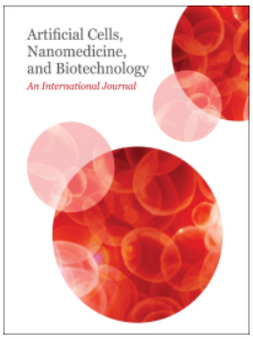

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Recent advancements in the cardiovascular drug carriers

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Abstract

Cardiovascular disease is the disease that affects the cardiovascular system, vascular diseases of the brain and kidney, and peripheral arterial disease. Despite of all advances in pharmacological and clinical treatment, heart failure is a leading cause of morbidness and mortality worldwide. Many new therapeutic advance strategies, including cell transplantation, gene delivery or therapy, and cytokines or other small molecules, have been research to treat heart failure. The main aim of this review article is to focus on nano carriers advancement and addressing the problems associated with old and modern therapeutics such as nonspecific effects and poor stability.

Keywords: cardiovascular disease, gene therapy, nanotechnological approaches, Nanoparticle

Introduction

Cardiovascular disease (also called heart disease) is that class of diseases which involves either one or both of the heart and blood vessels (arteries, veins and capillaries) (Anthea et al. 1993). ·Angina pectoris is a heart condition marked by chest pain resulting from low oxygen supply to the heart (Fuster and Kelly 2010). There are many causes of cardiovascular disease but atherosclerosis and/or hypertension are the most common. Additionally, with increasing age, a number of physiological and morphological changes modify cardiovascular function and lead to an increased risk of cardiovascular disease in the later years, even in healthy symptomless individuals (Dantas et al. 2012). Cardiovascular disease causes deaths worldwide on a large scale. Since the 1970s, cardiovascular mortality rates have declined in many developed countries (Fuster and Kelly 2010). Simultaneously, cardiovascular deaths and disease have increased at a fast rate in developing countries (Rosamond et al. 1998). The precursors of cardiovascular disease, especially atherosclerosis, start in early life, so we should adopt primary precautionary measures which are necessary, right from childhood (McGill et al. 2008). Therefore increased emphasis on preventing atherosclerosis by modifying and controlling risk factors, such as healthy eating, proper exercise, and elimination of smoking tobacco etc., is required.

Current statistics of cardiovascular disease

- Cardiovascular disease is the leading cause of death in the world. More people die every year from cardiovascular disease than any other disease (Alwan 2011).
- A disproportionate number of people are affected in developing countries, over 80% of deaths due to cardiovascular disease take place in developing countries and happen almost equally in male and females.
- The number of people who die from cardiovascular disease, mainly stroke and heart disease, will increase to reach 23.3 million by 2030. Cardiovascular disease is expected to remain the single leading cause of death in the world (Mathers and Loncar 2006).
- It was predicted that 17.3 million people would die from cardiovascular disease in 2008, representing 30% of all worldwide deaths. It was predicted that 7.3 million deaths were due to stroke and coronary heart disease.
- Cardiovascular diseases could be prevented by educating common people about the risk factors such as obesity, unhealthy diet, tobacco use, physical inactivity, high blood pressure, raised levels of lipids in blood, and diabetes.
- There are 9.4 million deaths each year (Mathers et al. 2008) . This includes 45% of deaths caused by coronary heart disease and 51% of deaths due to heart strokes (Lim et al. 2013).

Recent therapeutic strategies for cardiovascular diseases

Despite all the advancement in pharmacological and clinical treatment, heart failure is a leading cause of morbidity and mortality worldwide. Many new and advanced therapeutic strategies, including cell transplantation, gene delivery or therapy, and cytokines or other small molecules have been researched to treat heart failure (HF) (Arora et al. 2012b). Recent advancement in the study of those molecules that regulate the cardiac functions shows that they are key molecules to treating heart failure. Furthermore, a theory of the paracrine mechanism, which comes under the

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beneficial effects of cell therapy, leads us to search for new target molecules for pharmacological strategy (Chang 2011). Gene transfer therapy means delivery of genetic materials into cells to attain desired therapeutic effects. Recently, gene therapy in the cardiovascular system has seen great improvement and clinical research on several new therapeutic target genes has begun, with some desired good results already achieved. Among all the bioactive reagents, cytokines like granulocyte colony-stimulating factor and erythropoietin have been thoroughly examined, and a different number of clinical trials for acute myocardial infarction and chronic HF have been conducted. However, further research is desired in both preclinical and clinical areas into the means of molecular mechanisms, safety and good efficiency (Garg et al. 2014). Both gene therapy and cytokine therapy have a great possibility to open a new era in the treatment of HF (Gagandeep et al. 2014, Garg 2014). Various cardiovascular drug carriers are shown in Figure 1.

Tablets

Different drug delivery systems are present for cardiovascular (HF) drugs. Different kinds of receptor inhibitors, such as the angiotensin II receptor inhibitor, are frequently used on a regular basis in cardiovascular clinics. Many different routes of drug administration already exist. The conventional regular tablet or its novel liquid spray by sublingual or lingual administration is quickly absorbed. Specified oral tablets or transdermal patches, maintain longtime absorption via slow absorption through the digestive tract and skin. Some of the drugs have different types of tablets for attaining different pharmacodynamics. For example, nifedipine has 3 types of tablets which exhibit short, long and very long-term effects. Recent novel advances in the drug delivery system make it possible to deliver the drug to the small local area with the help of stent or balloon catheter in coronary interventional therapy (Miyake et al. 1998). There are currently four types of medicines used to treat angina: nitrates, beta blockers, calcium channel blockers, and ranolazine. However, some common side effects associated with these therapies include headache, constipation, rash, nausea, flushing, edema (fluid accumulation in tissues), drowsiness, low blood pressure, and dizziness. Sexual dysfunction, overgrowth of gums, and liver dysfunction also have been associated with calcium channel blockers. Diltiazem and verapamil worsen heart failure because they reduce the ability of the heart to contract and pump blood. Considering all of these adverse effects of tablets and the very slow absorption of sublingual tablets, nanoparticles are found to be the better and advanced approach towards curing of angina pectoris instantly, by direct intravenous administration into the patient's body.

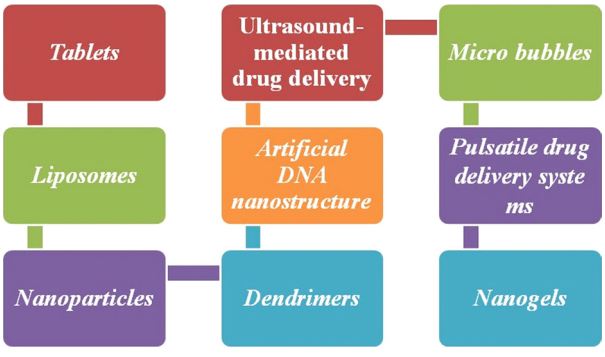

Figure 1. Cardiovascular drug carriers.

Liposomes

There are many different types of drug delivery vehicles, like polymeric micelles, liposomes, dendrimers, lipoprotein-based drug carriers, nanoparticle drug carriers etc. (Garg 2012). An ideal drug delivery vehicle should be biocompatible, non-toxic, non-immunogenic, and biodegradable and should avoid recognition by the host's defense mechanisms. Liposomes are made up of phospholipids and may comprise small amounts of other molecules. Liposomes can vary in the size from a low micrometer range to tens of micrometers in range. Unilamellar liposomes are in the lower size range with various other targeting ligands to their surface, which allows for their surface-attachments and accumulation in pathological areas for treatment of disease (Torchilin 2012). Liposomes are non-hemolytic, non-toxic, non-immunogenic, biocompatible and biodegradable in nature and could be designed to avoid clearance mechanisms such as chemical or enzymatic inactivation, renal clearance etc. Ligand-coated nanocarriers can store their payload in the hydrophobic shell or the hydrophilic inner part depending on the nature of the drug (Patel 2006). Liposomes supply both a lipophilic environment and aqueous environment in one system and are therefore suitable for the delivery of hydrophobic, amphipathic and hydrophilic drugs. Liposomes have the ability to protect their encapsulated drug from the external environment (propranolol). Liposomes can be formulated as a suspension, or in a semisolid form such as cream, gel and lotion, or they can be administered through most routes of administration including ocular, pulmonary, nasal, oral, intramuscular, or through the vein. Liposomes can encapsulate not only small molecules but also macromolecules like superoxide, erythropoietin, and interleukin-2 (Patel 2006). Liposomes reduce toxicity and increase the stability of the entrapped drug through encapsulation (amphotericin B). Liposomes also increase the efficacy and the therapeutic index of some drugs (actinomycin-D). Liposomes are helpful in reducing the exposure of sensitive tissues to toxic drugs. Liposomes alter the pharmacokinetic and pharmacodynamic property of the drugs (increased circulation life time, reduced elimination) (Nishiya and Chang 1994). They have the flexibility to couple with site-specific ligands to achieve active targeting (Patel 2006). One of the main disadvantages in the use of liposomes is their very fast elimination from the blood and the capture of the liposomal formulations by the cells of the reticuloendothelial system, mostly in the liver. There have been a few improvements meant to decrease this problem.

Immunoliposomes

To escalate liposomal drug accumulation in the chosen tissues and organs of the body, the use of directed liposomes with surface-attached ligands capable of identifying and binding to the target cells has been projected. IgG class immunoglobulins and their fragments are the most widely used

targeting moieties for liposomes, which could be attached to the liposomes without disturbing liposomal integrity or liposomal antibody properties. Covalent binding to the liposomal surface or hydrophobic insertion into the liposomal membrane improves in targeting ability. The majority of the immunoliposomes gather in the liver as a consequence of inadequate time for the communication between the target and directed liposome (Mobed and Chang 1998).

Long circulating liposomes

The use of improved liposomes with specific monoclonal antibodies against some components is a major characteristic of the cardiovascular system and tumor vascular system. Although there is vast literature about the characteristics and potential applications for the use of monoclonal antibodies to specifically target a number of varied carriers, an antibody-modified carrier system has not yet been approved for human use. When designing immune liposomes, antibodies work conjugated either to the liposomal surface or with the distal end of the liposomal PEG. Approximately 30 years ago, the specificity of myosin-specific antibody fragments localization was demonstrated in experimental myocardial infarction and the preservation of this antibody activity after covalent coupling to liposomes was shown. Later on, this group also demonstrated suppressed hypoxic cardiocyte death by sealing membrane lesions with antimyosin liposomes. Another interesting platform for immunoliposomes is their use as an acoustically reflective carrier that can be targeted for site-specific acoustic enhancement. There are some ultrasound parameters for enhancing the delivery of therapy-loaded echogenic immunoliposomes into the arterial wall for the treatment of atherosclerosis in an ex vivo mouse aorta model. This is done by using anti-ICAM-targeted echogenic liposomes and following the 1-MHz wave ultrasound. There is greater adherence of the targeted liposomes to the vascular endothelium and greater passage across the vessel wall. Targeting the liposomes to ICAM-1, fibrin, VCAM-1, fibrinogen, and TF, in addition to the application of ultrasound waves, was able to produce targeted enhancement in the vessel walls 5 min after intravenous administration of targeted liposomes (Chang 2012).

Advancement by long-circulating liposomes

Different approaches have been suggested to attain long-circulation of the liposomes *in vivo*, comprising coating the liposome surface with inert, biocompatible polymers like PEG, which form the defensive layer over liposome surface and slow down the liposome recognition by the opsonins and subsequent clearance of the liposomes. Long-circulating liposomes are now being examined in very much detail and are extensively used in biomedical *in vitro* and *in vivo* studies. Growing mole percent of polyethylene glycol on upper surface of liposomes by 4–10% expressively improved circulation time *in vivo* from 200 to 1000 min.

Improved liposomes

Liposome surface-modification chemistry

The formulation of the modified liposomes with controlled properties needs the chemical conjugation of the proteins, polymers, peptides, and other molecules to the liposomal surface.

Antibody-mediated liposome targeting to the body

The majority of the research in this zone relates to cancer targeting, which uses a variety of antibodies. Affecting antibodies are required to achieve the much improved therapeutic efficacy with the antibody-targeted liposomal drugs as is the case with B-lymphoma cells.

pH-sensitive liposomes

To attain the pH-sensitive release of liposome content, liposomes are made from pH-sensitive components. These fuse with the endovascular membrane as result of the lower pH in the endosome, and discharge their contents into the cytoplasm.

Diagnostic liposomes

Diagnostic liposomes are mainly used to target myocardial infarction and the relative importance of the liposome size, targetability of immune liposomes and continued circulation time on the efficiency of sealing hypoxia-induced plasma membrane harm to the cardiocyte is debated as a promising approach for therapy(Garg and Goyal 2014b).

Current approach

The current investigation on the PEG liposomes focuses on the achievement of PEG in a removable manner to facilitate the liposome capture by the cells. After PEG-liposomes accumulate at the target site, via enhanced permeability and retention effect, the PEG coating is separated by the action of local pathological conditions. Novel, detachable PEG conjugates have been defined, in which the detachment procedure is based on mild thiolysis of the dithiobenzylurethane linkage between the PEG and an amino-containing substrate. Table I represents liposomes as a drug delivery system for the treatment of angina pectoris.

Nanoparticles

Nanoparticles range in size from 1 nm to 100 nm. Because of their small size, they hold some unique properties compared to their larger matching counterparts. They are capable of easily crossing through the human body, cells and tissues. Depending upon the interactions that occur on their surface, they are able to alter tissue and cell physiology (Kaur et al. 2014b). When formulating nanoparticles for imaging or therapeutic applications, care must be put into choosing materials with proper molecular weight, chemical composition, and additional functional group modifications (Singh et al. 2014a) (Figure 2). These systems have very important applications for the delivery of poorly soluble drugs or the delivery of drugs with high toxicity to target areas, therefore alleviating possible side effects (Parnami et al. 2013). Cardiovascular diseases, mainly heart disease and stroke, are the chief causes of death and morbidity in commercial nations and are becoming a crucial health problem for all nations due to the unstoppable fashion in which an ageing and overweight population is growing. Due to the rapid growth of nanotechnology in recent years, the therapy

Table I. Liposomes as drug delivery system for the treatment of angina pectoris.

Types of liposomes	Drug	Inferences	References
Conventional liposomes	Amiodarone	Targeting the delivery of liposomal Amiodarone to ischemic/reperfused myocardium. Reduces the mortality due to lethal arrhythmia and the negative hemodynamic changes caused by Amiodarone	Takahama et al. (2013)
N-acetyl glucosamine liposomes	GlcNAc-Ls	These results demonstrated that GlcNAc-Ls can be specifically taken up by VSMCs both *in vitro* and *in vivo*. GlcNAc-Ls have potential for application in drug delivery targeted to injured blood vessels	Ise et al. (2011)
Conventional liposomes	ATP-2Na	ATP-2Na liposome increased ATP content of myocardium and liver in myocardial ischemic mice. ATP-2Na liposome might have an advantage in improving tissue energy state on myocardial ischemic animals	Pi et al. (2010)
Nano liposomes	Sirolimus	*In vivo* studies in balloon injured rat carotid arteries revealed the potential of SIR-loaded liposomes as efficient local and controlled drug delivery systems to reduce restenosis	Haeri et al. (2011)

available for cardiovascular diseases has been greatly enhanced, as it offers a solution for all the problems. Pharmaceutical nanoparticles have been developed as multilayered systems capable of recognizing and characterizing initial disease before the gross anatomical manifestations. Nanotechnology deals with four areas, in which cardiovascular diseases can be better battled with immediate impact. Cardiovascular disease (CVD) encompasses a class of diseases that involves the heart and vasculature. CVD has been one of the leading causes of mortality and accounts for virtually 1/3 of all deaths worldwide. So, there is a persuasive need to improve novel techniques such as nanotechnology, for the early detection and treatment of several types of CVD. Targeted therapeutics, tissue engineering, molecular imaging and diagnostics detect and treat problems inside the body. In pharmaceutical technology and biomedicine, nanoparticles are characteristically clear as the particles with diameter ranging from 1 to 100 nm, and have been exploited for both diagnostic and therapeutic purposes.

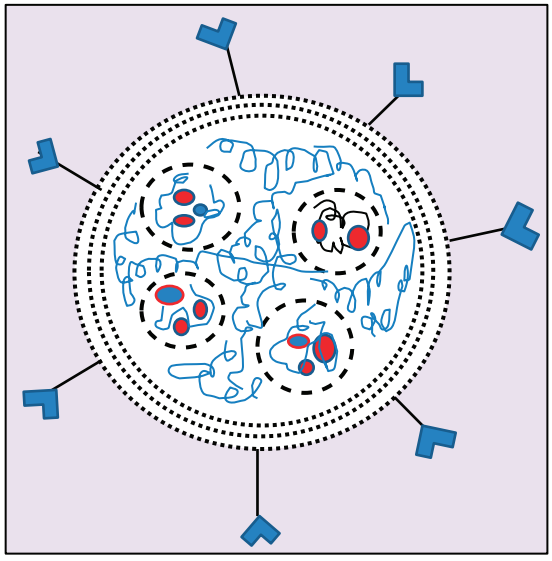

Figure 2. Structure of nanoparticles.

Nanoparticles can achieve controlled release of drugs, targeting and bioavailability of many diagnostic or beneficial therapeutic agents. Endothelial-selective delivery of therapeutic agents would provide a useful tool for modifying vascular function in various cardiovascular diseases and several research groups are interested in this targeting approach (Wang and Chang 2012).

Nanoparticles as drug delivery systems targeting atherosclerosis

Firstly, the use of polyethylene glycol (PEG) coated synthetic copolymers (Ding et al. 2006), conjugated with low molecular weight therapeutic mediators, can yield some drug delivery systems with interesting properties (Discher et al. 1999). PEG coating proves to be highly efficient as it forms a hydrated ring hindering protein interactions and reducing opsonization effects from macrophages (Photos et al. 2003). This results in increased circulation time and lesser activation of the host immune system (Moghimi et al. 2005). Another approach in the formation of drug delivery systems which preceded the use of the polymers, is the use of liposomes. Though liposome size does not range in nanometer area but rather in the micrometer range, their natural imitative properties enable control for drug delivery applications. Although liposome carrier circulation times are much lower compared to those of synthetic polymers, so much effort has been made into improving them (Christian et al. 2009). An interesting approach is the creation of protein conjugates, for example protein-protein, enzyme-antibody. Proteins propose a varied spectrum of responsive groups for conjugation, depending on the target desired (Shuvaev et al. 2004). These conjugates can undergo extensive chemical alteration, additionally varying their biochemical claims and expanding their area of applications (Wiewrodt et al. 2002). A significant advantage of these systems is that they are totally biodegradable, with most of the deprivation products eliminated via natural metabolic pathways. A slightly different approach for the treatment of atherosclerosis is the report of thromboresistant nano-membranes (Wilson

et al. 2007). These are able to exhibit antithrombogenic or thromboresistant properties via immobilizing athrombogenic molecules and creating resistant nano surfaces and immobilizing molecules shown to enhance endotheliazation (Jordan and Chaikof 2007). Finally, much effort is being put into creating nanoparticles that possess properties for both therapeutic and diagnostic applications. These nanoparticles can offer tremendous advantages for the treatment of atherosclerosis as they not only enable the delivery of therapeutic agents to the pathological tissues, but permit physicians to use imaging methods in monitoring the efficiency of the applied treatment (Janib et al. 2010). However, one of the key problems when employing nanoparticles is the toxicity, and the health risks related with the nanoparticle use are very tough to forecast; moreover, their small size and the large surface area to volume ratio makes them very reactive (Nel et al. 2006). Nanoparticle toxicity is affected by many parameters that is material composition, shape, size, and solubility (Magrez et al. 2006). Nanoparticles may produce increased amounts of reactive oxygen species and free radicals, resulting in oxidative stress, inflammation, and subsequent damage to RNA, DNA, and proteins. In addition, they can cross the biological membrane and increase access to tissues or cells, altering their physiology (Geiser et al. 2005). Kona et al. developed a novel nanoparticulate drug delivery system that mimics platelets binding to the injured vessel wall under physiological flow conditions. Glycoprotein Ib (GPIb) was chosen as the targeting ligand and conjugated to nanoparticles because its role in platelet adhesion to the vascular wall under high shear flow conditions is well-recognized. Dexamethasone-loaded biodegradable poly (d,l-lactic-coglycolic acid) (PLGA) nanoparticles were formulated using a standard emulsion method. The results demonstrated that conjugation of GPIb to PLGA nanoparticles increased particle adhesion onto targeted surfaces and increased cellular uptake of these nanoparticles by activated endothelial cells under shear stresses. In addition, these nanoparticles also provided a controlled release of the model drug. Therefore, these drug-loaded, GPIb-conjugated PLGA nanoparticles could be used as a targeted and controlled drug delivery system to the site of vascular injury, for treatment of cardiovascular diseases (Kona et al. 2012). Song et al. investigated the potential usefulness of biodegradable nanoparticles for the local intraluminal treatment of restenosis. NPs comprising an anti-proliferative, water-insoluble agent U-86983 were formulated from oil–water emulsions using biodegradable polymers such as poly (lactic acid) and detailed additives after the particle development, to improve arterial retaining efficacy using any of heparin, fibrinogen, didodecylmethylammonium bromide, or any combinations. The *in vivo* studies were conducted with a new dog model for the arterial angioplasty. The outcomes support the vision that improved nanoparticles along with optimized infusion conditions could enhance arterial wall drug concentrations of agents to treat restenosis (Song et al. 1998). In other studies, Fishbein and colleagues have prepared a Tyrphostin inhibitor comprising poly (DL-lactide) (PLA) nanoparticles for intra-arterial administration by spontaneous emulsification/solvent displacement technique for the treatment of restenosis. The results demonstrated that trans-catheter local delivery of the anti restenotic agent probucol-loaded PLGA particles, in rabbit iliac arteries, for enhanced retention, sustained release and increased therapeutic effects. Winter et al. performed studies to develop a prolonged antiangiogenesis therapy regimen based on theranostics αvβ3–targeting the nanoparticles. For this determination, fumagillin was combined into perfluorooctylbromide nanoparticles to elicit acute antiangiogenic effects. The impetus for this study is the observation in animal models that chronically high systemic doses of a water-soluble version of fumagillin resulted in a decrease in neovascularization and plaque development, thus a targeted nano agent may allow for localized delivery requiring decreased dosing (Gundogdu et al. 2014).

Dendrimers

Other types of drug delivery vehicles used are polymeric micelles and dendrimers.

Dendrimers are the smallest of the nanocarriers, which have their own multiple end groups appropriate for high degree of link targeting or the active agents. They symbolize polymer chains branching at regular splitting intervals, providing fast extension in the number of end groups with increasing molecular mass (Garg et al. 2011b). Dendrimers are typically very even in size, and can attain very high molecular masses beyond 1,000 kD. Because of their extremely branched structure, they tend to accept a sphere-shaped geometry. Dendrimers with a very low intrinsic viscosity and the very high surface-to-volume ratio, as related to linear polymers of the same molecular weight (Lee et al. 2005). They are prepared by certain amphiphillic co-polymers consisting of both hydrophilic and hydrophobic monomer units. These are used to carry those drugs which have poor solubility. Techniques that use reactive polymers alongside a hydrophobic additive to formulate a larger micelle that form a range of sizes have been developed. Dendrimers are also polymer-based drug delivery vehicles. Dendrimers have a core that branches out in regular intervals to produce a spherical, small, and very dense nanocarrier.

Artificial DNA nanostructures

The success of DNA nanotechnology lies in artificially constructing specially designed nanostructure systems for DNA computing. Artificial nucleic acid nano devices could be used to target delivery of drugs based upon sensing their environment (Singh et al. 2014b). These methods use the DNA as a chemical material and do not make use of its biological role as the carrier of genetic DNA information. Nucleic acid logic circuits that can be used as the core of the system which helps to release the drug only in response to a stimulus like a specific mRNA have been demonstrated (Singh et al. 2012a). In addition, the DNA "box" with a controllable lid has been formulated by using the origami method. This structure can encapsulate the drug in the closed state, and open to release it only in the response to the required stimulus (Singh et al. 2012b).

Ultrasound-mediated drug delivery

Ultrasound has been industrialized as both a valuable analytic tool and a potent promoter of valuable tissue for the

management and treatment of cardiovascular disease. These properties can be mediated by mechanical fluctuations of circulating micro bubbles, or ultrasound contrast agents, which might also encapsulate and guard a therapeutic agent in the bloodstream (Arora et al. 2012a). Oscillating micro bubbles can generate stresses directly on nearby tissue and induce fluid effects which result in drug penetration into vascular tissue, lyse thrombi and deliver drugs straight to the optimal sites for distribution (Sutton et al. 2013). The current review investigations provide proof for US-mediated drug delivery as a potent technique to deliver therapeutics to diseased tissue for the cardiovascular treatment. In particular, the attention will be on the investigations of specific aspects involving US-mediated drug delivery, such as delivery vehicles, drug transport paths, biochemical mechanisms and molecular targeting approaches (Sutton et al. 2013).

Gene therapy for cardiovascular disease mediated by ultrasound and micro bubbles

In these delivery systems, the delivery of a gene is highlighted to cure or treat the cardiovascular disease (Goyal et al. 2013a). Gene therapy provides an efficient approach for the treatment of cardiovascular disease to realize the beneficial therapeutic effect, both in the capable delivery to the target cells and in the sustained appearance of transgenes (Garg et al. 2011a). The ultrasound targeted micro bubble destruction (UTMD) technique has become a potential strategy for target-specific gene and drug delivery. Even though recent progress has been made in the remedy and analysis, cardiovascular disease remains the chief cause of death in so many countries (Garg et al. 2012a). Therefore, there is a strong impetus for more effective treatment and prevention. With increasing insight into the molecular mechanisms of cardiovascular diseases, gene therapy has been projected as the hopeful therapeutic tool for the treatment of cardiovascular diseases (Sharma and Garg 2012). To realize efficient delivery of therapeutic genes to the cardiovascular system, a sequence of barricades related to almost every aspect of cellular biology needs to be overcome (Goyal et al. 2013b). Primarily, the gene vectors must pass through endothelial barricades in the capillary walls when systemically inserted. By this time, the plasmid faces the risk of being ruined rapidly by DNAse in the serum before transfection. Thus, viral gene vectors are necessary to avoid immunoreaction in the blood circulation and transduction of non-targeted organs, mainly the spleen and liver. Then, as the plasma membrane and gene vector are negatively charged, the gene vectors have to diffuse by the myocardial membrane then fix to the cell surface but to resist from it (Kataria et al. 2014). Then the plasmid needs to escape being entrapped in the lysosome, where it will degrade. Then, the gene vector has to infiltrate the nuclear membrane to attain the goals of gene therapy. Still, suitable technologies can be used to create the gene vector itself with a wide-ranging target of the focus area, like a surgical operation or an injection catheter. Direct injection of the vector into the myocardium will lead to great local concentration of the gene vector. Optimized surface of the gene vector can realize directional transduction of vector into the cell(Kaur et al. 2014a). Nabel et al established gene therapy for the cardiovascular system in 1989. Since then, gene therapy trials for the cardiovascular diseases have been accomplished all over the world. However, progress in the region of gene therapy for cardiovascular disease is not very satisfactory because of the lack of gene delivery systems to transfer the therapeutic gene to the specific target to provide an adequate dose of a therapeutic gene. Gene delivery systems are mainly divided into two kinds, namely, viral systems and non-viral systems. Recently, researchers found that adenovirus expressing sarcoplasmic reticulum Ca^{2+} ATPase (SERCA2a) into coronary arteries could prevent ventricular arrhythmias in an ischemia reperfusion model. They also employed adenoviral and AAV vectors to attain high RNAi activities. They displayed that an adenoviral small hairpin RNA vector could silence phospholamban in cardiomyocytes and improve hemodynamics in heart failure. Then, they planned a dimeric cardio tropic AAV vector to intravenously deliver the RNA molecule to the heart for easy long-term therapy. Sometimes, viral gene therapy has been exposed to criticism due to its potential for uncontrollable and insertion mutagenesis. Non-viral systems consist of chemical methods (i.e. liposome, nanoparticle and polymers) and physical approaches (comprising electroporation, gene gun, particle Bo ultrasound, utilization). The advantages of a non-viral system include convenience, less instruction of immune system and cost effectiveness, and no limitation in size of transgenic DNA compared with the viral system, which have prepared them for intelligent application in gene delivery.

Pulsatile drug delivery systems

Oral controlled drug delivery systems signify the most popular form of controlled drug delivery systems for the obvious rewards of the oral route for drug administration. However there are some conditions for which such a release design is not suitable, like cardiovascular diseases, asthma, arthritis, diabetes mellitus, and peptic ulcer. In these cases, the pulsatile drug delivery system is cast off, which releases the drug on a planned pattern that is at a suitable time and at a suitable site of action. Pulsatile drug delivery systems are essentially time-controlled drug delivery systems in which the system controls the lag time self-governing of environmental factors such as pH, gastro-intestinal enzymes, motility etc. (Garg et al. 2012b). The principal rationale for the use of pulsatile release is for the drugs where a constant drug discharge, that is, zero-order release is not preferred. In chromo pharmacotherapy, drug administration is coordinated with the biological rhythms to yield maximal therapeutic effect and minimum damage for the patient (Garg and Goyal 2012). Theoretically, pulsatile drug delivery systems delivered via the oral route could be divided into two separate types, namely time-controlled delivery systems and site-specific delivery systems. In the recent pharmaceutical applications including pulsatile delivery, multi particulate dosage forms (e.g. pellets) are attaining much favor over single-unit dosage forms. Numerous pulsatile technologies have been formulated on the basis of methodologies, these include CODAS®, PRODAS®, SODAS®, ACCU-BREAK™, MINITABS®, DIFFUCAPS®, AQUALON, OROS® etc. Designing proper

pulsatile drug delivery will improve the patient compliance, promote ideal drug delivery to the target side and decrease the undesired effects. Table II shows the marketed formulations of the pulsatile drug delivery system. The benefits of the pulsatile drug delivery system are improved patient compliance, reduced side effects, reduction in dose size, dosage frequency, extended daytime or night time activity, reduction in the daily cost for the patient, protection of mucosa from irritating drugs, prohibition of drug loss by the extensive first pass metabolism, targetability of the drug to specific sites like the colon, and adaptability of the drug to suit circadian rhythms of body functions. However, there are some limitations associated with it, such as the lack of industrial reproducibility as well as efficacy, large number of development variables, need of advanced technology, higher cost of production, multiple formulation steps, and trained and skilled personnel requirements for manufacturing.

Advancement in pulsatile drug delivery system

Floating pulsatile drug delivery system

The floating approach has been used for gastric retention of the pulsatile dosage form. The floating-pulsatile model was useful to increase the gastric residence of the dosage form, having a lag phase followed by a burst release. Nowadays, chronotherapeutic formulations are established for precisely time-controlled release of dosage forms, in order to attain the maximum drug concentration in plasma at the peak time of symptomatology. To follow this standard, the dosage form should be taken at the suitable time which is before sleep, providing maximum drug release in the morning. The main difficulty of these systems lies in achieving the long residence time which is desired for diseases demanding morning medicine. With the conservative pulsatile release of dosage forms, the highly inconsistent nature of the gastric emptying procedure can result in *in vivo* variability and bioavailability problems. A combination of floating and pulsatile principles of drug delivery system would have the advantage that a drug can be released in the upper GI tract after a defined time period of no drug release. A pulsatile drug delivery that can be administered at bed time but releases the drug early in the morning would be a promising chronotherapeutic system. Table III represents a pulsatile drug delivery system for the treatment of angina pectoris. The development of floating pulsatile-release products is most challenging, as it means to get the right drug to the right place at right time. The novel FPDDS pays much consideration to the site and time-specific drug delivery. In these types of systems, there is the release of the drug after eroding or rupturing the polymer coating of the dosage form. Through the last two decades, technology has developed to ensure time-controlled floating pulsatile release of bioactive compounds. Significant progress has made toward attaining floating and pulsatile drug delivery systems in combinations that can effectively treat diseases with non-constant dosing treatments, such as hypertension.

Table II. Marketed formulations of pulsatile drug delivery system.

Formulations	Applications
PULSYS ™ Middlebrooks Pharmaceuticals	This company has developed a typical palsy's drug delivery format which is a tablet comprising multiple pellets with different release profiles
Orbexa® Eurand pharmaceutics	This company has the technology to produce beads that are of controlled size and density using granulation, by two techniques, that is spheronization and extrusion techniques
Diffucaps® Eurand pharmaceutics	The active drug is layered onto a neutral and then one or more rate-controlling, functional membranes are applied
Pulsincap® Scherer DDS, Ltd	A water impermeable capsule body with hydro gel plug. Plug length and insertion depth controls lag time

Nanogels

Nanogels are based on the cross-linked hydrophilic materials, developing insoluble gels that swell in water due to the hydrophilic nature of the inner volume and structural components. Nano gel loaded drugs remain in the native conformation. Uncontrolled accumulation is the technical challenge in the preparation of the injectable nano gels. The main necessity for any cardiovascular nano carrier is biocompatibility, which means they could be injected intravenously without the toxicity and side-effects including the stimulation of platelets, leukocytes, coagulation, complement, and kinins. Poor drug residence in arterial walls hinders the clinical implementation of the local drug delivery for the management of restenosis, but the problem has now been resolved by a new type of nanoparticle (Garg and Goyal 2014a). Figure 3 represents the basic pattern of drug release from nanogels.

Injectable cell constructs are fabricated via culture on a thermo responsive methylcellulose hydrogel system for the treatment of ischemic diseases. Cell transplantation via direct intramuscular injection is a promising therapy for patients with ischemic diseases. Sometimes, following injections, holding of transferred cells in the engrafted areas always remains problematic, and could be harmful to cell transplantation treatment. In the Evolvement Report, a thermo responsive hydrogel system composed of aqueous methylcellulose (MC) blended with phosphate-buffered saline is constructed to grow cell sheet fragments and cell bodies for the treatment of ischemic diseases (Garg et al. 2012c). They prepared the MC Hydrogel system which undergoes a sol-gel alterable transition upon heating or cooling at ≈32°C. Via this unique property, the cell sheet fragments (cell bodies) grown can be harvested without using proteolytic enzymes; consequently, their inherent extracellular matrices (ECMs) and integrative adhesive agents remain preserved. In the animal studies using pigs and rats with experimentally created myocardial infarction, the injected cell sheet fragments (cell bodies) become entrapped in the interstices of muscular tissues and adhere with engraftment sites, whereas a minimal number of the cells occur in the group receiving the detached cells. Furthermore, transplantation of the cell sheet fragments (cell bodies) significantly rises the vascular density, thus refining the function of an infarcted heart (Huang et al. 2014).

Liposome modified nanogels

Kono et al. have disclosed liposomes bearing succinylated poly (glycidol)s. These liposomes undergo a chain of

Table III. Pulsatile drug delivery system for the treatment of angina pectoris.

Polymer	Drug	Inferences	References
Hydroxypropyl methylcellulose (HPMC) K100 M, citric acid	Atenolol	The capability of the system with its prolonged residence of the tablets in the stomach and release of drug after a programmed lag time	Jagdale et al. (2013)
Polypyrrole	Dodecylbenzenesulfonate	The fabrication of electrically responsive nanoporous membrane used for emergency therapy of angina pectoris	Jeon et al. (2011)
Microcrystalline cellulose (MCC) and lactose	Isosorbide-5-mononitrate	The pellets achieved a lag time of 4.1 h *in vivo*, which had a good consistency with the in vitro results, and the relative bioavailability was nearly 100% comparing to the normal tablets	Liu et al. (2009)
PVP/HPMC	Atenolol	Upon exposure of the prepared tablets to the release medium it was found that the coating layer disintegrates first, followed by the immediate release of FELO from the active core	Karavas et al. (2006)

fusion reactions below the pH 5.5 which has shown to efficiently supply calcein to the cytoplasm. Liposomes anchored by or modified with poly(N isopropylacrylamide)-based copolymer groups are suitable for thermo- and pH-responsive nanogels, which are being investigated for transdermal drug delivery (Jung et al. 2009).

Micellar Nanogels

Polymer micellar nanogels can be obtained by the supramolecular self-assembly of amphiphillic block or graft copolymers in aqueous solutions (Garg et al. 2012d). They possess unique core-shell morphological structures, where a hydrophobic block segment in the form of a core is surrounded by hydrophilic polymer blocks as a shell (corona) that stabilizes the entire micelle. The core of micelles provides enough space for accommodating various drug or bio macromolecules by physical entrapment (Garg et al. 2013). Furthermore, the hydrophilic blocks may form hydrogen bonds with the aqueous media that lead to a perfect shell formation around the core of the micelle. Therefore, the drug molecules in the hydrophobic core are protected from hydrolysis and enzymatic degradation. Researchers have successfully developed highly versatile Y-shaped micelles of poly (oleic acid-Y-N-isopropylacrylamide) for drug delivery application. In this study, the delivery of prednisone acetate above its lower critical solution temperature (LCST) was demonstrated.

Conclusions

Nanocarriers and their advancement are a promising and innovative drug delivery system that can play a vital role by addressing the problems associated with old and modern therapeutics such as nonspecific effects and poor stability. A nanocarrier has overcome many challenges from blood-brain barrier to targeting diseases. Nanocarriers are improving molecular imaging to help improve diagnosis and treatment of cardiovascular disease. Future design and development of effective nanocarrier-based drug delivery systems for *in vivo* applications require a high degree of control over properties.

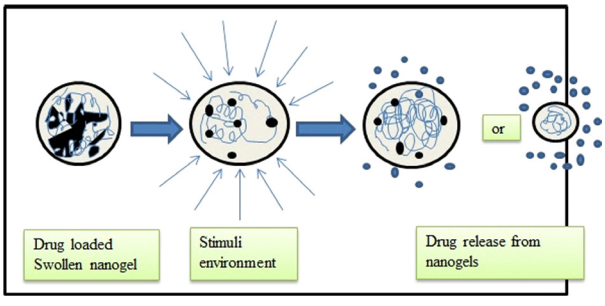

Figure 3. Pattern of drug release from nanogels.

Declaration of interest

The authors report no declarations of interest. The authors alone are responsible for the content and writing of the paper.

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References

Alwan A. 2011. Global Status Report on Noncommunicable Diseases 2010. Geneva: World Health Organization.

Anthea M, Hopkins J, Mclaughlin CW, Johnson S, Warner MQ, Lahart D, Wright JD. 1993. Human Biology and Health. Englewood Cliffs: New Jersey, pp 76–134.

Arora N, Bilandi A, Garg T. 2012a. Microneedle: as a future prospective tool. Int Res J Pharm. 3:102–105.

Arora N, Singh K, Garg T. 2012b. Areas of nanomedicine applications. Int J Univ Pharm Life Sci. 2:216–227.

Chang T. 2011. Nanobiotechnology-based blood substitutes and the cardiovascular systems in transfusion medicine. Nanomedicine Cardiovasc Syst. 77–98.

Chang TM. 2012. From artificial red blood cells, oxygen carriers, and oxygen therapeutics to artificial cells, nanomedicine, and beyond. Artif Cells Blood Substit Immobil Biotechnol. 40:197–199.

Christian DA, Cai S, Bowen DM, Kim Y, Pajerowski JD, Discher DE. 2009. Polymersome carriers: from self-assembly to siRNA and protein therapeutics. Eur J Pharm Biopharm. 71:463–474.

Dantas AP, Jiménez-Altayó F, Vila E. 2012. Vascular aging: facts and factors. Front Physiol. 3:325.

Ding B-S, Dziubla T, Shuvaev VV, Muro S, Muzykantov VR. 2006. Advanced drug delivery systems that target the vascular endothelium. Mol Interv. 6:98.

Discher BM, Won Y-Y, Ege DS, Lee JC, Bates FS, Discher DE, Hammer DA. 1999. Polymersomes: tough vesicles made from diblock copolymers. Science. 284:1143–1146.

Fuster V, Kelly BB. 2010 Promoting Cardiovascular Health in the Developing World: A Critical Challenge to Achieve Global Health Washington, DC: National Academies Press.

Gagandeep GARG, T., Malik B, Rath G, Goyal AK. 2014. Development and characterization of nano-fiber patch for the treatment of glaucoma. Eur J Pharm Sci. 53:10–16.
Garg T. 2012. An evolutionary approaches in development of needle free injection technologies. Int J Pharm Pharm Sci. 4:590–596.
Garg T. 2014. Current nanotechnological approaches for an effective delivery of bio-active drug molecules in the treatment of acne. Artif Cells Nanomed Biotechnol. 1–8.
Garg T, Bilandi A, Kapoor B. 2011a. Scaffold: tissue engineering and regenerative medicine. Int Res J Pharm. 2:37–42.
Garg T, Bilandi A, Kapoor B. 2012a. Evolutionary approaches for drug and gene delivery by micro and nano magnetic carriers. Indo-global Res J Pharm Sci. 2:182–189.
Garg T, Chanana A, Sharma G. 2012b. Pulsatile drug delivery systems: pulsincap system. IOSR J Pharm. 2:338–339.
Garg T, Goyal AK. 2012. Iontophoresis: drug delivery system by applying an electrical potential across the skin. Drug Deliv Lett. 2:270–280.
Garg T, Goyal AK. 2014a. Biomaterial-based scaffolds–current status and future directions. Expert Opin Drug Deliv. 11:767–789.
Garg T, Goyal AK. 2014b. Liposomes: targeted and controlled delivery system. Drug Deliv Lett. 4:62–71.
Garg T, Goyal AK, Arora S, Murthy R. 2012c. Development, optimization and evaluation of porous chitosan scaffold formulation of gliclazide for the treatment of Type-2 diabetes mellitus. Drug Deliv Lett. 2:251–261.
Garg T, Rath G, Goyal AK. 2014. Comprehensive review on additives of topical dosage forms for drug delivery. Drug Deliv.
Garg T, Singh O, Arora S, Murthy R. 2011b. Dendrimer—A novel scaffold for drug delivery. Int J Pharm Sci Rev Res. 7:211–220.
Garg T, Singh O, Arora S, Murthy R. 2012d. Scaffold: a novel carrier for cell and drug delivery. Crit Rev Ther Drug Carrier Syst. 29:1–63.
Garg T, Singh S, Goyal AK. 2013. Stimuli-sensitive hydrogels: an excellent carrier for drug and cell delivery. Crit Rev Ther Drug Carrier Syst. 30:369–409.
Geiser M, Rothen-Rutishauser B, Kapp N, Schürch S, Kreyling W, Schulz H, et al. 2005. Ultrafine particles cross cellular membranes by nonphagocytic mechanisms in lungs and in cultured cells. Environ Health Perspect. 113:1555.
Goyal AK, Rath G, Garg T. 2013a. Nanotechnological approaches for genetic immunization. In: Erdmann VA and Barciszewski J, Eds. DNA and RNA Nanobiotechnologies in Medicine: Diagnosis and Treatment of Diseases Berlin: Springer, pp. 67–120.
Goyal G, Garg T, Malik B, Chauhan G, Rath G, Goyal AK. 2013b. Development and characterization of niosomal gel for topical delivery of benzoyl peroxide. Drug Deliv.
Gundogdu E, Senyigit Z, Ilem-Ozdemir D. 2014. Nanomedicine for the diagnosis and treatment of cardiovascular disease: current status and future perspective. Cardiovascr Dis I.
Haeri A, Sadeghian S, Rabbani S, Anvari MS, Boroumand MA, Dadashzadeh S. 2011. Use of remote film loading methodology to entrap sirolimus into liposomes: Preparation, characterization and in vivo efficacy for treatment of restenosis. Int J Pharm. 414:16–27.
Huang CC, Liao ZX, Chen DY, Hsiao CW, Chang Y, Sung HW. 2014. Injectable cell constructs fabricated via culture on a thermoresponsive methylcellulose hydrogel system for the treatment of ischemic diseases. Adv Healthc Mater.
Ise M, Ise H, Shiba Y, Kobayashi S, Goto M, Takahashi M, et al. 2011. Targeting N-acetylglucosamine-bearing polymer-coated liposomes to vascular smooth muscle cells. J Artif Organs. 14:301–309.
Jagdale SC, Sali MS, Barhate AL, Kuchekar BS, Chabukswar AR. 2013. Formulation, development, and evaluation of floating pulsatile drug delivery system of atenolol. PDA J Pharm Sci Technol. 67:214–228.
Janib SM, Moses AS, Mackay JA. 2010. Imaging and drug delivery using theranostic nanoparticles. Adv Drug Deliv Rev. 62:1052–1063.
Jeon G, Yang SY, Byun J, Kim JK. 2011. Electrically actuatable smart nanoporous membrane for pulsatile drug release. Nano Lett. 11:1284–1288.
Jordan SW, Chaikof EL. 2007. Novel thromboresistant materials. J Vasc Surg. 45:A104–A115.
Jung H, Jang M-K, Nah J-W, Kim Y-B. 2009. Synthesis and characterization of thermosensitive nanoparticles based on PNIPAAm core and chitosan shell structure. Macromol Res. 17:265–270.
Karavas E, Georgarakis E, Bikiaris D. 2006. Application of PVP/HPMC miscible blends with enhanced mucoadhesive properties for adjusting drug release in predictable pulsatile chronotherapeutics. Eur J Pharm Biopharm. 64:115–126.
Kataria K, Sharma A, Garg T, Goyal AK, Rath G. 2014. Novel technology to improve drug loading in polymeric nanofibers. Drug Deliv Lett. 4:79–86.
Kaur M, Garg T, Rath G, Goyal AK. 2014a. Current nanotechnological strategies for effective delivery of bioactive drug molecules in the treatment of tuberculosis. Crit Rev Ther Drug Carrier Syst. 31:49–88.
Kaur M, Malik B, Garg T, Rath G, Goyal AK. 2014b. Development and characterization of guar gum nanoparticles for oral immunization against tuberculosis. Drug Deliv.
Kona S, Dong J-F, Liu Y, Tan J, Nguyen KT. 2012. Biodegradable nanoparticles mimicking platelet binding as a targeted and controlled drug delivery system. Int J Pharm. 423:516–524.
Lee CC, Mackay JA, Fréchet JM, Szoka FC. 2005. Designing dendrimers for biological applications. Nat Biotechnol. 23: 1517–1526.
Lim SS, Vos T, Flaxman AD, Danaei G, Shibuya K, Adair-Rohani H, et al. 2013. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet. 380: 2224–2260.
Liu Y, Liu S, Dai Q. 2009. Design and evaluation of pH-independent pulsatile release pellets containing isosorbide-5-mononitrate. Chem Pharm Bull. 57:55–60.
Magrez A, Kasas S, Salicio V, Pasquier N, Seo JW, Celio M, et al. 2006. Cellular toxicity of carbon-based nanomaterials. Nano Lett. 6:1121–1125.
Mathers C, Fat DM, Boerma J. 2008. The Global Burden of Disease: 2004 Update. Geneva: World Health Organization.
Mathers CD, Loncar D. 2006. Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med. 3:e442.
McGill HC, Mcmahan CA, Gidding SS. 2008. Preventing Heart Disease in the 21st Century Implications of the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Study. Circulation. 117:1216–1227.
Miyake F, Kamogawa A, Sakakibara M. 1998. [Drug delivery system in cardiovascular drugs]. Nihon Rinsho. 56:737–741.
Mobed M, Chang TM. 1998. Comparison of polymerically stabilized PEG-grafted liposomes and physically adsorbed carboxymethylchitin and carboxymethyl/glycolchitin liposomes for biological applications. Biomaterials. 19:1167–1177.
Moghimi SM, Hunter AC, Murray JC. 2005. Nanomedicine: current status and future prospects. FASEB J. 19:311–330.
Nel A, Xia T, Mädler L, Li N. 2006. Toxic potential of materials at the nanolevel. Science. 311:622–627.
Nishiya T, Chang TM. 1994. Toxicity of liposomes containing low mol% of dienoyl phosphocholine to blood: use of carboxymethyl chitin to reduce toxicity. Artif Cells Blood Substit Immobil Biotechnol. 22:883–888.
Parnami N, Garg T, Rath G, Goyal AK. 2013. Development and characterization of nanocarriers for topical treatment of psoriasis by using combination therapy. Artif Cells Nanomed Biotechnol.
Patel SS. 2006. Liposome: a versatile platform for targeted delivery of drugs. Pharmainfonet. 5:1–5.
Photos PJ, Bacakova L, Discher B, Bates FS, Discher DE. 2003. Polymer vesicles in vivo: correlations with PEG molecular weight. J Control Release. 90:323–334.
Pi F, Tu X, Wu Y. 2010. [Preparation of ATP-2Na loaded liposome and its effect on tissues energy state in myocardial ischemic mice]. Yao Xue Xue Ba.*o*, 45:1322–1326.
Rosamond WD, Chambless LE, Folsom AR, Cooper LS, Conwill DE, Clegg L, et al. 1998. Trends in the incidence of myocardial infarction and in mortality due to coronary heart disease, 1987 to 1994. N Engl J Med. 339:861–867.
Sharma G, Garg T. 2012. Importance and application of gene therapy. Int J Institut Pharm Life Sci. 2:205–220.
Shuvaev VV, Dziubla T, Wiewrodt R, Muzykantov VR. 2004. Streptavidin-biotin crosslinking of therapeutic enzymes with carrier antibodies. In: Niemeyer CM, Ed. Bioconjugation Protocols. Berlin: Springer.
Singh H, Sharma R, Joshi M, Garg T, Goyal AK, Rath G. 2014a. Transmucosal delivery of Docetaxel by mucoadhesive polymeric nanofibers. Artif Cells Nanomed Biotechnol.
Singh K, Arora N, Garg T. 2012a. RFID: A trustable security tool in pharmaceutical industry. Am J Pharm Tech Res. 2:113–127.
Singh K, Arora N, Garg T. 2012b. Superbug: antimicrobial resistance due to NDM-1. Int J Institut Pharm Life Sci. 2:58–66.

Singh O, Garg T, Rath G, Goyal AK. 2014b. Microbicides for the treatment of sexually transmitted HIV infections. J Pharm. 1–18.

Song C, Labhasetwar V, Cui X, Underwood T, Levy RJ. 1998. Arterial uptake of biodegradable nanoparticles for intravascular local drug delivery: results with an acute dog model. J Control Release. 54: 201–211.

Sutton JT, Haworth KJ, Pyne-Geithman G, Holland CK. 2013. Ultrasound-mediated drug delivery for cardiovascular disease. Expert Opin Drug Deliv. 10:573–592.

Takahama H, Shigematsu H, Asai T, Matsuzaki T, Sanada S, Fu HY, et al. 2013. Liposomal amiodarone augments anti-arrhythmic effects and reduces hemodynamic adverse effects in an ischemia/reperfusion rat model. Cardiovasc Drugs Ther. 27:125–132.

Torchilin VP. 2012. Multifunctional nanocarriers. Adv Drug Deliv Rev. 64:302–315.

Wang Y, Chang TM. 2012. Nanobiotechnological nanocapsules containing polyhemoglobin-tyrosinase: effects on murine B16F10 melanoma cell proliferation and attachment. J Skin Cancer. 2012:673291.

Wiewrodt R, Thomas AP, Cipelletti L, Christofidou-Solomidou M, Weitz DA, Feinstein SI, et al. 2002. Size-dependent intracellular immunotargeting of therapeutic cargoes into endothelial cells. Blood. 99:912–922.

Wilson JT, Cui W, Sun X-L, Tucker-Burden C, Weber CJ, Chaikof EL. 2007. In vivo biocompatibility and stability of a substrate-supported polymerizable membrane-mimetic film. Biomaterials. 28:609–617.