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REVIEW ARTICLE

Review: Role of Tissue Engineering in Cellular Therapies for Myocardial Infarction

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Yuan-Kun (Aden) Wu[a] and Jiashing Yu[b]

Current medical treatments of myocardial infarction (MI) face a serious shortcoming in that they cannot reverse the detrimental effects of ischemia induced necrosis. In searching for novel solutions to this medical problem, great focus has been placed on cardiac tissue engineering. Recently much progress has been made using cellular approaches, with multiple studies undergoing clinical trials. Non-cellular approaches to constructing engineered cardiac tissue have also achieved some major breakthroughs, although drawbacks remain. In this review article, an update on the progress of non-cellular approach is discussed with major focus on the two main scaffold types: implantable cardiac patches and injectable cardiac hydrogels. The design properties, cell sources, and material properties are briefly described. will be discussed.[11]

Introduction

Myocardial infarction (MI) is defined as the death or necrosis (usually coagulative) of myocardial tissue. With its limited repair capabilities, myocardial tissue is incapable of reversing ischemia or post-reperfusion damage after surpassing a critical threshold for an extended period of time, which results in apoptosis of cardiac tissues. The implications and severity of MI and its related sequelae have been well emphasized in many cardiac tissue engineering works.[1-4] Upon infarction, myocardial tissue soon develops necrosis due to a critical imbalance between oxygen supply and demand of the myocardium, which leads to the loss of contractility and conduction functions of cardiac tissue. While several studies have shown the presence of endogenous cardiac stem cell populations,[6-8] their limited innate regeneration ability cannot, however, efficiently replace damaged myocardial tissue mass that has undergone severe fibrotic changes with non-contractile scar tissue formation. In concurrent medical practices, insertion of pacemakers, surgeries, and medicinal control of the post-MI patient have shown limited regenerative effects, and the results worsen with poor patient compliance.[9, 10]

In this review, we will focus mainly on the engineering of cardiac tissue. We will first describe the design criteria for biomaterials in cardiac tissue engineering, and then the cell source of engineered cardiac tissue as well as the two main non-cellular approaches towards cardiac tissue regeneration, implantable cardiac patches and injectable cardiac hydrogels,

[a] No. 1, Section 1, Ren'ai Road, Zhongzheng District, Taipei City, 100
[b] No. 1, Section 4, Roosevelt Rd, Da'an District, Taipei City, 10617
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Design criteria for biomaterials in cardiac tissue engineering

In addition to the influence of the ECM microenvironment on cardiac stem cells, as well as the regular harvested cardiac cells, the choice of material may also greatly affect the outcome of transplantation. Another important key consideration in cardiac tissue engineering design is the application of the resultant product. Material and product design therefore varies greatly, depending on whether the engineered tissue is a scaffold for cell delivery, a functional material implanted to maintain normal ventricular geometry, or a vehicle to deliver cells. Despite the diversity of the criteria that needs to be met, there are some fundamental design criteria that must be satisfied[4, 12-15]: biocompatibility, biodegradability, and mechanical support. All of which will be briefly discussed in the following.[5, 12-15]

Biocompatibility

As in the case of other transplantable devices (prosthetics) or tissues (bone marrow transplants and skin grafts), the biocompatibility of biomaterials or engineered tissues is the ability of the product to perform with an appropriate host response.[16] In the case of engineered cardiac tissue, two main factors require much scrutiny when designing these transplantables: immunogenicity and coagulability.[4, 12, 14, 17] The host immune response must be minimized, that is, both humoral and cellular immune reactions (although mitigation is almost impossible unless under total immunosuppression). Regarding coagulability, any formation of blood clots which may cause proximal or distal tissue infarction should also be minimized; dislodgement of engineered cardiac tissue is clearly a contraindication. However, in some cases, directed

immunogenicity or cellular recruitment of engineered tissue may benefit the healing process of infarcted tissue, such as the recruitment of stem cells through bi-specific antibodies (BiAb) or increased accumulation of reparative M2 macrophages over the cytotoxic M1 macrophages.[18-20]

Biodegradability

Implanted materials are broken down in vivo via various mechanisms, including bioerosion, bioresorption, and biodegradation. Bioerosion is mediated by hydrolytic mechanisms; bioresorption is the degradation of a material through cellular activity; biodegradation is degradation through enzymatic activity. Biodegradability is therefore the susceptibility of the implanted material to breakdown.[4, 12, 14, 15, 21] Although biocompatibility and biodegradability are distinct concepts, it is of utmost importance to consider both simultaneously, not only of the designed material, but also of the degraded products. Biodegradability of engineered cardiac tissue gives the advantage of minimizing host immune response (as discussed under biocompatibility), as opposed to non-degradable tissues.[22] Both naturally and synthetically derived materials have been utilized in engineered cardiac design.

In organs as terminally differentiated as a heart, each tissue is uniquely optimized for its specific organ system, and offers innate biocompatibility. Naturally derived tissue therefore offers the optimum composition that enhances the ECM microenvironment for various cell types.[2] One important source of naturally derived material is decellularized ECM of animal tissues. Biomaterials obtained from process of decellularization readily fit both biocompatibility and biodegradability criteria (e.g. collagen, chitosan, and gelatin), as they contain the required molecular components for the acclimation of different cell sources (especially in the case of stem cells)[23]. Not only can the molecular properties be easily reproduced, but mechanical properties can also be adjusted to meet different demands for various designs.[24, 25] However, despite the compatibility of these naturally derived materials, surface antigens from various sources may generate antigenicity.

Synthetic biomaterials in engineered cardiac tissue consist primarily of polymers. Durability and strength are the main benefits of synthetic materials, although their biocompatibility issues may cause complications (as mentioned above). One of the major concerns with synthetic materials is the release of toxic byproducts into the bloodstream upon degradation. Therefore chemically inert materials serve as the primary sources for this type of biomaterials. Common synthetic materials include self-assembling peptides,[26] polyethylene glycol (PEG),[27] polyethylene terephthalate (PET),[2] and polyurethane (PU).[2]

Mechanical properties

The mechanical properties of the biomaterials contribute to two functions: withstanding the mechanical demands of the designed engineered cardiac tissue, and altering the phenotypical presentation of the seeded cells in the scaffolds.[28, 29] Mechanical demands depend on the ultimate envisioned application. For example, human myocardium ranges in stiffness (Young's Modulus) from 22 kPa at the end of the diastole up to 500 kPa at the end of the systole,[30] while the stiffness of rat myocardium ranges from 0.1 to 140 kPa.[13-15] Engineered cardiac tissue that is designed to thicken the ventricle wall and maintain ventricular geometry should utilize biomaterials with a stiffness in the high end of the range, whereas a design that will be injected and provide a temporary matrix for cell transplantation requires a stiffness in the lower end of the range. Surgical glues have been developed to increase the strength of junctions between the engineered cardiac tissue and the original heart tissue, increasing the success of full thickness repair.[31]

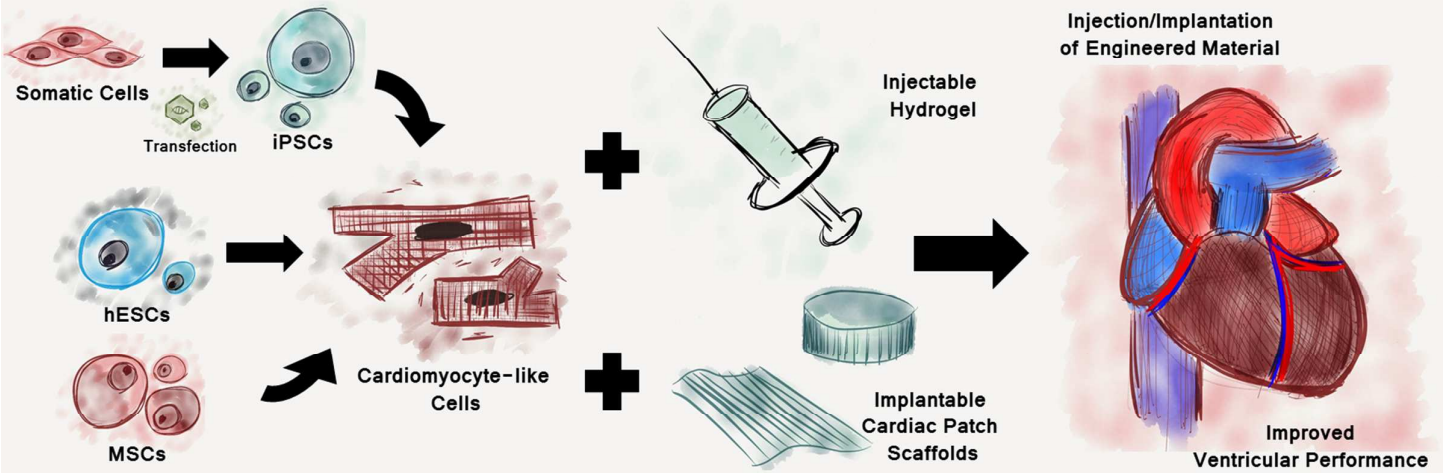
Phenotypical presentation of cells seeded in the scaffolds/engineered tissue varies greatly with different mechanical properties. For example, extracellular stiffness close to that of native myocardium (10 kPa) significantly enhances their maturation as reflected by aligned sarcomeres, greater mechanical force (examined using traction force microscopy), and the largest calcium transients and sarco/endoplasmic reticulum calcium-ATPase 2a expression.[29]

Naturally derived materials have batch-to-batch and source-to-source variability, while synthetic materials are more consistent between batches. Mechanical properties are therefore more easily managed and precisely controlled using synthetic materials.

Cell source of engineered cardiac tissue

Interestingly, while cardiomyocytes occupy most of the heart volume, it only account for 20-40% of the cell count.[28] Early approaches to engineered cardiac tissue focus mainly on purifying cardiac cells to obtain a pure harvest of cardiomyocytes. It is now an established concept that purified cardiomyocytes have limited ability to remodel their microenvironments, leading to inferior functional properties of the engineered cardiac tissue. Cardiac fibroblasts and endothelial cells are now included in engineered cardiac tissue to secrete ECM and form myocardial vasculature, respectively.[32, 33] Although one may witness how fibroblasts overgrow cardiomyocytes in traditional monolayer two-dimensional culture, they do not overgrow in three-dimensional scaffolds.[34] Several factors may influence such phenomena, including: presence of topography, appropriate mechanical properties of engineered cardiac tissue, or contact inhibition of a three-dimensional matrix. By providing a monolayer two-dimensional culture condition with intermediate stiffness of a rat cardiac tissue (22kPa and 50kPa), enhanced contractile properties and phenotypical presentation may be observed.[30]
Usually under laboratory investigations, neonatal or adult rat/mice cardiomyocytes are harvested without too much

Fig. 1 Overview of creation of injectable hydrogel/implantable scaffolds



difficulty. However, under clinical settings, finding suitable cell sources is one of the biggest challenges in tissue engineering. Sources with more potential for human cardiac tissue engineering include human embryonic stem cells (hESCs), human induced pluripotent stem cells (hiPSCs), and mesenchymal stem cells (MSC).

hESCs are usually induced to cardiomyocytes using medium-containing activin A and BMP4.[35] In numerous studies it has been found that hESCs do improve ventricular function in many small animal MI models.[36-39] In a recently published study by Chong et al., hESC derived stem cells were shown to remuscularize myocardium. Molecular features (calcium transient electrophysiological properties) were also shown to be normal. However, ventricular arrhythmias were observed several days after the injection. At the same time, the same group demonstrated that non-fatal ventricular arrhythmias were observed in smaller animal models (such as the mice, rats, and guinea pigs) using the same technique.[40]

hiPSCs can be induced from human somatic cells through viral transfection of transcription factors (either Oct4, Sox2, Klf4, and c-Myc or Oct4, Sox2, Nanog, and Lin[28]). Strong advantages of utilizing hiPSC include the nonexistence of ethical issues and autologous source of somatic cells is unlimited. Recent studies have shown that fibroblasts can be transformed directly into cardiomyocyte phenotypical cells by using three transcription factors (Gata4, Mef2c, and Tbx5).[41] Lepperhof et al. demonstrated the viability of iPSC-derived cardiomyocytes transplanted in syngeneic mice recipients.[42] iPSC-derived cardiomyocytes have also been transplanted in minipigs. However, relatively few human cardiomyocytes persisted, and the underlying functional improvements were probably due to paracrine effects of higher VEGF and bFGF levels released from the cell sheet.[43] It is important to note that hESC derived cardiomyocytes are allogenic and would give rise to immune response. Along with other limitations, iPSC and hESC cardiomyocytes have not been translated to clinical applications yet. Future prospects for iPSCs are highly promising.[44, 45]

MSCs have not been shown and lack the intrinsic ability to differentiate into beating cardiomyocytes. Improved functionality found in vivo is attributed to the paracrine factors that subsequently direct a number of restorative processes including myocardial protection, neovascularization, cardiac remodeling, and differentiation. [46, 47] Mesenchymal stem cells play a major role in some clinical trials.[48] Most of these focus on cell replacement through bone marrow mesenchymal stem cells,[49-51] mononuclear stem cells,[50, 52, 53] and cardiosphere-derived cardiac progenitor cells (CADUCEUS).[54]

Implantable cardiac patches

Implantable cardiac patches are designed according to the classical tissue engineering paradigm: cells and scaffolds are combined and cultivated in a bioreactor to reach desired degree of characteristics before implanting in target tissue (see Fig. 1).[3, 55] A general summary of the advantages and disadvantages of various types of implantable cardiac patches is provided in Table 1. The morphology and function of myocardium require precise imitation when designing engineered cardiac tissue replacements. In addition to the mechanical properties mentioned above, they should also have conductibility of electrical impulse propagation (around 25 cm/s). Typically, cell quantities depend on the animal model utilized[48]: 10^6 cells in a mouse MI model (0.15 g heart),[40, 56] 10^7 in rat models (1 g heart),[38, 39] 10^8 cells in guinea pig models (3 g heart),[57] and 10^9 for non-human primates (37-52 g heart).[40] Cardiac cells would then be anisotropically aligned (achieved by micro-fabrication, soft lithography, and patterning of synthetic materials) with a designed vasculature system of inter-capillary space not exceeding 20μm for sufficient diffusion.[58-62] Under these criteria, multiple groups have

Table 1: General summary of the advantages and disadvantages of various types of approaches to engineered cardiac patches. Properties of different subtypes may vary with types of materials and cells introduced.

	Advantages	Disadvantages
Implantable cardiac patches		
Synthetic scaffolds[4, 5, 45]	1 High versatility in design 2 More control over physical and mechanical properties of scaffold	1 Limited generated active force 2 Limited vascularization 3 Transportation of nutrients and wastes 4 Inevitable inflammatory response 5 Invasive implantation procedure
Cell regulated synthetic/ biopolymer[5, 33, 46, 55, 64-68]	1 High versatility in design 2 Maximized development of active force 3 Higher biocompatibility 4 Enhances phenotypic presentation of seeded cells	1 Less control over physical and mechanical properties 2 Limited vascularization 3 Transportation of nutrients and wastes 4 Inevitable inflammatory response 5 Invasive implantation procedure
Cardiomyocyte monolayer patches[56]	1 Less foreign body introduced 2 Transportation is efficient with only single layer of cells 3 Possibly less inflammatory response 4 High cell density and organization	1 Only cardiomyocyte introduced 2 Limited vascularization 3 Invasive implantation procedure 4 Challenging surgery introduction 5 Fragile patch
Injectable cardiac hydrogels[70-82]	1 High versatility in design (pre-injection or post-injection gelation) 2 Less invasive procedure and collateral damage 3 Relatively easier introduction of angiogenic molecules 4 Physical shape versatility to mod into the area of infarction	1 Less control over final transplanted material post injection 2 Less control over cellular concentration post injection

attempted to manipulate the microenvironment to facilitate cell assembly. Under the most ideal conditions, autologous cardiomyocytes and self-extracted ECM can be used to minimize the immune response upon implantation (as discussed above). Synthetic materials such as poly(glycolic acid)/poly-L-lactide, poly(glycerol sebacate) (PGS)[2, 63, 64] as well as natural materials (alginate,[65] collagen,[66] and chitosan[67]) are often used. The use of hydrogels has also been reported.[68]

Another design property that requires more attention, particularly for implantable cardiac patches, is the thickness of the scaffold design. Scaffolds need to be in the millimeter scale, that is, the biomaterial of choice must be capable of supporting the cultivation of tissue up to ~10mm for full thickness cardiac grafts.[58] However, the limitation of oxygen diffusion within a metabolically active tissue (under a cell density $\sim 10^8$ cells/cm^3 mentioned above) is around $200\mu m$. As a preforming vascular network is a prerequisite, sufficient nutrient exchange to the center of the scaffold has proven to be a major setback for transplantable cardiac patches, [69] as will be discussed later in this section.

It has already been demonstrated in the late 1990s that contractile engineered cardiac tissue can be created under laboratory settings and be utilized in rat MI models.[70, 71] In a study by Zimmermann and Eschenhagen, collagen type I was mixed with ECM and neonatal rat cardiomyocytes to form a circular lattice. By changing the medium content and providing continual mechanical stimulation, engineered cardiac tissue with spontaneous and synchronous contractions were generated after one to two weeks of culture. Interestingly, anisotropy of cardiomyocyte alignment was achieved by simply providing cyclic mechanical force.[70] Furthermore, this group later demonstrated that cardiac function can be improved through implantation of stacked engineered cardiac tissue. [72] This pioneering research set the foundation for the engineered cardiac tissue field.

With the increasing knowledge of the effects of micro-environment and mechanical properties, material, and cell source, engineered cardiac tissues can match various desired properties, which is a major advantage for implantable cardiac patches [4]. Although highly malleable, implantable cardiac patches with pre-formed porous or fibrous scaffolds may limit the generated active force of the seeded cells. [73] In response to this critique, hydrogels that were remodeled by cells were utilized to maximize the development of active force and used for implantable cardiac patch preparation. The design of cardiomyocyte monolayer patches was pioneered by Okano's group. Release of an undamaged monolayer of cardiomyocytes was made possible by seeding cardiac cells on poly(N-isopropylacrylamide)-grafted polystyrene dishes and then lowering the temperature to 20°C to induce a hydrophobic/hydrophilic switch of the surface. Transplantation of this cardiomyocyte monolayer was shown to improve ventricular function.[74] Other scaffold free approaches have recently emerged: human embryonic stem cells were cultured

in rotating orbital shakers, differentiated into cardiomyocyte (using activin A and BMP4), and aggregated into cellular discs;[75] cell sheets were harvested directly from myocardium, cultured, and stacked together before transplantation to allow free capillary growth within the graft tissue for sufficient perfusion.[76]

Synchronous contractility can be achieved by mechanical stimulation or suprathreshold electrical field stimulation with monophasic pulses. Utilizing suprathreshold electrical field stimulation may also result in the anisotropic alignment of cardiomyocytes in porous collagen scaffolds.[77, 78] The ideal electrode material was found to be carbon, with the highest charge-injection capacity and yielding cardiac tissue with the best structural characteristics and contractile force.[79]

One of the major challenges of implantable cardiac patches is the survival of cardiomyocytes and other cardiac cells in the tissue patch. Cell survival is highly dependent on the transport of nutrients and waste, particularly through the vasculature. Several strategies have been utilized to enhance this feature: changing the structure of the scaffold (especially scaffolds porosity or topography); incorporating angiogenic factors in the scaffold, and incorporating pre-existing vasculature into the engineered tissue constructs.[34, 80-82] Regarding structure, our study group has reported microbubbles produced using microfluidic techniques to generate a scaffold with adjustable pore sizes. Neonatal mice cardiomyocytes were shown to retain their phenotype for a prolonged period of time with molecular presentations matching those of *in vivo* cardiomyocytes.[34] Another well-established method is the usage of electrospun hydrogels or biopolymers to create scaffolds with patterns, which enhances the phenotype of seeded cardiomyocytes and allows higher flexibility in material choice and density at which fibers are stacked up (hence varying the mechanical properties). [5, 83] Scaffold designs have utilized angiogenic molecules such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) to improve angiogenesis. Chiu et al. have demonstrated increased endothelial proliferative effects of covalently linked VEGF and angiopoeitin-1 on scaffolds (using carbodiimide (EDC)). Upon implantation in the rat heart, scaffolds with covalently immobilized VEGF increase endothelial angiogenesis, which in turn improves the outcome of scaffold implantation and cardiac repair.[58, 84, 85] Another study demonstrated the effect of decellularized and processed coronary artery tissue flaps with patent vasculature, reseeded with retrograde aortic perfusion. [82] There is no obvious consensus on which method is the most effective for cardiac tissue engineering. However, in order to create clinically relevant thick tissue cardiac patches, the issue of perfusion needs to be addressed.[4]

Injectable cardiac hydrogels

Materials designed for direct injection into the myocardium lack the control over cell and biomaterial organization offered by implantable cardiac patches. However, injectable cardiac hydrogels can be delivered via catheter without the risk of surgery or general anesthesia. In fact, through catheter delivery, hydrogels may reach the subendocardial region, the layer in the myocardium that is most susceptible to ischemic damage, with the least collateral damage to the surrounding myocardium.[12] Injectable scaffolds are prepared in vitro by mixing liquid forms of the hydrogel with cells and biochemical factors. A general overview of the advantages and disadvantages of the injectable cardiac hydrogels is summarized in Table 1.

Injectable hydrogels, depending on their desired functions, can be classified into three categories.[4] As discussed above, hydrogels can be designed to 1) prevent adverse remodeling and recruitment of endogenous cells for repair and 2) act as a temporary matrix for cell transplantation and exogenous repair (as in the case of hESC and primate MI mentioned above); On the other hand, hydrogels can also be designed to 3) act as bulking material to support the failing ventricle and restore normal heart geometry to improve functional outcomes. All three categories are not exclusive of one another; in fact, most injectable cardiac hydrogels have overlapping features of more than one category.

Material choice has been based on the optimization of cell survival and remuscularization effect of injected cardiac scaffolds. Growth factor reduced Matrigel mixed with hESC induced cardiomyocyte was shown to remuscularize primate-MI model. Not only was the infarcted space regenerated with myocardium, but the vasculature was also shown to grow into the injected mass. The graft was shown to couple with the host's ventricular contraction, despite episodes of arrhythmias several days post-injection (another major challenge in the field of cardiac tissue engineering)[40]. Other materials such as fibrin glue,[86-88] polyethylene glycol (PEG),[89] and poly(N-isoproylacrylaminde) (PNIPAAm)[90] have been shown to improve cell survival. Further material usage is summarized in other review articles.[2, 3]

Biochemical factors such as growth factors (such as bFGF, VEGF, PDGF, IGF-1, SDF-1, TGF-β1, and HGF) can also be added to injectable hydrogels to enhance cellular survival. Similar to how growth factors can be covalently bounded to implantable cardiac patches, hydrogels may entrap these biochemical factors through ionic or covalent interactions. By varying the properties of these hydrogels, such as degradation rate or density of the solidified hydrogel, the rate of release and concentration of these growth factors can be controlled. Since mass transport of any engineered tissue is one of the most important factors in keeping the cells alive, the addition of angiogenic agents may stimulate capillary growth from peripheral tissues into the injected gel.[3, 91]

Gelation can be induced prior to administration of hydrogels, or hydrogels may utilize physiological parameters such as temperature, pH, and chemical composition to solidify post-

administration. This important characteristic brings us to a unique design property of injectable cardiac hydrogels: injectability. Injectability is characterized as something that can pass through a fine gauge needle ($\sim$27G), to ensure minimally invasive delivery into the myocardium. As mentioned, gelation can be achieved with several approaches, but gelation time is also vital, usually on the order of minutes to tens of minutes to prevent regurgitation of gel content into the blood stream.[14] External induction of cross-linkage such as that by light has also been demonstrated.[92]

Wall et al. hypothesized that injecting bulking material into zones of ventricle wall post-MI may alter the geometry and reduce local wall stresses.[93] Such mechanical support may improve ejection fraction and functional value of the left ventricle. An N-isopropylacrylamide(NIPAAm), acrylic acid(AAc), and hydroxyethyl methacrylate–poly(trimethylenecarbonate) (HEMAPTMC)-based synthetic hydrogel was developed to prevent progressive remodeling post-MI. Six weeks post injection, significant LV function preservation was observed.[94]

The exact mechanism of how injectable cardiac hydrogels improve cardiac function is still unknown, although several studies have suggested that mechanical support plays a major role in relieving stress.[95, 96] However, mechanical support alone is insufficient to prevent dilatation of ventricles post-MI. PEG and non-degradable PEG-vinyl sulfone hydrogels, both inert biomaterials without any biochemical or angiogenic properties, were injected into a rodent-MI model in attempt to increase infarct wall thickness in two studies. Despite an observed increase in wall thickness, ventricles continued to dilate and ventricular function continued to deteriorate.[97, 98] These results suggest that mechanical support alone is insufficient to prevent post-MI deterioration of ventricular function, and that biochemical interaction is also required. Promising results were demonstrated in a recent publication by Ye et al.. In their report, tri-lineage (cardiomyocytes, endothelial cells, and smooth muscle cells) implantation in a 3D fibrin patch loaded with insulin growth factor encapsulated microspores in MI porcine model has demonstrated improved left ventricular function, myocardial metabolism, and arteriole density. In addition, infarct size, ventricular wall stress, and apoptosis were shown to decrease, and interestingly, no signs of arrhythmias were observed.[99]

Future directions and conclusions

The major goal of tissue engineering is to compensate for what current medical practices cannot provide: regeneration of damaged tissues that do not have self-renewability. Cardiac tissue has received much focus over the past decades due to its lack of renewability and shocking epidemiology. Over the decades, much focus has been on mimicking innate cardiac tissue properties by changing the biochemical and mechanical characteristics of engineered materials, improving vasculature/mass transportation of nutrients and wastes, and evaluating *in vivo* performances. Although several laboratory settings have shown promising animal model results, translation into clinical settings requires further improvements: costliness, time of preparation (culture and preparation time is still far too long), safety (side effects such as arrhythmias, collateral tissue damage, and further necrosis due to immune reactions), and ease of application (surgery versus catheter introduction).

The availability of materials for scaffolds has not been as troublesome as that of cell source. Synthetic biomaterials or laboratory-produced natural materials are readily available for the production of engineered cardiac tissues. However, cell source has always been the major challenge in the field of tissue engineering. Fortunately, with the discovery of iPSs, the age of endless stem cell source maybe imminent. However, a more solid rebuttal of the potential risks of using hiPSCs, such as the development of teratoma or other carcinogenic side effects, is required for greater confidence. hESC-derived cardiomyocytes have shown great promise with the successful remuscularization of myocardium in primates, albeit with some observed arrhythmias. Future studies on the mechanistic properties of the induced arrhythmias could improve this problem.

We should also bear in mind that non-cellular tissue engineering is not the only approach to this problem. Molecular strategies and cellular therapies are promising and under multiple clinical trials.[100] However, cardiac patches and hydrogel injections offer greater control and versatility for the treatment designs and more opportunities for novelty. We should not limit studies of cardiac tissue engineering solely to repairing necrotic heart tissues, but for the ultimate goal of complete restoration of cardiac function and finally for organogenesis.

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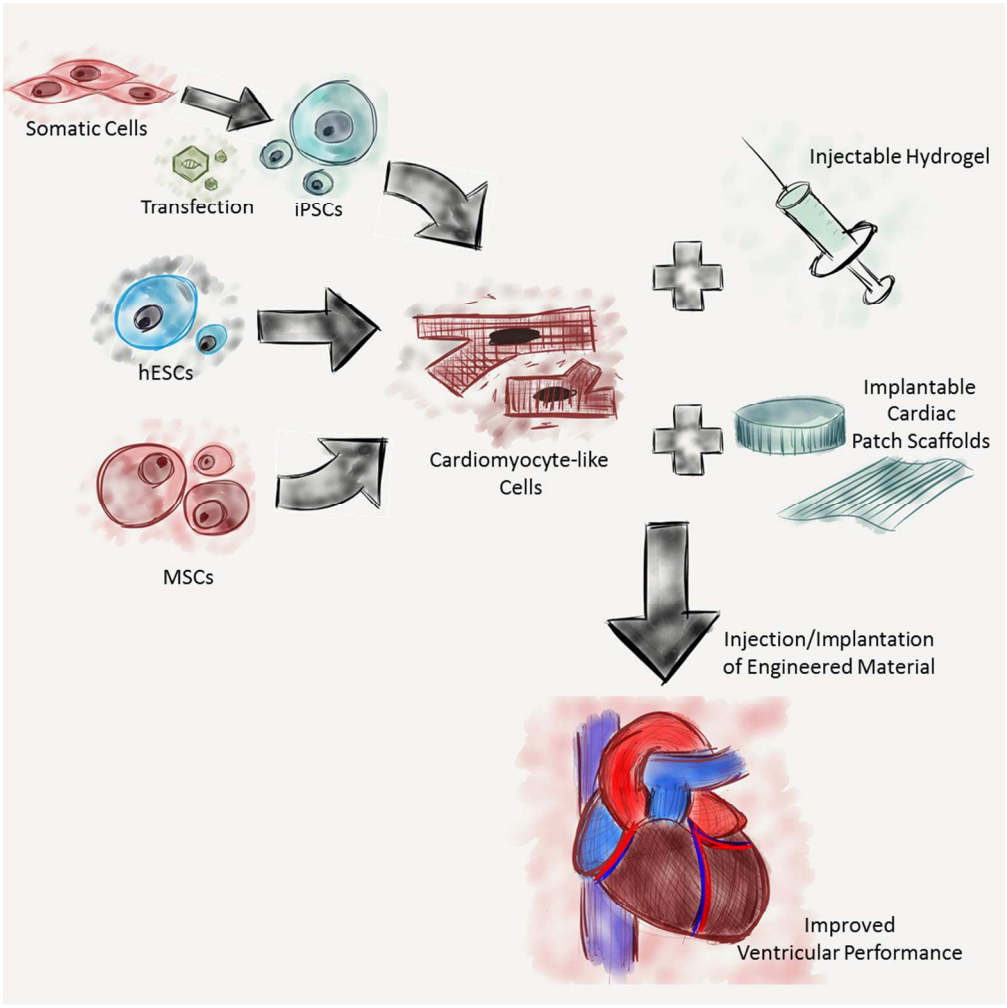

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