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Mini Review

Strategies in cell therapy for cardiac regeneration

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Cardiac regenerative medicine is emerging as a new approach to treat severe cardiovascular diseases that are resistant to conventional therapies. To achieve fair engraftment and efficient outcome, the method of cell transplantation is important, as the efficacy of engraftment after simple needle injection is relatively poor. Using biomaterials (e.g. collagen, fibrin, gelatin or matrigel) as a scaffold of the transplanted cells is an effective method, and various attempts to control cell distribution for the creation of tissue-like structure have been made. In this regard, scaffold-free cell sheet technology using temperature-responsive culture surface is another promising method because it bears potential for generating three-dimensional tissue-like structure *in vitro*. Furthermore, the cell sheet system enables us to elucidate the cellular mechanisms for cardiac regeneration. Combination of cell therapy and sustained release of growth factors, such as basic fibroblast growth factor, is another valuable approach for cell engraftment and augmentation of the potential of cell transplantation. Herein, we review various engraftment strategies of the transplanted cells to achieve more efficient outcome in cardiac cell therapy. We expect that these advanced modalities with bioengineering technology would largely contribute to cardiac regenerative medicine.

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Introduction

Cardiovascular disease remains the leading cause of death worldwide. A boundary for conventional severe heart failure treatments exists in Japan due to the shortage of heart donors[1)]. This health problem has prompted research into new therapeutic approaches including cardiac regeneration[2)].

With the discovery of various stem cell populations possessing cardiogenic potential, and the subsequent ability to isolate and expand these cells, the notion of a restor-

ative therapy using stem cells has begun to take shape. Acute ischemic injury and chronic cardiomyopathies lead to permanent loss of cardiac tissue and following heart failure. For these pathologic conditions, cell transplantation is thought to be an ideal therapeutic method for replacing the lost myocardium[3,4], and stem cell research and clinical trials for cardiac cell therapy are now being prioritized[2,5].

In spite of the substantial knowledge gained through numerous basic research studies, significant barriers to true cardiac regeneration remain, and the field still lacks sufficiently conclusive results to support full-scale implementation of such therapies. A major reason for the inadequate results would be the poor engraftment of the transplanted cells. Results from these researches have reached the conclusion that stem cells may be beneficial in the treated hearts but act primarily through paracrine mechanisms, including angiogenesis, apoptosis prevention and promotion of healing, rather than through direct differentiation as initially expected[2]. The low level of grafted cell survival and engraftment diminishes their potential, and is a potent technical limitation for stem cell therapy[6]. It is reported that more than 70% of the cells die during the first 48 hours after needle injection, being progressively lost during the following days due to the hypoxic, inflammatory, and/or fibrotic environment[7]. Another report indicates that only 5.4 to 8.8% of microspheres directly injected into the beating myocardium remain just after the injection due to massive mechanical loss[8]. Thus, new strategies like combination of the cells with bioengineering techniques have been developed and are being subjected to intense research, suggesting that new strategies may improve the efficiency of stem cell therapies. In this review, we introduce transplantation technologies for effective engraftment of the transplants and attempts to increase the efficacy of cell transplantation. The approaches reviewed here are summarized in Table 1.

Table 1 Approaches to improve engraftment of transplanted cells

	References
● Scaffolding with biomaterials	
Cell injection with collagen / fibrin / gelatin / Matrigel	9)
Cellular patches using biomaterials	11), 13)
3D contractile cardiomyocyte-loops using collagen	12)
Organized construct as parallel channels	14)
● Scaffold-free approaches	
Cardiac tissue patches	15)
Cell sheets Temperature-responsive culture dishes Fibrin polymer coated culture dishes Magnetic force-based cell sheets	 16), 17), 18) 19) 20)
3D cardiosphere	24)
● Combinatory approaches with cell transplantation	
Sustained release of cytokines (bFGF etc.)	21), 22), 23)
Pericardium wrapping	25)
Omental wrapping	26), 27), 28)

3D: three-dimensional, bFGF: basic fibroblast growth factor

Biomaterials scaffolding the transplanted cells

Initial experiments were performed by combining the cells with injectable biomaterials such as collagen, fibrin, or gelatin. Matrigel or other factors providing a favorable environment rich in cytokines and growth factors were also tested. In general, these early studies showed an increased survival of the transplanted cells and a greater improvement of the cardiac function of the transplanted hearts[9]. However, these approaches did not assure complete cell retention or an adequate distribution of the grafted cells. Techniques, like the creation of cell sheets or patches as microtissues, are now being developed in order to allow, together with a greater cell survival, a more homogeneous and organized distribution of the cells[10].

The creation of cellular patches has been developed by using biomaterials which act as a delivery platform for the cells, assuring their engraftment and interaction with the tissue. With hydrogel/extracellular matrix (ECM)-based matrices, the cells are usually embedded in soluble hydrogels matrices that can condensate after temperature changes, forming a cellularized patch that can be applied to the heart pericardium. The creation of a patch with mesenchymal stem cells (MSCs) entrapped in a collagen-I matrix was reported, and the application to rat infarcted hearts induced an increase of cell engraftment and a functional improvement, due to the trophic effect of the MSC potentiated by increasing their survival in the tissue[11]. Three-dimensional (3D) contractile loops of mixed collagen and neonatal cardiomyocytes, a more-sophisticated approach, have been successfully created. Implantation of these loops could support the contractile function of the damaged heart[12]. On the other hand, porous biomaterials,

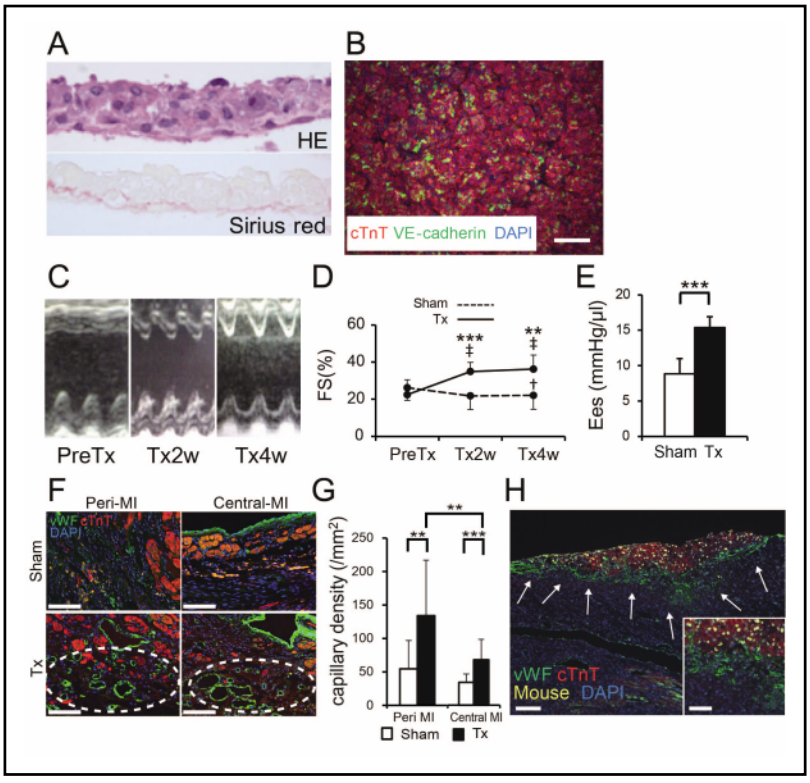

Fig.1 The improvement of infarcted heart function after transplantation of cardiac tissue sheets bioengineered with mouse ES cell-derived defined cardiac cell populations

(A): Cross-sections of the sheet. Upper panel: H&E staining showing cell appearance of the sheet. Lower panel: Sirius red staining showing intact extracellular matrix. (B): Immunostaining of sheets for cTnT (red), VE-cadherin (green), and DAPI. (C,D): Echocardiogram (n=9). (C): Representative M-mode image. Note that infarct anterior wall started to move 2-4 weeks after transplantation (Tx). (D): Fractional shortening (FS). (E): LV pressure-volume loop study 4 weeks after Tx (n=8). Ees: End-systolic elastance. (F,G): Capillary formation at Tx-d28. (F): Double staining for vWF (ECs, green) and cTnT (cardiomyocytes [CMs], red) at peri-MI and central-MI areas. Note that newly formed capillaries are clearly observed in transplantation group (dotted circles). (G): Quantification of capillary density (capillary number per square millimeter). Peri-MI area (left panel) and central-MI area (right panel) (15 views each). (H): Triple staining for vWF, cTnT, and species-specific fluorescent in situ hybridization (mouse nuclei, yellow) (Tx-d3). Most of the accumulated vWF-positive cells are negative for mouse nuclear staining (arrows). Inset: higher magnification view.

$^{**}p < 0.01$; and $^{***}p < 0.001$ (unpaired *t* test), $^{\dagger}p < 0.05$ and $^{\ddagger}p < 0.01$ (vs. PreTx, paired t test). PreTx; Pretransplantation, Tx2w, Tx4w; 2 and 4 weeks after transplantation, respectively. Scale bars: 200 μm in (B), 100 μm in (F) and (H) (main panel), 50 μm in (H) (inset). HE, Hematoxylin and Eosin; cTnT, cardiac troponin-T; DAPI, 4,6-diamidino-2-phenylindole; vWF, von Willebrandfactor; MI, myocardial infarction. (quote from ref. 18 with revision)

such as alginate or polymers like poly-glycolide-colactide, have also been tested as cell scaffolds. Application of cardiomyocytes derived from embryonic stem (ES) cells has been reported, where improvements in heart remodeling and function were observed after transplantation[13]. However, their use still presents some drawbacks such as the lack of control for a homogenous seeding and distribution of the cells. To solve this problem, new strategies like microtemplating or electrospinning have been incorporated in order to create scaffolds that mimic the natural heart extracellular matrix. A study has reported the creation of a poly(2-hydroxyethyl methacrylate-co-methacrylic acid) hydrogel construct organized as parallel channels that can direct an aligned cardiomyocyte distribution[14].

Cell sheet technology with temperature-responsive culture surface

Another promising approach for fair engraftment and construction of 3D tissue-like structure is the creation of cell sheets or patches without scaffold support. With this approach, inflammatory reactions against the biomaterials constituting the scaffolds would be avoided. Stevens et al. reported a transplantation experiment of scaffold-free and vascularized human cardiac muscle tissue patches including human embryonic stem cell (ESC)-derived cardiomyocytes and endothelial cells which were created by rotating orbital shaker-based cell culture[15].

The generation of cell sheets using two-dimensional cell culture is more promising because of larger scalability and accessibility. This technique has been made possible by using a culture dish covalently grafted with temperature-responsive polymer poly (N-isopropylacrylamide) (PIPAAm) which enables the preparation of cell sheets without enzymatic digestion[16]. The beneficial potential of this technique has been demonstrated by many experiments of stem cell therapy such as the transplantation of a monolayer of adipose tissue-derived MSCs to the infarcted rat heart[17]. Recently, we have reported a transplantation study of a three-

layered cardiac tissue sheet bioengineered with ESC-derived defined cardiac cell populations in the infarcted heart (Fig.1)[18]. In both cases, an increase in tissue neovascularization together with a positive attenuation of heart remodeling responsible for the improvement in cardiac function has been demonstrated. Furthermore, our report showed a cell sheet-based method for prospective elucidation of the cellular mechanisms of cardiac restoration. The combinations of cell types composing the transplanted cell sheets enabled us to elucidate the regenerative function of each cell type (for example, the comparison of cell sheets with or without cardiomyocytes is helpful for the elucidation of the cellular function of cardiomyocytes). This cell-type controlled analysis led us to identify one of the cellular mechanisms of cardiac restoration following cell therapy, namely, that cardiomyocytes are essential for the functional improvement through neovascularization (Fig.2). These results indicate that the tissue-like cell sheet system is useful for the elucidation of cardiac regenerative mechanism as well as for therapeutic purposes. The cell sheet transplantation would be one of the best approaches for cardiac restorative therapy, at least for sub-acute myocardial infarction which might be restored through potent paracrine effects.

Another promising technology for collecting scaffold-free cell sheets is reported using fibrin polymer coated culture dishes. The fabricated cell sheets from neonatal rat cardiomyocytes were transplanted onto the heat-injured rats, and demonstrated successful electrical integration between host heart and cell sheet postoperatively[19]. A novel magnetic force-based cell sheet technology was also developed, and human MSC-derived cell sheet recently showed therapeutic effects for mouse hind limb ischemia[20].

Combination of cell therapy and sustained release of growth factors

The beneficial effects of cell therapy must be further advanced before this therapy attains its full potential. The combination of cell therapy and local protein administration which induces paracrine mechanisms, such as angiogenesis, is one direction for the enhancement of its therapeutic potential. Tabata et al. have developed a sustained release system of angiogenic cytokines, such as basic fibroblast growth factor (bFGF), with biodegradable material, gelatin hydrogel, which enables us to control the release of cytokines during required periods for efficient clinical outcome[21]. The sustained release system of bFGF was applied together with the transplantation of cardiosphere-derived cardiac progenitor cells for porcine MI model with the enhanced functional benefit[22], and with the ongoing clinical trial, ALCADIA[23]. Thus, the drug delivery system using biodegradable biomaterials would be a promising strategy for the advances of cardiac regeneration with cell therapy.

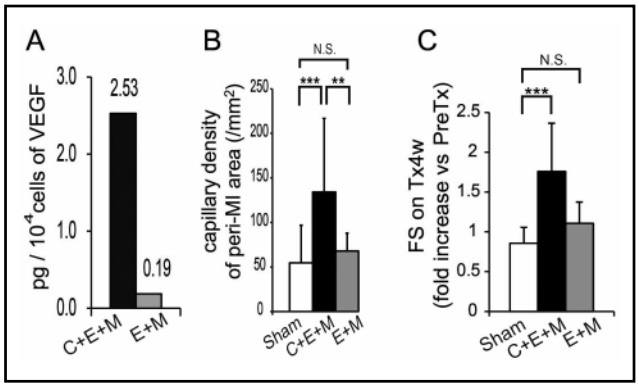

Fig.2 Cell type-controlled sheet analyses

(A): ELISA for VEGF secretion (picogram per10^4 cells) in culture supernatants of C+E+M and E+M sheets. (B,C): Transplantation of sham operation (n=9) versus C+E+M sheets (n=9) versus E+M sheets (n=3) (Tx-d28). (B): Capillary density in peri-MI area (capillary number per square millimeter). (15 views each). (C): Fractional shortening (FS) on echocardiogram (fold increase vs. PreTx). $**p < 0.01$, and $***p < 0.001$ (unpaired *t* test). C: cardiomyocytes, E: endothelial cells, M: vascular mural cells. N.S., not significant; VEGF, vascular endothelial cell growth factor. (quote from ref. 18 with revision).

Other approaches

It has been recently shown that transplantation of in-vitro created 3D cardiospheres improves engraftment of the cardiac progenitors and the in vivo differentiation towards cardiac and vascular cells[24]. Furthermore, the use of decellularized tissues as scaffold for cell transplantation has also been explored. Different tissues such as the bovine pericardium[25] and omental wrapping[26, 27] have been also used as a support for different cell types like the mesenchymal cells, with the purpose to improve their paracrine effect. Suzuki et al. reported that the concomitant omental wrapping with myocardial cell sheet transplantation for rat myocardial infarction model enhanced the effects of cell transplantation mainly due to promoted neovascularization[28]. However, hurdles still remain for achieving cardiac cell sources with no immunological risk, and for creat-

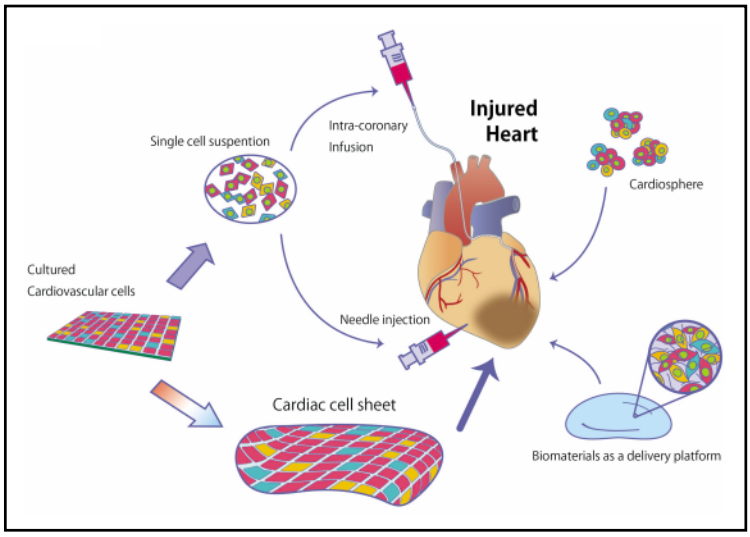

Fig.3 Various methods for cardiac cell transplantation

Efficient cell delivery is essential to maximize the therapeutic potential of cell transplantation.

ing patches/organs that can mimic the structure and function of the heart.

Conclusion

In this review, we have introduced various strategies for fair engraftment of the transplanted cells after cardiac cell therapy to achieve more efficient outcome (Fig.3). We sincerely expect that these advanced modalities with bioengineering technologies would largely enhance the efficacy of cardiac cell therapy and further contribute to cardiac regenerative medicine.

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Disclosure of potential conflicts of interest

None

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