Nanodevices for the prevention and treatment of cardiovascular diseases

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Abstract

Currently, cardiovascular disease represents the main cause of morbidity and mortality worldwide; the application of nanotechnology holds great promise for its prevention and treatment. Nanodevices ("smart drugs") are currently being developed for directed and controlled delivery of drugs to specific sites in the body, such as cells, tissues, blood vessels and the heart, as well as for diagnosis and early detection of cardiovascular conditions and patient-individualized treatment. Another application of nanodevices is the delivery of drugs to correct defective protein bad coupling or binding. With potent super-effects, nanoparticles should be able to elicit therapeutic effects at lower doses and prolonged periods. The manufacture of nanodevices and nanocarriers should be with a comprehensive approach that takes general properties into account in order to assess for biocompatibility and, therefore, avoid adverse and toxic effects. Intensified research in this field will help to significantly reduce morbidity and mortality caused by cardiovascular disease.

KEY WORDS: Nanomedicine. Nanocarriers. Nanodevices. Nanotechnology. Drug delivery systems. Cardiovascular diseases. Smart drugs.

Introduction

Cardiovascular disease (CVD) affects human body's heart and blood vessels; according to the World Health Organization, it constitutes the leading cause of morbidity and mortality in developed and developing countries. Each year, more people die from CVD than from any other disease: 23 million people died from CVD in 2011; 30 % of total deaths recorded in the world.¹ CVD is predicted to continue to be the main cause of death by 2030;^{1,2} a dark scenario that has to be tackled by means of nanotechnology advantages and advances.

Nanotechnology is defined as the synthesis of particles and the manufacture of devices at a scale that enables them to act at the molecular level. Owing to their properties deriving from their size, nanoparticles offer the possibility for developing new tools for CVD diagnosis and treatment. Nanomedicine involves the

use of nanoparticles at a scale ranging from 10 to 100 nm for diagnostic, treatment and prevention purposes.³ Potential clinical applications include targeted and controlled delivery of drugs to maximize therapeutic activity and minimize adverse effects. Most investigations are focused on CVD diagnosis and treatment using nanoparticles (NP) because it is the most common cause of morbidity and mortality. However, researchers are studying NPs for use in acute coronary syndromes, revascularization procedures, aortic aneurism, heart failure, stroke, hypertension, thrombosis and other cardiovascular conditions. The design and characterization of nanosystems, preclinical and clinical validations and toxicological analyses are examples of ongoing studies, the purpose of which is to prevent the appearance of atheromatous plaque and stroke by means of imaging and preventive treatment.⁴

Owing to their size and adaptation physicochemical properties, nanomaterials are structures with

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biomedical application in imaging, diagnosis, treatment and regenerative medicine.⁵

CVD current treatment includes drugs, coronary percutaneous intervention with stents and coronary revascularization surgery. Most complications are derived from these therapeutic modalities, such as drug-induced systemic toxicity or thrombosis with coronary percutaneous intervention. Nanomedicine has emerged as a strategy to prevent them.⁶

The present work describes some of the most recent advances of nanotechnology in CVD. In the short term, there is the possibility for developing nanocarriers both for administration, targeted and controlled delivery of drugs and for diagnostic procedures aimed at preventing, attenuate and heal. In the long term, robots for nanosurgery and the use of NP to prevent or correct defects and protein defective coupling. Research in these fields might reduce CVD morbidity and mortality.

In addition to their capability to act at low doses and increase the therapeutic window, NPs can elicit responses to cell stress that compensate systemic mechanisms for longer time.⁷ Nanodevices might enter into the cardiac tissue and blood circulation in order to directly deliver the active drug substance to specific sites, which would further reduce toxicity and increase therapeutic effects. Finally, protein binding and defective coupling in nanoaggregates that vary in morphology and size represent a relatively new area of research. Several intracellular metabolites that control protein coupling, as well as interactions with other proteins, can be targets for NPs.^{5,8} Basic understanding of the molecular processes that lead to deficient coupling and self-aggregation of proteins implied in CVD appearance might provide further information on their functioning, which would aid the design of new treatment strategies.

Aortic aneurism

Identification of the patient with abdominal aortic aneurism is useful for treatment. Arterial wall and intraluminal thickness and form are highly important for the prediction of abdominal aortic aneurism rupture. The definition of aortic wall internal and external contours enables distinguishing between the vessel wall and the intraluminal thrombus. This method works in a model of active form and texture statistical information.⁹ There is high correspondence between vessel wall areas automated and manual measurements. Nanodevices facilitate this procedure.

Acute myocardial infarction and heart failure

Heart failure is a complex syndrome resulting from multiple inherited or acquired causes involving the cardiac structure or function. Acute myocardial infarction (AMI) is the cause of heart failure and continues to be a growing problem in terms of morbidity, mortality and costs. Drug treatments have demonstrated to be efficacious in the decrease and regression of cardiac hypertrophy, myocardial remodeling and morbidity and mortality reduction;¹⁰ however, the prognosis is poor.

Magnetic NPs can be multifunctional for extended and selective delivery of drugs. Polyfunctional NPs with poly lactic-co-glycolic acid (PLGA) have been designed with an armored core structure that acts as a robust vector for peptide and protein encapsulation and delivery. In addition, vascular endothelial growth factor could be specifically delivered to infarcted myocardium cardiac tissue in order to help regenerating the vasculature and mesenchymal stem cells in AMI.¹¹

In patients with exercise-induced stable chest pain, microvascular angina is the most probable diagnosis. Clinical findings and diagnostic workup results suggest that angina pectoris is caused by microvascular coronary dysfunction, but definitive diagnosis is required for deterioration due to microvascular coronary dysfunction or increased vasoconstriction to be demonstrated.¹² The use of nanoparticles is highly useful in analysis, diagnosis and treatment.

Acute and chronic inflammation

Nanocarriers can be used for the treatment of inflammation in blood vessel endothelial cells lining. Nanocarriers can be used to target endothelial cells and, therefore, to facilitate penetration in this tissue. Several endothelial surface markers, including peptidases and adhesion molecules, have been identified as targets for this purpose.¹³ Nanocarriers' binding to these molecules enables drug selective release across and within the endothelial layer, providing therapeutic effects that are unreachable for their non-targeted counterparts (Fig. 1).

There are specific receptors that can mitigate inflammation by modulating the expression of key inflammatory genes. These receptors and their ligands are attractive targets for vascular endothelial tissue inflammation attenuation by means of polymeric NPs.^{14,15} Strategies of targeted and controlled release of drugs or contrast agents at specific sites with NPs

Figure 1. Comparison between a normal and a swollen vessel that was possible with the use of nanoparticles.

based on biodegradable polymers can be designed for inflammatory CVD.

In addition, some anti-inflammatory agents (e.g. biotherapeutic drugs) require precise release within sub-cellular compartments. Optimal release on desired sites might improve anti-inflammatory agents' effect; for this purpose, various nanocarriers for their release in endothelial cells have been designed and explored.¹⁸ A rigorous analysis is necessary in order for therapeutic adverse events, cost-benefit and clinical usefulness in the management and control of acute and chronic inflammation to be quantified.

Currently, superoxide dismutase conjugated with antibodies has been reported to provide targeted release within endothelial endosomes, mitigating endothelial inflammatory activation by cytokines and Toll-like receptor agonists.¹⁷ There is evidence that intracellular superoxide main role is mediating endothelium pro-inflammatory activation. Nanocarriers would help to elucidate this.

Atherosclerosis

Atherosclerosis is a chronic, progressive systemic inflammatory disease that starts due to endothelial

dysfunction and subsequently is amplified by oxidative stress, lipid deposit and monocyte recruitment. This interaction occurs between different cells, chemotactic factors, adhesion molecules and low-density lipoproteins in the sub-endothelium. Nanoparticulate systems with diagnostic and therapeutic applications for targeted and controlled release at atherosclerotic lesions specific sites, as well as adequate nanomaterials for cardiovascular implants can offer solutions to current therapeutic deficiencies. Various events have shown nanomedicine potential for atherosclerosis prevention, identification and treatment,^{18,19} which range from "nanotheranostic" strategies for vulnerable plaques to nanopores and nanoparticles based on drug-releasing stents, with the purpose to achieve a balance between efficacy and potential toxicity of systems manufactured with nanotechnology.^{20,21}

One alternative is the development of targeted apoptosis with a NP that mimics high-density lipoprotein and carries contrast agents for vulnerable plaque early detection, which would enable patients to receive a preventive treatment that would potentiate high-density lipoprotein vascular protecting effects.²² Cardiovascular molecular imaging studies have been applied in studies in vivo using nanocarriers that are

delivered and targeted towards components of the immune system that participate in the development of the atherosclerotic plaque.²³

Polymeric micelles, which are self-assembled amphiphilic polymer structures with a hydrophobic nucleus and a hydrophilic armor, constitute a class of delivery vehicles that have been investigated for several biomedical applications. Sugar molecule-based amphiphilic polymers (SBAP) have been used for the delivery of anticancer drugs via physical encapsulation within SBAP micelles and chemical conjugation to form SBAP prodrugs able to form micelles. SBAPs are excellent delivery systems for liposomal stabilization and have a single bioactivity, which might be useful for atherosclerosis treatment and prevention.²⁴⁻²⁶

Carotid atheroma

Nanodevices are currently used for the study of patients with carotid atheroma.^{4,27}

Hypertension

Combined biological materials have been developed for specific medical applications. One area of research is specific detection of DNA sequences for early diagnosis of genetic diseases by using NPs that contain gene nanosensors. Typical genic sensor nanodevices employ bio-recognition electrodes that contain immobilized probes to capture DNA, which are able to hybridize with specific targeted DNA sequences. Sandwich-type efficient genosensors based on gold AND-NP complexes can be efficiently applied for the detection of systemic arterial hypertension gene polymorphisms.²⁸

Three-dimensional imaging

There is a new area known as “3D image-guided drug delivery” (IGDD), which currently combines the potential of radiological sciences with drug targeted delivery and promises to meet the challenges of personalized medicine.

Currently, IGDD is used to deliver drugs around tumors and this way increase their effect, as well as to quantify different biomarkers that might put patients at risk. IGDD main goal is to use imaging in order to maximize damaged tissues effective treatment and minimize drug exposure with the purpose to reduce their toxicity.²⁹

3D imaging modalities take advantage to the last inch of the energy spectrum. These modalities include magnetic resonance imaging, X-ray computed tomography, ultrasound and light-based methods (endoscopy and optical coherence tomography). Modalities for research include several light microscopy techniques (confocal, multiphoton, total internal reflection, super-resolution microscopy), electron microscopy, mass spectrometry imaging, fluorescence tomography, bioluminescence, chemoluminescence, optical coherence tomography and optoacoustic imaging, among others.³⁰

Currently, NPs for molecular imaging are used in drug-releasing stents and in techniques for in vivo and ex vivo early detection.³¹ Contracted myocytes can be analyzed by 3D imaging with high spatial and temporal resolution optical microscopy with the heart beating, which enables sarcomeres independent visualization and cardiomyocyte contractile cycle individual measurement, which has been possible due to efficient tissue stabilization, a prospective approach to real-time cardiac synchronization, an algorithm to capture images in motion free of artifacts during the entire cardiac cycle and a membrane fluorescent staining fluorescence protocol.³² Cardiomyocyte contractile function quantification in vivo enables various possibilities for research in myocardial diseases and therapeutic intervention at cellular level.

Intravital microscopy imaging (e.g., microscopic-resolution imaging in live animals) has become an essential tool to study cell microdynamics in CVD. High spatial and temporal resolution, combined with deep penetration and multi-reported visualization capacity makes fluorescent intravital microscopy to be highly convenient in cardiac 3D imaging; however, tissue movement caused by cardiac contraction and respiration considerably limit its use. As a result, cell preparations in vitro or non-contractile cell culture models are more widely used. The methods are based on mechanical stabilization, on image-processing algorithms, on obtained/triggered acquisition approaches or a combination of both.³³ Their use will enable increasing the understanding on cardiac biology and elucidating cardiomyocyte function within the body in vivo and thereby improving CVD prevention and treatment.

Methods and techniques

Currently, computed tomography imaging enables coronary lesions assessment, but it lacks the

Figure 2. Techniques for CVD treatment by means of nanoparticles.

capability to measure coronary scar transmural extent.³⁴ Another method is polymeric peptides conjugation and physical encapsulation, which is divided in techniques based on surfactants and techniques based on polymeric carriers. The former predominantly employ liposomes, micro-emulsions and lipid-solid NPs.^{25,27} Nanotargeted systems, such as nanocarriers covered with polymers, albumin or lipid-solid particles, have been used as carriers *in vivo* in animal models; drugs trapped within the carriers are selectively released on their target or deposited on the surface of cardiac cells' lipid layers or on blood vessels (Fig. 2).

Drug-releasing nanocarriers

There are two great challenges for effective systemic nanorelease: limited penetration through the vascular reticulum and uptake by the reticulo-endothelial system, which hinder NP release efficacy within tissues. Although the design of NPs with prolonged half-life in the circulation is essential, it is not sufficient for NPs crossing the vascular endothelium. Current nanorelease systems are founded on passive trans-vascular exchange and tissue accumulation. High doses are required to create large gradients that passively carry the NPs through the tissue-blood

circulation interface. So far, passive accumulation has resulted in a NP fractional dose that penetrates the target tissue, which inevitably decreases therapeutic efficacy and worsens potential adverse effects. Targeted NPs active release through the endothelium might significantly increase the therapeutic index and decrease adverse effects of NP-based drug-release systems.³⁵ Trans-endothelial transport routes, such as caveolae, might provide an effective solution both to NPs specific destination and effective release (Fig. 3).

The use of nanoparticulate drug delivery systems (NDDS) to increase drug effectiveness *in vivo* is well established. The development of multifunctional NDDS that are sensitive to stimulation is an active area of research. These NDDS can have prolonged circulation times, direct to specific sites of disease, increase intracellular drug release and respond to local stimuli such as, for example, release of an entrapped drug or spilling from a protecting layer, which would facilitate interaction between nanocarriers of the loaded drugs and target cells or tissues.³⁶ In addition, the rests of formation and contrast of images can be connected to these carriers to follow their biodistribution in real time, as well as their accumulation in target cells or tissues.

Figure 3. Use of nanoparticles for targeted drug release.

On the other hand, gold colloids have fascinated scientists for more than one century and today are used in chemistry, biology, engineering and medicine. Currently, these materials can be synthesized in an easily reproducible form apparently unlimited functional chemical groups and, in certain cases, accurately characterized at the atomic level. There are several examples of highly sensitive and selective tests based on gold nanoconjugates. The therapeutic properties of such materials have been explored in the past few years. Structures that behave as gene regulation agents, drug carriers, agents for imaging and photosensitive therapeutic agents have been developed and studied at cellular level and in common diseases such as CVD.³⁷ These structures' physical and chemical structures confer them substantial advantages with applications at the cellular and molecular level.

There are multiple barrier throughout the body that limit xenobiotic substances migration to tissues; hence, one goal in drug administration and release is to create transport strategies that mimic those of viruses and bacteria.³⁸ Packaging of drugs within micro and nano-scale vehicles should enable changing pharmacologically active compounds biodistribution and permanence times. Therefore, the development of nanodevices for drug administration typically involve in vitro release characterization, assuming that performance will approach to in vivo circumstances. However, the devices are increasingly complex, and in sequential drug-release models it is important to confirm that in vivo properties are correlated as expected with those "programmed" and obtained in vitro.³⁹

Antioxidant enzymes (AOE) for the treatment of oxidative stress-related diseases are promising. Several

nanocarrier systems are developed to address and correct two great challenges to their clinical application: their poor localization and stability. In most cases there has been a compensation between the released mass of the loaded enzyme and carriers' capability to protect the enzyme from catalytic degradation.⁴⁰ One potential method to overcome this limitation is the use of mesoporous silica nanomaterials arranged as potential AOE nanocarriers.

Even when AOE, catalase and superoxide dismutase are known to detoxify harmful reactive oxygen species, AOE therapeutic usefulness is hindered by its inadequate release. AOE modification by means of polyethylene glycol and encapsulation of liposomes covered with this compound increases AOE bioavailability and enhance its protecting effects in animal models. Pluronic formulation-based micelles formed with AOE show higher protective effects. Nanocarriers based on PEG copolymers protect encapsulated AOE from proteolysis and increase their release in target cells, such as the endothelium that lines the vascular lumen. Antibodies such as conjugated endothelial determinants for AOE or AOE carriers provide intracellular targeting and release. Targeted liposomes, conjugated proteins and magnetic NPs release AOE in sites with presence of oxidative stress in the cardiovascular system. Advances in nanodevices for EAO delivery will provide the bases for an understanding with a focus on the clinical field.^{27,41}

Cardiac arrest and stroke

Nanotechnology application in tissue engineering is highly promising. NPs are used to monitor grafted cells or to increase the visualization of tissues by means of a non-invasive imaging modality by magnetic

resonance, computed axial tomography and positron-emission tomography scanning. Different agents can be used for the formation of images by contrast, from iron oxide, perfluorocarbonated compounds, cerium oxide or platinum NPs to quantum dots. Carbon nanotubes can act as scaffolds for the treatment of cardiac arrest and stroke by means of stem cells.⁴²

Thrombosis

One strategy for drug administration and biomimetic release by nanocarriers could be useful in blood vessels obstructed by thrombosis or embolism. One biophysical approach could consist in targeting nanocarriers during the friction force caused by vascular narrowing (in the same way platelets do), which would decrease drug doses, minimize their adverse effects and potentiate their therapeutic efficacy.⁴³ In addition, nanocarriers have demonstrated in vivo and in vitro to significantly reduce the dose of the used thrombolytic drugs.⁴⁴

Difficulties in the management of patients with high thrombosis and hemorrhage risk continue to exist in antithrombotic pharmacotherapy. Antithrombotic agents' usefulness in these scenarios is limited by an inadequate pharmacokinetics and narrow therapeutic index. The use of advanced drug delivery systems (ADDs) might help to correct these problems. Several nanocarriers, ligands with affinity and covered polymers provide ADDs with the potential to optimize antithrombotic agents' pharmacokinetics, targeted drug delivery to thrombotic sites and detection of pathologic changes in the vascular microenvironment, such as altered hemodynamic forces, expression of inflammatory markers and structural differences between mature homeostatic clots and growing pathological clots.

The release of antithrombotic agents by means of synthetic biomimetic carriers, host blood cells and recombinant activated fusion proteins in sites where thrombi develop has been encouraging in preclinical models. On the other hand, ADDs development and translation into separating homeostatic fibrin clots holds promise for extending antithrombotic agents' usefulness to thrombophylaxis.^{45,46}

The design of adhesion molecules expressing on the surface at rest and in pathologically altered endothelial cells will enable the release of drugs on the endothelial surface (preferably for thrombolytic agents) and in intracellular compartments (preferably for antioxidants). Platforms for drug targeted release—including conjugated proteins, recombinant fusion constructs

Figure 4. Nanoparticles application and their effects on endothelial cells.

and polymeric carriers—at endothelial level are already being cautiously used.⁴⁷

Vascular endothelium

Endothelial alterations are implied in several pathologies, including common acute diseases with high morbidity and mortality owing in part to the fact that drugs and drug carriers have no natural affinity to the vascular endothelium. Molecule ligands exposed to the bloodstream on the endothelial surface will enable the design of specific therapeutic targeted agents at the endothelial level.

Target molecules and binding to epitopes must be accessible to drug carriers, which should be free of harmful effects; target site selection provides the desirable direction at the subcellular level for drug delivery. The list of candidate target molecules for endothelial nanomedicine application includes peptidases and other enzymes, cell adhesion molecules and integrins, located at different endothelial plasma membrane domains and differentially distributed throughout the entire vascular system. Conferring carriers with affinity to specific endothelial epitopes will enable an unprecedented level for drug release accuracy and control: binding to selected endothelial cell phenotypes, cell conduction and therapeutic effect duration. Nanocarriers characteristics will depend on the chosen epitope, on the control of ligand release and on the effect of specific endothelial nanomedical agents. The selection of optimal binding sites and nanocarriers characteristics are key factors in studies in vitro and preclinical experimental models in vivo. Nanomedical agents targeting the endothelium provide antioxidant and anti-inflammatory effects, as well as other therapeutic effects in animal models⁴⁸ (Fig. 4).

On the other hand, targeted and individualized nanotechnology holds promise to find clinical use in diverse medical areas. Several endothelial surface markers, including peptidases (e.g., ACE, APP and APN) and adhesion molecules (e.g., ICAM-1 and PECAM) have been identified as key targets. Nanocarriers binding to these molecules should be able to allow drug delivery and subsequent penetration into the endothelium. The analysis of diverse aspects of nanomedicine should include:

- Circulation and targeting of carriers with different geometries.
- Multivalent interactions of carrier with endothelium.
- Anchoring to multiple determinants.
- Accessibility to binding sites and cell response to their involvement.
- Role of the cell phenotype and microenvironment in the selection of the target site.
- Optimization of targeting by lowering carrier avidity.
- Endocytosis of multivalent carriers via molecules not implicated in ligand internalization.
- Modulation of cell uptake and trafficking by selection of specific epitopes at determinant sites, carrier geometry and hydrodynamic factors.⁴⁹

Refinement of these aspects will improve vascular pathophysiology understanding and should be applied at the clinical level for the steering of nanocarriers in the vascular endothelium.

Multidimensional protein recognition technology is used to identify several proteins (with 3 or more spectra)⁵⁰ in luminal endothelial cell plasma membranes isolated from animal microvascular endothelial cell cultures.

Finally, endothelial vasculature mapping and characterization using proteomics offers the opportunity to better understand the molecular stages and mechanisms in vascular development and angiogenesis. Proteomics has important advantages over genomics, especially in protein expression determination. It is necessary focusing on proteomic principles and methods, with particular interest on their applications to characterize the vascular endothelium (normal and malignant), as that existing *in vivo*.⁵¹

Conclusions

Various methods have been designed for nanocarriers innocuous access into blood vessels and cardiac tissue, in such a way that drugs can be selectively directed and delivered, which will increase their

therapeutic efficacy and minimize adverse effects. The use of nanocarriers and nanodevices has been intensified in animal models *in vivo* and *in vitro*, which in a near future should translate into their clinical application significantly decreasing CVD morbidity and mortality.

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