated by these promising results.

However, Murry *et al.* introduced a new system for determining cell transdifferentiation to cardiomyocytes using genetic methods without a histological detection system, and clearly showed that no HS cells differentiated into cardiomyocytes after their transplantation to infarcted myocardium [1]. Moreover, Balsam *et al.* failed to reproduce the transdifferentiation of Lin- and c-kit+ bone marrow cells to cardiomyocytes *in vivo* [26]. Several groups clearly revealed that BMCs can spontaneously fuse with other cells, such as liver cells [27], cardiomyocytes, and Purkinje neurons [28], and that the BMCs can obtain the phenotype of the cells with which they have fused [29]. This special ability of BMCs to undergo cell fusion can explain the apparent transdifferentiation of BMCs to cardiomyocytes [30]. In addition, Laflamme *et al.* pointed out that the apparent transdifferentiation to cardiomyocytes evaluated by histological detection systems was owing to misleading artifacts in confocal microscopy. in his review [31]. However, Kajstura *et al.* [32] and Yoon *et al.* [33] offered counterarguments to the lack of HS cell transdifferentiation to cardiomyocytes. Thus, there is presently no consensus about whether HS cells transdifferentiate to cardiomyocytes *in vivo*.

BMCs contain two components, HS cells and mesenchymal stem cells (CD 34−, present in the bone marrow stroma). Several reports revealed that mesenchymal stem cells (MSCs) can differentiate into cardiomyocytes *in vitro* under certain conditions [34-36], while the incidence of their transdifferentiation to cardiomyocytes *in vivo* is quite low [37,38]. In spite of the low incidence of differentiation to cardiomyocytes and small amount of newly developed cardiomyocyte tissue, MSC [39] implantation induces a marked improvement in cardiac function.

2.2.2. Clinical Achievements

In spite of the low evidence for BMC differentiation to cardiomyocytes and unclear mechanism, intracoronary

