Nanodevices that acts as nanocarriers for controlled and directed drug delivery to select cells, organs or tissues in cardiovascular diseases

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Abstract

Cardiovascular disease, which today represents the main cause of death worldwide, is a likely candidate for the application of nanotechnology in the near future. Nanocarriers are currently being developed to deliver medicine (smart drugs) to selected targets in cells and tissues of blood vessels and the heart, as well as to aid in diagnosis and screening for early detection and individualized treatment. Other applications of nanotechnology hold promise for the long run, such as using nanodevices for drug delivery or correcting the misfolding of proteins. With super-potent effects, nanoparticles should be able to evoke therapeutic effects at a lower dose and for longer periods. The development of nanodevices and nanocarriers must take an integral approach that considers many properties—physical, chemical, biological, biochemical, anatomical, morphological, physiological, pharmacological, toxicological, mechanical, electrical, magnetic, thermodynamic, and optical—in order to evaluate biocompatibility and therefore avoid toxicological and/or other adverse effects. Intensified research in relation to nanocarriers and other nanotechnology could help reduce morbidity and mortality in cardiovascular disease.

KEY WORDS: *Nanomedicine. Nanocarrier. Nanodevice, Nanotechnology. Drug delivery system. Cardiovascular disease. Smart drug.*

Resumen

Las enfermedades cardiovasculares actualmente representan la principal causa de muerte en el mundo, y son importantes candidatas para la aplicación de la nanotecnología en un futuro próximo. Se están desarrollando nanotransportadores para la administración de medicamentos (fármacos inteligentes) en sitios específicos en células y tejidos de los vasos sanguíneos y el corazón, así como para realizar el diagnóstico, la detección temprana y el tratamiento individualizado. Otras aplicaciones de la nanotecnología están contempladas a largo plazo, como el uso de nanodispositivos para el suministro de fármacos o para corregir el mal acoplamiento de las proteínas. Con efectos superpotentes, las nanopartículas deberán ser capaces de provocar efectos terapéuticos a menor dosis y durante periodos más largos. La elaboración de nanodispositivos y nanotransportadores debe adoptar un enfoque integral que considere integralmente las propiedades físicas, químicas, biológicas, bioquímicas, anatómicas, morfológicas, fisiológicas, farmacológicas, toxicológicas, mecánicas, eléctricas, magnéticas, termodinámicas y ópticas, con el fin de evaluar la biocompatibilidad y, por tanto, evitar efectos tóxicos y no deseados. La intensificación en la investigación en relación con los nanotransportadores y otras nanotecnologías podrá ayudar a reducir la morbimortalidad provocada por las enfermedades cardiovasculares.

PALABRAS CLAVE: *Nanomedicina. Nanotransportadores. Nanodispositivos. Nanotecnología. Sistema de liberación de fármacos. Enfermedades cardiovasculares. Medicamentos inteligentes.*

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Introduction

Cardiovascular disease (CVD) is the principal cause of mortality in the world, according to the World Health Organization (WHO). Each year more people die of heart disorders than any other cause. For example, 23 million people died of CVD in 2011, which represents 35% of the total deaths registered in the world[1]. It is projected that CVD will continue to be the principal cause of death in 2030[1,2], a bleak scenario that should be confronted by taking advantage of recent advances in nanotechnology.

Nanotechnology can be defined as the synthesis of particles and the manufacture of devices in the nanometer range that operate at the molecular level. Due to their unique size-dependent properties, nanoparticles offer the possibility of developing new diagnostic and therapeutic tools (Annex 1). Among the many potential applications in clinical medicine and research, one of the most advanced techniques is targeted drug delivery systems to maximize therapeutic activity and to minimize adverse effects[3].

The present study summarizes the recent developments in nanomedicine with the aim of proposing accelerated research efforts. In the short run, there is great promise for the development of nanocarriers for both targeted drug delivery and diagnostic procedures aimed at preventing, attenuating, and curing CVD (Annex 2). The long-term possibilities include the development of nanodevices for drug delivery, robots for nanosurgery, and nanoparticles for impeding or correcting the misfolding of proteins. An intensified research effort in these fields could possibly reduce the incidence and mortality of CVD in the near future (Annex 3).

Nowadays, there are a lot of the multifunctional nanomaterials, which are widely used for diseases treatment and diagnosis[4]. For example, a tissue-microenvironment-responsive nanoparticle, especially well-designed pH/redox-sensitive ones for therapy or diagnosis, is a new direction for constructing smart drug delivery systems, in which therapeutics or diagnostic agents could be selectively delivered to the organ lesion site instead of normal organ, resulting in less side effects induced by administered agents. The sheet-like nanomaterial MnO2 is one kind of inorganic material, which is currently under extensive investigation for its capacitance properties[5]. Recently, its use for drug delivery systems is reported for its relatively high drug-loading capacity and stimuli-responsive magnetic resonance imaging (MRI) property. Another example is the use of well-defined nanostructures to modify a drug's pharmacokinetic profile for improved treatment. This carrier-based drug delivery strategy aims to improve the drug's specificity to target sites for minimized side effects, and also to circumvent the possible drug resistance mechanisms that tumor cells may develop over the course of the treatment[6].

One of the biggest impacts that the nanotechnology has made on medicine and biology has been in the area of drug delivery systems (DDS). Many drugs suffer from serious problems concerning insolubility, instability in biological environments, poor uptake into cells and tissues, sub-optimal selectivity for targets, and unwanted side effects. Nanocarriers can be designed as smart DDS to overcome many of these drawbacks. One of the most versatile building blocks to prepare these nanocarriers is the ubiquitous, readily available and inexpensive protein, serum albumin[7]. Also, nanocarrier-based DDS offer new opportunities to enhance therapeutic efficacy due to their improved bioactivity and enhanced bioavailability of drugs[8-12]. To further improve pinpoint therapeutic efficacy, various efforts have been devoted to explore stimuli-responsive[13,14] and imaging-guided DDS[15-18], which are specifically stimulated to release drugs within target sites and achieve real-time imaging-guided therapeutics. A broad range of stimuli have been explored to design smart DDS, including external triggers, such as light[19,20], ultrasound[21], magnetism[22,23], temperature[24], and electric field[25], as well as internal stimuli such as pH[26,27], redox potential[28], oxidative stress[29,30], enzyme[31,32], and glucose levels[33,34]. Imaging-guided DDS have been rapidly developed by inorganic nanosystems with contrast properties made of carbon dots[35], quantum dots[36], metals[37], and metal oxide frameworks[38]. In addition, conjugated polymer nanoparticles, as special organic nanosystems, have remarkable photophysical properties, which are also suitable for biological fluorescence imaging[39-44]. One more example is the biodegradable polymer microspheres, which have emerged as cell carriers for the regeneration and repair of irregularly shaped tissue defects due to their injectability, controllable biodegradability, and capacity for drug incorporation and release. Notably, recent advances in nanotechnology allowed the manipulation of the physical and chemical properties of the microspheres at the nanoscale, creating nanostructured microspheres mimicking the composition and/or structure of natural extracellular matrix (for example, in cardiac muscle). These nanostructured microspheres, including nanocomposite microspheres and nanofibrous microspheres, have been employed as cell carriers for tissue regeneration and could be used in cardiac muscle and vascular lesions.

They enhance cell attachment and proliferation, promote positive cell-carrier interactions, and facilitate stem cell differentiation for target tissue regeneration[45].

Site-specific drug targeting using particle drug carrier systems has made substantial progress and today is set for rapid expansion into a wide variety of applications[46]. Specific functional groups can be incorporated into polymers to make them responsive to environmental stimuli such as pH, temperature, or varying concentrations of biomolecules. Nanoparticles acquire unique size-dependent biological, physiological, chemical, thermal, optical, electromagnetic, thermodynamic, and atom-like quantum properties. They exhibit high surface-adsorptive capacity for other substances, thus enhancing drug bioavailability by improving the ability to cross otherwise impermeable barriers, including the blood-brain barrier and cell membranes. The fusion of such "intelligent" biomaterials with nanotechnology has led to the development of powerful therapeutic and diagnostic platforms[47]. Advances in the design, manufacture, and application of such platforms hold great potential for the prevention, early detection, diagnosis, and treatment of CVD.

Obviously, the potential interaction with tissues and cells, as well as potential toxicity, greatly depend on the composition of the particular nanoparticle[46]. It is important to evaluate potential toxicity issues related to the chemical properties, size, and shape of nanoparticle materials[34]. In this sense, knowledge of particle toxicity (e.g. inhalation toxicity) is key for evaluating potential hazards. The toxicology of nanoparticulate matter, like that of other substances, depends on their solubility in biological matrices, which in turn greatly influences their potential exposure to various internal organs[46].

For instance, the different methods of polymer-peptide conjugation and physical encapsulation are divided into surfactant-based techniques and polymer carriers. Surfactant-based techniques predominantly employ liposomes, micro-emulsions and solid-lipid nanoparticles[48]. Nano-targeted systems, such as nanocarriers that are coated with polymers, albumin and/or solid lipid particles, have been used as transporters with *in vivo* animal models. This allows drugs to be selectively delivered to their target because they are either trapped within carriers or deposited in subsurface fatty layers of heart cells and blood vessels. This selectivity could make drug delivery much more efficient and at the same time minimize side effects and toxicity.

Magnetic nanoparticles can be multi-functionalized to provide a prolonged and selective release of drugs[49]. Poly(lactic-co-glycolic acid)-polycation nanoparticles were recently engineered with a core-shell structure that acts as a robust vector for the encapsulation and delivery of proteins and peptides[50]. Moreover, vascular endothelial growth factor has been delivered in a targeted manner to myocardial infarcted heart tissue to help regenerate vasculature, in support of mesenchymal stem-cell therapy, in a rat model of myocardial infarction[51].

Nanocarriers may also be used to treat inflammation in the lining of endothelial cells on the luminal surface of blood vessels. Such inflammation can lead to ischemia, thrombosis, stroke, and other cardiovascular conditions. Nanocarriers are also used to target endothelial cells and thus facilitate the penetration of this tissue. Several endothelial surface markers, including peptidases and adhesion molecules, have been identified as key targets in this sense. Binding of nanocarriers to these molecules enables the selective delivery of drugs into or across the endothelium layer, offering therapeutic effects that are unattainable by their non-targeted counterparts[52].

There are specific receptors that can attenuate inflammation by modulating the expression of key inflammatory genes. Thus, these receptors and their ligands are particularly attractive targets for the attenuation of inflammation in vascular endothelial tissue by polymeric nanoparticles. Site-directed drug delivery with biodegradable polymer-based nanoparticle platforms may be engineered to load multiple drugs and/or contrast agents as potential alternatives for tracking and treating inflammatory CVD[53,54].

Lack of early detection is a grave problem with atherosclerosis, allowing macrophage apoptosis to contribute to the instability of atherosclerotic lesions. One attractive alternative is the development of an apoptosis-targeted high-density lipoprotein (HDL)-mimicking nanoparticle to carry contrast agents for early detection of vulnerable plaques. Such detection would enable patients to receive timely preventive therapy that exploits the vascular protective effects of HDL[55]. Apart from early detection, nanodiagnostics has the potential of assisting in a personalized treatment of CVD[56], finding specific therapies that best suit individual needs[57]. In the same sense, preclinical cardiovascular molecular imaging has been applied to an *in vivo* study of targeted nanocarriers specifically directed toward immune system components that drive atherosclerotic plaque development. These nanocarriers may be used for precision diagnostics as well as efficient therapy for atherosclerosis and its ischemic complications[58].

A biomimetic targeting strategy may be useful for nanocarriers to deliver drugs to blood vessels obstructed

by thrombosis or embolism. Nanocarriers could target high shear stress caused by vascular narrowing (in the same way platelets do). This biophysical strategy for drug targeting could lower required doses and minimize side effects, while maximizing drug efficacy in treatment of life-threatening diseases that result from acute vascular occlusion[59]. Additionally, *in vitro* and *in vivo* nanocarriers have proved to significantly lower the dose of thrombolytic drugs[60].

Some approaches for treating cancer have potential for use in CVD therapy[61]. Many indirect approaches deliver therapeutic agents to molecular targets that are overexpressed on the surface of tumor cells. By enhancing permeability and retention (in passive therapy) or by functionalizing the nanocarrier surface (in active therapy), the carriers transport the therapeutic agents to tumors, avoid normal tissues, and reduce toxicity in the rest of the body. Furthermore, they protect cytotoxic drugs from degradation while increasing their half-life, payload and solubility[62]. Sustained delivery of polyethylene glycol (PEG)-conjugated liposomal nanoparticles may serve as a delivery platform for treating heart injuries through sustained bioavailability.

Newly developed non-viral gene delivery approaches have shown improved anticancer efficacy, suggesting that RNAi-based therapeutics can provide novel opportunities to elicit gene silencing and induce regression of tumor growth[63]. Successful clinical application of nanocarrier drug delivery devices has been limited mainly due to the lack of control of sustained drug release from the carriers. A wide range of sophisticated approaches aims to employ the formation of crosslinkable or non-crosslinkable stimuli-responsive polymer nanocarriers in order to enhance their delivery efficiency[64].

Anticancer drugs are hydrophobic and require carriers to solubilize them in aqueous environments. Gene-based therapies (e.g. siRNA or pDNA) require carriers to protect the anionic genes from enzymatic degradation during systemic circulation. Polymeric micelles, which are self-assemblies of amphiphilic polymers (AP), constitute one class of delivery vehicles that has been investigated for many biomedical applications. Having a hydrophobic core and a hydrophilic shell, these polymers have been used as drug carriers. Sugar-based amphiphilic polymers (SBAP) have been employed in anti-cancer drug delivery via physical encapsulation within SBAP micelles and chemical conjugation to form SBAP prodrugs capable of micellization. SBAPs are excellent for stabilizing liposomal delivery systems and have unique bioactivity that may be useful for managing atherosclerosis[65].

Advances have also been made in the composition and functional properties of exosomes released by cells during the fusion of multi-vesicular bodies with the plasma membrane. While their envelope reflects a cellular origin, their surface membrane and internal contents include important signaling components. As functional biomarkers, exosomes could provide insights into CVD-related conditions, thus assisting in the diagnosis and prognosis of heart disease[66].

New opportunities will most certainly appear in relation to fighting disease as nanotechnology develops. Apart from the capacity of nanoparticles to lower drug doses and increase the window of effective treatment time, they may evoke cellular stress responses leading to compensatory systemic mechanisms that can alter the impact of nanoparticles over time[67]. Additionally, nanodevices could be developed that are capable of entering heart tissue and blood circulation to directly deliver active ingredients to the target without the need for a pharmaceutical vehicle, which would further reduce toxicity while maximizing therapeutic effects. Finally, the folding and misfolding of proteins in nano-aggregates varying in morphology and size represents a relatively new area of research. Various intracellular metabolites that control protein folding as well as interactions with other proteins may be a useful target for nanoparticles. A fundamental understanding of the molecular processes that lead to misfolding and self-aggregation of proteins involved in various CVDs should certainly provide critical information to help design new therapies[68,69].

In this study we propose to reduce/eliminate the adverse effects caused by nanodevices during their administration by different routes into the body through controlled drug-delivery systems directed to specific sites, in comparison with current conventional systems, also administered by different pathways, where the drugs act in more than one site, consequently causing side effects. Here is the underlying relevance and impact of this study: the implementation of nanocarriers to obtain a significant decrease in morbidity/mortality elicited by CVD. Developing smart carriers with the capacity to deliver drugs specifically to the microenvironment of diseased cells with minimum systemic toxicity is the goal[70-76].

Conclusions

Methods have been designed for the innocuous entry of nanocarriers into blood vessels and heart tissue, enabling drugs to be selectively targeted. This in turn increases efficacy and minimizes or eliminates adverse effects. The development of nanocarriers and

nanodevices for this purpose must take an integral approach that encompasses their physical, chemical, biological, biochemical, anatomical, morphological, physiological, pharmacological, toxicological, mechanical, electrical, magnetic, thermodynamic, and optical properties[13] (Annex 4). Experimental work has already been reported with *in vitro* and *in vivo* animal models, meaning that intensified research efforts in nanotechnology could possibly have a significant impact in the morbidity and mortality of CVD in the near future.

Future perspectives

The recent advances with nanocarriers and nanodevices for diagnosis and drug delivery to cardiac tissues and blood vessels have created the groundwork for rapid development in this field. With a concerted research effort, these possibilities may soon become a clinical reality. Nanocarriers should soon be ready for clinical use in diagnostics, targeted drug therapy, and individualized patient treatments in the short term. The long-term possibilities include the development of nanodevices that could pass through blood vessels, micrometer-sized nanorobots for precisely controlled heart surgery, and new techniques for intervening in protein folding.

Disclosure of interest

The authors report that they have no conflicts of interest in relation to any of the techniques or materials mentioned in this work.

Author contributions

J.M.V.R. designed the project, wrote the paper, and directed the research.

J.J.V.A. interpreted data, contributed to the study strategies, and coordinated the project.

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Annex 1. Techniques, tools and instruments for the design, construction and application of nanodevices used as carriers for targeted and controlled release of drugs for prevention and remediation of cardiovascular disease

AFM:	atomic force microscopy	RIE:	reactive ions envelope
CLSM:	confocal laser scanning microscopy	SE:	spectroscopic ellipsometry
FESEM:	field-emission scanning electron microscopy	SEM:	scanning electron microscopy
MALDI-TOF:	matrix assisted laser desorption/ionization	UHPLC-ESI-MS:	ultra high performance liquid chromatography ElectroSpray ionization tandem mass spectrometry
MRCS:	micro Raman confocal spectroscopy		
NI:	nanoindentation		
NMR:	nuclear magnetic resonance	XRD:	X-ray diffraction
PSXRI:	photoelectron spectroscopy X-ray induced		

Annex 2. Studies and laboratory tests

Coronary angiography, coronary angiogram, angioplasty, coronary arteriography, coronary bypass, cardiac catheterization, intravascular echocardiography, echocardiogram, electrocardiogram, phlebography, phonocardiogram, cardiac scintigraphy, Holter, nuclear magnetic resonance image, 3D imaging, pacemaker, myocardial perfusion, vascular magnetic resonance imaging, stent, computerized tomography, cardiac transplant, ultrasonography, cardiac ventriculography, vascular ventriculography.

Annex 3. Classification of cardiovascular diseases

- Heart diseases: arrhythmias, cardiac hypertrophy, cardiac remodeling, cardiac palpitation, myocardial contusion, myocarditis, endocarditis, pericarditis, coronary spasm, cardiac tumor, cardiac ischemia (angina pectoris), heart failure, acute myocardial infarction, syncope, cardiogenic shock, sudden death.
- Changes in tension: hypotension, hypertension, preeclampsia.
- Vein diseases: phlebitis, thrombophlebitis, varicose veins, fibrosis, hemorrhage, gangrene.
- Artery diseases: atheroma, atherosclerosis, thromboembolism, atherothrombosis, angiopathy.
- Joint diseases: fibrosis, peripheral vasculopathy, fibrillation, atherosclerosis.
- Congenital diseases: valvulopathy, cardiac murmur, congenital heart disease, aortic coarctation, aneurysm, stenosis, valvular failure, aortic dissection.
- Cerebrovascular diseases: cerebral stroke, cerebral embolism, cerebral thrombosis, apoplexy, ictus.

Annex 4. Evaluation and characterization of properties for both, nanodevices and environment where these will be used (into cardiovascular system)

- Physical: color, smell, flavor, strength, boiling point, melting point, mass, volume, texture, weight, specific weight, capillarity, divisibility, porosity, inertia.
- Chemical: reactivity, acidity, alkalinity, specific heat, combustion, corrosion, decomposition, dissociation, hydrolysis, addition, condensation, redox, flammability.
- Anatomical: constitution, permeability, fluidity, mobility, molecular transport.
- Morphological: shape, structure, architecture, composition.
- Physiological: excitability, motion, nutrition, growth, evolution, proliferation, differentiation, signaling, self-repair, self-regulation, transport of substances.
- Pharmacological: pharmacokinetics, pharmacodynamics, intracellular and intercellular interactions.
- Toxicological: toxicity, mutagenicity, teratogenicity, carcinogenicity, harm, impair, oxidative stress.
- Mechanical: hardness, elasticity, flexibility, acceleration, motion, power, work, ductility, malleability, flexion, compression, torsion, traction, rigidity, strain, distortion, stress, toughness, elasticity, plasticity, surface tension, resistance, fragility, penetrability.
- Electrical: conductivity, intensity, brightness, luminosity, electric resistance, capacitance, electric charge, electric field.
- Magnetic: dipole moment, dipolar current, magnetic field, permeability, reluctance, magnetic flux, amperage, intensity current, electromagnet, magnetic susceptibility, magnetic permeability, magnetization, magnetic excitation, intensity of magnetic field, paramagnetism.
- Thermodynamic: pressure, volume, temperature, density, viscosity, internal energy, enthalpy, entropy, heat, work.
- Optical: reflection, refraction, diffraction, radiation, absorption, absorbance, transmission, emission, scattering, dispersion, brightness, luminosity, luminescence, photoconductivity, thermoluminescence, chemiluminescence, triboluminescence, polarization.