Functional Nanomaterials in Visualization of Cardiovascular Injury

Subjects: Biology

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Acute myocardial infarction is a major global health problem, and the repair of damaged myocardium is still a major challenge. Myocardial injury triggers an inflammatory response: immune cells infiltrate into the myocardium while activating myofibroblasts and vascular endothelial cells, promoting tissue repair and scar formation. Fragments released by cardiomyocytes become endogenous “danger signals”, which are recognized by cardiac pattern recognition receptors, activate resident cardiac immune cells, release thrombin factors and inflammatory mediators, and trigger severe inflammatory responses. Inflammatory signaling plays an important role in the dilation and fibrosis remodeling of the infarcted heart, and is a key event driving the pathogenesis of post-infarct heart failure. At present, there is no effective way to reverse the inflammatory microenvironment in injured myocardium, so it is urgent to find new therapeutic and diagnostic strategies. Nanomedicine, the application of nanoparticles for the prevention, treatment, and imaging of disease, has produced a number of promising applications.

Keywords: Multimodal Imaging ; Nuclear Imaging ; nanoparticles ; Cardiovascular Injury

1. Application of Functional Nanomaterials in the Visualization of Cardiovascular Injury

Imaging technology allows noninvasive cardiovascular testing, disease staging, and monitoring of response to treatment, providing a powerful tool for the diagnosis and treatment of cardiovascular injury. Traditional imaging techniques include CT, PET, ultrasound, and optical imaging to obtain anatomical definitions and functional information, such as vascular reactivity and myocardial perfusion, activity, stiffness, and contractility, by identifying changes in the physical characteristics of lesions. Since it is difficult to fully understand the disease process at the molecular level, the pathophysiological characteristics of the disease cannot be directly revealed, which greatly reduces the information and accuracy of diagnosis. The development of molecular imaging techniques has provided an approach beyond anatomy for the diagnosis of cardiovascular disease by observing in vivo processes related to myocardial injury, such as inflammation, angiogenesis, apoptosis, oxidative stress, and fibrosis. Molecular imaging technology relies on the affinity or interaction between the contrast agent and target and has excellent specificity. However, the traditional small molecule contrast agent has a single function and rapid metabolism, which makes it difficult to meet the complex clinical needs in specific use.

The versatility of nanoparticles (NPs) provides a basis for increasing the long cycle, improving the targeting and biocompatibility, and improving the signal intensity of the unit target, which is expected to make up for the deficiency of traditional contrast agents. NPs have excellent radio-tagging efficiency and rapid synthesis and purification strategies, which are highly desirable for nuclear imaging applications using short-lived radioisotopes. In addition, nanoparticles with very high specific surface areas can achieve efficient loading of contrast agents and targeted modification, which has attracted extensive attention in the field of molecular imaging. In recent years, a large number of functional nanoparticles have been designed to visualize the inflammatory microenvironment during cardiovascular injury.

2. Nuclear Imaging

The nuclear imaging technique is a new technique combining nuclear technology and modern image theory. These methods include X-ray tomography (XCT), positron tomography (PET), nuclear magnetic resonance computed tomography (NMR–CT) and single photon emission tomography (SPECT). The common principle of the various nuclear imaging technologies is to obtain detailed internal information of the object to be studied by using the attenuation law or distribution characteristics of physical quantities related to nuclear radiation in the object to be measured, then use a computer to process this information at a high speed, and finally reconstruct an image of the object to be studied. Among

them, PET, SPECT, and MRI are the most advantageous imaging methods in cardiovascular imaging. Because they can play roles in functional imaging, they are expected to achieve early detection before the occurrence of organ lesions.

PET and SPECT are 3D tomography techniques with high sensitivity for nanomolar or even picomolar detection. They are widely used as noninvasive clinical imaging tools. However, they also have limitations, such as low spatial resolution (1–4 mm) and ionizing radiation risk. A further challenge is that the requirements of a cyclotron for radioisotope production and its short half-life (typically a few hours) may reduce the time available to perform the required quality control. In addition, the commonly used contrast agents, such as 18FDG, may be absorbed and create a large amount of background due to metabolically active myocardium during PET detection of inflammatory myocardial injury rich in macrophages, which reduces the specificity and sensitivity of detection.

Multifunctional contrast agents based on nanoparticles provide a new strategy for detecting and diagnosing cardiovascular injury. Nanoparticles have unique sizes and physicochemical properties and can be loaded with a variety of radioactive tracers using different synthesis strategies. One of the main advantages of radioactive nano contrast agents is the presence of a large number of radioactive atoms in a uniform nanoparticle. The unique surface chemistry of nanoparticles also allows for improved targeting of disease sites using a variety of probes. This improves the contrast of the disease site relative to other normal tissues and improves the sensitivity and specificity of PET. Ueno et al. [1] used dextran nanoparticles doped with the isotope Cu-64 to track the immune-regulatory effects of angiotensin-converting enzyme inhibitor therapy on allograft bone marrow cells. The experimental results showed that the PET signal of nanoparticles in allografts was much higher than that of the background. The target-background ratio was also increased in allograft recipients, reflecting lower uptake of nanoparticles by non-inflammatory hearts. Keliher et al. [2] reported that a modified poly-glucose nanoparticle could achieve PET imaging of ischemic heart disease due to its high affinity for macrophages. The nanoparticles were enriched in cardiac macrophages to increase PET signaling in infarcted myocardium in mice. In the field of PET imaging, the most thoroughly studied nanomaterials include liposome carriers, magnetic ions, quantum dots (QDs), polymerized nanoparticles, silica nanoparticles, dendritic macromolecules, inorganic metallized nano-formulations, and carbon nanotubes [3].

MRI is a noninvasive clinical diagnostic tool that utilizes nonionizing radio waves. With high spatial resolution (~100 μm) and unlimited tissue penetration, MRI is capable of simultaneously imaging cardiovascular anatomy, physics, and molecular events. Contrast enhancers, such as gadolinium (Gd^{3+})-based drugs, are commonly used to enhance MRI imaging. Magnetic nanoparticles (MNPs) have lower toxicity and better contrast performance than gadolinium MRI imaging agents, and are playing an increasingly important role in imaging cardiovascular injury. MNPs can exude into the infarcted myocardium and aggregate in the target area, thus achieving angiography effects and greatly improving the early diagnosis of acute myocardial infarction.

Recent studies have found that MNPs targeting inflammation have a better detection effect. Park et al. [4] found that MNPs had a better detection effect than gadolinium-enhanced and manganese-enhanced MR in acute myocardial infarction models and could provide information on the inflammatory response in AMI mouse models. Cuadrado et al. [5] reported that magnetic nanoparticles targeting EMMPRIN could achieve visualization of acute myocardial infarction. EMMPRIN was targeted using paramagnetic/fluorescent micellar nanoparticles conjugated with EMMPRIN binding peptide AP-9 (NAP9). Cardiac magnetic resonance (CMR) scans revealed enhanced signaling in the left ventricle of mice injected with NAP9 compared to mice not injected with NAP9. These results suggest that magnetic nanosystems have potential applications in myocardial injury, such as myocardial infarction.

3. Optical Imaging

Optical imaging is widely used in preclinical studies for cell, subcellular and whole animal imaging due to its low cost and high detection sensitivity (picomolar level). Fluorescence imaging is one of the most commonly used optical imaging techniques, in which light of an appropriate wavelength is used to excite endogenous or externally introduced fluorescent parts and to detect light emitted at a longer wavelength. However, the effects of light scattering and tissue attenuation limit the application of visible fluorophores in deep tissue imaging. The fluorophore activity in the near infrared region can reduce the tissue attenuation effect and increase the penetration depth up to several centimeters, showing a certain prospect for in vivo detection.

Lu [6] et al. developed photodynamic selenium nanoparticles (SeNPs) targeting inflammatory macrophages. They coated selenium nanoparticles with chitosan (CS) via Se-S bonds, combining RB-CS-GSH with the photosensitizers Bengal red Rose (RB) and glutathione (GSH). The RB-CS-GSH layer was combined with catalase for imaging and photodynamic therapy. Second, they conjugated the carboxyl groups of hyaluronic acid (HA) and folic acid (FA) with the amine groups of

ethylenediamine (EDA) to form an HA-EDA-FA mixture as the second layer of the nanoparticles. HA and FA are used as binding ligands of folate receptor β (FR-β) and CD44 on the surface of inflammatory macrophages. In vitro studies have shown that SeNPs provide a stronger RB fluorescence signal in lipopolysaccharide (LPS)-stimulated macrophages than in nonstimulated macrophages. This suggests that SeNPs can specifically bind to active macrophages via folic acid and HA coating. Inactive macrophages are not damaged by these nanoparticles. The results show that these nanoparticles have potential therapeutic and imaging applications.

Kosuge et al. [7] designed a single-walled carbon nanotube (SWNT) functionalized with Cy5.5 dye for near infrared (NIR) imaging and photothermal ablation of inflammatory macrophages. In vitro NIR imaging showed a strong signal after ligation of the left carotid artery in SWNT-treated mice, but no NIR signal in non-SWNT-treated mice. In general, single-walled carbon nanotubes have been shown to be therapeutic nanomaterials capable of fluorescence imaging and photothermal ablation of vascular macrophages. Ziegler et al. [8] constructed ROS-responsive self-assembled fluorescent nanoprobes for imaging and treatment of cardiac ischemia–reperfusion injury. Reactive fluorescent ROS nanoprobes were evaluated in a mouse model of myocardial infarction and found to be highly specific for the ischemic/reperfusion myocardium in the first 24 h after reperfusion. Sun [9] et al. developed a novel nanoparticle based on three-function viroid anthropoid virus 40 (SV40) to deliver hirudin. The nano-system is also loaded with near-infrared quantum dots and labeled with a cyclic peptide (CGNKRTRGC) to target the P32 protein on macrophages. In ApoE$^{-/-}$ mice, the fluorescence signal of the targeted nanoparticles in the plaque was three times stronger than that of the nontargeted nanoparticles. However, it is limited by fluorescence quenching and photobleaching. For deep cardiovascular tissue, fluorescence imaging is also limited by its penetrating ability.

Photoacoustic imaging (PAI) is a new optical imaging technology that uses light as an excitation source to generate thermal plucking by using the photo-thermal effect of target molecules, and it generates light absorption images by detecting the transient acoustic signals generated by the target through ultrasonic transducers. It provides high spatial resolution (50–500 μm) and penetration depth of information up to 5 cm. Both intrinsic tissue molecules (such as hemoglobin, lipids, water, and melanin) and exogenous photo-thermal agents (such as ICG, GNPs, etc.) can be used as contrast agents for imaging, and multi-wavelength imaging can be realized due to the differences in their absorption peaks. Therefore, photoacoustic imaging has gained wide attention in cardiovascular diseases.

Qin et al. [10] demonstrated for the first time that a class of photoacoustic nanoparticles (PANPs) containing semiconductor polymers (SPs) can be used as contrast agents for photoacoustic imaging (PAI) of HesC-CMS transplanted from living mouse hearts. In a recent study, Zhang et al. [11] used photoacoustic imaging of myocardial infarction regions in a rat myocardial ischemia–reperfusion model with noninvasive fibrin-targeted nanoparticles.

In another interesting study, Zhao et al. [12] synthesized ultrasmall NPs via single-stranded DNA (ssDNA)/metal ion complex self-assembly. Animal experiments showed that the ultrasmall NPs could significantly enhance the PA signal intensity in the area of myocardial infarction. Gifani et al. [13] recently constructed super-selective nanoparticles targeting LY-6Chi monocytes and foam macrophages, and combined them with clinically feasible photoacoustic imaging (PAI) to accurately and specifically image inflammatory plaques in vivo in a mouse model simulating human vulnerable plaques. In conclusion, photoacoustic imaging has good application prospects in the field of cardiovascular injury and is expected to be translated into clinical practice.

4. Contrast Ultrasound Imaging

Molecular ultrasound imaging offers the possibility of real-time, noninvasive visualization of molecular markers of cardiovascular disease using clinical ultrasound imaging systems. The technique uses particles or nanoparticles to target binding to functional specific epitopes after systemic injection. These bound particles (contrast agents) are acoustically active in the presence of ultrasound, resulting in backscattered signals from the molecular events of interest, which can be located and imaged by a two-dimensional ultrasonic scanning system. Contrast agent ultrasound imaging of inflammation has been achieved by targeting contrast agents to activated immune cells or to endothelial cell adhesion molecules that regulate leukocyte trafficking and adhesion. Myocardial contrast echocardiography is used for tissue perfusion imaging, extending the concept of ultrasonic red blood cell tracking to visualization of intracardial microcirculation blood volume. During echocardiographic imaging, microvascular transport of microbubbles leads to a transient increase in myocardial tissue video intensity, which can be quantified and regionally mapped to myocardial perfusion.

This method has been applied to the detection of coronary artery disease and the evaluation of myocardial activity after acute myocardial infarction. Several commercial microbubble formulations for perfusion imaging are currently being evaluated in multicenter clinical trials. The application of microbubble molecular imaging takes advantage of these

properties, namely, its intravascular position and acoustic responsiveness, to specifically detect molecular signatures on the endothelial surface. Unlike free-circulating microbubbles used for ventricular sedation and perfusion imaging, targeted microbubbles adhere to endothelial cells through specific ligand–receptor interactions pre-specified in the microbubble design. Targeted ligands attach to the surface of micro-vesicles, causing them to adhere to specific endothelial markers. This adhesion is shown in two-dimensional ultrasound images as an increase in the video intensity of the molecular target location region, and the adhesion persists even after the circulating microbubbles are washed out.

In recent years, ultrasonic microbubble contrast agents have been rapidly developed, and a wide variety of components have been reported, often including perfluorocarbons or nitrogen in shells made of phospholipids, albumin, or biodegradable polymers. Microbubbles are small enough to remain in the vessel, so the molecular target must be luminal. Other ultrasound contrast agents for non-gas-encapsulated microspheres include liposomes and liquid perfluorocarbon emulsion nanoparticles, which can exit the intravascular space and have the potential to acoustically identify extravascular targets, such as atherosclerotic plaque components.

Microbubble contrast agents are detectable because the frequencies used by the ultrasound imaging system will cause the expansion and contraction of the microbubbles. At high acoustic power, the oscillations of the microbubbles become asymmetric, and at a high enough acoustic power, the microbubbles may be induced to rupture. This behavior, known as nonlinear resonance, causes the microbubbles themselves to become intense ultrasonic emitters that produce unique acoustic signals based on their unique spectrum and profile. Clinically available ultrasound machines can distinguish nonlinear signals from tissue backscattering. The imaging system varies with the incident ultrasonic frequency, pulse waveform, sound power, whether the microbubbles are induced to burst, and the detection frequency. On two-dimensional images, all system configurations display processed microbubble signals with increased intensity. Micro air bubbles are used for the expansion of molecular imaging in the existing echocardiography applications. Their application by intravenous injection of microbubbles, and their rheological equivalent to that of red blood cells in blood vessels, creates a turbid blood pool to delimit the endocardial boundary and allows for a more accurate assessment of left ventricular systolic function. Two inflatable microsphere ultrasound contrast agents are currently in use for indications approved by the FDA.

5. Multimodal Imaging

Ultrasound is widely used, safe, and inexpensive, but has insufficient penetration for noninvasive imaging of deep blood vessels, including coronary arteries, with high spatial resolution or sensitivity. SPECT and PET have high sensitivity, but their spatial resolution is limited. Another disadvantage is the use of radioactive materials. In contrast, MRI is slightly less sensitive than SPECT and PET and requires a longer imaging time, but it is safe and has good resolution (~10 μm with a strong magnetic field). In contrast, CT has the advantages of a fast scan time and superior performance in coronary angiography at the cost of limited sensitivity, the use of nephrotoxic drugs, and ionizing radiation. Optical imaging techniques, such as near-infrared fluorescence reflection, or fluorescent molecular tomography, have excellent sensitivity and temporal resolution and allow for precise determination of the tissue distribution of the probe with an in vitro fluorescence microscope. However, until now, this technique has only been used noninvasively to monitor superficial structures due to the limited ability of light to penetrate tissue.

Because there is no single imaging method that ideally meets all research and medical needs, multiple imaging agents can be integrated into a unified platform. Multimodal nanoparticles with complementary imaging agents provide highly sensitive detection, anatomical localization, and data validation from different imaging modes. Nanoparticles combining optical, MR, and PET imaging agents have been reported. Imaging of macrophage-labeled multimodal nanoparticles by PET and MRI has been demonstrated. The availability of multimodal imaging devices that combine MRI, CT, optics, PET, and ultrasound facilitates simultaneous anatomical and molecular imaging. Functional PET imaging has been combined with CT to pinpoint radioisotope-labeled macrophages. Similarly, functional 19F MRI and fluorescence combined with anatomic MRI and CT, respectively, have been reported. Chen et al. [14] prepared a targeted nano-probe (called IMTP-Fe_3O_4-PFH NPs) with enhanced ultrasound (US), photoacoustic (PA), and magnetic resonance (MR) properties for direct and noninvasive visual imaging of ischemic myocardial models in rats. The probe has the characteristics of nanoscale, good stability, ADV, and safety. It has an obvious targeting effect on hypoxia-damaged cells and rat heart models. After NPs were injected into the tail vein of model rats, the in vivo imaging results showed that the US/PA/MR signal was significantly enhanced, indicating that this nano-probe is highly feasible for distinguishing ischemic myocardium. This makes up for the shortcomings of different imaging methods and provides a new idea for accurate diagnosis.

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