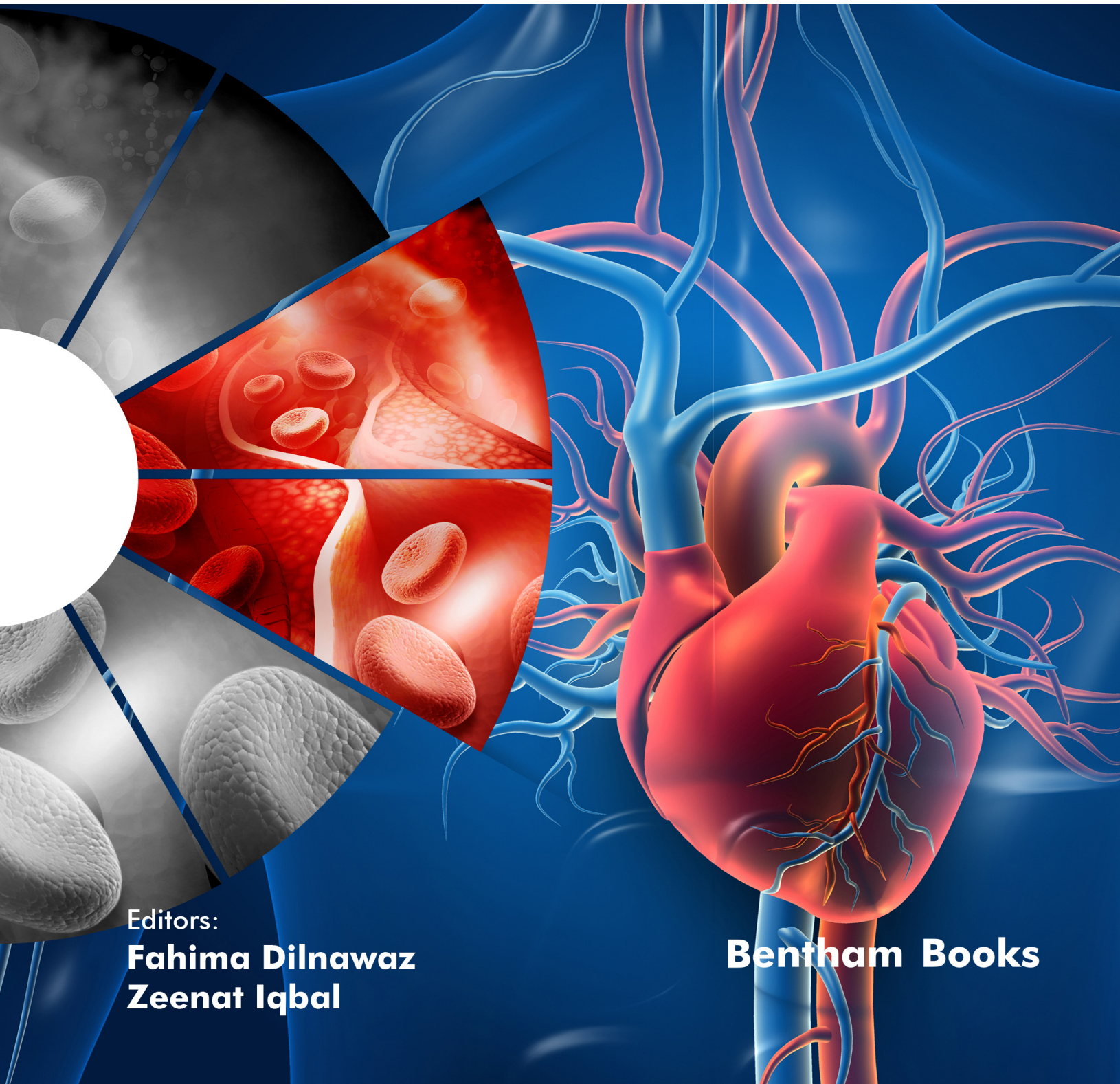
NANOMEDICINAL APPROACHES TOWARDS CARDIOVASCULAR DISEASE



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Nanomedicinal Approaches Towards Cardiovascular Disease

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ISBN (Online): 978-981-4998-21-5

ISBN (Print): 978-981-4998-22-2

ISBN (Paperback): 978-981-4998-23-9

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FOREWORD 1

It is my immense pleasure to write the foreword to this book. The book deals with various aspects of nanotechnology based application for the treatment of cardiovascular disease.

Across the globe cardiac diseases remain the major cause of mortality and morbidity despite current pharmacological advancements, however, a complete cure for the disease has not been accomplished. In cardiac therapy nanotechnology-based therapeutic application has illustrated remarkable progress. Immense usefulness of nanotechnological application towards therapy, it is highly anticipated that nanomedicine may fill the huge gap by initializing the new avenues to meet the existing therapeutic demands of cardiovascular diseases. And it may provide a subtle solution with better prognoses along with a reduced side effect profile.

This abreast book is quite informative as it deals with the understanding of the cardiovascular disease and current progression of nanotechnological mode for the holistic approach of the therapeutic improvement. This book gives the surfeit information about various nanocarriers, biomaterials for cardiac tissue regeneration which is highly beneficial for the students, academicians and clinicians. The simple language with pictorial illustration of the book will surely help for the upgraded understanding of the subject.

I am convinced that my colleagues *Dr. Fahima Dilnawaz* and *Dr. Zeenat Iqbal* have done a great, focused and detailed job in compiling and editing this cutting edge and comprehensive book on nanomedicine and cardiovascular disorders written by subject experts. I wish this extraordinary book to entice extensive readership, and I hope the readers will thoroughly enjoy the assortment of recent scientific facts as much as I did!

Farida Khan
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FOREWORD 2

It is a pleasure to write the foreword to this book. It presents a comprehensive review of various aspects of nanotechnology in the treatment, drug delivery and amelioration of cardiovascular disease.

Up till now, cardiac diseases remain the major cause of mortality and morbidity across the globe and despite the recent therapeutics' options and pharmacological advancements, a complete cure for cardiac diseases has not yet been achieved. However, in recent years, a tremendous explosion has been seen in the advancement of nanotechnology, where nanomedicine has brought a remarkable improvement in cardiac therapy. And it is expected that nanomedicine will fill the remaining gap by opening new frontiers for inventing newer therapies and addressing the unmet needs of cardiovascular diseases.

This up-to-date book incorporates both the underlying science of cardiovascular diseases and the advancements in the field of nanotechnology in such a balanced fashion that led to the holistic development in the next generation of disease treatment. From nanocarriers to biomaterials for cardiac tissue regeneration, this book gives a plethora of information that will be definitely beneficial for the students and clinicians. Also, simple language and pictorial presentations of the various concepts will help in the better dissemination of knowledge and hence a better understanding of the book.

I am convinced that *Dr. Fahima Dilnawaz* and *Dr. Zeenat Iqbal* have done a great, focused and detailed job in compiling and editing this cutting edge and comprehensive book on nanomedicine and cardiovascular disorders, written by subject matter experts from academia. I wish this extraordinary book attracts a broad readership and I hope the readers will enjoy this collection of scientific facts as much as I did.

Shamarez Ali Mohammed
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PREFACE

The last decade has witnessed a tremendous change in the way disease therapy has been impacted by nanotechnology. It has indeed birthed and nurtured a newer branch of advanced therapy and diagnostics vis a vis "**nanomedicine**" . The uniqueness and propensity of adapting itself to diagnosis and treatment of plethora of diseases makes "**nanomedicine**" a key area of pharmaceutical research. Although at its nascent stage "**the nanomedicine approaches as an intervention for cardiac diseases**", is fast attracting the attention of researchers and practitioners alike. Cardiac diseases are often associated with myriad complications during the lifetime of a patient and continue to remain the cause of frequent deaths worldwide. The disease segment is still on a lookout of well-equipped treatment tools and is poised to explore nanomedicine armamentarium wherein, cardiovascular complications could be diagnosed at the molecular level and its treatment is delivered at the cellular level. This, precisely would make a fit case for early detection and diagnosis followed by quicker remedy for the cardiac ailments. This would indeed be a welcome step as most cardiac diseases often reduce the window of survival time for the patients.

The book entitled "**Nanomedicinal approaches for cardiovascular disease**" by **Dr. Fahima Dilnawaz and Dr. Zeenat Iqbal** illustrates the application of nanomedicine and nanotechnology in the diagnosis and treatment of cardiovascular diseases. This book indeed is a humble attempt to present the various approaches of nanomedicine in cardiovascular ailments to the broad readership, including academicians, researchers, scholars and clinicians. The authors have made concerted efforts towards inviting various reputed contributors who have been working in the domain of nanomedicine for quite some time.

This book is divided into separate sections such as: Section 1: *Introduction to cardiovascular diseases and need of nanomedicine and regenerative nanomedicine: Nanomedicine aspects in cardiovascular diseases, Role of Nanomedicine in the diagnosis of cardiovascular diseases, Nanotechnology based molecular imaging in cardiovascular disease, Nanocarriers for therapeutics delivery of cardiovascular diseases, Nanocarriers for theranostics delivery of cardiovascular diseases, Nanocarriers for biologicals delivery to cardiovascular delivery and Ethics and regulations for cardiovascular diseases*. Section 2: *Biomaterials for cardiac regeneration, Biomimetic materials design for cardiac tissue regeneration, Nanotechnology based direct cardiac reprogramming for cardiac regeneration, Smart nanomaterials for cardiac regeneration therapy, Stem cell engineering ability to promote cardiac regenerative activity.*

Each section gives a plethora of novel information on a particular field. Summarily, the book is a collection of quality information on various applications of nanomedicine that can be applied for the successful treatment of cardiovascular diseases. The lucid textual and pictorial presentation of the various sections and chapters is primarily in simple language that will support easy dissemination of knowledge.

The authors are hopeful that the collaborative efforts invested in the writing of this book on a very dedicated area of nanomedicine in cardiac diseases would attract good readership.

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CHAPTER 1

Introduction to Cardiovascular Diseases and The Need for Nanomedicine and Regenerative Nanomedicine

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Abstract: Worldwide, cardiovascular diseases claim a number of lives; however, some of them are preventable with an early and proper management. Still, the treatment of cardiovascular diseases is limited as it deals with prescribed medicines administered orally and under critical condition with invasive surgery. Due to this, there exists an enormous gap in the area of medicine for the development of therapies for better patient outcomes. In this regard, recently, nanotechnological aspects of the development of medicines are sought, which may provide a solution for more effective treatment of disease, having better therapeutic outcomes with reduced side effect profile. Further, the regenerative nanomedicine therapeutic approach opens up a paradigm that deals with the repair of damaged heart tissue and future potential use of such systems.

Keywords: Bioavailability, Cardiomyocytes, Nanomaterials, Nanomedicine, Nanoscience.

INTRODUCTION

Cardiovascular diseases (CVDs) are one of the major causes of mortality and morbidity globally and include primarily hypertension and coronary artery diseases and their associated diseases like atherosclerosis, myocardial infarction, cardiac arrythmia, angina pectoris and chronic heart failure. CVDs are being accounted for the death of ~17.8 million people in 2017 and ~ 30% of all deaths occurring globally [1]. Of these deaths, around 7.3 million people died due to coronary artery disease and 6.2 million cases of deaths were due to stroke. Heart problems, which are often associated with reduced physical and mental health, lead to a decreased quality of life [2, 3]. Treatment for various CVDs includes

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non-invasive therapy such as prescription medications and lifestyle modifications or invasive or surgical procedures such as bypass surgery, angioplasty *etc*.

During the past recent years, several advancements have been made in the field of diagnosis and treatment of various diseases with rapid expansion in nanoscience, which includes detailed molecular level understanding of diseases and use of sophisticated technologies in the nano range, in the field of medicine. Nanomedicine has emerged as a novel tool for the diagnosis and therapy of various Cardiovascular diseases [4]. National Institute of Health defines Nanomedicine as "the application of nanotechnological aspects for the diagnosis, treatment, monitoring and control of biological system." Nanotechnology is a collective term that refers to scaling down the particles to nanometer range (less than 1000 nm) [5]. The nanomaterials possess a relatively larger surface area compared to the same mass of materials, which makes the materials chemically reactive. Recent advances in nanoscience lead to the construction of new materials and devices that are used in molecular diagnostics and manufacturing of nanopharmaceuticals. These nanofeatured structures address the underlying cause of the cardiovascular disease that can improve the detection of early stage diseases so as to decrease premature mortality and enhance patient compliance for treatment [6, 7]. Nanomedicine serves to deliver a valuable set of research tools and clinical devices in the near future. Therapeutic delivery to the cardiovascular system may play an important role in the successful treatment of a variety of disease states, including atherosclerosis, ischemic-reperfusion injury and other types of microvascular diseases, including hypertension. Nanoformulated drugs are designed to protect against systemic degradation, thereby reducing toxicity, immunogenicity, and increasing half-life, bioavailability and precise biodistribution [4, 8]. Further to attain therapeutic selectivity to the heart, functionalization with targeting moieties allows specific accumulation in the diseased heart [6, 7]. In CVDs, thrombotic events occur in ischemic stroke, myocardial infarction, pulmonary embolism, and venous thrombosis, where thrombolytic therapy are used to break up the blood clots. Recombinant tissue plasminogen activator (tPA) is actively used as therapeutic molecule for the treatment of acute ischemic stroke [9]. Multifunctional nanoliposomes are used for highly specific binding to activated platelets whilst minimising undesirable side effects [10, 11]. Many nanoformulations are undergoing clinical trials; some are in the pipeline. CVN should be focused on disease-driven approach rather than formulation-driven approach to strengthen the significant potential and to overcome physiological barriers and improve therapeutic outcomes in patients. However, nanoformulation approach is still in infancy; great efforts are being made by the researchers for improved outcomes in the patients.

Regenerative nanomedicine emerged as another aspect of therapy where it demonstrates a considerable capacity for repairing damaged heart tissue [12]. As injuries to the heart are often permanent due to the limited proliferation and self-healing capability of cardiomyocytes [13]. In this regard, the development of patient specific cardiac cells is recognized as a useful strategy to overcome this problem. Engraftment of the therapeutic cells illustrates little turnout, due to cell rejection activity of the immune system. To overcome this, compatible biomaterials are used, which display exctracellular matrix activity (ECM). Stem cell based therapy has broad applications in cardiac regenerative medicine. To replenish the functional cells to the heart induced pluripotent stem cells (iPSC) and iPSC-derived cardiomyocytes (iCM) presents a better opportunity. The regenerative aspect of the cardiac cells can be further addressed with developed functional biomimetic engineered cardiac tissues through precise control over cell-cell and cell-ECM interactions can mimic the biological properties of the native environment in some way. The ongoing activities for cardiac regeneration are emerging fast and demonstrating promising outcome in preclinical studies [14].

Plasmonic nanoparticles for cardiovascular disease are quite specific and useful at different wavelengths of irradiation. These nanoparticles are responsive to various optical response and exhibit important changes which are strongly influenced by surface plasmon resonance (SPR) which are extremely useful in biomedical applications [15, 16]. The clinical application of nanomedicines in CVDs is currently under various clinical trials. Functional restoration of the vessel wall is very challenging; in this regard, nanoburning technique is implemented which can demolish and reverse the plaque, especially in combination with stem cell technology. In a 5 year clinical cohort study: nanomedicine in the real-world clinical practice, wherein, NANOM first-in-man trial was evaluated with an intention-to-treat population (nano *vs* ferro *vs* stenting) to demolish and reverse the plaque, especially in combination with stem cell for promising functional restoration of the vessel wall by the process of nanoburning. Outcome of this trial demonstrated high safety with a better rate of mortality, target lesion revascularization, major adverse cardiovascular events at the long-term follow-up when compared with everolimus drug eluting coronary stent XIENCE V® [17]. A clinical trial using gold nanoparticles with silica-iron oxide shells *versus* stenting was evaluated for the treatment of atherosclerosis. For which bioengineered structure NANOM-PCI was used, which effectively showed the ability for high-energy plasmonic photothermic burning under the near-infrared laser irradiation on the lesion and reduces the volume of the plaque with most optimal long term approach compared to stenting [18]. In the following section of chapters: **section -1**, we discuss various aspects of nanomedicinal approach towards CVDs along with ethical issues pertaining to it. In **section-2**: we discuss the regenerative

CHAPTER 2

Nanomedicinal Aspects in Cardiovascular Diseases

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Abstract: There is hardly any approved drug product for a cardiovascular ailment that utilizes nanotechnology. Although there are a few products in this category, they do not claim to be nano-therapeutics. An exhaustive evaluation of clinical trial databases indicates that in the near future, we may see some drug products in the market in this category. There are several similar investigational products by different groups across the globe. A comprehensive collection of published literature augurs the inclination of scientists in this field. The use of nanotechnology in cardiovascular is beneficial owing to Critical Quality Attributes that can be imparted based on the size, spatial arrangement of drug molecules, release profile, *etc*. In some cases, drug release characteristics have to be in sync with circadian rhythm, which can be easily obtained using this technology. The section of the book tries to highlight some of the aspects related to the exploration of nanotechnology in the case of cardiovascular treatment.

Keywords: Cardiac ischemia, CT, MRI, Nanomedicine, PET.

INTRODUCTION

According to the WHO's records, cardiovascular diseases are found to be the top most leading cause of death. Approximately 17.9 million people die every year worldwide due to the severity of cardiovascular disease and incurs (*i.e.* 31%) of all deaths. Out of these deaths, 85% are due to heart attack and stroke [1]. Annually by the year 2030, nearly 12 million deaths are expected to be caused by coronary atherosclerosis, which includes acute coronary syndromes like ST segment (*i.e* interval between ventricular depolarization and ventricular repolarization) or non ST segment elevation myocardial infarction. Cardiac ischemia might not reverse promptly; leading to the initiation of irreversible cell death, contractile dysfunction and scars tissue development [2].

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Despite the era of advanced technology in our scientific and clinical area, the death rate of heart diseases still remains high. Nanomedicine is the transitional science of nanotechnologies in healthcare, which involves the mechanism that leads to the development of new pathways at the molecular stage for the development of novel therapeutics and diagnosis of cardiovascular disease (CVDs) [3]. Nanomedicine has various applications in therapeutics; nanotechnologies such as nanoemulsions, nanoparticles or nanodevices are used to penetrate the biological barriers. The nano-devices can contain encapsulated active molecules for the locoregional delivery of the targeted area. These are also used to cross the biological barrier for the systemic effect [4]. The advance of treatment modalities include pharmaceuticals, reconstitution by surgery and implantation of devices. Although these conventional treatment technologies provide a better quality of life but still needs improvement in therapeutics, or else it requires other alternative therapeutic approaches. Recent technologies of nanostructured systems, nanomedicines, nanoscience and nanotechnology have provided unique properties that can potentially overcome the limitations of conventional cardiovascular pharmaceutical medicines through the development of novel pharmaceutical nanomedicines and biomedical devices. Bioengineering perspective towards the diagnosis of atherosclerosis and other cardiac disorder provides exclusive opportunities for the diagnostic and management of these disorders. Further, studies on molecular engineering have provided new pathways that can potentially serve for diagnostic and therapeutic targeted delivery **(Fig. 1**).

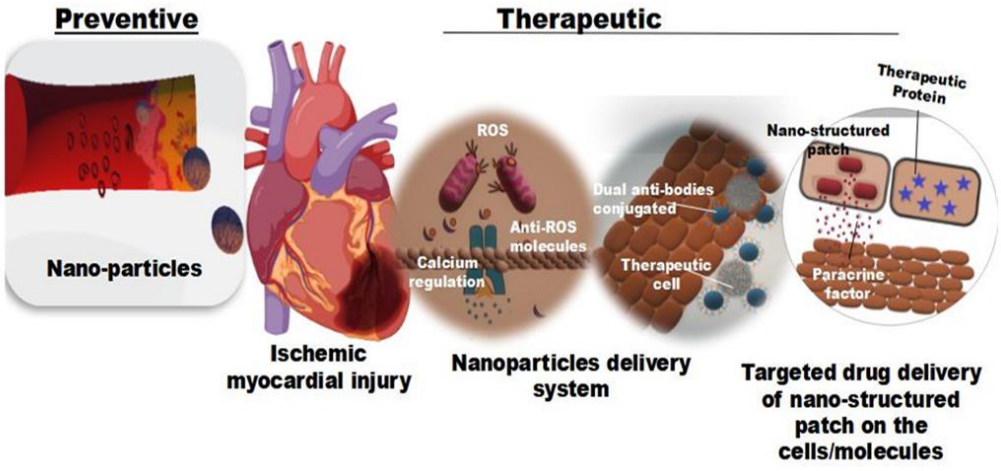

Fig. (1). Nanoparticles or nanostructured biomaterials can be used for delivery of cells and molecules and for targeted therapy of artherosclerosis or Ischaemic myocardial injury.

Radiotracers images obtained by various nuclear imaging techniques, such as single-photon emission computed tomography (SPECT) and positron emission, respectively (PET), are used to determine the cardiac disorder *via* exogenous administration whereas, for the anatomical imaging of arteries, advanced nanomedicine techniques are used including magnetic resonance imaging (MRI), ultrasonography (US) and computed tomography (CT) [5]. Drugs are commonly delivered either *via* oral route or needle based routes, such as intramuscular, intravenous or subcutaneous, which are painful for the patients. In the current technology platform, nanoparticles can be administered *via* intranasal route to treat cardiac disorder. As we know, inhalational delivery is a common route for a respiratory disorder. In one study, peptide –loaded nanoparticles are administered *via* inhalation for cardiac targeted therapy [6]. This study, reported that inhalation therapy is suitable for carrying peptide based nanoformulation to the cardiac disorder as it deals with biomineralized inspired technique without any toxic effect and lacks interference in the functional activity of the cardiac myocytes. For imaging aspect, nanoparticles are formulated with the contrast agent that are either attached on the particles surface, or encapsulated with fluorescent dyes in the matrix or a combination of both.

ADVANCES OF NANOMEDICINE FOR DIAGNOSIS OF CVDS

Vascular physiology under normal condition was found to be tight (< 2 nm) junction of endothelial membrane, which is responsible for preventing the penetration of nanoformulations. However, endothelial dysfunction creates gap in-between the cells, which enables the microparticles or nanoparticles to penetrate from the blood vessels at topical sites, in turn, unabling to get cleared owing to its lymphatic impairment. Development of contrast generating nanomaterials for the use of radioactive imaging, fluorescent, para/super-paramagnetic, LSP (light scattering particles) and electron dense method were sought by combining multiple contrast agents at nanoscale for the detection and analysis of cardiovascular disorders at an early stage. MRI for cardiovascular imaging requires powerful magnetic fields or radiofrequency waves for the diagnosis *via* internal structures scanning. The size dependent imaging properties of fluorescent nanoparticles enable the detection level from ultraviolet to mid-infrared range and its enhancement of emission wavelength are correlated with the particle size [7]. These multistage nanoparticles provide images of the target areas where macrophages are accumulated. Nanocontrast agents like ^{18}F-CLIO (^{18}F-cross linking with iron oxide) are used for the detection with PET and MRI. Three iron oxide nanoformulations such as AMI-121 (Ferumoxsil™); OMP50 and AMI-25 (Feridex) are approved for imaging by FDA [7].

CHAPTER 3

Role of Nanomedicine in the Diagnosis of Cardiovascular Diseases

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Abstract: Various lifestyle related factors are primarily responsible for the increase of cardiovascular diseases. The development of numerous diseases such as acute myocardial infarction, stroke and thrombosis need multiple therapies. These therapies are based on synthetic active ingredients, which in long-term usage, give adverse side effects. Currently, a lot of attention has been focused on nanotechnology based drug formulation, which can provide sustained drug release, increased half-life, and in turn, can circumvent limitations of conventional therapies. With the advent of the nanomedical approach, the survival length of the patients can be prolonged. This chapter mostly focuses on widely used nanomedicines in therapy and imaging of cardiovascular diseases.

Keywords: Dendrimers, Liposomes, Micelles, Nanocarrier, Nanomedicine, Polymeric.

INTRODUCTION

Cardiovascular diseases (CVDs) are one of the prominent reasons for the death of millions of lives. It is reported by WHO that cardiovascular complications claimed 17.7 million deaths in 2015 and the number is estimated to rise to 23.6 million by 2030 [1]. Dietary factors such as high intake of salt, sugar, saturated fat-rich diet as well as sedentary lifestyle increase the chances of CVD. Some other well-known contributors to the disease are genetic predisposition, age, diabetes, stress and obesity. With the disappearance of traditional cultures such as the consumption of organic food, stress-free life and heavy physical activity, the incidence of CVD is on the rise. Nevertheless, the epidemic, although declining in

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developed countries, is growing in developing countries. CVDs are a pool of heart diseases comprising congestive heart failure, coronary cardiac failure, myocardial infarction, inflammatory heart disease, deep vein thrombosis, *etc.* that result in tissue death and, eventually, mortality.

The rising numbers of statistics clearly indicate the need for newer treatments and technological developments to fight CVDs. The first stage in the pathogenesis of CVD complications is atherosclerosis. Atherosclerosis is the hardening of fat, cholesterol, calcium or macrophage cells overtime to form plaques. These plaques, which get deposited in the vasculature, result in congestion that severely hampers the blood flow and, in severe cases, it can even rupture the blood vessels leading to ischemia [2]. The introduction and approval of coronary stents by the US FDA in 1994 have seen major breakthroughs in the area. Other surgical methods, including bypass surgery and angioplasty even though can be modified for refinement but their efficiency is still questionable. Decades later, medicine still relies upon 'blockbuster' therapies such as beta-adrenergic blocking agents, diuretics and HMG-CoA reductase inhibitors for the treatment of vascular diseases. These treatments aim at reducing the build-up in the blood vessels and restore normal blood flow. Again, these drugs also present shortcomings such as significant adverse effects, poor response and lack of patient compliance [3]. Nanotechnology could be a promising potential for the delivery of drugs and genes for the management of cardiovascular problems (Fig. **1**).

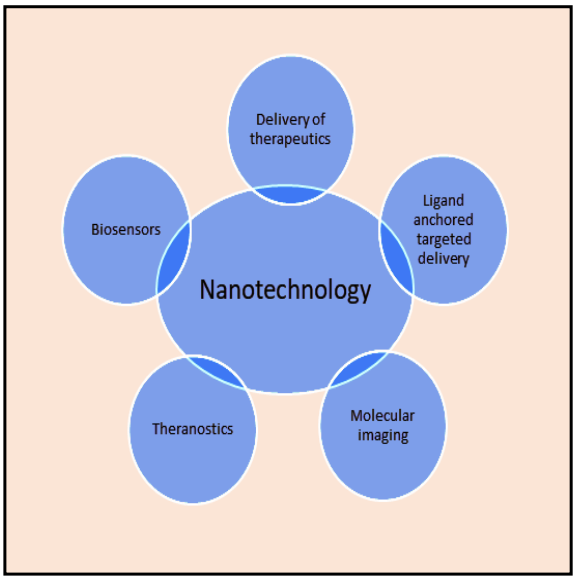

Fig. (1). Applications of nanotechnology in cardiovascular medicine.

Combination therapies encompassing delivery of multidrug in the same carrier are becoming increasingly popular. The various advancements in nanotechnology have led to reduced toxicity, reduced side effects and prolonged delivery of drugs [4]. Additionally, the technology can also be used to improve the performance of cardiac stents by providing nanomaterial coating or controlled release of therapeutics. The area of nanotechnology has also witnessed advances in target-specific molecular imaging. The early identification of a CVD is very crucial as it helps improve prognosis. Targeting can be achieved either through conjugation of a therapeutic molecule to a ligand or by coupling with a high molecular weight polymer that will enhance the penetration in vascular tissues [5]. These include liposomes, micelles, polymeric nanoparticles, dendrimers [6], *etc.,* which will be explained in detail in the following section. The different delivery systems used in nanotechnology have been figuratively detailed (Fig. **2**).

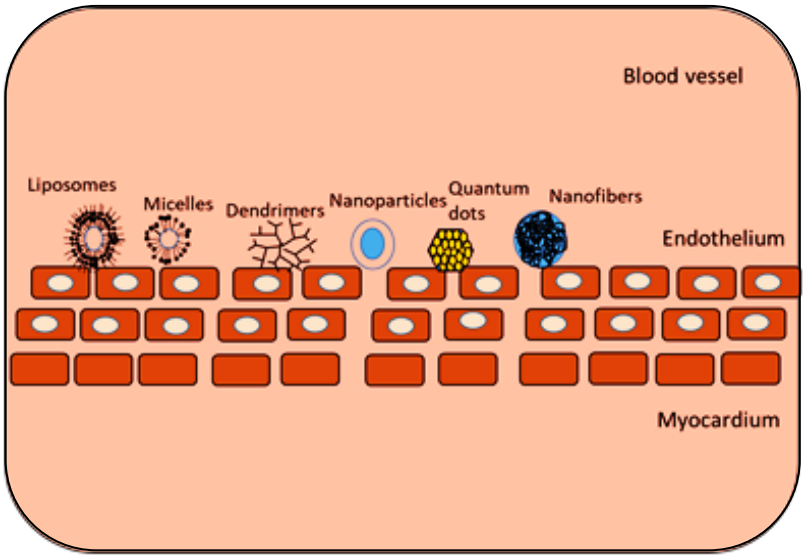

Fig. (2). The various nanodelivery systems used for the treatment of cardiovascular complications.

LIPOSOMES

Liposomes are essential vesicles, made up of phospholipids and cholesterol in their outer layers surrounding the hydrophilic core in sizes of 50-200 nm [7]. The delivery system can be used for the encapsulation of both lyophobic and lyophilic drugs. The type of liposome is small or large unilamellar vesicles and multilamellar vesicles are small-sized single lipid bilayer vesicles, large-sized single lipid bilayer vesicles or multiple layered bilayer vesicles, respectively affect the drug loading [8]. Therapeutic delivery of CVD drugs faces a number of

CHAPTER 4

Nanotechnology-Based Molecular Imaging in Cardiovascular Disease

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Abstract: Nanoparticulate formulations have been valuable imaging tools in preclinical cardiovascular disease research. Nanocarriers' distinct properties are useful to carry out imaging with significant functional versatility, which is not achieved by traditional small-molecule agents. Various cardiovascular diseases (CVDs) require molecular and cellular mechanisms understanding, which will provide valuable insight towards theranostic (diagnostic and therapeutic) applications. Nanocarriers and radiolabeled nanoparticulate probes demonstrate their utility in several CVDs applications such as blood pool imaging and molecular imaging of ischemia, angiogenesis, atherosclerosis, and inflammation. Further, these emergent technologies need to address safety, toxicity and regulatory obligations for their clinical translation.

Keywords: Microbubble, Nanoparticles. Imaging, Quantum dots.

INTRODUCTION

Recently emphasis has been driven towards the primary or secondary prevention mode of treatment of cardiovascular disease. Consequent improvement of human health would lead to a quality life. Shifting towards the prevention of disease invites new challenges for translational research and stimulates the technological revolution of existing diagnostic procedures. For an easy and accurate diagnosis and therapy, molecular imaging plays a crucial role and has evolved as a fast-growing research field. Through this approach, vital information of physiologic, anatomic and molecular aspects of the disease can be obtained [1]. Currently, nanoparticle-based formulations have garnered enormous attention for the application of cardiovascular imaging and therapeutic delivery due to their better pharmacokinetic and biodistribution performance than that of small molecules [1].

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Outstandingly, nanoparticulate imaging agents are very helpful in signal amplification; these agents can be functionalized with functional entities such as targeting ligands for precise specificity. Imaging platform with the aid of nanoparticulate agents plays a significant role in imaging clots, thrombus, apoptosis-linked gene expression *etc*, which can be visualized by tracking. In imaging, the prime application is to image clogged blood vessels, defective valves, damaged heart muscles, *etc*. Till now, nanoparticle-based imaging agents have achieved inadequate clinical access as they require additional developmental features to overcome various functional limitations and related safety concerns. Further nanoparticles display the capability of cellular guidance in certain cardiovascular applications (Fig. **1**).

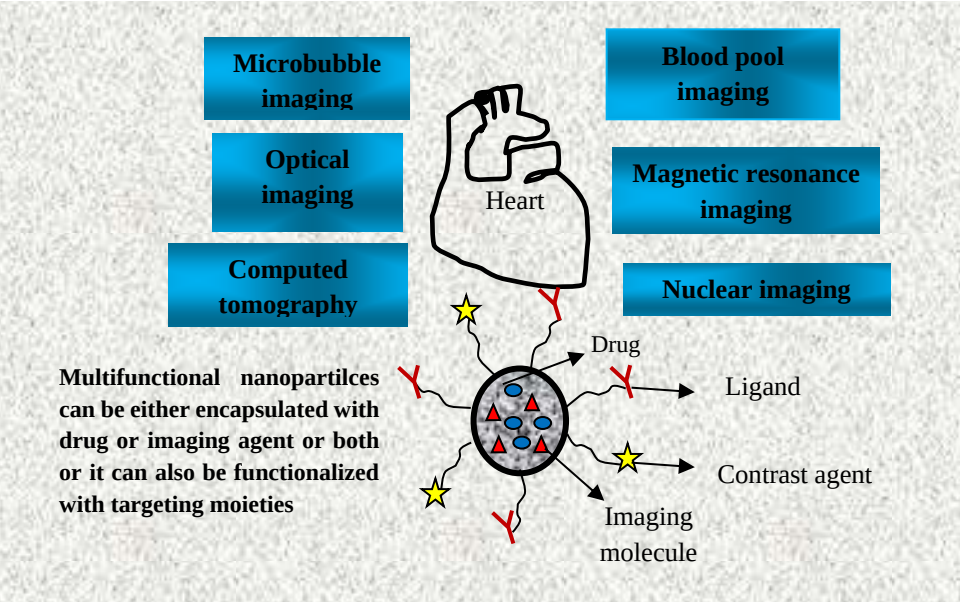

Fig. (1). Usage of multifunctional nanoparticles for various molecular imaging facility.

Nanoparticle-based cardiovascular imaging through Computed Tomography (CT), Magnetic Resonance Imaging (MRI), Positron Emission Tomography (PET) and Single Photon Emission Computed Tomography (SPECT) and its radiolabeled nanoparticulate probes have illustrated preclinical applications in blood pool imaging and molecular imaging of ischemia, angiogenesis, atherosclerosis, and inflammation [2 - 4]. In this chapter, various imaging modalities mediated through nanoparticles are discussed.

MICROBUBBLE BASED IMAGING

Microbubbles are the small gaseous particles between 1 and 10 μm, that are

scattered with ultrasound waves giving enhanced contrast to the image during an echocardiogram [5]. An echocardiogram is a sonographic based imaging technique, which is an exquisite tool for the non-invasive and real-time diagnosis of physiological tissues and requires contrast agents for providing backscatter of sound waves to scanner head, which are usually gas-filled microbubbles (1-5μm in diameter) that are re-stabilized either with lipid, protein, polymers, or a mixture of these [6, 7]. Looking at the potentiality, researchers have engineered microbubbles to enable drug loading, improve circulation time, increase stability as well as molecular targeting [8]. Recently to provide multifunctionality, nanoparticles and microbubbles are complexed together. This complexion stabilizes the bubbles by modifying the interfacial tension and diffusivity of a gas bubble in liquid [9]. In an attempt, Dixon *et al*., conjugated gold nanorods onto the lipid shell of the microbubbles through the gold–thiol linkage under the presence of the (pyridyldithio)propionyl group for *in vivo* photoacoustic imaging [10]. Ke *et al*., complexed quantum dots and microbubbles *via* the electrostatic layer-by-layer technique that responded to the medical ultrasound to act as a contrast-enhanced agent [11]. In another study, Yang *et al*., developed dual contrast agents of magnetic resonance and ultrasound imaging by embedding superparamagnetic iron oxide nanoparticle-microbubbles for imaging [12]. Molecular imaging is of paramount importance as it provides access to the identification of specific cell-surface tissue functionality receptors with targeted contrast agents. Lanza *et al*., used a ligand-targeted acoustic nanoparticle system to identify the angioplasty-induced expression of tissue factor by smooth muscle cells within the tunica media. The result illustrated that the nanoemulsion was able to infiltrate into arterial walls after balloon injury and localize the expression of overstretch-induced tissue factor within pig carotid arteries proving to be a prognostically important predictor of subsequent restenosis [13]. Hamilton *et al*., developed targeted echogenic immunoliposomes (ELIPs) for enhancement of intravascular ultrasound imaging of atherosclerosis. By functionalizing with anti-ICAM, anti-fibrinogen, anti-fibrin, and anti-VCAM antibodies (Ab/Abs), early and later atheroma components can be targeted by imaging [14]. Tiukinhoy-Laing *et al*., entrapped tissue-plasminogen activator (tPA) for fibrin-targeted, ultrasound-directed and enhanced local delivery of a thrombolytic agent. After administration of tPA–ELIP the clot images are highlighted when compared to control [15]. VEGF peptides have angiogenic potential and resulted in therapeutic effectiveness. Hwang *et al*., developed chitosan hydrogel nanoparticles loaded with vascular endothelial growth factor (VEGF) for the treatment of myocardial ischemia. Administration of chitosan peptides reduced the intensity of perfusion defects and increased vascular density as compared to control [16].

Optical Imaging: It is a non-invasive technique that uses light to probe cellular and molecular function in the living body. The most fruitful nanotechnology

CHAPTER 5

Nanocarriers for Therapeutics Delivery of Cardiovascular Disease

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Abstract: Cardiovascular diseases are presently the leading cause of death worldwide. Prescribed drugs in terms of therapeutic modalities of clinical management have treated patients, but still a limitation exists. This administered drug displays unwanted health adversity due to the side effects. In this regard, the current focus has been drawn towards nanomedicine-based drug formulation, which illustrates better therapeutic ability, sustained release, bioavailability and less toxicity. To address this, various nanocarriers are developed and are being involved in and studied for clinical application. In this chapter, the potential application of different nanocarrier-based therapeutic delivery has been discussed.

Keywords: Coronary artery disease, Dendrimers, Liposomes, Nanomedicine, Polymeric.

INTRODUCTION

Cardiovascular diseases (CVDs) and administered medicine in the form of nanosize is called cardiac nanomedicine. With the advent of nanoformulation, the focus has been driven towards the establishment of innovative solutions to meet the challenge of current CVD treatments. Nanomedicine is the fastest emerging research area that can revolutionize the CVDs care system. The major emphasis of designing nanoformulation is to improve the pre-existing drugs' bioavailability, stability and safety. The nanoformulative drugs are continuously being improved to achieve protection from systemic degradation, to demonstrate reduced toxicity, immunogenicity, improved pharmacokinetics and increased half-life, bioavailability and precise biodistribution [1]. For targeted therapeutic application, these nanoparticles can be further functionalized with different classes of targeting moieties for selective delivery to the site of interest [2].

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Various administrative routes, such as inhalation, oral administration, or intravenous injection, can be taken into account for CVDs. These nanocarriers can enter into the system through various strategies (Fig. **1**).

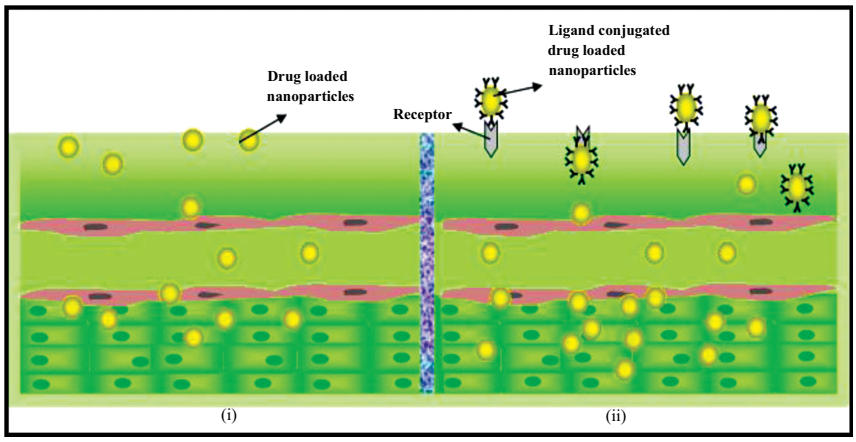

Fig. (1). (i) Passive targeting: In CVDs the chronic inflammatory process the increase of vascular permeability permits the drug loaded nanoparticles to pass through the blood vessels and reach the target site, **(ii)** Active targeting, the ligand conjugated nanoparticles are captured by the overexpressed receptors and are released at the site of action.

PASSIVE TARGETING

The passive targeting is diffusion mediated, as the microvascular endothelial cell space in normal tissue is dense and intact, but in the case of a tumor, the endothelial cells are richly vascularised, which has poor structural integrity and impaired lymphatic drainage system [3, 4]. In this case, the nanosized particles pass easily through the vascular wall and remain endocytosed in the tumor tissues, where they are largely accumulated due to the enhanced permeability and retention (EPR) effect [5]. The EPR effect is not only applicable to tumor tissues, but it can also be used in various CVD, such as the occurrence, and development of atherosclerosis (AS) is a chronic inflammatory process, where vascular permeability is often increased, which is very similar to that of solid tumors. The vascular endothelial permeability offers an effective means for the delivery of nanocarrier to deliver from the lumen side to the interior of the plaque. In another strategy, the nanocarriers can also enter the circulation and get ingested by the inflammatory cells (monocytes or macrophages) and further, these nanocarriers can migrate to plaque inflammation, allowing drugs to be delivered [6]. Additionally, for longer circulation in the blood-stream, the nanocarriers are PEGylated to decrease the immunogenicity, opsonization, and phagocytosis [7].

ACTIVE TARGETING

During the course of the development of CVDs, various vascular endothelial cells are in an inflammatory activation state, where certain molecules, such as intercellular cell adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), integrins, selectins, *etc*. are often overexpressed. These molecules can be functionalized with the nanocarriers for active targeting. These targeting moieties can bind effectively to the receptor sites of inflammation before they were taken up by endothelial cells [8]. Whether it is passive targeting or active targeting, the ultimate targeting efficiency is highly dependent upon the physicochemical properties (such as, particle size and distribution, targeting unit types, surface chemistry, morphology and density) of the nanoparticles [9]. Inside the body, different intrinsic biological factors (such as development stage, type as well as location of CVD and tumor, vascular wall shear rate, blood composition and its fluid type) also play an important role which greatly affects the targeting efficiency [10]. Moreover, the active targeting of the nanocarriers in clinical diagnosis and therapy is enormously attractive, but still, it faces great challenges because of the limitation of the discovery of an ideal target. Secondly, to combat many bottleneck problems of targeting, the formulation parameters need to be designed effectively.

NANOCARRIERS FOR THERAPEUTICS DELIVERY IN CVDS

Nanocarriers are the transport moiety, which is designed for drug loading and ligand functionalization. Most commonly used nanocarriers are micelles, polymeric, carbon-based materials, liposomes, dendrimers and other substances. Widely three types of nanoparticles (Fig. **2**) are used for cardiovascular study, which is being discussed below.

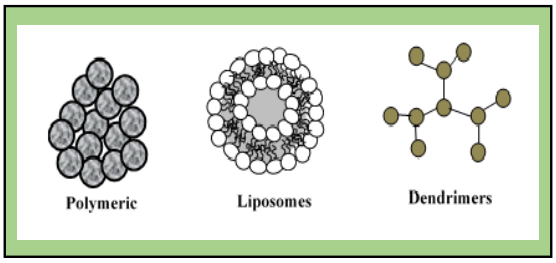

Fig. (2). Nanoparticles widely used for Cardiovascular disease.

POLYMERIC NANOMEDICINE

Ischaemic cardiac complication occurs when loss of blood supply to the tissue is lost, leading to cell death. With due course of time, the damage spreads, and the

CHAPTER 6

Nanocarriers for Theranostics Delivery of Cardiovascular Diseases

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Abstract: Cardiovascular diseases (CVDs), predominant global disabilities, are solely responsible for causing a significant number of deaths annually. The arising issues of conventional therapeutics, such as high insufficiency in reducing disease progression, unpleasant side effects, *etc.,* have made CVDs a significant clinical challenge. Henceforth, the exploration of newer technologies and strategies for CVD management has become a need of present times. Recently, CVDs have become a major area of focus for medical, scientific and technological development. One such area of particular interest is an advancement of nanoparticle drug delivery systems for targeting CVDs, which offers a bouquet of positive attributes such as a high - targeted approach for specific disease sites, drug bioavailability and functional payloads. The present article mainly emphasis on the growing concept of 'theranostic nanoparticles or nanotheranostics' in the field of CVDs. The term 'Theranostic' combinedly refers to the union of diagnostics and therapeutics, with the purpose of enhancing the safety and efficacy of the treatment. Although it is still in its infancy stage for cardiovascular complications, the idea of theranostics has already been applied in the field of oncology and is giving fruitful results as well. The present chapter gives its description in the field of diagnosis as well as therapeutics, which may improve the status of nanomedical research in CVDs.

Keywords: Drug delivery, Nanomedicine, Nanoparticles, Nanotheranostics, Theranostics.

INTRODUCTION

Theranostics is a novel approach to clinical medicine that combines therapeutics and diagnostics with the purpose of enhancing the safety and efficacy of the treatment, such as nanoparticle drug delivery systems (NDDS) [1]. In simple terms, theranostics can be defined as an advanced diagnostic process that is

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equipped with a therapeutic moiety (drug)/device for improving site-specific targeted approach, drug bioavailability/safety/efficacy, functional payloads and to reduce the related steps and incurred costs of the treatment. Presently, theranostics have garnered wide acceptability and expectations for medical, scientific and technological development because of its multipronged approach [2]. In nanotheranostics, nanoparticles can themselves act as diagnostic probes (imaging/contrast agent) and get conjugated with therapeutic or diagnostic molecules/moiety or vice versa; thus, they can be cited as one of the best examples of theranostics (Fig. **1**).

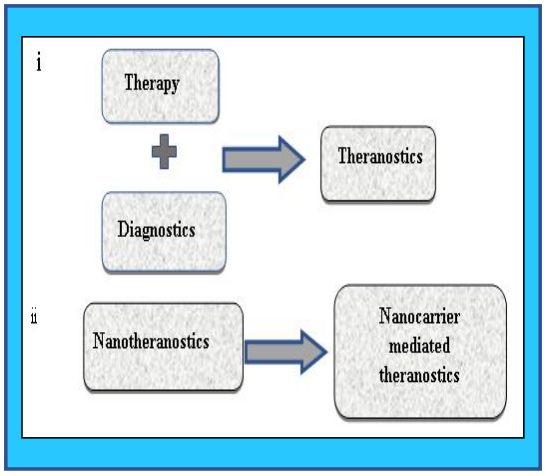

Fig. (1). (i) The model describes the combination of therapy and diagnostic leading to the formation of thernostics **(ii)** the nanotheranostics can be used with *via* nanocarriers.

The advancement of nanobased therapeutics has garnered worldwide attention in major disciplines, such as engineering, chemistry, biology, pharmaceutical science and medicine. Moreover, the synergistic combination of a nanoparticulate approach with theranostics has yielded a novel premise for research and development in the aforementioned field, which is known as 'Nanotheranostics'. Presently, it is cited as a multidisciplinary as well as multipronged approach owing to its great potentials in the field of human healthcare and well-being. It also has played a prominent role in disease treatment and its characterization *via* the development of various biomarkers and personalized medicines. The novel approach of theranostics nanoparticles allows for the simultaneous determination of drug localization, release and efficacy in a variety of conditions such as atherosclerosis [2]. Atherosclerosis is a prevalent cardiovascular complication that can only be detected after the onset of clinical symptoms; hence there is an emerging need to diagnose as well as to treat it as early as possible. The various targets for imaging the plaques are endothelia, fibrin, macrophages as well as

various markers of angiogenesis. In atherosclerosis, theranostics nanoparticles imaging capabilities validate the drug to reach the targeted site of action and screen its effect on a molecular level. It also designs dosage regimens and identifies the population that responds to a particular therapy [2]. Although the concept of theranostics has not been applied too much in cardiovascular complications in the near future, it may yield excellent results in the diagnosis as well as therapeutics, which may improve the status of nanomedical research. The 3 verticals of the theranostics nano-approach (Fig. **2**) are introduced below.

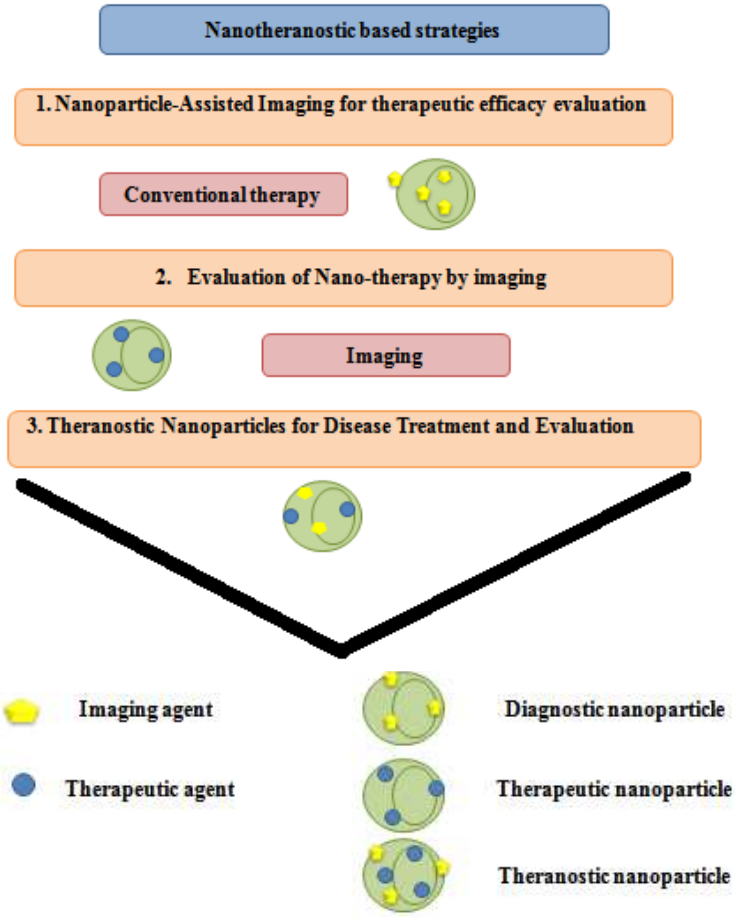

Fig. (2). Outlines of nanotheranostic based approach.

THERANOSTICS IN NANOMEDICINAL RESEARCH

Nanoparticles (NPs) are a widely known nanosized particulate system having wide applications for various diseases. The combination of NP with theranostics opens up a wide array of research owing to its high no. of positive attributes. One

CHAPTER 7

Nanocarriers for Biologicals Delivery to Cardiovascular System

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Abstract: As cardiovascular diseases remain the leading cause of mortality worldwide, a large number of clinical trials are under development, investigating the safety and efficacy of RNA therapeutics in clinical conditions. Nanomedicine based drug delivery systems are currently the new avenue for the treatment of CVDs, providing great advantages to the treatment regime of CVDs. Currently, antisense therapy DNA- and RNA-based and microRNAs are widely applied therapeutic strategies to regulate gene expression and its effect on CVDs. In this review, different biological-based targeting therapies for cardiovascular diseases and their outcomes are discussed.

Keywords: DNA, miRNA, Nanomedicine, Nanoparticles, RNA interference, siRNA.

INTRODUCTION

Cardiovascular diseases are the leading cause of death and disability, surpassing infectious diseases due to lifestyle changes in developing as well as developed countries. Prescribed medicines do provide enormous benefits to the patients, but the adverse effect on the kidney and liver can never be negated. In this regard, advanced studies in RNA biology have been achieved with the help of microRNAs and short interfering RNAs, which regulate various cellular processes across the eukaryotes. RNA interference (RNAi) offers the possibility to silence every defectively expressed gene in a given disease. The structural similarities of siRNA and miRNA have been well explored for cardiovascular disease applications. In the mammalian system, the miRNAs are encoded in the genome, processed in the nucleus by the cell of origin, whereas siRNAs are exogenously delivered.

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While siRNAs lead to degradation of target mRNA, miRNAs lead to either degradation or translational inhibition of target mRNA (Fig. **1**).

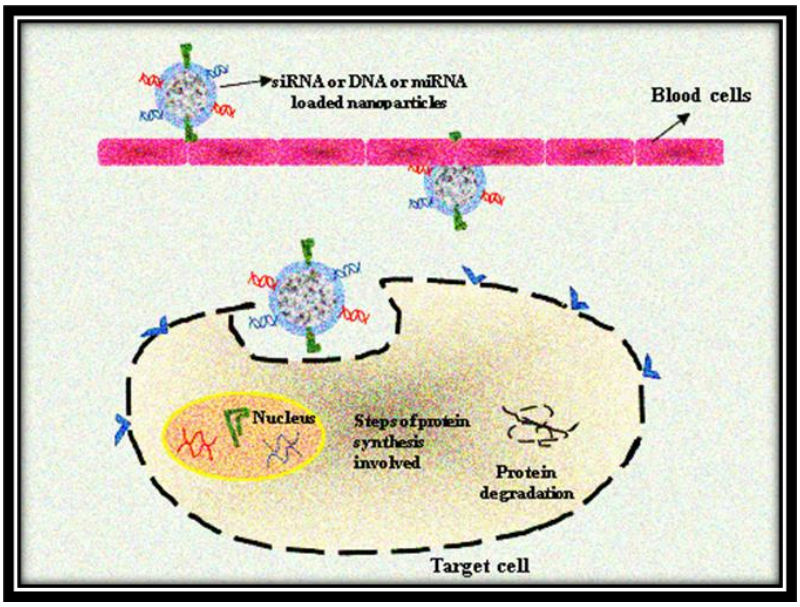

Fig. (1). General process of entry of siRNA, DNA or miRNA through the blood vessels to the target cells and degradation of its target protein.

Systemic delivery of siRNA nanovehicles has been explored for cardiovascular disease applications. Nanomaterials-based delivery of siRNA (or miRNA) hold great promise for gene therapy in cardiac diseases. For cardiac application, siRNA (or miRNA) therapy-based cardiac targeting still remains an issue, and several strategies are used for the improvement of cardiac uptake. siRNA (or miRNA) therapy strategies are used for the improvement of cardiovascular disease with encouraging results. A growing understanding of cardiac science can open up new facets for the successful functioning of siRNA and miRNA for better therapeutic outcomes. With the help of suitably designed siRNA, the RNAi machinery can be utilized for silencing any gene in the body and providing a better therapeutic effect than other typical small drug molecules [1]. Various studies have documented the efficacy of the synthetic siRNA's knocking down targets *in vivo* . Therefore, methods are applied to target only the cardiovascular system, which has shown minimal side effects. But for achieving the desired clinical outcomes, a safe and effective delivery system for siRNA is of paramount importance. Over the last decades, progress in nanotechnology has been implemented to develop nanomedicine for CVD therapies. A variety of nanoparticles-based formulations have been developed containing drugs, proteins, and biologics have been tested

pre-clinically to provide some encouraging results for cardiovascular-specific applications, such as hypercholesterolaemia [2], hepatitis [3], liver cirrhosis [4], bone cancer [5] and ovarian cancer [6].

SIRNA BASED APPLICATIONS

Small interference RNA (siRNA) technology effectively attenuates specific proteins *in vivo* by the degradation of mRNA [7]. However, siRNA being a large molecule with a negative charge faces problems while crossing the cell membrane [8]. To combat these challenges, nanoparticles mediated delivery vehicle is chosen. Various delivery systems have been developed for targeting the siRNA in cardiovascular diseases. Li *et al*. developed an amino-acid based nanoparticle HB-OLD 7 for local delivery of siRNA targeting NOX2 to the arterial wall. In an atherosclerotic rat model after angioplasty, the siRNA nanoparticles were successfully transferred into regional carotid artery walls. The Cybb gene expression was reduced to > 87% compared to angioplastic controls [9]. In another study, Frank-Kamenetsky, Maria *et al*. developed cross-species, siRNA proprotein convertase subtilins/kexin type 9 (PCSK9) and targeted it to non-human primate-like murine rat. PCSK9 regulates low-density lipoprotein receptor (LDLR). For the study, they formulated siRNA lipidoid nanoparticles and studied their targeting efficacy in mice and rat models. The liver-specific siRNA silencing decreases the PCSK9 mRNA levels by ~ 50-70% that isassociated with a reduction in plasma cholesterol concentration of ~ 60%. They suggested that the targeting of PCSK9 can be a treatment strategy for hypercholesterolemia treatment [2]. Gene silencing stent is a promising approach for regeneration of vascular endothelial cell wall and inhibition of restenosis. Gene silencing stents consist of specific siRNAs which are released to the vascular endothelial cell wall. For combating coronary diseases, Hossfeld *et al*. developed a layer-by-layer technology of multilayers of chitosan and hyaluronic acid (HA) nanoparticles. The coated stents were evaluated in an ex-vivo model with porcine carotid arteries, which provoked the uptake of siRNA /chitosan nanoparticles into the artery walls and long-term siRNA release, illustrating it as a powerful technique to prevent restenosis [10]. Silencing C-C chemokine receptor type 2 (CCR2) provides cellular specificity for this emerging therapeutic target. Therefore, the use of nanoparticles can be an effective strategy for siRNA to prevent the inflammatory cycle of atherosclerosis. Monocytes and macrophages participate critically in the inflammatory activity of various major diseases of atherosclerosis and cancer. For the reduction of inflammation, either monocyte-chemotactic protein (MCP)-1 or CCR2 genetic deletion can result in a profound reduction of inflammation in various disease models [11 - 13]. In a study, Leuschner *et al*. prepared siRNACCR2 lipid nanoformulation for systemic delivery. The results revealed silence of CCR2 gene along with significant reduction of plaque [14]. In

CHAPTER 8

Ethics and Regulations for Cardiovascular Diseases

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Abstract: Ethics deals with human values and conduct, with respect to right and wrong in certain motives. The rapid development of therapeutics has raised a number of ethical issues pertaining to philosophical, legal, religious and moral beliefs. With the advancement of science, inclusion of ethical values is of absolute necessity in different aspects of clinical practice. The introduction of nanotechnology has helped in fighting many complex illnesses, including cardiovascular diseases. Nanotechnology may provide a solution for better prognoses and a reduced side effect. In this regard, cardiovascular therapeutic involvement needs a testimony with respect to societal benefits for developing a code of ethics.

Keywords: Autonomy, Beneficence, Ethics, Morality.

INTRODUCTION

Cardiovascular diseases (CVDs) are a group of disorders related to the heart and blood vessels. CVDs have become one of the common causes of death due to unhealthy diet, physical inactivity, alcohol and tobacco use, leading to 31% of deaths worldwide [1]. Apart from that, coping up with urbanization, intense involvement in industrialized work and related lifestyle changes have been associated with high blood pressure, raised blood lipids, high blood glucose levels leading to overweight and obesity, and an increased risk of developing a heart stroke, heart failure and related complications. The related severe complications of CVDs are a very frequent reason for the hospitalization of patients. However, modern treatments have intensely improved the quality of life of cases with

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hypertension, valvular heart disease, coronary artery disease, and mild heart failure.

Patients with chronic CVD problems, such as congestive heart failure, need continued medical support with the intervention of expensive medical care for their long-term survival [2]. Beyond medication, technology has advanced with the supply of medical devices to improve symptom burden and prolong the patients' survival. The CVDs therapeutic activity has broadened from being principally curative to being preventive [3, 4]. The identification of various factors responsible for CVD diseases is required to predict the risk associated with CVD diseases and implement diagnostic methods for early detection and prevention. In clinical research trials, many invasive cardiologists are involved. Frequently, invasive cardiologists usually perform dual roles as a clinical researcher as well as a treating clinician. Often this is programmed by the industry to evaluate their products and enroll patients in their trials for which they provide a variety of incentives and financial aids to the clinical researchers. While doing this activity, clinicians have to remember all the time that their first commitment is towards their patient. However, during the course of treatment, if the clinician gets to know that there is another avenue for the patient, then the clinician-researcher should withdraw the subject (enrolled patient) from the research trial supporting subject's best interest. For doing this kind of dual-mode research and treatment, the patients must be fully informed. Further, if critically ill patients are involved, then great caution should be taken into account [5].

Cardiovascular diseases are multivarious in nature; therefore, several guidelines have been established for their prevention in clinical practice while taking into the account their scientific understanding. In the case of symptomatic treatment, the drugs are advised by the clinicians for purely preventive purposes, whereas with the advancement of the disease, sometimes the intervention of specific medical devices pertaining to the ailment is needed. This may pose some kind of ethical dilemma. In that case, the cost-effectiveness of the treatment for the patient can somehow increase the pressure on the medical community. Therefore, the medical fraternity should take into consideration the financial burden while evaluating the potential benefit as well as the long-term consequence of the therapeutic interventions. While dealing with the increased number of cardiac-related medical cases, complex societal bound medical care and patient's budgetary restrictions sometimes remainone of the major decisive factors. In this case, preventive medicine should remain as one of the choices in clinical practice. The principal values of medical ethics and their application in different aspects of secondary and primary CVDs prevention are discussed in the following section.Since the earliest recorded history of medicine, "Codes of medical ethics" have existed. With time, the medical practice has evolved and modified. The American Medical

Association (AMA) first adopted a general code of ethics in 1847 and revised it most recently in 1997 [6]. In medicine, "medical ethics" is the study of moral values and judgements. The medical fraternity treating CVDs should set the Code of Ethics in their clinical practice and should have certain ethical obligations to their patients, as well as to the community and world at large. The clinicians being a member of the medical profession must respect, recognize, and adhere to these obligations [7, 8]. The main principles that are laid down in "Code of Ethics" are mentioned below: (i) Beneficence; actions in the best interests of patients (ii) Primum non nocere: first of all, do no harm to patients (iii) Autonomy; right of the patients to choose or refuse their treatment (iv) Informed consent; truthfulness and honesty and (v) Justice; fairness in the distribution of health resources [9, 10].

GENERAL ETHICAL IMPLICATIONS

Actions in the best interests of patients (Beneficence): (i) The ethical principle of 'beneficence' states that the clinicians should make an attempt for the improvement of patient's well-being with a provision of maximal clinical benefits while minimizing clinical harms. (ii) First, do no harm (primum non nocere; non-maleficence): The principle of 'non-maleficence' urges the clinicians not to cause any intentional harm for incurring greater benefits; rather, they should respect patients' clinical concerns. (iii) In the case of autonomy, the patient has the absolute right to choose or refuse their treatment and can make a rational uninfluenced decision whether to get treated under a particular clinician or not. (iv) truthfulness and honesty (informed consent). The principle of informed consent is applicable to the patients, where the patient is fully informed regarding the understandability of the potential benefits and risks of their choice of treatment, whereas an uninformed person can be at risk of mistakenly making a choice of not knowing the facts. (v) fairness in the distribution of health resources (justice). In this case, the clinicians are the best advocate to suggest what type of treatment is necessary for the patient, and accordingly take decision as to what kind of treatment should be given to whom after looking at the availability of the health resources. These principles can be applied when the benefits and risks for interventions are clear, but in uncertainty, it can be challenging. In this regard, patient-centered approach can be applied to involve (vi) the ethical principle 'respect for persons' in which the clinicians are required to respect the autonomy of patients and to function as a chaperone in accordance with patients' values, beliefs and preferences.

CONSENT FOR PUBLICATION

Not Applicable.

CHAPTER 9

Biomaterials for Cardiac Regeneration

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Abstract: Globally, cardiovascular disease is one of the predominant clinical conditions, which accounts for about 50% of human mortality and morbidity. No doubt pharmacological and surgical interventions have dramatically improved the quality of life of patients with cardiovascular diseases. However, the demand for new therapeutic interventions as well as regenerative strategies is currently increasing. Biomaterials, both natural and synthetic, have exhibited great potential in cardiac regenerative therapy. Therefore, the development of biomaterials based extracellular matrix, grafts or stents, *etc*. would be highly beneficial for supporting the natural function and physiology of heart tissues.

Keywords: Biohybrid vascular grafts, Bioresorbable stents, Bioceramics, Cardiac patches, Extracellular matrix, Hydrogels, Implants, Scaffolds.

INTRODUCTION

Heart is one of the major organs of the human body which starts functioning in the first three weeks of gestation. Any disease or condition either relating to or acting on the heart is medically termed as 'Cardiac'. Normally, the human heart pumps approx. 175–224 millionliters of blood throughout the body with the help of a unique subset of cells, cardiomyocytes, present in the heart muscle (myocardium). These cells are intimately connected to one another through gap junctions and produce a synchronous contractile response to electrical stimulations. The human heart is composed of a diverse cellular structure, mainly cardiomyocytes (contractile elements), smooth muscle cells, fibroblasts, blood vessels, nerves and the extracellular matrix (ECM) components (collagen). Cardiomyocytes normally have the limited ability to proliferate (average proliferation rate < 1% annually). Any impairment or disability of the tissues and

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cells may lead to irreversible heart damage such as ischemic/ hypertensive/ valvular heart disease, myocardial infarction, *etc*., which can subsequently lead to a condition of heart failure (impairment of blood pumping efficiency) [1, 2].

Therefore, novel treatment approaches are urgently needed to control and regulate the rampant prevalence of cardiac diseases. Conventional treatment modalities such as medications, surgical interventions (bypass grafts, valvular change, stenting, *etc*.) are marred with various challenges. Further, in acute cases, heart transplants are highly unlikely due to the chronic shortage of human tissue donors. In order to expand the patient care options, alternative therapies based on biomaterials are utilized for repairing, reconstructing or regenerating damaged/impaired heart tissues or cells [3 - 7]. Biomaterials (BM), in general, can be defined as foreign, non-drug material that is accepted to be used inside the body for the purpose of enhancement of tissue/organ functions, as well as to repair the damaged/impaired organ(s) in a variety of clinical conditions. BMs can either be synthetic or natural and are mostly biocompatible in nature so that they can have safe interaction with the biological system (body fluids, soft tissues, *etc*.). The term biocompatibility generally signifies its patient compliance, mechanical as well as biological properties in varied processes of implantation, cardiovascular repair/regeneration, tissue replacement, *etc*. The BM should possess the properties of durability, strength, flexibility and minimum ability to elicit any adverse reactions. They are generally integrated into a device or can be used on their own for replacement purposes in order to mimic the characteristics of the required heart tissue. Whereas, their biological properties are anti-thrombogenicity, hemostasis, endothelialization, non-calcification, non-immunogenicity, non-toxicity and inertness [8, 9]. Historically, the use of BMs for medicinal purposes is a widely evolving global concept. Ancient Phoenicians have exploited, for dental work, gold wires like BM to hold artificial teeth with the real ones [1]. Further, in the era of the 19th century, artificial bone plates were introduced to help to stabilize bone fracture and to facilitate the healing process. Currently, the use of BMs has been extended to the development of cardiovascular components such as heart valves, blood vessels, scaffolds, gauges, *etc*., thereby marking their efficiency in the field of cardiac tissue repair and regeneration [10]. The chapter explores the future prospects in terms of biomaterials-based regenerative medicines and also highlights the bioethical aspects of safety, regulatory and ethical issues.

TYPES OF BIOMATERIALS

Biomaterials can be broadly classified into four main categories, depending upon the nature of the source as presented below:

Natural

These are mainly those BMs which are derived from plant or animal sources such as collagen, alginate, coral, keratin, cellulose, *etc*. Naturally derived BMs can also be obtained from humans, either as auto-graft, allograft or iso-graft. They can be either used on their own or in conjugation with a device. Most often, natural derived BMs are basically used for healing purposes rather than replacement therapy [9 - 11]. Natural BMs could be either (i) Xenogenic (animal-derived), which can be used for developing pericardium, collagen patch, or for septal defect repair [12]. (ii) Allogenic (same-species donor), which can be employed for creating pericardium, fibro serous sac surrounding the mammalian heart, for reconstruction, valve repair and aortic root enlargement [13] or (iii) Autogenic (same individual), wherein the BMs can be used for valve replacement [13, 14].

Metal-based Biomaterials

Metals are the most widely used material for BMs that can be either of pure metals or alloys. Due to various intrinsic properties of metals such as tensile strength, relative inertness, malleability and ductility, and higher resistance to mechanical wear, they are used to construct a wide array of parts such as wires, screws, stents, bone plates, prosthetic limbs, and joint replacement, among others. The most commonly used metals are stainless steel, titanium, gold and silver which are mostly non-reactive and biocompatible in nature. Alloys such as titanium and cobalt are also used. However, these metal-derived BMs have the drawback of corrosion, which limits their use for long-term therapy [9 - 11]. A few common examples of metal BMs and their applications are presented below: (i) Metals stents for the purpose of opening lumen in obstructed heart vessels. The classic examples are titanium and stainless steel; however, owing to their comparative greater strength, the present stent design also utilizes cobalt-chromium, platinum-chromium and nickel-titanium alloys [15 - 17]. (ii) to minimize the risk of blood clot formation, mechanical replacement of heart valves using stainless steel or titanium is utilized [18, 19].

Ceramics-based Biomaterials

Ceramic biomaterials or bioceramics are refractory polycrystalline compounds that are known to be widely used in dentistry over very long periods. Usually, they are inorganic, highly inert, hard, brittle, and compressive with good electric and thermal insulation strength. Additionally, the aesthetic appearance of bioceramics highly compliments the aforementioned attributes, making them a better choice for dentistry applications. However, drawbacks like poor fracture, toughness and biocompatibility issue limit the incorporation of bioceramics in cardiac-related treatments. The most common examples of bioceramics are

CHAPTER 10

Biomimetic Materials Design for Cardiac Tissue Regeneration

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Abstract: Globally, heart failure is among the principal cause of death. Heart transplantation is the only way out to replace the diseased or damaged heart. Since this technique has several disadvantages, newer therapeutic approaches are required for cardiac repair. One such approach is cardiac tissue engineering, which helps in designing and developing biomimetic materials that mimic the microenvironment of the myocardium. Approaches for cardiac tissue engineering consists of cell injection, tissue patch implantation, replacement of the valve, and injection of acellular materials. Biomaterials are designed to support stem cell expansion, protection, and differentiation. They also facilitate cell retention, cell survival and provide mechanical support. Advances in nanotechnology have made the biomimetic material design more advanced as it can deliver bioactive factors, manipulate surface topography, control cell behavior, and align cells and tissues properly. Furthermore, electrical conductivity and mechanical stiffness can be modulated as well. Overall, biomimetic materials are the new therapeutic approaches in the field of cardiac regenerative medicine, demonstrating their potential in treating heart disease.

Keywords: Biomimetic, Cardiomyocytes, Electrospinning, Nanofibers, Nanolithography, Scaffolds.

INTRODUCTION

Cardiovascular diseases (CVDs) are a class of diseases affecting the heart and blood vessels, which include two events; heart attack and stroke and are acute in nature and mainly caused by blockage of the blood vessels towards the brain and heart [1]. Heart failure is still the leading cause of death worldwide, killing almost 17.9 million people, which represents 31% of the world's total mortality. In 2015,

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treatment of heart failure alone, amongst the patients suffering from CVD, caused an economic burden of $560 billion [2].

Despite the encouraging use of conventional treatments for heart diseases, the last stage of heart disease is often transplantation or replacement of failed heart. However, this process has several limitations like immune rejection, blood loss, surgical difficulties, and unavailability of the organs for transplantation. These caveats have urged scientists to develop alternative techniques to repair the damaged heart and re-establish its functioning [3]. Cell therapy has also been used as a therapeutic approach to repair the failed heart. In this method, single cells or collection of cells are directly injected into the cardiac muscles, and changes in the heart rate are observed. But this approach limits the treatment of the wounded heart as the rate of cell survival by this method is significantly less, and the transplanted cells are poorly retained. Further, any inappropriate cell source may lead to arrhythmias after cell transplantation [4]. Recently, cardiac tissue engineering has become a great therapeutic approach for cell transplantation in terms of cell injection, cardiac patches, valve replacement, stimulation of endogenous repair mechanism (Fig. **1**).

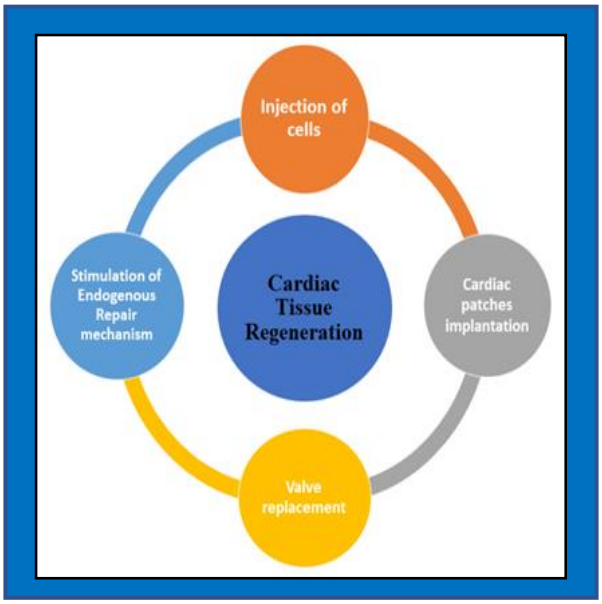

Fig. (1). Various modes of cardiac tissue regeneration.

The development of engineered scaffolds has led to the regeneration of the damaged heart tissue as they provide a microenvironment to the heart cells for

expansion and differentiation. They also protect the injected cells into the heart, and their implantation requires surgeries [5]. Tissue engineering has helped in the design and development of biomaterials that mimic the extracellular matrix (ECM) of the heart tissue, and these biomaterials provide features such as molecule adhesion, sequestration, and release of growth factors [6].

Nanotechnology has emerged as an interdisciplinary field involving all the medical doctors, cell biologists, and pharmaceutical scientists for the design and development of biomimetic scaffolds for tissue engineering. Nanotechnology has played a significant role in the development of engineered scaffolds for tissue regeneration. Nano-fabrication approaches have identified goals for nanotechnology, such as functional vascularization, scaffold engineering, and surface-mediated cell assembly [7]. There are distinct advantages of nanotechnology in the preparation of biomimetic materials that may successfully be used for cardiac tissue engineering [7, 8]. Surface manipulation of the individual cell and its structure and function can be regulated by nanoscale topographical features. The chemistry behind the formation of the extracellular matrix can be easily controlled and functionalized. Nanotopography helps in identifying the cellular junction gaps, which in turn helps in cytoskeletal organizations, as a result, the immune response is reduced. Scientists and researchers have taken a step forward in the advancement of nanotechnology as it is capable of manipulating the differentiation of the cell and its behavior by activating the signaling pathways and tracing the differences between the non-functional cells and the cardiac cells. MRI imaging is a key tool in tracking cell migration and development. Liao *et al*. used superparamagnetic iron oxide nanoparticles for *in-vivo* MRI imaging [9]. Different uptake ratios by the embryoid bodies of gold particles are detected through Raman scattering, so they can be used to differentiate between the diverse stages of cardiac differentiation [10]. Furthermore, cardiac biomarkers help stem cell detection by binding to the specific sites, as investigated earlier. NaYF4 nanocrystals were conjugated with the primary antibodies, and cellular junction gaps were detected with the help of near-infrared emission spectra [11]. PEG-PLA-CPLA nanofibers prepared by electrospinning showed two times increased in the expression level of α-myosin biomarker when compared *in-vivo* in an ES-cell derived cardiomyocytes without the nanofibers [12]. PLA nanofibers prepared by rotary extrusion were cultured on neonatal rat ventricular myocytes. These nanofibers showed robust sarcomeric alignment [13]. To form a soft lithography mold, gold-palladium particles were sputtered on a polystyrene sheet in order to coat the mold. PDMS substrates were formed, which directly influenced the orientation of junction proteins like connexin-43 and N-cadherin in cardiac cells derived from stem cells [14].

CHAPTER 11

Nanotechnology-Based Direct Cardiac Reprogramming for Cardiac Regeneration

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Abstract: Cardiovascular diseases are the main reason for morbidity in developed countries, and congestive heart failure represents the major health burden. Although clinical application of cardiac regeneration is still in its infancy, studies on rodents have proven its feasibility. Also, the technique of direct cardiac reprogramming has unveiled new paths for the success of cardiac regeneration, wherein one cell type can be directly converted into the cardiac myocytes without involving the pluripotent intermediate cell. Firstly designed for the management of cancer, nanotechnology has opened up newer vistas for direct cardiac reprogramming for cardiac regeneration. This chapter discusses cardiac regeneration and the limitations of current approaches to cardiac regeneration in brief. Direct cardiac reprogramming involving both *in-vitro* and *in-vivo* trials has been duly explored to enlighten the readers. An attempt has been made by the contributors to elaborate the various approaches of nanotechnology such as nanomaterials and stem cells in regenerative medicine and their impact on direct cardiac reprogramming. This chapter involves an exhaustive effort of the contributors to enlighten the understanding of a broad readership about the nanotechnology-based direct cardiac reprogramming for cardiac regeneration.

Keywords: Cardiac regeneration, Cardiac reprogramming, Cardiomyocytes, Cardiac fibroblasts, Nanotechnology, Stem cell therapy.

INTRODUCTION

A possible association between nanotechnology and the area of cardiac reprogramming is not limited to textbooks alone. Although still in its infancy, conversion of putative *in-vitro* techniques to *in-vivo* scenarios seems promising and is much required. The limited literature available is suggestive of unravelling certain areas that need to be touched before initiating an effective *in-vivo* evaluation of cardiac reprogramming. These primarily include 1). Identification

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and characterization of the ideal cardiac resident cell for direct reprogramming. 2). Selective targeting of the injured myocardial tissue with a therapeutically effective dose of reprogramming factor and 3) to achieve the proper *in-vivo* functioning of regenerated cells [1].

The first issue to be resolved is the identification of the ideal cardiac cell for reprogramming. These cells must be in sufficient amount in the heart and easily targetable in the infarcted zone. Furthermore, selective targeting by the nanocarriers, thorough characterization of these cells is the utmost requirement. As after myocardial infarction, infarcted tissue performed a high degree of remodelling process wherein activation of cardiac resident fibroblast takes place, and this attracted the researchers to conduct the study on these cells [2]. Studies have supported these cells as the ideal candidates for direct cardiac reprogramming due to their characteristics, but still, there is a need for further characterization for targeting the nanocarriers [3].

The most suitable combination for reprogramming should be based on the use of chemical entities for which a valid safety profile is available. In order to explore the potential of chemical entities for cardiac reprogramming, a thorough understanding of the chemistry and efficient handling of the customized delivery strategies is needed so that proper administration of compounds with a pre-defined rate can be achieved at the pre-identified target cell. To fulfill the objectives, we need to begin with the identification of material for the engineering of suitable nanocarriers. Biocompatible polymers can be promising materials for the designing of selectively targetable nanocarriers. Moreover, for *in-vivo* efficiency, a more selective strategy of active targeting is required, which can be achieved only after a thorough understanding of the ideal cell candidate. Therefore, the generation of reprogrammed functional cardiomyocytes is the outcome of a combinational strategy involving a cautious selection of the candidate cell and a suitable chemical entity delivered through a suitable nanocarrier [4].

CARDIAC REGENERATION AND LIMITATIONS OF CURRENT APPROACHES FOR CARDIAC REGENERATION

From the last five decades, the center of cardiovascular research lies in the fact that the heart is a terminally differentiated organ, and it cannot replace the functional myocytes after myocardial infarction [5]. In the past 30 years, the focus of research in molecular cardiology revolves around the identification of genes and signaling pathways involved in the hypertrophic reactions of cardiac myocytes during pathologic states [6]. The potential of an adult cell to generate other types of cells beyond its tissue boundary is called developmental plasticity.

Bone marrow cells (BMC) are the most versatile and characterized cell for developmental plasticity in both *in-vitro* and *in-vivo* conditions [7]. Studies were conducted to identify the potential of BMC for tissue regeneration and simultaneously revealed that injury to a tissue encourages the multiplication of alternate stem cells. Based on this, bone marrow cells were injected into the infarcted area of the myocardium or were injected along with cytokines into the systemic circulation. Both the experiments resulted in the repairment of the infarcted tissue and gave rise to functionally active myocardium [8, 9]. Clinical trials have been conducted on human beings for assessing the therapeutic efficacy of BMC in ischemic and non-ischemic cardiomyopathy, and promising results were obtained [10].

By this time, several approaches have been explored to repair the infarcted heart. This includes BMC, fibroblasts, fetal cardiomyocytes, bone marrow-derived immature myocytes, endothelial cells derived from the embryo, skeletal myoblasts, endothelial progenitors, and smooth muscle cells [11]. All of the above-mentioned approaches result in the formation of passive graft by decreasing the negative remodeling, thereby reducing the hardening in the scarred portion of the heart wall. Hence the variable degree of improvement was observed in cardiac performance. However, in a few cases, the dynamically active graft was also observed, which contributed to myocardial contractility [8, 9, 12]. The implanted cells may also exhibit the paracrine response by activating the growth of resident progenitor cells [13, 14].

Recently researchers have identified and characterized the cardiac stem cell in the heart of mice, rats, and dogs [15, 16]. As resident cardiac stem cells involuntarily differentiate into myocytes and vascular structure, these are the preferred cells to be assessed for cardiac repair. C-kit-positive are the self-renewing, multipotent and clonogenic cells having the fundamental characteristics and properties of stem cells [16, 17]. When these c-kit-positive cardiac stem cells with or without local activation factors were injected intramyocardially in Fisher rats, they resulted in significant regeneration of the infarcted heart. In comparison, the IV injection of stem cell antigen 1-positive cell (Sca1) after reperfusion insult resulted in a limited effect on myocardial regeneration [15]. However, it is unclear that the variation in response was due to the route of administration or the distinct progenitor cell. It can be said that myocardial repair requires the regeneration of cardiac myocytes and coronary blood vessels, and this criterion cannot be fulfilled by a cell already programmed for the myocyte lineage. In the absence of blood vessels, myocytes would not survive. Also, the use of cells programmed for exclusively generated coronary blood vessels had not given the significant results of tissue regeneration [18]. For myocardial regeneration, administration of more primitive, multipotent cells is required that can differentiate into variable type of

CHAPTER 12

Smart Nanomaterials for Cardiac Regeneration Therapy

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Abstract: Cardiovascular disease (CVDs) have been observed as the major cause of death worldwide. During the cardiac attack, the blood flow slows down, by which the pumping gets affected. For getting the heart functional, sometimes several surgeries are done that weakens the heart. In ultima cases, loss of heart cells led to a heart attack. The therapeutic options that are adapted for preventing CVDs patients are often being treated with invasive cardiac surgery. Lack of solution to heart troubles and its underlying mechanism led towards the drive of regeneration therapy. Tissue engineering and regenerative strategies goal serves a dual purpose, firstly to stop disease progression and secondly to reverse disease effects to regain and restore heart function. Nanotechnology has been a revolutionary step towards cardiac therapy. Through nanotechnology, there has been a great paradigm shift in the treatment of coronary heart diseases, heart injury, muscle cells improvement, normal functioning of the heart after massive injuries. Tissue-engineered therapeutics are basically delivered to the heart by two approaches, such as cardiac injections and cardiac patches. Engineered nanoparticles for the specific purpose of cardiac ailment play a vital role in heart cure and biomedical application.

Keywords: Biomedical application, Biomaterials, Nanotechnology, Nanomaterials, Tissue engineering.

INTRODUCTION

Cardiovascular disease (CVD) is one of the main causes of disability and death worldwide [1]. CVD accounts for approximately 40% of all human mortality [2]. It has been estimated that about 1.8 million from the UK, 5 million from the US, and around 25 million people worldwide are suffering from cardiovascular diseases.

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Poor prognosis with 12 months of diagnosis leads to 40% mortality thereafter, causing around 10% annual mortality. Annually, in the US and UK, this disease causes an economic burden of more than around $33 billion and £700 million, respectively [3]. CVDs mainly means to a group of pathological syndromes found in blood vessels, cardiac and valvular tissues linked with the heart [4]. Myocardial infarction (MI), atherosclerosis, ischemic cardiomyopathy, coronary heart disease, heart failure, atrial fibrillation, angina pectoris, and endocarditis are the major type of heart diseases found throughout the world population. CVD is primarily characterized by the formation of atherosclerotic lesions in small and medium-sized arteries initially that eventually leading to narrowing of vessels and possibly require revascularization. Mostly, these CVDs occur due to augmented workloads in the lining of the blood vessels and muscles of cardiac tissues [5]. Presently, different approaches, such as the use of b-1 receptor blockers, surgical intervention like Coronary artery bypass graft (CABG) surgery, are some of the treatment options available for CVDs management [6]. However, the use of these approaches is limited up to some extent. Due to the limitations of the present treatment modalities and the poor regeneration capacity of the myocardium or cardiac tissue, researchers have now moved to develop advanced approaches aiming at improving the function of the myocardium. They anticipate that promoting the proliferation of cardiomyocyte and heart regeneration might be a promising approach to combat the CVDs to a great extent.

In this regard, nanotechnology has shown promising efficacy in the field of regenerative medicine both in preclinical and clinical settings for biomedical research applications [7]. Till now, the presently existing synthetic materials are lacking in clinical applications because of their lower biocompatibility and lacking in standard physiological responsiveness. A continuous effort of both bio-engineers and tissue engineers, along with their strong understanding of cardiovascular diseases, has directed the investigators towards developed regenerative strategies, emphasizing new materials for formulating better temporary scaffold-like structures that enhance the self-regeneration of naïve tissues [8]. Mostly, these materials include biocompatible and biodegradable polymers that can synthetically be prepared or occurred naturally. These biopolymers have scaffold-like characteristics that provide a microenvironment resembling the extracellular matrix of native tissues [9]. Among different polymers Poly (lactic-co-glycolic acid) (PLGA) is one of the most commonly used synthetic polymers approved for human use because its byproducts such as lactic acid and glycolic acid can easily be metabolized in humans. PLGA establishes a striking podium equally as a scaffold material for cardiovascular disease grafts or stents and also as a polymeric drug delivery carrier system [10]. Similarly, alginate is an abundantly used natural polymer that has shown promising results clinically. This is highly accepted for various medical

applications in humans because of its non-toxic, biocompatibility, non-thrombogenic, and non-immunogenic nature. Alginate-constructed hydrogels offer good mechanical properties and an aqueous atmosphere essential for metabolic exchange, lead to effective pilot clinical trials of alginate-based injectable scaffolds for treating the left ventricular remodeling after myocardial infarction [11]. Thus, in this chapter, we will discuss the CVDs and their treatment by using smart nanomaterials.

FATAL IMPACT OF CARDIOVASCULAR DISEASES (CVD)

Cardiovascular diseases are remained to be the number one cause of death globally. CVD has already been declared as the leading cause of death by the World Health Organization (WHO). CVD usually affects both men and women in equal proportion. It has been estimated by WHO that around 23.6 million people will die by 2030 due to CVD conditions such as stroke and heart disease [12]. Although these circumstances continue predominant in universal mortality rates, people can initiate steps to avoid them. The devastating and regular fatal difficulties of CVDs frequently occur in middle-aged people or elderly men and women [13]. Usually, CVDs refer to a number of conditions as the most prevalent one is heart disease, which is linked to a process known as atherosclerosis. This condition refers to a condition that when a substance known as plaque gets deposited in the walls of the arteries, which leads to the narrowing of the arteries, subsequently blocking the blood flow [14]. Moreover, atherosclerosis mainly leads to the development of coronary, cerebral and peripheral artery disease. It usually begins early in life then progresses slowly to adulthood. It is generally asymptomatic for a long time. The degree of development of atherosclerosis is mostly affected by cardiovascular risk factors like the use of tobacco, sedimental lifestyle and unhealthy diet, high blood pressure, blood lipid, and blood glucose level [15]. Continuous exposure to the mentioned risk factors leads to the formation of plaques, blood vessel narrowing and blockage in blood flow to vital organs such as the brain or heart. This leads to a heart stroke or attack. Maximum individuals stay alive after their first heart attack and lead a normal life living for many more years with productive activity. However, the onset of heart attack does not ask the necessity of changes. The lifestyle changes and medications recommended by the doctor mostly depend on the extent of your heart damage and the degree of heart attack caused by the heart disease. Another type of CVD is an ischemic stroke caused due by blockage of blood supply by the blood vessel to the brain, mostly from a blood clot [16]. Heart failure, sometimes called congestive heart failure, is a type of CVD where the heart will not pump the blood. Arrhythmia is a type of CVDs which is related to the abnormal type of heart rhythm, such as the rhythm can be too slow, too fast or irregular [17]. One more type of CVD is the heart valve problems in which the heart valves do not

CHAPTER 13

Stem Cell Engineering Ability to Promote Cardiac Regenerative Activity

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Abstract: The heart of adult humans is usually less capable of regeneration; several strategies have been used for the repairment of a damaged heart and to recover its function. However, stem cell technology showed a promising approach for cardiac therapy. More advanced strategies are used for improved stem cell-mediated cardiac regenerative therapy and to develop vascularization between scaffold (established by 3D engineered techniques) and host hearts. Through understanding the cellular and molecular mechanisms regulating heart regeneration, considerable progress has been made, which offers potentiality in controlling cardiac remodeling and redirecting the adult human heart towards a regenerative state.

Keywords: Cardiac regeneration, Cardiomyocytes, Mesenchymal stem cells, Pluripotent stem cells, Scaffold.

INTRODUCTION

The main reason behind heart failure is the irrecoverable damage to contractile heart muscles. To date, medicinal or surgical modalities are the only essentially available options for heart diseases [1]. In the year 1990, transplantation of syngeneic organism's cardiomyocytes (CMs) has shown to improve the function of the left ventricle in animal models of myocardial infarction [2]. Still, clinically there was no useful source of contractile cells. The maintenance of mammalian cardiomyocytes requires sophisticated routine protocols to keep alive for several days and always require the dissociation of neonatal rodent myocardium. Although isolation of adult CMs has been documented the cultivation of human CMs has been criticized a lot leading to futile research.

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Mostly, the donor CMs are of allogeneic origin that raises the problem of immune rejection. Thus, the concept of using skeletal myoblasts for replacing CMs has become the topic of interest. However, the idea of using skeletal myoblasts failed because these cells are lacking with cardiomyocytes specific ion channels that are crucial for the electrical link with native CMs [3]. Till now, many investigations have reported the vast research on the derivation of CMs from numerous adult stem or progenitor cells [4, 5]. However, these are mainly disappointing at preclinical and clinical experiments. As compared to the presently used therapy, stem cell-based therapy has shown tremendous potential in enhancing and supporting the process of cardiac repair. Thus, stem cells afford the basis for complete regeneration of damaged cardiac tissue [6]. Although stem cells use in the context of cardiac tissue regeneration is highly appreciable. However, there exist many challenges and problems in cell-based therapy for CVDs. The analysis of current trials depicts that there exist a number of reasons behind the least success in cell-based therapy that includes patient's inconsistency *i.e.* variation in cell population choice, lower number isolation of cells and slow replicative potential. Moreover, the capacity of regeneration decreases with age, and also the delivery of enough numbers of the cell to the injury site is also a difficult task [7]. Thus, there is an urgent need for the improvement in the therapeutic properties of the used cell-based strategies in order to enhance the process of cardiac regeneration. To deal with the issue, stem cell engineering approaches provide solutions to overpower the present limitations. Particularly, the major goal of the engineering approaches has uniquely able to regulate the biochemical and biophysical characteristics of materials used in stem cell-based therapies [8]. Bioengineering strategies fill an exclusive environment for stem cell research by observing the dynamic behaviour of stem cell populations, which can be defined and manipulated strategically with the help of computational models in order to achieve desirable properties [9]. The eventual goal of stem cell bioengineering is to develop approaches to activate the populations of stem cells and allow probable control in regulating the fate of stem cells. This will set strategic engineering of the microenvironment for getting robust results using stem cell population for cardiac regeneration (Fig. **1**).

Moreover, bioengineering approaches can be useful to get a broad systems-level knowledge that is functional within the stem cells. It also helps in the understanding of rules that guide intracellular and cell-environment interactions that lead to the development of tissues and organs. Bioengineering strategies also help in improving stem cell survival and engraftment. The design of advanced materials can help in achieving the regulatory standards that increase the scale needed for commercialization. In this chapter, we will describe the role of stem cells in cardiac regeneration and how the stem cells are engineered for improving the stem cell-based therapies for cardiac tissue regeneration therapy.

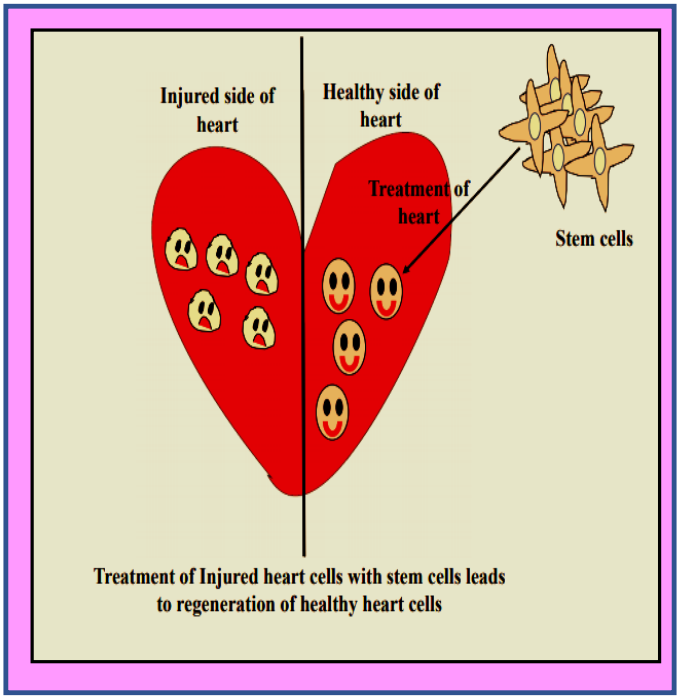

Fig. (1). Graphical illustration of stem cells based regeneration of injured heart.

STEM CELLS IN CARDIAC REGENERATIVE THERAPY

The therapeutic strategies for tissue regeneration have widely developed to combat the problem of damage or injury in different tissues or organs, which is caused due to any physical factors or the physiological ageing process in order to reduce the onset of its undesirable or negative effects. In this regard, stem cell based therapy has undoubtedly shown promising efficacy for the efficient management of a wide range of human diseases. Stem cells help in repair, replace or regeneration of damaged cells, tissues and organs that help in restoring its reduced function, which arises due to the effect of disease, ageing, trauma and defects [7]. Thus, stem cells have remained one of the major choices of clinicians and investigators for tissue regeneration therapy. Although many challenges exist in the clinical application of stem cell therapy, as evidenced from limited success obtained for human disease treatment till now [10]. The experimental and clinical study results are mainly challenged by a number of issues. Most significantly, a thorough understanding of stem cell biology has also shown the potential role of vesicular systems formed by stem cells such as exosomes and microvesicles. These vesicular systems are presently considered as a major form of wireless transformation of biological information between the microenvironment and the stem cells [11]. Regarding cardiac regenerative therapy, mainly two different

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