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REVIEW: INJECTABLE TISSUE PATCHES FOR HEART REPAIR

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ABSTRACT

Background: Injectable tissue patches provide effective minimally invasive approach for recovery of heart muscles. The aim of this paper is to study the advantages and disadvantages of injectable tissue patches and highlight their potential applications for myocardium repair. **Overview:** Cardiovascular disease is prime cause of death worldwide. Since postnatal cardiomyocytes displays no regenerative capacity, myocardium damages often develop into scar tissues and causes heart dilatation and failure. Except for heart transplantation, there are no sufficiently good treatments available for heart failure. Since heart muscles do not have any replacement alternatives like heart valves, there is need to create/regenerate engineered heart muscle using cardiovascular tissue engineering. Due to declining donor supply, the gap between supply and demand for heart replacement therapies has been increased. As a result a alternative invention was generated in the field of drug delivery to the heart. **Conclusion:** Injectible tissue patches help to repair diseased and injured heart. Different approaches have been used for scaffold synthesis and fabrication. The patches thus produced through this approaches showed significant improvement in the functioning of heart.

KEYWORDS: Engineered tissue, Cardiac patch, Decellularized tissue, Induced pluripotent stem cells, Cardiomyocytes, Extracellular matrix.

INTRODUCTION

Cardiac tissue engineering promises to revolutionize the treatment of patients with the end stage heart failure and provide new solution to the serious problem of heart donor storage.[1,2] Recent advances enable engineering of highly sophisticated heart tissues that accurately reproduce the functional and structural properties of native cardiac tissues.[1]

Thus a broad approach of tissue engineering i.e. creation of engineered heart tissues (EHTs) in the form of scaffolds serves as a promising alternative therapy for heart repair. Tissue engineering involves the construction of tissue equivalents from donor cells seeded within three-dimensional polymeric scaffolds, then culturing and implanting of the cell seeded scaffolds to induce and direct the growth of new healthy tissue of heart.[1-3]

The goal of tissue engineering is to repair or replace the damaged organ or tissues by delivering functional cells, supporting scaffolds, growth promoting, and signal molecules or DNA encoding these molecules to areas in need.

The classic tissue engineering strategy is to isolate specific cells through a biopsy from a patient, to grow them on a three-dimensional (3D) biomimetic scaffold under precisely controlled culture conditions. In order to achieve successful regeneration of damaged organs or tissues based on the tissue engineering concept, several critical elements should be considered, including the biomaterial scaffold that serves as a mechanical and biological support for cell growth and differentiation.

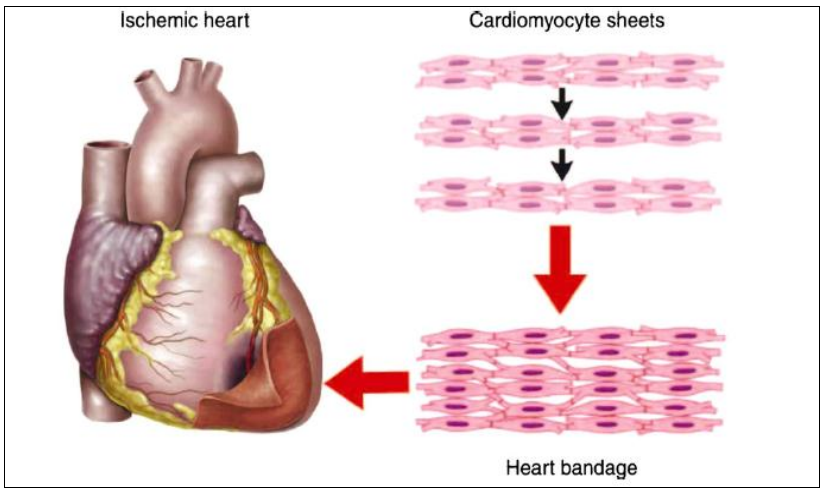

Figure 1: Transplantation of a myocardial cell-sheet graft(heart bandage).

The ongoing approaches for treating patients with heart failure are-

1. Pharmaceutical therapy

2. Interventional therapy-such as surgery or implantation of pacing devices to control electrical/mechanical asynchrony.

(1) Reduction of the heart volume

(2) Implantation of a pace-maker

3. Heart transplantation- Option to end-stage heart failure.

4. Tissue engineering strategy

(1) Cardiomyoplasty (active systolic assist)

(2) Cell-based therapy (isolated cell-delivery) - Cells are injected to the infarct region via the pericardium, coronary arteries, or endocardium.

(3) Pharmacological therapy-focuses on reduction of work load and protection from the toxic humoral factors which are overactivated in heart failure.

(4) Scaffold-free cell-sheet implantation

(5) Heart patch implantation (passive diastolic constraint and cell delivery)

(6) 3D tissue engineering construction (scaffold + cells + macromolecules)

Rebuilding 3D cell networks in vitro and implanting cell bandages on to the damaged heart is one such approach to deliver cells to the infarct region which in turn may improve the site accuracy of cell delivery. The complication of cardiovascular damage is faced by 30% of the patients who undergo cardiac surgery. In prevention and treatment of such cases of heart damage, drugs reducing vascular inflammation and those that improve vascular dysfunctioning and bioavailability prove to be efficacious. To study the effects of drugs, observational and prospective studies are being done. This enabled use of minimally invasive techniques which helped to reduce recovery time and other negative side effects.

1.1 In vitro and in vivo approaches

The in vitro approach provides good control on construct shape, size, cell sources, development, and function, it is limited by the ability to create robust muscle and the risk of tissue necrosis after transplantation. The objective of the in vivo approach is replacing the tissue in natural milieu.

1.1.1. Culturing tissue in vitro in a dish or bioreactor

(a) Creation of engineered cardiac graft from cell seeded scaffold.

(b) Creation of engineered cardiac graft from cell seeded biomaterial gel.

(c) Creation of cell film from cardiac cells and biomaterial sheet.

1.1.2 In vivo tissue engineering (in situ generation)

(a) Cell transplantation.

(b) Cell seeded scaffold implantation.

(c) Unseeded scaffold implantation and recruiting endogenous cells.

(d) Injectable scaffold with or without cells.

(e) Promotion healing and self-repair by delivery of active molecules.

Scaffold characteristics

- Patch must possess a property of non immunogenicity, easy to harvest, proliferative, and should have the power to differentiate into mature cardiomyocyte.
- The scaffold should be able to provide not only a physical support for the cells but also the chemical and biological cues needed in forming functional tissue.
- It should be able to crosstalk, on the molecular level, with the cells in a precise and controlled manner.
- It must be biocompatible and non-foreign body reaction forming.
- It should be resistant to stress and strain.
- It should be sterlizable and match the biochemical characteristics of the tissue it is replacing.
- Material degradation and resorption should be analyzed. Degradation products must be non-toxic and readily evacuated from the body.
- From macroscopic perspective, scaffold should be porous with interconnecting pore structure to enable the accommodation of large number of cells into a functioning tissue.
- Pore size of at least 50 micrometer is needed to allow vascularization of the scaffold.
- Scaffold should be able to release growth factors, gene signals and other bioactive proteins in a time-dependant fashion.

2. MATERIALS AND METHODS

2.1. Biomaterials

The biomaterial itself or its degradation/dissolution products are used to stimulate local tissue repair. Bioactive materials release chemicals in the form of ionic dissolution products, or growth factors. In the case of large infarctions, attempts have been made to replace infracted cardiac tissue with tissue-engineered cardiac patches made of biocompatible and bio-absorbable materials providing sustained release or controlled release at a rate compatible with the repair process. Suitable materials include extracellular matrix, or other biodegradable hydro gels or polymeric materials providing sustained or controlled release of drug at the site of application.[5,6] The ideal material for cardiac patches should be sufficiently strong to withstand the force of repeated contraction in the myocardium. Three-dimensional cardiac patches have been engineered using gelatin mesh, collagen gel, alginate, e-

caprolactone-co-L-lactide sponges reinforced with knitted poly-L-lactide fabric, and polyglycolic acid.[7,8]

2.1.1 Classification

Scaffold biomaterial for tissue engineering and regeneration can be divided into two categories-

(a) Synthetic materials- synthetically derived materials allow for the control over properties such as molecular weight, mechanical properties and hydrophilicity/hydrophobicity ratio.

Examples-Degradable polyesters composed of lactide (PLA) and glycotide(PLG) and their copolymers(PLGA).

Drawback-Use of PGA mesh within the heart results in a intense inflammatory response.

(b) Biologically derived materials- Biologically derived materials are also known as natural polymers. These include ECM proteins and derivatives (e.g. collagen) and materials derived from plants and seaweeds.

Polymers derived from ECM:-e.g. type I collagen and fibronectin.

Polymers obtained from seaweeds:-e.g. alginates.

2.1.2. Selected biomaterial for tissue engineering

1. Synthetic polymers

(a) Bulk biodegradable polymers-

Aliphatic polyesters, Poly(lactic acid) PLA, Poly(D-lactic acid) PDLA, Poly(L-lactic acid) PLLA, Poly(D,L-lactic acid) PDLLA, Poly(glycolic acid) PGA, Poly(lactic-co-glycolic acid) PLGA, Poly(e-caprolactone) PCL, Poly(hydroxyalkanoate) PHA, Poly(3 or 4-hydroxybutyrate) PHB, Poly(3-hydroxyoctanoate) PHO, Poly(3-hydroxyvalerate) PHV, Poly (p-dioxanone) PPD or PDS, Poly(propylene fumarate) PPF, Poly (1,3-trimethylene carbonate) PTMC, Poly(glycerol-sebacate) PGS, Poly (ester urethane) PEU.

(b) Surface bioerodible polymers-

Poly(ortho ester) POE, Poly(anhydride) PA, Poly(phosphazene) PPHOS, Polyurethane PU.

(c) Non degradable polymers-

Poly(tetrafluoroethylene) PTFE, Poly(ethylene terephthalate) PET, Poly(propylene) PP, Poly(methyl methacrylate) PMMA, poly(N-isoproplylacrylamide) PNIPAA.

2. Natural degradable polymers

Polysaccharides, Hyaluronan (HyA), Alginate, Chitosan, Starch, Proteins, Collagen, Gelatin, Fibrin.

2.2. Sources for myocardial tissues

1. Fetal cardiomyocytes
2. Skeletal myoblasts
3. Mesenchymal stem cells
4. Smooth muscle cells
5. Endothelial progenitor cells
6. Crude bone marrow
7. Umbilical cord cells
8. Fibroblasts
9. Human embryonic stem cells and Cloned cells

Collagen is a protein present in the extracellular matrix, which is predominantly used along with with other cells for fabricating the cardiac patches. Additionally, delivery of human bone marrow– derived cells with a collagen scaffold increased the number of vessels however, there was no significant differentiation into cardiomyocytes.[9,10] Applying collagen scaffold seeded with human mesenchymal stem cells (MSCs) showed increase in fractional shortening (FS) in the patch-treated group.[11] Animals that received the cell sheet and seeded cell scaffold showed significant improvement in cardiac functioning. In some studies, fibrin alone caused improvement in function similar to that of injection of fibrin with cells, whereas in others cases, an enhancement of cardiac function was seen by adding cells to the biomaterial. One potential concern with fibrin, however, is the 2-component system made of fibrinogen and thrombin, making minimally invasive delivery a challenge. As with cardiac patches, the use of decellularization as a technique for preparation of biomaterials is growing at a rapid pace. Injection of decellularized extracellular matrix promoted cell migration and improvement in cardiac function. In contrast to biologically derived materials, synthetic materials allow for control over properties such as degradation, stiffness, porosity and do not suffer from the batch-to-batch variability that occurs with bio-derived materials.

2.2. Methods

In the preferred embodiment, the drug is mixed with the material forming the matrix and the matrix formed then administered. This is the preferred method for materials such as

extracellular matrix material matrices and polymeric meshes, which are sutured to the tissue to be treated at the time of implantation, typically during surgery. Alternatively, for example, in the case of a fibrinogen matrix, the drug is mixed with the fibrinogen and thrombin added, and the polymerizing mixture applied to the heart tissue.[5,6] Following are the steps involved in making of the tissue scaffold-

2.3.1. Fabrication and characterization of the shape-memory scaffold

The scaffold biomaterial being an elastomer is capable of supporting thousands of stretch cycles without deforming or impeding the heart contraction and relaxation. A polymer is selected. This polymer should have tunable mechanical properties which must be suitable for micro fabrication, should biodegrade by hydrolysis, and should even exhibit non-toxic and minimal inflammatory properties in vivo.[3] Synthesis of prepolymer is done via a polycondensation reaction and its chemical structure was confirmed using FTIR and 1H-NMR spectroscopy. Then using a combination of soft-lithography and injection moulding scaffolds are fabricated. The diamond-like design was made over the oval designs because the straight lines and grooves present in the diamond-like design would improve cell guidance and elongation compared with the oval designs.[7,10,13-14] The anisotropic (directionally dependent) elasticity of native heart tissue as a parameter should be taken into consideration so as to incorporate the tissues into the design of the shape-memory scaffold.[12]

2.3.2 Engineering cardiac patches in vitro

Injectable cardiac patches are cultivated in custom-made bioreactors fitted with carbon electrodes. The scaffold is firmly hooked in place in the holder and maintain its structure during cell culture. During injection, the applied force is stored in the scaffold as strain energy (or stiffness energy), which then becomes responsible for the scaffold's return to its original shape. The empirical data quantifying the success rates for injection and unfolding calculates strain energies per unit area of the scaffold mesh confirmed higher anisotropic energy storage in the diamond-like mesh when compared with the control.

2.3.3. Preparation of patches from different cells

Example 1: Preparation of scaffold using POMAC

Considering a example of an ultraviolet (UV)-crosslinkable and elastomeric poly(octamethylene maleate (anhydride) citrate) (POMAC) elastic polymer. This polymer was synthesized via poly condensation reaction and its chemical structure was confirmed

using Fourier transform infrared spectroscopy (FTIR) and 1H-nuclear magnetic resonance (1H-NMR) spectroscopy.[12]

Engineering cardiac patches in vitro

Polydimethylsiloxane (PDMS) posts in the holder were used to firmly hook the scaffold in place and maintain its structure during cell culture and stimulation, preventing undesired tissue rolling and compaction.

An array of short PDMS posts in the holder enabled CMs to wrap around the scaffold struts from all sides. The tissue contracted synchronously causing in-plane compression and macroscopic movement of the scaffold. There were no significant differences between the percentage of viable cells before and after the injection of CM based tissues in vitro. The POMAC scaffold mesh exhibited auto-fluorescence enabling clear delineation of the scaffold structure and the viable (green) tissue filling the diamond-shaped lattice.[12]

Functional properties of the tissue were unaffected by injection. There was only a 30% decrease in the measured scaffold area of the injected scaffolds compared with the surgically implanted, manually flattened scaffolds.

Example 2: Using combination human iPSC-derived cardiovascular cells and decellularized heart matrix

In this a fixed ratio of human iPSCs-derived cardiomyocytes and fibroblasts with pieces of decellularized natural heart ECM were combined for preparation of engineered human cardiac patches. This method helped to generate human cardiac patches of any desired shape and size with well distributed cells. Further, decellularized natural heart ECM improved maturation of human iPSCs-derived cardiomyocytes. Resulting cardiac patches were cultured. Using decellularized natural heart ECM and human iPSCs-derived cardiac cells, it is feasible to generate individual-specific human cardiac patches of different sizes and shapes.[5,6]

Derivation and culture of hiPSCs

The cells were assayed for the expression of pluripotency markers by immunofluorescence and alkaline phosphatase staining. Derived hiPSCs were maintained on tissue culture dishes.

Differentiation of hiPSCs

Spontaneous differentiation of hiPSCs were carried out for obtaining CD90+ cells. Briefly, hiPSCs were digested from culture plates and floating embryoid bodies (EBs) were formed.

The spontaneously differentiated EBs were then dissociated and subjected to fluorescence activated cell sorting (FACS) using anti-CD90 antibodies.[5,6]

Generation of 3D cardiac patch-

In this process the decellularized heart ECM was cut into pieces of desired shape and size using a surgical scissor under sterile condition. The mixture of hiPSC-derived cardiac cells (75% hiPSC-derived cardiomyocytes and 25% hiPSC-derived CD90+ cells) were then seeded onto the ECM sheet.

Advantages

- Seeding the hiPSC-derived cardiac cells on a layer of natural heart ECM piece may lead to a more homogeneous distribution of the hiPSC-derived cardiac cells on the scaffold.[5,6]
- Cells were seeded on the endocardial side of the natural heart ECM, leaving the epicardial side intact. This provided a better pattern of cardiac patch formation, because only one side of the patch contains cells. When this patch is transplanted/ patched on the heart, the epicardial part of the natural heart ECM will act as a cover for and protect the cellular contents of the patch from exposing to the outside.

Disadvantages

- Obstacle in this field is how to make stem cells without them developing out of control and growing into tumors.
- The hiPSC-derived cardiac cells are not mature and as aligned as the adult heart tissue. This is because the current best cardiac differentiation protocol of hiPSCs still generates cardiomyocytes similar to human fetal cardiomyocytes . The maturation of the hiPSC-derived cardiomyocytes still needed to be improved.
- Although a ratio of hiPSC-derived 75% cardiomyocytes and 25% CD90+ nonmyocytes were used as the seeding cells for the patch formation, it may not represent the optimal combination achieving the best treatment effect *in vivo.*

The reason to choose hiPSC-derived CD90+ cells as supporting fibroblasts was that they are from the same individual so as to further minimize the immunogenicity of the engineered

human cardiac patches for future clinical use. CD90+ nonmyocytes expresses relative high levels of adhesion proteins which promote cell-cell contact and may facilitate maturation signaling for the heart tissues. The beneficial effects of this type of patch showed that it improved function of the heart after patching on top of the infarct area. It is feasible to generate individual-specific human cardiac patches of different sizes and shapes using decellularized natural heart ECM and human iPSCs-derived cardiac cells. These human cardiac patches exhibited normal contractile and electrical physiology in vitro and improved heart function in vivo when patching on the infarct area. The engineered human cardiac patches can be of great value for drug screening and study of inherited heart diseases, as well as meeting the demands for individual-specific (personalized cardiac cells, size and shape) EHTs for personalized regenerative therapy of myocardial damages.

Example 3: Patches engineered with bone marrow-derived mononuclear cells

In this study poly[glycolide-co-caprolactone] (PGCL) scaffold which is fairly elastic, is employed to engineer a patch for mechanically dynamic environments such as the heart. Transferring mechanical signal to the seeded cells is a one of the mechanical elastic property which is a necessary for good cellular interaction in a scaffold. Good elastic mechanical properties and cellular interaction are the properties possessed by PGCL scaffolds. Under this study investigations were carried out to see whether tissue engineered cardiac patches incorporating PGCL scaffolds laden with bone marrow-derived mononuclear cells (BMMNCs) could prevent cardiac remodeling.

BMMNCs were seeded onto PGCL scaffolds and implanted on the epicardial surface over infracted areas and adjacent normal myocardium. After implantation, LV function was examined with echocardiography and left ventricular end-diastolic pressure (LVEDP) analysis by catheterization, and tissue regeneration and neovascularization were analyzed by histological and immunohistochemical staining.[7]

Evaluation of patches

The patches were evaluated using scanning electron microscopy (SEM). Patches were plated and cultured for 2 days and were fixed in 1% (v/v) buffered glutaraldehyde and 0.1% (v/v) buffered formaldehyde for 30 min and 24 h respectively, dehydrated with a graded ethanol series, and dried. The dried samples were mounted on aluminum stubs and sputter-coated with gold.

Characteristics of BMMNC-seeded scaffold

The seeded BMMNC adhered well to the PGCL scaffolds. SEM examination and histological analysis showed that a high density of BMMNC was present on the PGCL scaffolds.

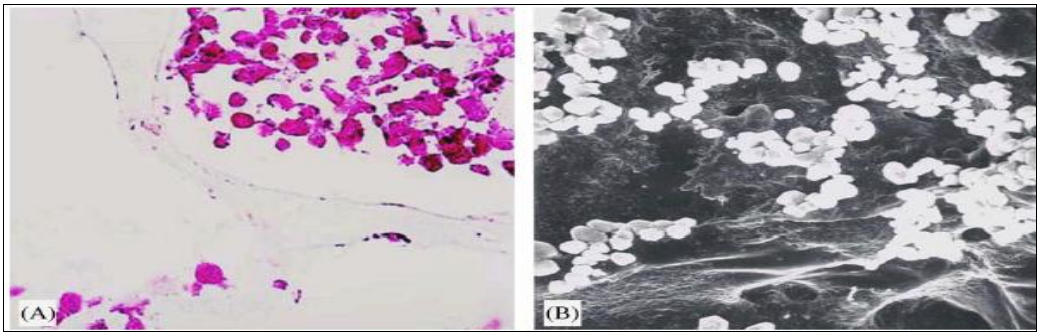

Figure 7: (A) BMMNCs (red) attach well to the PGCL scaffold (white amorphous area) as shown by H&E staining.

(B) The PGCL scaffold was seen to be covered with abundant numbers of BMMNC in SEM

Example 4: Patch constructed from MSCs-

The creation of thick and viable cardiac tissues by growing three-dimensional scaffolds on layers of seeded cells is a crucial task. To achieve this goal, a bioengineered cardiac patch (the MSC patch) composed of a sliced porous biological scaffold inserted with multilayered mesenchymal stem cells (MSCs) was developed. After culture, sliced layers of the scaffold were stuck together and seeded MSCs were redistributed throughout the scaffold.[11]

Multilayered MSCs incorporated in the porous biological scaffolds (MSC patch)

The above-mentioned multilayered MSCs were inserted into the sliced biological scaffold under aseptic conditions and then cultured for 7 days for further animal studies.

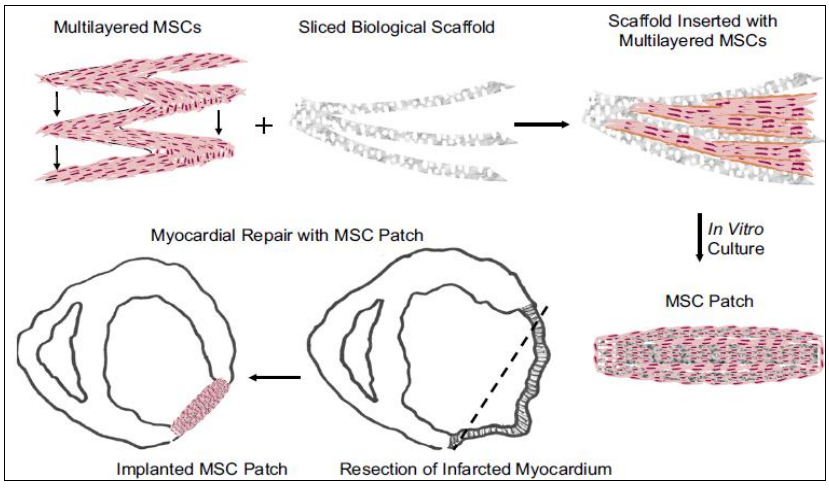

Figure 8: Construction of the MSC patch.

Characteristics of the MSC patch

The biological scaffold used in the study was made from decellularized bovine pericardia, which are composed of native ECM proteins that can serve as scaffolds for cell attachment, migration and proliferation. This can be a large advantage over synthetic materials. It is generally accepted that a tissue-engineering scaffold must be highly porous for a sufficient cell density to be seeded in vitro, for blood invasion to occur in vivo and for oxygen and nutrients to be supplied to cells.

3. Advantages of cardiac patches

- The decellularized heart ECM preserved the natural ECM components and thus was optimal for the attachment and growth of cardiac cells.
- The natural heart ECM can be cut by surgical scissors to generate pieces of any desired sizes and shapes, thus avoiding making sophisticated molds of fixed shapes and sizes for EHTs.
- Seeding of cardiac cells on a layer of natural heart ECM piece lead to more homogenous distribution of cardiac cells on the scaffold.
- A biologically active scaffold can provide a suitable microenvironment in addition to serving as a support.
- Injection of cells with the nanofibers into the patch improved both diastolic and systolic function.
- Increase in wall thickness by cardiac patches can lead to a decrease in wall stress, preventing infarct expansion.
- Feasibility of using it with a catheter based approach to avoid the need for any surgical thoracotomy.

4. Disadvantages of tissue engineered patches

- It is critical to create an at least 1cm thick and strong muscular patch i.e. this method remains the inability to generate patches with sizable thickness due to diffusion limitation.
- Cell transfer efficiency and survival rate in recipient myocardium through the patch is very limited.
- Simple cell supply or direct myocardial injection on the patch has proved deficient due to lack of a mechanical barrier.

- Although biomaterials in the patch may function as a structural support to prevent wall thinning, the timely degradation of these polymers may allow for cellular infiltration.
- Injection of a polymer can improve cardiac function at early time points, but at later time points, that effect is lost.
- In the case of a synthetic degradable scaffold, the scaffold functions as a temporary support for the cells before they integrate into the host myocardium.
- Modification of cells during the processes of cell amplification or differentiation is one of risk associated with this method and also the research and development costs are extremely high.

5. Applications

- Three-dimensional constructs that contain disease-specific or normal cardiomyocytes aims to develop the treatments for heart valve deficiency, ischemic heart disease and a wide range of vascular diseases.
- This research helps to improve the patient's quality of life, progressing towards developing cures, rather than treatments.
- It is helping to develop new ways for engineering bio-artificial organs or tissue parts that the body will adopt as its own.
- Tissue engineered patches helps to promote the establishment and differentiation of induced pluripotent stem cells (iPSCs), scaffolds from decellularized tissue that may produce more highly differentiated tissues and advance clinical translation, improved methods to promote vascularization, and novel in vitro microphysiological systems to model normal and diseased tissue function.

CONCLUSION

Heart muscle engineered scaffold synthesis aims to regenerate functional myocardium to repair diseased and injured heart. Several strategies and their approaches have been established. Different approaches have different requirement on biomaterials for scaffold synthesis and fabrication. Implanting engineered functional tissues for organ repair still requires an invasive surgical approach despite of great progress in this area of research. Thus research of designing micro-fabricated scaffold using biodegradable polymer and different human induced cells is been done. The patches significantly improved cardiac function. Scaffolds provide structural support, tunable chemistry, and suitable elasticity for cells. The interconnected scaffold lattice maintained the physical integrity of the cardiac patch, enabling

synchronous tissue contraction and the shape-memory property of the living tissue. Although each patch is relatively small, covering of larger areas is possible by sequential delivery of multiple patches and thickness is increased by stacking prior to delivery.

A polymeric scaffold was designed to produce flexible cardiac patches for minimally invasive delivery in vivo, while maintaining cell viability and function. Implantation of organically structured stem cells on a biomaterial patch offers several advantages over direct injection of dissociated cells into infarcted cardiac tissue. Although biomaterials may function as a structural support at early time points to prevent wall thinning, the timely degradation of these polymers may allow for cellular infiltration. As minimally invasive surgeries move towards standardization and lower cost, the designed scaffold should aid in translational studies.

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