Gaikwad et al. World Journal of Pharmacy and Pharmaceutical Sciences

DRUG AND CELL DELIVERY FOR CARDIOVASCULAR REGENERATION SYSTEM

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ABSTRACT

Cardiovascular diseases (CVD) are fast spreading common diseases found worldwide in all age’s of peoples. After major myocardial infarction; heart should be effectively repair its weakness or inability. Continuously, an extensive research has in progress for finding the appropriate strategies to induce myocardial regeneration and repairing. A major weakness of known clinical approaches is how to improve patient survival by replacing or regenerating the damaged myocardium after infarction. Number of regenerative strategies has developed in recent years for curing heart disease shifting from conventional approaches. Effective and targeted administration of bioactive agents directly to the diseased tissue is the key in regenerative strategies unlocking, which could improve current conventional treatments. This chapter offers some important findings in these areas and updates the development of new strategies in myocardial regeneration and repairing.

KEYWORD: Biomaterials, Cell therapy, Heart failure, Medical device, Drug delivery.
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I. INTRODUCTION

In developing countries, CVD is growing very rapidly because of urbanization and lifestyle changes. Myocardial infarction (MI) is the major cause of heart failure in almost all countries on this earth responsible for the death of patents results from temporary or permanent occlusion of the main coronary arteries. MI mainly causes reduction in blood supply to the beating heart muscle, particularly at left ventricle. A significant portion of myocardium has suddenly lost decreases contractile function of heart. An increase in left-ventricular volume can compensate these changes, which enhance contractility of non-infarcted myocardium through Frank-Starling mechanism to compensate temporary loss of myocardium contractility. Cardiac remodelling is the healing and repairing process occurs after different types of left ventricle (LV) injury like MI. It includes adverse changes in LV geometry and increases LV mass due to altered hemodynamic conditions. These facts have been correlated changes in LV geometry and hypertrophy with elevations in angiotensin II and other neurohormones post-MI. Cardiac tissues such as, cardiac myocytes, fibroblasts and
endothelial cells produces angiotensin locally. This angiotensin II production is an important contributor in LV remodelling process.\[^1\]

Unlike other human body tissues, heart tissue has ability to repair itself effectively after myocardial infarction (MI). Pharmacological agents have been successfully used to augment the life of patients suffering from MI. Myocardial infarction is the cellular death and necrosis processes after the occlusion of a coronary vessel that supplying blood to a specific area of the myocardium. Lowering of coronary flow results in myocardial necrosis and leads to functional impairments in the compromised region. Moreover, the major myocardial necrosis can lead to severe dysfunction and makes the heart unable to maintain perfusion of vital organs, with subsequent decompensation and death. In chronic myocardial dysfunction, alternative therapies like stem/progenitor cell transplantation are found to be more effective rather than existing thrombolytic therapies and primary angioplasty.

Subsequent therapies have been reported after MI has targeted regression of the LV remodelling process. Cell therapy had been given in support after MI is derived because of insufficient regeneration in injured heart tissue. These cells can replace or repair damaged vascular and cardiac tissue and resulted in number of clinical trials worldwide.

II. Biomaterial-based Controlled Delivery for Myocardial Regeneration

A new strategy has emerging combining both bioactive molecules and biomaterials to achieve effective therapy. In this strategy, biomaterials performing ECM replacing platforms and local depots for controlled bimolecular delivery to achieve myocardial regeneration. Various strategies have been developed for incorporation of bioactive molecules in different biomaterial systems to achieve protection and sustained presentation in the infarct zone. Bioactive molecules growth factors, cytokines, and stem cell mobilizing factors were continuously used in therapeutic myocardial regeneration. Different effects exerted by these molecules cover all target in the regeneration strategies.\[^2-4\]

At cellular level initially, some affected myocytes respond to diminished oxygen supply and start apoptosis, 4 h after MI.\[^5-6\] Further, cardiac cells breakdown, deteriorate the microenvironment and initiates the mass necrosis 12 h after MI. Animal studies confirmed the possibilities of apoptosis and necrosis after MI through p53-upregulated modulator deletion).\[^7\] An improvement in circulating heat shock proteins (HSP) levels were correlated with cytokines level in circulation, activate monocytes and myocardial damage after MI.\[^8\]
However, hyperthermia causes an increase in the circulating HSPs levels, played a cardioprotective role with significant reduction in infarct size.\cite{9}\ The necrosis mass deteriorate the microenvironment and causes a significant inflammation in infarct region.\cite{5-6}\ This inflammation releases cytokines IL-6 and IL-8 results in the accumulation of tissue infiltration by white blood cells (WBCs) such as neutrophils, lymphocytes and macrophages. In addition, the myofibroblasts infiltrate the damaged tissue to help in wound healing. Once the WBCs arrive at injury site, they phagocytose the dead cells and start the remodelling process.

After immune response, the left ventricle muscle mass lost due to dead cells phagocytosis and resulted in weakening of the ventricle wall. To maintain structural rightness of ventricular wall granulation tissue replaces the lost cardiac tissue with strong fibrillar collagen types I and III in 3-28 days after MI.\cite{10}\ Structural integrity of infarcted heart wall is maintained because the remodelling process culminates in the formation of scar tissue exhibiting little contractile function.\cite{6,11}\ Regeneration of cardiac tissue become difficult due to densely packed fibrillar scar and the cardiac tissue is unlikely to recover its normal function.\cite{12}\ In addition, transmural MI also involves in microenvironment deterioration through the extracellular matrix proteolysis, vasculature and nerves. Tissue repairing process does not involve any significant microenvironment regeneration. For example, some angiogenesis/vasculogenesis naturally started in first 3 days after MI. But because of higher injury, restoring of lost vasculature become difficult, which is an essential microenvironment component.\cite{13-14}\ 

A basic function of the heart is blood pumping, which can be compromised by disease and/or injury, particularly ischemia. Several pharmacological and/or invasive therapies are in used to regulate blood circulation of diseased heart. In interventional ischemic heart disease treatment and its complications several clinical trials provided a validation. The common treatments used are diuretics Lasix™ and vasodilators Isosordil™, which reduces left ventricular filling pressure and volume. Angiotensin converting enzyme (ACE) inhibitors successfully lowered the blood pressure and attenuating the angiotensin II effects in cardiac remodelling process. Metoprolol™ Beta-blocker blocks the receptor sites of noradrenaline, with positive actions on LV remodelling, adrenergic activity and oxidative stress. Although, aspirin is used as current therapeutic agent for such disorder. Advances in antiplatelet therapy allowed the development of new antiplatelet regimens like clopidogrel and 2b3a glycoprotein inhibitors.
to improve the short and long-term outcomes among patients having stent coronary intervention.\textsuperscript{[15]} To restore essential coronary perfusion, invasive therapies like coronary bypass surgery and percutaneous catheter interventions, rescue the myocardium risk. Whereas, these interventions does not restore myocardial function in long term and only moderately recover LV function post-MI.\textsuperscript{[16]} Many new drugs and invasive treatments are under development prevents aberrant cardiac remodelling and repair the lost microenvironment post-MI.

Recently, randomized controlled trials (RCTs) were suggested for global benefits observed in cardiac function might be very small and the effect of cell therapy is restricted to infarct-related regions only.\textsuperscript{[17-18]} Targeted or systemic pharmacological interventions are important strategies in heart disease treatment. These emerging treatments has potential to lower heart failure rate and effectively administered resulting in restoration of LV function after MI. Long acting Ca 2+ -channel blockers injected directly into the heart improved the ejection fraction by 42%.\textsuperscript{[19]}

In last few years, curing of heart diseases through engineering replacement myocardium and its supporting microenvironment using cell-based therapy has developed. Stem cells are self-renewing have the potential to regenerate cardiac tissue lost after MI. various type of stem cells including embryonic, bone marrow-derived stem/progenitor cells and skeletal myoblasts were used to repair impaired myocardial tissue.\textsuperscript{[20]} Embryonic stem cells are totipotent having the potential for differentiation into cardiac muscles. Unfortunately, this therapy is not in implemented due to the some ethical consensus regarding their use.

5 years ago first Phase of clinical trials were carried out using bone marrow stem/progenitor cell therapy for MI.\textsuperscript{[21-25]} Even though it was not designed to test the efficacy of intervention, the initial trials showed a promising improvement in several clinical outcomes and cardiac function, and suggested the safe intervention. Preclinical studies suggested that the mononuclear cells of bone marrow contributes the ischaemic revascularization in infarcted myocardium.\textsuperscript{[26-27]} Stem or other mature cells within the graft may exert a paracrine effect and serve as a reservoir for vascular progenitors and cardiomyocytes.\textsuperscript{[28]} or supports for endogenous cardiac stem cells.\textsuperscript{[30]} Adult stem/progenitor bone marrow cells differentiate between cardiomyocytes and endothelial cells and improve damaged heart function by induction of myocardial angiogenesis and/or infarcted scar regeneration.\textsuperscript{[31-33]} Clinically, transplantation of bone marrow-derived cells results in small (2.9 – 5.5%) and significant
improvements in ejection fraction. Bone marrow hematopoietic/endothelial progenitor stem cells are very rare. These are most capable stem cells used to treat ischemic diseases due to their vasculature production ability. Myoblasts are the sufficient source of cells having the potential to regenerate infarcted myocardium and are extremely resistant to ischemia.

High migration affinity of mesenchymal stem cell (MSC) at injury site without T-cell response makes them not only potential donor cells for myocardial regeneration, but also used to transfer gene at targeted cardiac tissue. In addition, heme-oxygenase-1 may increase cell survival in ischemic myocardium through anti oxidant and anti-apoptotic activities. Gene delivery to infarcted heart using VEGF transfected skeletal myoblasts increased angiogenesis in ischemic recipient myocardium as compared to non-transfected animal cells. Gene therapy provides a powerful therapy for salvaging damaged myocardium. Delivery of survival and engraftment promoting factors at proper time significantly affects the viability of implanted stem cells for regenerating lost cardiac tissue. Pro-angiogenic VEGF administration to ischemic heart may encouraged for new blood vessel formation before or in combination with stem cell therapy. In addition, possible side effects have hampered the revascularization of infarcted myocardium due to systemic delivery of proangiogenic VEGF and basic fibroblast growth factor (bFGF) It indicates that the targeted drug delivery play a key role to provide an microenvironment in regeneration of cardiac tissue lost by MI.

**Fig:-1** Current access routes for cell-based therapies to the heart include transvascular delivery, intracoronary perfusion, epicardial delivery and endocardial delivery.

Cell survival and proliferation requires enough development of vascularization at early stage of implantation. Fibrosis and mass transfer limitations of foreign body results in non-sufficient transportation of nutrient leading to implant necrosis. An oxygen supply to thick and resistant new tissue produced on tissue-engineered cardiac grafts (TECGs) has
demonstrated by several peoples [50-54]. They found an increase in oxygen concentration in vitro TECG, improved the production of cardiac muscle.\[^{51,55}\] By increasing endothelialization oxygen availability is increases and myocardial patches are directly applied to an infarcted area in in vivo experimental models to provide ventricular mechanical support. Thus, rapid formation of high-density local vasculature is crucial for tissue regeneration. Highly porous and spongy structure provides sufficient cell seeding density and ease mass transfer of nutrients and oxygen within the cell-seeded scaffolds. However, such type of neovascularization is insufficient and rare to support insufficient tissue formation with massive cell death observed in the early-phase of implantation.\[^{56-57}\] Therefore, inducing angiogenesis into the inner pore space of scaffolds is essential. Incorporation of angiogenic growth factors such as basic fibroblast growth factor (bFGF) and VEGF, among others, into scaffolds for controlled release has shown to promote local angiogenesis.\[^{46,58}\] The sustained pro-angiogenic in appropriate tissue could be fostered by the release of specific growth factors leading to a rapid vessel sprouting and collateralization. Moreover, growth factors and other types of molecular signals addition to scaffolds result in the generation of a molecular device for guided cell differentiation and tissue regeneration. Trophic cutaneous lesions related to diabetic or venous insufficiency-related ulcers could benefit for skin reconstruction following tissue loss in vascular field. This concept becomes more relevant in cardiac tissue engineering to promote tissue restoration. Sudden oxygen supply suppression at cellular level triggers the molecular cascades and other dramatic biological events. Inflammation of wound healing early-phases is characterized by a high regenerative and proliferative capacity of both cardiac and mobilized stem cells. Adaptive mechanisms, sustained by neurohormonal activation, further lead to a deleterious counterpart of fibroblast proliferation and ECM deposition to compensate tissue loss and prevent ventricular dilation. At this stage proliferative capacity and actual viability of myocardiocytes are dramatically reduced with the non-functional scar generation. Simultaneously it could generate an environment to maintain the proliferative capacity of the cells surrounding the infarcted area providing a molecular pathway to promote cell differentiation.

III. Cardiovascular Drug Delivery Scaffolds

Biomaterials play a vital role in various strategies of tissue engineering and regeneration. In these strategy developments, it is essential to know about biomaterials selection, use of natural and synthetic polymer scaffolds and their fabrication methodology.
Heart disease and atherosclerosis are the leading causes of morbidity and mortality worldwide. Development of heart disease and high risk of heart failure are still challenging for scientist community. Angioplasty is found to be more effective treatment on MI which decreases the mortality rate by half. But unfortunately, it becomes the treatment of choice where this service is available. In 90% of the MI patients, primary percutaneous coronary intervention (PCI) regulates the normal flow of infracted arteries. After MI, early reperfusion of occluded artery prolonged the life and improved long-term prospects of patients. A wide range of biodegradable and synthetic materials have been reported by different scientists. Natural materials collagen,[59–61] fibrin,[62] hyaluronic acid,[63] involving tissue decellularization.[64-65] was evaluated.

To improve tissue-engineering vascular graft management a variety of local drug delivery systems have been developed. Some of them are discussed as follows.

i. **Tissue Engineering**

In tissue engineering several cell types autologous have been used to start the construction of particular endothelial cells obtained from saphenous vein.[66] Recently, bone marrow stem cells were seeded into porous biodegradable polymer scaffolds and implanted to regenerate or heal injured tissues.[67] A 3D collagen type I matrix had been seeded with human umbilical cord blood mononuclear cells and grafted onto infarcted ventricles in ischemic heart model with remarkable improvement in left ventricular and myocardial remodeling.[68] A similar approach has successfully used in clinical trial (MAGNUM Trial), with a bone marrow cells seeded collagen sponge applied on the top of scarred aiming at regenerating myocardial cells and restoring the extracellular matrix (ECM) which altered following myocardial infarction.[69-70]

ii. **Polymeric Scaffolds in Cardiovascular Drug Delivery**

Biodegradable polymers are the ideal scaffolds disappear from the host on tissue regeneration and restoring the normal function. Biodegradable scaffolds do all these through polymeric degradation via hydrolysis, enzymatic cleavage or by dissolution of the matrix. It should be keep in mind that the degradation products would be biocompatible and resorbed by the body or removed via excretion with urine. Scaffold fabrication should be mild process done using safe reagents and not affecting the material properties. On use as scaffolding for cells, the porous matrix should be interconnecting and construct vascularization after implantation. Polymers used in scaffold fabrication are categorized as per their origin (natural or synthetic).
and by their chemical structure (peptides/proteins, polysaccharides, polyesters, and others). Usually natural polymers are biocompatible, easy to modify chemically and physically and processed into various structures. Hyaluronan and collagen possess cell recognition patterns enabling to stimulate cell response. However, their use in human therapy is limited because of pathogen transmission and immune rejection risks associated with natural polymers. Synthetic polymers are designed with versatile properties like mechanical strength and biodegradation rate. A disadvantage of synthetic polymers is the lack of biological cues in promoting cell responses.

Inherent diversity of structures makes polymers useful in controlled release applications. However, it is beneficial as highlight common properties within groups of polymers. Polymers are mainly classified as either biodegradable or nonbiodegradable. Biodegradable systems have gained much attention because of their use in drug delivery systems. In the realm of degradable polymers, their classification is based on erosion mechanism. The term “degradation” refers to bond breaking, whereas “erosion” refers to depletion of material. Degradation is a chemical process; whereas, erosion is a physical phenomenon based on dissolution and diffusion processes. Two mechanisms of polymer erosion can be identified, surface and bulk erosion. Furthermore, the magnitude of erosion may change simply by changing the surface area of drug delivery device. Both of biodegradable polymer classes possess highly labile groups to ensure rapid hydrolysis of polymer chains encountering water molecules. Water permeation is restricted by introducing hydrophobic monomer in polymer. Polysaccharides like starch, cellulose, and chitosan are naturally occurring biodegradable polymers. They are called as biopolymers; and their synthesis is limited to the manipulation of bulk material to enhance their viability. Because of natural material physicochemical limitations there is significant exploration of synthetic materials tailored to offer properties for specific applications. The ability to design biomaterials with specified release, mechanical, and processing properties has opened opportunities for synthetic chemists in the controlled release arena. Different way of mechanism has described for temporal and distribution controlled release of drugs using polymers. This diversity is essential for different drugs commanding various restrictions on type of the delivery system employed. An important consideration in designing polymers for controlled release mechanism is the fate of polymer after drug release. Naturally excreting polymers are desirable for controlled release applications. These polymers may excrete directly via kidneys or may be biodegraded into
smaller molecules and then excreted. Nondegradable polymers are acceptable in which the delivery system can be recovered after drug release.

A need of artificial grafts is increased in comparison to natural materials because of unavailability of appropriate autologous grafts. Tissue engineering promises as a new approach for replacement or repairing of congenital defects and/or diseased tissues. Available artificial grafts have significant limitations, including thrombosis, infection, limited durability and inability to grow. To avoid these shortcomings, tissue-engineered cardiovascular vascular grafts (TECVG), constructs using synthetic biodegradable polymer scaffold have been developed.\textsuperscript{[71-72]} Both pre-clinical and clinical studies have demonstrated the probability of constructing large-diameter vascular grafts from autologous vascular cells seeded onto a biodegradable tubular matrix.\textsuperscript{[73–75]}

Several types of polymers have been used in vascular grafts. Poly(lactic-co-glycolic acid) (PLGA) and poly-L-lactic acid (PLLA) polymers are most commonly used. These polymers have advantageous mechanical properties, plasticity and biocompatibility.\textsuperscript{[76]} These biodegradable polymers aspire for \textit{invivo} vascular cell remodelling and to perform the structural role also. The degradation process further pursued to avoid aneurysmal degeneration and rupture, as it does confer adequate strength to the conduit. Porous biodegradable polymer scaffolds are used to develop temporal templates for tissue regeneration, prosthetic devices and stents (Table 1).

\textbf{Table 1 Biomaterial used for cardiovascular applications [Ref. No. 77].}

<table>
<thead>
<tr>
<th>Source</th>
<th>Biomaterial</th>
<th>Application</th>
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<tbody>
<tr>
<td>Natural</td>
<td>Matrigel</td>
<td>Cardiac</td>
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<tr>
<td></td>
<td>Matrigel</td>
<td>Cell differentiation</td>
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<td></td>
<td>Collagen</td>
<td>Cardiac</td>
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<td></td>
<td>Collagen</td>
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<td></td>
<td>Hyaluronic acid</td>
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<td></td>
<td>Alginate</td>
<td>Cell differentiation</td>
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<td></td>
<td>Fibrin</td>
<td>Cardiac</td>
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<td></td>
<td>Fibrin</td>
<td>Vascular</td>
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<tr>
<td></td>
<td>Decellularized vessel</td>
<td>Vascular</td>
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<td></td>
<td>Decellularized small intestine submucosa</td>
<td>Vascular</td>
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<tr>
<td></td>
<td>Poly-L-lactic acid (PLLA)</td>
<td>Cell differentiation</td>
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<td></td>
<td>PLLA</td>
<td>Vascular</td>
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<td></td>
<td>Poly(lactic-co-glycolic acid) (PLGA)</td>
<td>Cell differentiation</td>
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Several techniques have been developed to construct vascular scaffolds including solvent casting/particulate leaching, phase separation, emulsion freeze drying, fibre extrusion and fabric forming processing, gas foaming and the electrospinning technique. Moreover, polymers engrafted with cytokines and drugs have been developed as drug releasing systems for localized delivery. Local sustained delivery of epidermal growth factor, transforming growth factor (TGF), vascular endothelial growth factor (VEGF) and bone morphogenic proteins (BMPs) within the biodegradable scaffolds has used to stimulate the seeded cells of specific organized tissues. Because of their biochemical properties, growth factor microspheres were encapsulated within polymeric materials to achieve the sustained release.

Fig.1 S.E.M. microphotographs of electrospun poly-L-lactic acid polymer for vascular tissue engineering. Electrospinning technique provides a useful mean to generate a native extracellular matrix like structure to mimic the native histoarchitecture thereby facilitating cell attachment, proliferation and differentiation. Bottom right: vascular prosthesis realized by electrospinning [Ref. No. 77].

A sustained VEGF delivery through polymer 85:15 poly(lactide-co-glycolide) (PLG) increases angiogenesis and improve perfusion in an ischemic murine hind limb model.
Angiogenic process was regulated by the polymer-derived VEGF delivery and results in more mature vasculature in muscle surrounding the implanted area and within the scaffold. Platelet-derived growth factor (PDGF) has promoted wound healing through the formation of granulation tissues. Despite its vital role in tissue regeneration enhancement, clinical application of PDGF encounters some hurdles basically represented to maintain sufficient therapeutic concentration. To avoid these shortcomings, biomaterials engineering developed a microsphere-based scaffold release system of PDGF-BB encapsulated in PLGA microspheres incorporated with biodegradable PLLA scaffold. Polymeric delivery systems allow localized and sustained release of therapeutic agents may avoid the limitations of growth factors delivery. Alginate hydrogels can be used for localized VEGF delivery. Recently biodegradable alginate hydrogel used in sustained and localized release of VEGF at desire concentration has developed. It is important to focus on biocompatibility of the materials currently used does not imply in its significance when drugs and growth factor are involved in experimental arena. Angiogenic local response in severe combined immunodeficiency SCID and immunocompetent mice (C56BL/6) to a porous PLG engineered to provide a sustained delivery of VEGF. The VEGF delivered from this system maintained at least 90% bioactivity and induced sprouting angiogenesis from surrounding host vasculature, which infiltrates the highly porous scaffold, resulting in the rapid formation of neovasculature vivo. The release profile of bFGF from heparin containing PEUU scaffolds was found to be similar to PLGA scaffolds and methylidene malonate polymer. A bFGF-loaded PLGA scaffold
fabricated using supercritical CO2 exhibited 45% initial burst release in 1 day.\textsuperscript{[101]} The release of bFGF from methyldene malonate polymer films had over 30% initial burst release in the first day.\textsuperscript{[102]}

Self-assembling peptide nanofibres has designed for prolonged delivery of insulin-like growth factor 1, a cardiomyocyte growth and differentiation factor, to the myocardium, using a ‘biotin sandwich’ approach.\textsuperscript{[103]} Control on intramyocardial environment by delivering growth factors to injured myocardium may prevent heart failure and improve the endogenous regenerative response providing a chemo attractant signal to promote stem cell migration.

Poly (N-isopropylacrylamide) (PIPAAm) has used in the creation of intact cell sheets. On the culture surface an attachment and detachment of cells are simply controlled by temperature change. Cells are non-invasively harvested as intact cell sheets along with their deposited ECM, and attached to host tissue. Using this technique, several cell sheets could be layered on the top of each other to create multilayered three-dimensional cell constructs.\textsuperscript{[104-105]} Biodegradable poly(\(\alpha\)-hydroxyacid) [poly \(\varepsilon\)-caprolactone (PCL), polyactic acid (PLA), polyglycolic acid] and their copolymers have been mainly used as solid macroporous scaffolding for cardiac patch reconstruction.

Hydrogels are prepared from natural and synthetic polymers by physical/ionic interactions (alginate) or via chemical cross-linking (collagen, HA, and others). Cells are incorporated/encapsulated in the hydrogel during fabrication. Due to their resemblance to ECM texture, hydrogels are extensively being investigated as ECM replacements for damaged ECM after MI. They are delivered either by intramyocardial injection or by catheter-based techniques through the intracoronary route.\textsuperscript{[106-111]}

iii. Growth Factors Delivery

Growth factors localized delivery using implantable DDSs has successfully used in site-specific pharmacological effects neo-angiogenesis using VEGF and bone growth using BMPs. Allowing Localized and sustained release of therapeutic agents through polymeric delivery systems may bypass the current limitations of growth factors delivery.\textsuperscript{[90]} Alginate hydrogels are used in localized VEGF delivery.\textsuperscript{[91-92]} Recently sustained and localized release of VEGF of desired concentration allowed through injectable biodegradable alginate hydrogel.\textsuperscript{[93]} This system provides a spatiotemporal factor bioavailability leading to angiogenic response in ischemic hind limbs. The biocompatibility of materials currently used
does not imply in its significance and not exert any collateral effect in vivo setting, when drugs and growth factor are involved in the experimental arena.

iv. Steroids Delivery
To reduce the foreign body response graft implantation and vicious interrupt lead to the failure of the biomedical device. Thus, corticosteroids have been used in such scaffolds fabrication. A sustained release of dexamethasone biodegradable scaffold inhibits its effect on smooth muscle cell proliferation.\textsuperscript{[112]} PLGA microsphere/PVA hydrogel composite DDS has developed to release a constant amount drug delivery. Dexamethasone loading on device has used with cytokines as a stimulating agent for the differentiation of stem cells into bone cells.\textsuperscript{[113-114]} For instance, a scaffold implanted in vivo at bone defect site colonized by bone marrow stem cells recruited and migrated from the local tissue.

v. Gene Delivery
Genomic biomaterials have been has always of medical interest including muscle and bone regeneration. In vivo sustained release of plasmid DNA encoding PDGF gene with PLGA matrix has reported to enhance matrix deposition and blood vessel formation.\textsuperscript{[15-116]} The polymer scaffold used is capable for controlled release of intact and functional plasmid DNA to enhance and regulate gene transfer within a developing tissue and avoid the use of viral vectors.

vi. Heparin Delivery
Heparin is widely used in various cardiovascular issues including myocardial infarction, deep venous thrombosis and to circumvent thrombotic phenomena arising from bare metal stent implantation in coronary arteries or other vascular procedures. Heparin has also accompanied in various type of vascular repairing. Heparin, rapidly inhibits DNA and RNA synthesis in cultured vascular smooth muscle cells, restricts leucocyte adhesion to injured endothelium and restores endothelial integrity. It also interacts with vascular growth factors involved in their signalling cascade. After vascular interventions, to reduce intimal hyperplasia heparin is the best alternative.\textsuperscript{[117-120]} Recently, in association with biodegradable scaffolds of PLGA, heparin has used in local and sustained bFGF delivery.\textsuperscript{[121]} In this study, heparin was immobilized on PLGA scaffolds surface through covalent conjugation between carboxylic groups of heparin and amino groups of PLGA scaffolds. This study induced the formation of blood vessels at implant site. Local and sustained delivery of angiogenic growth factor for tissue regeneration has achieved by surface modification of porous scaffolds with heparin.
vii. **Nitric Oxide Delivery**

Nitric oxide plays a vital role in maintaining the vascular smooth muscle in a non-proliferative state and preserving blood vessels from adverse vascular remodeling.\[^{122}\] Nitric oxide constitutes an alternative to ameliorate biomaterial properties for cardiovascular applications. Polyethylene terephthalate and ePTFE grafts are currently used as vascular substitutes, facing several limitations like concerning thrombosis, intimal hyperplasia and thus, failure in small diameter applications. Moreover, nitric oxide releasing polyurethane, inhibits smooth muscle cell growth, reduced platelets adhesion as revealed by a mepacrine-based assay. Recently, diazeniumdiolate nitric oxide incorporation or into the polyurethane backbone containing both Polyethylene glycol (PEG) and the cell adhesive YIGSR peptide sequence has performed to provide nitric oxide local release during graft endothelialization period.\[^{123}\] PEG has become resistant to protein adsorption, platelet adhesion and bacterial adhesion. Numerous methods were developed to integrate PEG into biomaterials, including polyurethanes, through co-polymerization and surface modification.\[^{124\textendash}129\] Nitric oxide generating, cell adhesive polyurethane-PEG copolymer reduced platelet adhesion and smooth muscle proliferation enhancing endothelial cells attachment and proliferation. However, the therapeutic potential of nitric oxide generating polyurethane remains high and suitable for small-diameter vascular grafts.

viii. **Protein Delivery**

Proteins are highly organized complex molecules maintained their structural and chemical integrity properly. Moreover, on administration the solid protein are hydrated and exposed to physiological temperature within the delivery device for long time period. Polymer matrix microenvironment and polymer degradation products reduced the protein stability.\[^{130}\] Thus, proteins are always in stresses including formulation, longterm storage, and release in vivo. Drugs entrap within and releases subsequently from polymers or liposomes led a new approach to treat number of diseases. Despite all these advances, majority of delivery systems are still used for small molecules only taken either orally, transdermally, parenterally, or through the nose or lung.\[^{131}\] Development of proteins controlled-release system involves more challenges. An important issue is the instability of the proteins themselves. Unlike small drug molecules, proteins cannot be easily delivered orally or through the skin and have short half-lives in vivo. As a result, most of them are administered via injection.\[^{132}\] In addition, to reduce dosage of injections, controlled-release formulations of proteins need protection from in vivo degradation, diminished toxicity, improved patient comfort and
compliance, localized delivery to a particular site, and more efficient use of the drug resulting in lower dosages.

Poly(DL-lactide-co-glycolide) (PLGA)-based delivery system has exhibited to rise the acidity on polymer degradation, leading to distinct deleterious reactions in encapsulated proteins. Consequently, several acid-labile proteins—bovine serum albumin (BSA), basic fibroblast growth factor, and bone morphogenic protein-2 were stabilized in the PLGA formulations, as BSA in the PLGA microsphere injectable formulation during long-term release. In this case, stability is affected by controlled-release system microstructure including porosity, size and degradation of PLGA. The neutralization of acidity process has utilized for polymer decomposition used in medical applications rather than protein stabilization.

Fig 3. Scanning electronic micrograph (SEM) of protein-containing poly(lactic-co-glycolic) acid (PLGA) microspheres (Ref. No.134).

IV. Nanoparticulate Cardiovascular Drug Delivery

Nanoparticles are solid colloidal particles having a size from 10 to 1000 nm (1 µm). They consist of macromolecular materials entrapped/encapsulated drug or biologically active material. Nanomedicine are medically useful nanodevices functioned inside the body. It must act only on the target organ, and not in other places where it could cause harm to the organism. The science of drug delivery; transporting medications encompassing the field of nanotechnology and nanofabrication to improve drug delivery in which the drug is only act at target area of the body and sustained release the drug over a period of time in a controlled manner. Nanoparticles are useful active drugs carriers, when coupled with targeting ligands, work as a ‘magic bullet’. In late 1960s Prof. Speiser developed first nanoparticles for drug
delivery purposes. Tetanus and diphtheria infections require multiple injections to build up antibody for sufficient protection. Sustained drug release from nanocapsules was the objective to circulate in the blood after intravenous injection. To test sustained release feasibility from such capsules, Speiser developed nanoparticles for vaccination purposes.\[137\] In cardiology field, nanoparticles have much more advantages over microparticles as they are suited betterly for intravenous (IV) delivery. Designing liposomal and polymer-based nanocarriers are the main issues for drug targeting to endothelial cells. Functions of nanocarriers include: 1) optimization of a drug’s pharmacokinetics in the bloodstream and protection of drugs against inactivation and premature activity enroute to the target; 2) controlled drug-release kinetics; 3) providing multivalent affinity to binding sites and enhancing effectiveness on target cells; and 4) modulation of subcellular drug delivery.\[138\]

Local drug delivery system has an important advantage of decreasing the total drug burden at target site.\[139\] Various polymer-based drug delivery systems allowed prolonging drug at delivery site and making possible the rapid degradation on administration.\[140-143\] Figure 3 illustrates the structural design of some currently available nanocarriers.\[144\]

\[145-146\] Cardiac targeting moieties generally used include lectins, proteins and antibodies.

\[144\] V. Liposomal Cardiovascular Drug Delivery

Various targeting moieties are used to improve delivery by targeting at specific location, increasing local concentration and cellular internalization. An essential step in targeted drug delivery is to achieve desired therapeutic effects of pharmaceutical agents and genetic materials. In vascular delivery, targeting ligand reduces washout by encouraging adhesion to vessel walls or atherosclerotic lesions.\[145-146\]
In 1965 Liposomes were firstly reported by Bangham and his coworkers. They are artificial vesicles made of an aqueous core surrounded by lipid bilayers. Liposomes are composed of naturally derived phospholipids or synthetic amphiphiles, and incorporated with cholesterol to affect membrane permeability. They are prepared by thin-film hydration involving dissolved lipid components in organic solvent, drying in rotary evaporation and rehydrating in aqueous solution, as well as by freeze-drying, reverse-phase evaporation and ethanol injection.

Liposomes are used as delivery vehicles to carry drugs, genetic material, and imaging agents. Because of their partial hydrophilic (aqueous core) and partial lipophilic (lipid bilayer) nature, liposomes used to encapsulate active agents, irrespective of solubility and physical properties. Encapsulation into liposomes reduced systemic toxicity by minimizing dosage requirements and protects or controls release of an active drug or genetic material. Liposome helps to overcome biological barriers, to deliver their payload and exert the desired pharmacological effect. Main drawback includes a surface chemistry which attracts proteins and liposomes for rapid clearance from circulatory system.

Stable liposomes were prepared them by coating with hydrophilic polymers, polyethylene glycol (PEG), or other polyelectrolytes designed specifically to increase circulation time and reduce clearance in vivo. As compared to nanoparticulate delivery vehicles, liposomes have ability to load both hydrophilic and hydrophobic components in same system. Liposome-based research has underway to develop liposomal drug and gene delivery devices and imaging agents for cardiovascular disease (CVD) applications.

Certain CVD forms like atherosclerosis impaired blood flow and make transportation of therapeutics difficult. Liposomes are well-suited to overcome these challenges since factors such as size, charge as well as the inclusion of targeting moieties were adjusted to improve delivery efficiency. In blood flow blockages overcoming, Caride and Zaret (1977) were firstly suggested that positively charged liposomes accumulate in myocardial infarction area. Further studies focused that liposomes enhanced permeability and retention effect in certain areas of the vasculature. It could be exploited to influence the distribution of therapeutics in the cardiovascular system. Early studies proposed that liposomes can serve as direct treatments, by forming a ‘plug’ and sealing damaged endothelial membranes. Furthermore, liposomes are accommodating a wide range of cardio-specific adhesion
molecules and polymers on its surface to increase adhesion with vascular tissues or cells [Figure 4].\cite{158}

![Fig 4. Schematic representation of (A) immunoliposomes and (B) selected targets to damaged endothelium and (C) schematic depicting the proposed ‘plug and seal’ mechanism of immunoliposomes to ischemic cardio-myocytes [Ref. No. 158].](image)

**VI. Liposomal Targeting Cardiovascular System**

Liposomes can precisely customize at stipulated conditions by varying the formulation and/or processing steps in vascular drug delivery. In such delivery system; active targeting is exploited to improve payload delivery and residence time within the body. Regarding the passive targeting of liposomes, size, charge, and polymeric surface coatings affect blood clearance, cellular uptake and distribution throughout the cardiovascular system.\cite{159-160} Liposome size was changed for passively targeted cells. Large liposomes were found to be more phagocytosed by macrophages, whereas, smaller liposomes can be readily taken up by fibroblasts.\cite{161} One of the main drawbacks of liposome used is the rapid clearance by liver and reticuloendothelial system. Liposomes used to improve blood circulation time and avoid clearance are characterized by the incorporation of PEG into the liposomal formulation through adsorption, conjugation or covalent linkage.\cite{162} PEG can increase liposomal circulation times by reducing activation of the complement system through steric interactions and enhancing stability. Although, the results are totally depends on molecular weight and grafting density of the polymer. Immunoliposomes (liposomes conjugated with antibodies) are predominately used to target the cardiovascular system.
VII. Immunoliposomes

In seventies middle, Gregoriadis et al. (1975) proposed the concept of liposomes to target cells by attaching antibodies to their surface [163]. Since afterward, immunoliposomes have been employed to treat variety of diseases, including CVD. It is known that, an important aspect of cardiovascular targeting research lies on the pathways during each stage of disease evolution or healing. Particularly, information of cell types or the expression of receptors helps to elucidate selection of antibodies to target the liposomal vectors at desired location. More importantly, selection of antibodies should base on availability and specificity of given target. While selecting the antibodies following important things has to keep in mind: i) targets should be accessible by the vascular system; ii) targets should selected based on cell surface glycoproteins or receptors; iii) antibodies must be able to attach to the liposomal surface without undergoing any deleterious effects on targeting activity; and (iv) antibodies should elicit no immunogenic effect.

In atherosclerosis, vascular cell adhesion molecule-1 (VCAM-1) can be targeted since they are expressed on the surface of endothelial cells. Selection of targeting cell population or adhesion molecules, and antibody depends on type of drug or imaging agent to be delivered. Endothelium is the main site of inflammation and cellular infiltration in atherosclerotic lesions development. Thus, the prime targets for cardiovascular therapeutic delivery and imaging is to activate endothelial lining. During activation, glycoproteins endothelial-leukocyte adhesion molecule ELAM-1 or E-selectin), VCAM-1 and/or intercellular adhesion molecule-1 (ICAM-1), regulate and express the surface of cells. Depending on disease or injury severeness, these molecules serve as potential targets for liposomes with appropriate monoclonal antibodies. For example, for activated endothelial cells using in vitro model, Lim et al. (2011) observed liposomes loaded with celecoxib and designed to target VCAM-1, successfully increased liposomal uptake as compared to unconjugated liposomes. Homem de Bittencourt et al. (2007) in vivo study, atherosclerotic mice were subjected to a dose of anti-VCAM-1 immunoliposomes. The presence of anti-VCAM-1 antibodies improved the distribution to thoracic aorta, while reducing accumulation in the spleen and kidneys. These results indicate that antibodies directed toward inducible cell surface glycoproteins could aid in localizing liposomes to vascular disease site and improving cellular uptake.

From above discussion it is clear that antibodies provides degree of active targeting to improve liposomal retention at given site and also improves therapeutic outcomes in CVD. In
this way, the required dosage can be reduced and nonspecific accumulation in healthy areas in the body will be also avoided. Ultimately, immunoliposomes can play a pivotal role in improving the drug efficiency, gene and imaging agent delivery to treat and diagnose CVD, in conjunction with ligand and cardiac biomarkers.[169]

VIII. Liposomal therapeutic delivery to the cardiovascular system

Routine CVD treatments are suffered from number of drawbacks from administration point of view. Systemic delivery of active pharmaceutical agents requires high concentrations and short half lives in vivo. These can lead to unsustainable drug levels causing drug resistance and other side effects. Liposomal technology can offer alternative delivery carriers to controlled therapeutic delivery, to target specific body tissues and improve cellular internalization rates. Liposomes protect the active agent from degradation, improve residence time in vivo.[170] shield the body from toxicity.[171] offer control over pharmacokinetics,[172-174] and accommodate ligands to target specific areas of the vasculature.

IX. Liposomal CVD of Genetic material

Local and sustained molecules production with DNA or oligonucleotides genome is offered by gene therapy. Such genetic carriers can offer superior rates of transfection efficiency. Genetic material encapsulated in liposomes improves transportation across the biological membranes by increasing residence time and reducing degradation in vivo. Cationic liposomes commonly been used to promote gene transfer as they can condense plasmid DNA to form stable complexes, called lipoplexes.[175] Gene therapy has widely used to treat CVD.[176] Majority of gene carriers used for CVD research and clinical trials are viral vectors.[177] however, a number of groups have also utilized liposomes, owing to their non-immunogenic and relatively low toxicity profiles in the body.[176] In another study, the inducible nitric oxide synthase transgene was delivered by liposomes to a chronic myocardial ischemia porcine model.[178] A moderate recovery was reported compared with control procedures in ischemic region, suggests the need of further study. A liposome mediated transfer of genes has used for the treatment of restenosis and ischemia.[179-180] In recent study liposomes are found to be vastly inferior to viral vectors in terms of transfection efficiency of gene carriers used in cardiovascular clinical trials.[181] Complexes formed between liposomes and viral components were investigated to improve the transfection capabilities.
X. CONCLUSION
Several Cardiovascular Drug Delivery Systems has reported by various peoples. However, CVDS is still challenging issue to scientist community as the mortality rate due to myocardial infarction is very high. There is a need to develop more CVDS for the survival of peoples from heart diseases.

XI. REFERENCES


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