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Nanotechnology For Regenerative Medicine In Cardiovascular Diseases: An Updated Review.

K. Kavitha, A. Deevan Paul*, B. Shrivastava, and Pankaj Sharma.

School of Pharmaceutical Sciences, Jaipur National University, Jaipur (India)

ABSTRACT

Recently, a wide range of nanotechnologies has been approached for material modification by realizing the fact that the extracellular matrix (ECM) consists of nanoscale components and exhibits nanoscale architectures. Moreover, cell-cell and cell- ECM interactions actively occur in the nanoscale and ultimately play large roles in determining cell fate in tissue engineering. Nanomaterials have provided the potential to preferentially control the behavior and differentiation of cells. The present paper reviews the need for nanotechnology in regenerative medicine and the role of nanotechnology in repairing, restoring and regenerating damage body parts, such as blood vessels and the heart. The field of nanotechnology has witnessed and explosion in research efforts over the past decade. However, nanotechnology in regenerative medicine is still at an infant level and is a complicated interdisciplinary field which needs the collective collaboration of physicists and clinicians. To help mature the exciting field of nanotechnology, researches must unravel the mechanisms of cell-biomaterial interactions at the nanoscale and develop unique nanotechnology applications in regenerative medicine.

Keywords: Nanotechnology, regenerative medicine, theranostic, Vascularization

Corresponding author

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INTRODUCTION

The current challenges in the cardiovascular diseases (CVD) usage of Nano-technology will provide greater scope for providing treatment. Kong, in his report stated that says Nano-technology helps in detecting and providing therapy by improving the ex-vivo and in-vivo detection and imaging of biomarkers, and it helps in upgrading the delivery of drugs and tissue regeneration. The current study will provide an in-depth understanding on the recent developments in nanotechnology for the detection and therapy of cardiovascular diseases (CVD) focusing on nanoparticles [1].

Regenerative medicine holds the great promise for restoring the normal, healthy functions of human tissues after damage. Its Potential to treat a broad range of degenerative and ischemic diseases in tissues or organs has been improved with significant progress in understanding biological mechanisms. Many research studies have used nanoparticles as a Nano-carriers with varied surface characteristics and examined as therapeutic and theranostic agents for restenosis [2]. The researchers have examined by delivering growth factors, cytokines and drugs to the infarcted cardiac cells in two ways either by direct injection or by injecting biomolecule-loaded Nano-particles to the left ventricle (LV). However, these methods are not effective, due to lack of retention of the factors or micro particles in the desired area. Therefore, it is necessary to develop an approach that effectively deliver the bio-molecules into the infarcted area.

NANOTECHNOLOGY TOWARDS CARDIOVASCULAR DISEASES

Earlier most of the biomedical application of nanotechnology was in the field of cancer, later the study was extended in almost all fields of biomedical research.

In the current scenario, Cardiovascular Nano medicine is gaining importance in diagnosis, drug delivery, stem cell therapy, tissue engineering, stent surgery. In addition to these the most promising area is improving clinical imaging, for instance nanoimaging of cardiovascular diseases. Cardiovascular imaging is one of the most reliable diagnostic tools for cardiovascular diseases. Many research studies have hypothesized that nanoparticles are the unique contributors in the field of the medical imaging, with their special characteristics as [3].

(i) Biocompatible size distribution*:* The micro size of Nano particles helps in accommodating with different bio components even inside the subcellular organelle.

(ii) High penetration power: This is an important aspect fulfilled by nanoparticles for bio-medical imaging.

(iii) Image contrasting ability: Paramagnetic nanoparticles are magnetic resonance imaging (MRI) contrast agents. Iodinated nanoparticles can be used as computed tomography (CT) contrast agents, whereas quantum dots can act as fluorescent enhancers [4].

(iv)Surface tunable property*:* Nano surface can be modified with the molecules according to the choice. Due to this property, it is possible to conjugate a nanomaterial with multimodal entity, for example, target specific molecules (targeted delivery), imaging probes and/or therapeutic molecules.

(iv) Stability: Contrast enhancer nanomaterials are more stable than chemical image probe.

(v) Half life: In case of carrier nanoforms, used as image contrast agents, the half life of the chemical image probes is also increased due to their conjugation with nanoparticles [5,6]. Thus, atypical size distribution, target specific delivery, high contrast capability, increase lifetime are the key features that make nanomaterials indispensable in the future medical imaging.

Nano based cardiovascular imaging can analyses the live physiological system in a noninvasive manner, with almost no pain. This live imaging helps in both proper diagnosis and also beneficial for the basic understanding of the pathological conditions, which in turn helps us to develop future advanced techniques. Most of the Nano-based cardiovascular imaging modules are in the field of diagnosis, and has entered in the domain of therapy and surgery due to the advancement in technology [7].

In most of the cases, the Nano based imaging are not discrete, but are inter-connected between the fields of diagnosis, therapy or surgery. Thus, for the better understanding, Nano-cardiovascular-imaging can be broadly divided into four categories depending on the site of detection.

i) Thrombus imaging*:* Acute coronary syndrome (ACS) is one of the major cause that leads to many deaths in today's world scenario. Atherosclerotic plaques that are in humans contains various bio-components that are heterogeneous in nature such as macrophages, smooth muscle, endothelial cells, other undefined mesenchymal cells [8]. Therefore, proper detection of plaques in fore hand and in a non-invasive way is significant and this kind of detection procedure is demanding in treating the diseases accurately. Thus, modern physics found different non-invasive imaging techniques that can detect plaques through contrast agents. The choice of these contrast agents totally depends on the kind of the technique used. Most of the contrast agents that applied clinically pose two significant effects. They may show toxic effects or it may get distributed to the whole body through circulation due to absence of any target specificity. Due to these effects, the Nano-based imaging system plays a role of rescuer over the conventional clinical imaging techniques. The atherosclerotic plaques are classified as two types, stable plaques (fibrous plaque) and unstable plaques (lipid plaque). Unstable plaques mainly causes thrombosis, also called as vulnerable plaques. Vulnerable plaques constitute of large amount of lipids, wrapped up by a thin fibrous cap. Destruction of this fibrous cap makes the plaques unstable and these become detached from the endothelial layer [9]. This phenomenon triggers and activates the circulating resting platelets and results in formation of platelet rich thrombus. Thrombus blocks the artery, prevents local circulation and results in muscle necrosis. Hence detecting these vulnerable plaques is essential to initiate diagnosis or therapy for this disease. Macrophages is one of the vital components of atherosclerotic plaque that play a decisive role in plaque destabilization. These get cling to the thin fibrous cap of the plaques and secrete proteolytic enzymes that dissolve the fibrous cap81. Therefore, conceptually it has been explained by various literature that these macrophages are good identifiers of vulnerable plaques. Phagocytic activity is considered to be one of the key characteristic of macrophages that have been used in identifying the vulnerable plaque. Therefore it has been found that macrophages can effectively take up a wide range of nanomaterials, including contrast enhancing nanoforms. Therefore, nanoform loaded inflammatory macrophages on the plaque can be easily identified by non-invasive imaging techniques. Now, the choice of imaging techniques is based on the constituent of nanomaterials, or vice versa [10].

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generated by atherosclerosis induced inflammation. Under certain circumstances these oxidizing species can neutralize local antioxidant defenses, thus leading to oxidative stress and tissue injury. These oxidation reactions are mainly catalyzed by myeloperoxidase (MPO), a heme protein secreted from activated phagocytes in human atherosclerotic lesions. Though *in vivo* imaging of ROS/RNS has significant clinical impact, yet there is no conventional method for their detection. The oxazine Nano based imaging method has been developed to monitor hypochlorous acid (HOCl/OCl) formation by peroxynitrite, a reactive nitrogen species and myeloperoxidase (MPO), thereby identify the oxidative damage by atherosclerosis [11].

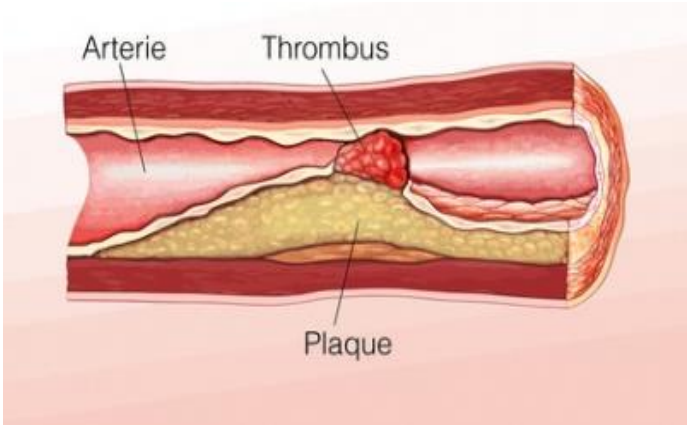

Figure No 1: Tissue constriction due to vascularization

(ii) Theranostic approach: 'Theranostic' is a new term used in clinical medicine that deals with a treatment strategy which combines both the therapeutics and diagnostics. It can be defined as a modified diagnostic

procedure equipped with therapeutic molecules. Theranosis has made a huge expectation in medical sciences because of its multimodal applications. It can reduces the steps and costs of both diagnosis and therapy. In addition, nanoparticles can also act as diagnostic probes (image contrast agent) and get conjugated with therapeutic or diagnostic molecules or vice versa.

Nanoimaging mediated cardiovascular theranosis is a newly introduced area. Simultaneous detection and volume reduction (thrombolysis/fibrinolysis) of thrombus is one such approach. The sole component of the thrombolytic / fibrinolytic pathway is plasminogen, which gets converted into plasmin (serine proteinase) by plasminogen activators, *i.e.* tissue-type PA (tPA) and urokinase type PA (uPA). This plasmin reduces fibrin and different extracellular matrix proteins (fibronectin, laminin, proteoglycan, and type IV collagen), results in reducing the plaque volume. Now Recombinant tissue plasminogen activator (rTPA) is recognized as an effective therapeutic molecule that dissolve plaque. In this context, a Nano-based theranostic approach can be conceptualized to analyse as well as to reduce plaque volume. It has been already found in previous studies that iron oxide nanoparticles tagged with rTPA can efficiently dissolve clot. A real time monitoring on thrombolytic effect has been done using fluorophores coated nanoparticles. Therefore, diagnosis of plaque and its volume reduction can be carried out with the help of nanotechnology [12,13]. Another theranostic approach is detection and inhibition of angiogenesis. Angiogenesis is an essential phenomenon during the development of atherosclerotic plaque. Neovascularization is associated with risk of plaque rupture; plaque progression therefore the consequence is myocardial infarction105. Integrin αvβ3 is only expressed in angiogenic vasculature, not in mature vasculature; hence can act as a marker of active angiogenesis. To get molecular image (MRI) of angiogenesis, ultra small paramagnetic iron oxide nanoparticle has been developed to target integrin αvβ3 receptor. The research in this context has further led to the "detection and quantification of early angiogenesis" (through MRI) by integrin αvβ3 targeted per fluorocarbon. The ultimate nanotechnology based theranostic approach shows that fumagillin (potent angiogenic inhibitor) incorporated with paramagnetic nanoparticle not only detects early angiogenesis, but also effectively inhibits it [14].

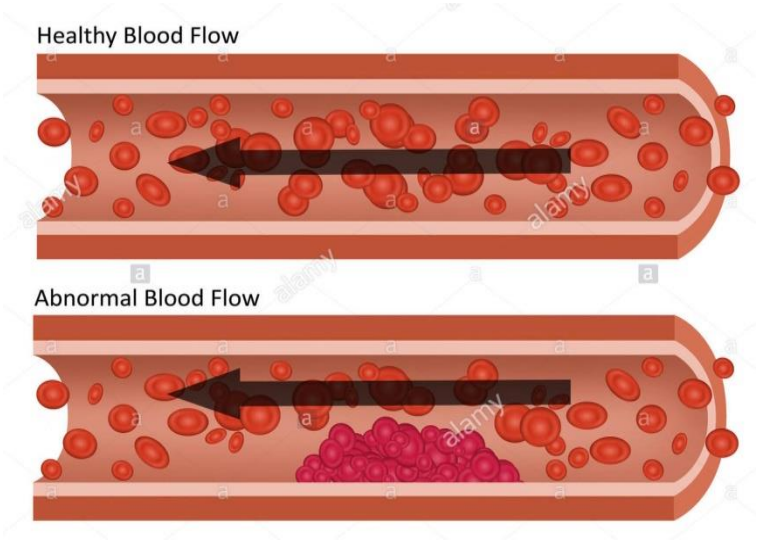

Figure No 2: Coronary artery flow reduction in the absence of Pharmacologic therapy.

(iii) Stem cell imaging: The most important approach is an in-depth investigation, that provides hope in using stem cell technology while treating of cardiovascular diseases. Infarcted myocardium can't be replaced, because the human cardiomyocytes are post-mitotic cells, therefore cannot proliferate after birth. Recent findings show that mesenchymal stem cells (MSCs) are the bone marrow stromal cells which can differentiate into cardiomyocytes in an appropriate condition. Most interesting fact revealed in recent studies that, "transplantation of MSCs can improve cardiac activity in patients with myocardial infarction (MI)".Therefore proper implementation of the MSCs transplantation in terms of fraction (%) of cells reached to the infracted myocytes that is of great importance to prognosis of the disease. So far, Super paramagnetic iron oxide nanoparticle (SPION) are found efficient in this regard. Super paramagnetic iron oxide nanoparticle labelled stem cell tracking and targeting has been piloted effectively in animal models with chronic MI. Cellular

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magnetic resonance imaging is found to be the effective method for the study of SPION guided delivery of MSCs to the infarcted muscle [15,16].

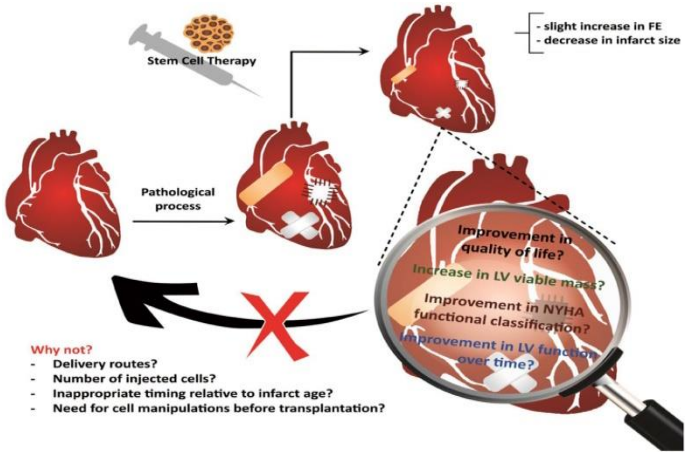

Figure No 3: Stem cell therapy of Pathological process

(iv) Graft imaging: Heart transplantation is the only treatment for patients with an end-stage heart failure or severe coronary artery disease. Even after heart transplantation, patients have to undergo repeated endomyocardial biopsies to see transplant graft rejection. This procedure has significant risk, prone to sampling error and can induce fibrotic tissue build up at the site of biopsies. A recent nanotechnology based approach has shown that fluorophore tagged iron oxide nanoparticle can efficiently diagnose this pathological condition. Macrophages and cathepsin (protease) play a key role during graft rejection; therefore, these are attractive molecular imaging targets. These fluorescent conjugated magnetic nanoparticles have been used as a marker for macrophages with phagocytic activity [17,18].

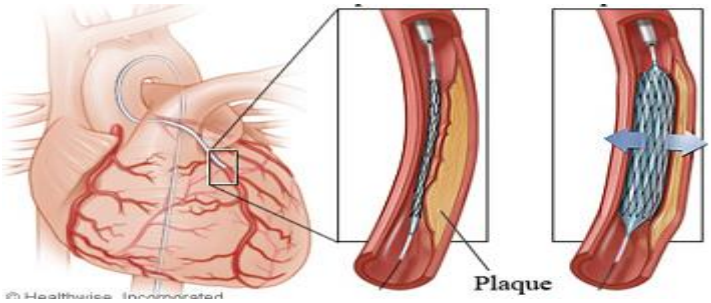

Figure No 4: Mechanical Stress, Lesion Vulnerability, and Rupture

A limited number of biomechanical and imaging studies started to emerge in the early 1990s addressing the role of hemodynamic shear stress in the destabilization of vulnerable plaques.

The underlying premise is based on observations that atherosclerosis is a focal point, where rupture occurs more frequently at the proximal side of the stenosis near flow dividers, an area where secondary wall shear stress is assumed to be the highest. Consistent with these observations, blood flow–induced shear stress may also present a significant influence on processes that govern fibrous cap morphology and composition, where increased peak circumferential stress is greater in thinner fibrous caps. Therefore, regions of high shear stress typically exhibit high strain, thus supporting the notion that mechanical stress applied to a weakened fibrous cap may precipitate rupture, particularly in the presence of macrocalcification[19-21].

CARDIOVASCULAR STENTS

Coronary stents represent one of the most rapidly growing applications of implantable cardiovascular biomaterials. Stents have been shown to reduce restenosis compared with balloon angioplasty, but in – stents restenosis (re-narrowing within the confines of the stent) is also a problem, occurring in 10-60% of patients.

The placement of stent is bale to smooth the luminal surface following creation of an arterial dissection after angioplasty and to prevent elastic recoil leading to the suboptimal expansion if the flow lumen. Restenosis migration of smooth muscle cells into the neointima [22-26]. The smooth muscle cells ultimately produce significant amounts of matrix protein, leading to a mostly collagen- containing lesion and neointimal thickening. Initial studies have shown that coating stents with drugs such as paclitaxel or sirolimus may result in a 70-80% reduction in necessity for repeat procedures for restenosis. However, these drug -eluting stents aimed at inhibiting the cell cycle also inhibit the normal remodeling of the vessel that results in the stent becoming implanted in the vascular wall. Because of this, the exposed stents are prone to thrombus formation, and patients must given antiplatelet drugs to prevent clotting. Additionally, there are several other stent coatings currently under investigation, including polytetrafluoroethylene, 7-hexanolytaxol, and rapamycin-eluting microporous stents. Nanotextured stent coatings (hydroxyapatite and Titania) are currently under development with the aim of enhancing vascular cell attachment and proliferation (to promote re-endothelization of the vessel wall). An innovative alternative to the problem of neointima formation after balloon injury proposed by Wong et al. biased on the use of submicroscopic spheres to provide a sustained release of elastase that creates a chemoattractant gradient across arterial walls, directing smooth muscle cell migration away from the lumen. It is also possible that this technology can be utilized to direct cell migration in other areas, such as to injury sites for repair purposes or away from injury to decrease macrophage infiltration [26-32].

AN OUTLOOK ON CHALLENGES AND FUTURE OPPORTUNITIES

One of the most significant advances has been the rapid development of optical and ultrasound nanoparticles [33]. Especially the introduction of quantum dots, as well as the use of near-infrared probes and highly sensitive detectors, have now enabled imaging of deeply seated tissue structures [34], allowing clinical optical imaging [35]. The availability of calcium-sensitive agents, for instance, allows an in vivo imaging approach that bridges the gap between conventional single-cell electrophysiological recording and macroscopic activity recording, such as functional MRI [36]. Light-sensitive theranostic nanoparticles can also be used to monitor reaching a treatment site, with a specific light wavelength triggering the release of drug in just this area, hence providing a very targeted treatment [37]. These approaches further lend inspiration to the development of probes for other modalities, such as MRI, that currently still dominate the clinical arena. However, optical imaging is currently seeing a more rapid development of nanoparticles than any other biomedical imaging modality. The shift beyond near infrared reduces tissue light scatter and greater organ coverage will eventually dominate biomedical imaging in smaller species to drive a deeper understanding of biology. Still, it remains unclear if optical imaging can indeed deliver on whole-organ imaging in larger species, such as primates and humans. Modalities, such as MRI and SPECT, might hence remain the dominating nanoparticle-based clinical imaging techniques. Further challenges to clinical applications are the growing considerations for toxic side effects of nanoparticles, the so-called nanotoxicity [38]. Many of the constituent parts of nanoparticles do not exhibit toxicity in their bulk form, but due to the emergent properties at the nanoscale (e.g., increased cell membrane permeation), cytotoxic effects can become apparent [39]. However, there is also support to indicate that the Nano size by itself is insufficient to determine toxicity and that a more detailed general consideration of particle toxicity is needed [40, 41]. The combination of nanoparticles with biologicals, such as stem cells, further raises concerns as to their potential to induce unwanted side effects that might only become apparent over time [42, 43]. An unanswered question remains if materials should be biodegradable and cleared over time or if biological inertness is more desirable [44]. Indeed, these issues raise concern regarding a premature clinical translation and what framework of evidence is needed to ensure safety [45]. Beyond the regulatory framework, the potential for scale-up and cost-efficient production at an industrial scale will also require further investment and might refi ne quality control procedures, especially in relation to monitoring potential adverse effects [46].

CONCLUSION

The role of nanotechnology in analytical, diagnostic and therapeutic modalities related to cardiovascular pathologies is expanding. With the continued innovations in imaging, biomaterials, tissue -targeted nanoparticles, biosensors and personalized medicine, nanotechnology has the potential to offer cardiologists and vascular surgeons new ways to improve patient care, and to diagnose, monitor and treat cardiovascular events more efficiently and effectively. These approaches further lend inspiration to the development of probes for other modalities, such as MRI, that currently still dominate the clinical arena.

However, optical imaging is currently seeing a more rapid development of nanoparticles than any other biomedical imaging modality. Emerging nanotechnology – based therapies can potentially help manage the escalation of atherosclerosis and prevent severe cardiovascular episodes. Nanotechnology has enhanced stem cell-based regenerative therapy, aiding with the ex vivo culturing and differentiation of stem cells as well as with in vivo cell tracking and monitoring. Future advances in nanotechnology will likely produce significant changes in the methods and practice of regenerative cell therapy.

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