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Modified Nanoparticles for the Diagnosis and Treatment of Ischemic Stroke

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Abstract. Stroke is a disease that will affect both physical health and mental health of people. Scientists are developing different methods to detect and cure this disease. Nanotechnology has achieved successful results in diagnosis as well as treatment of ischemic stroke. Stroke will cause blood-brain barrier dysfunction which provides chances for detection and treatment to process. Researchers have developed and evaluated a variety of nanoparticles in vivo or in vitro, including gold nanoparticles, perfluorocarbon nanoparticles, PLGA nanoparticles and iron oxide nanoparticles. These nanoparticles are being modified and achieve good results in diagnosis including magnetic resonance imaging, microwave imaging and fluorescence imaging, and treatment including thrombolysis and stem cell transplantation. Nanotechnology has several benefits such as tiny size and ease to modified which will fasten future development in stroke detection and treatment. This review paper will outline several different modification methods of various nanoparticles in laboratory.

Keywords: Nanotechnology, Ischemic Stroke, Diagnosis, Treatment

1. Introduction

Stroke, a central nerve complication [1], is caused by blood flow being stopped in the brain which will cause death of brain cells and affect motion. There are two types of stroke, the first one is ischemic stroke which is caused by a clot which decreases the blood supplying or stenosis (narrowing of blood vessel) [2]. The second one is hemorrhagic stroke which is due to the damage of blood vessels [3]. Symptoms of stroke includes depression, feeling tired, being anxious, changing personalities and even mania [4]. Therefore, it is urgent to diagnose and treat stroke. This paper will focus on the most common ischemic one.

Stroke was first found by Hippocrates from 460 to 370 before the Common Era. He used a Greek word “apoplexy” to describe a condition which a person lost control of his body and fell down after an injury occurred to his head. In the late 20th century, Moniz and Seldinger invented a new method called Angiography help people understanding more about anatomy of blood vessels. This led to a breakthrough in visualization of pathological changes in blood vessels before people die and new treatment methods for stroke. Hounsfield and Damadian invented Magnetic Resonance Imaging (MRI) and Computerized Tomographic (CT) scan in the 1970s which are widely used in diagnosis of stroke [5].

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Some nanoparticles have been found helpful in both detecting and therapy of ischemic stroke. Nanoparticles are used due to several reasons: (1) they have tiny size, in nanometer scale; (2) They are simple to be modified and synthesized; (3) They can release probes or biomedicines which they carried when they meet specific environments in stroke; (4) They can act as contrast agents in imaging techniques; (5) They have direct targeting ability [6]. This paper will describe gold nanoparticles, iron oxide nanoparticles, perfluorocarbon nanoparticles and poly-D, L-lactide-co-glycolide nanoparticles modifications and the mechanisms to detect and treat the ischemic stroke.

2. Nanomaterials for the diagnosis of stroke

Blood brain barrier (BBB) is one special membrane structure which has high selectivity on blood entering central nervous system (CNS) [7]. It consists of endothelial cells, non-neural astrocytes which maintain tight junctions close to one another, and pericytes that cover the endothelial cells [3]. BBB will diffuse the circulating blood, letting small non-polar and hydrophobic molecules into CNS while rejecting the large and hydrophilic molecules [8]. BBB is a problem scientists should cover in the design of biosensor as well as the drugs in positioning and treatment of stroke. However, in ischemic stroke the ROS production will cause the first dysfunction of BBB by affecting the arrangement of tight junction as well as the astrocytes for up to several hours after stroke happens. The second longer dysfunction of BBB is up to several days due to the refilling of blood at the stroke sites. The opening of some channels enables lots of substances usually be stopped by BBB including nanomaterials [9]. The limited opening time of BBB forces scientists to develop more quicker and precise detecting methods to detecting the position of blood clots. Several successful nanotechnology examples have achieved success in MRI, MI and fluorescence imaging in laboratory.

2.1. Nanoparticles for magnetic resonance imaging

Magnetic resonance imaging (MRI), has increased resolution than CT, is now being used in detecting position of ischemic stroke. Nowadays, MRI becomes more sensitive which means it can detect the ischemic stroke earlier [10]. PFC, iron oxide and PLGA nanoparticles is now proven useful in MRI detecting ischemic stroke.

A highly paramagnetic liquid perfluorocarbon (PFC) nanoparticle contrast agent which is used in standard MRI. The reagent consists of PFC emulsions encapsulated in liposomes. Small chelates (Gadolinium) are attached to the PFC nanoparticles. These nanoparticles can target different epitopes. The result can be analyzed from the mass of Gadolinium on different places [11]. Neuroinflammation describes the inflammations happens in the brain, peripheral nervous tissue and spinal. Neuroinflammation will occur due to so many reasons like Multiple sclerosis, stroke etc. [3]. In ischemic stroke, endothelial cells will be activated then lose their functions like alter blood flow, changing frequency of blood vessel contraction and relaxation [12]. Some scientists have developed several probes using nanotechnology which aim at this feature of ischemic stroke and results will be achieved via MRI (Figure 1B and 1D). A new magnetic nanoparticle-P-selectin binding with peptide (MNP-PBP), which is made from iron oxide nanoparticles coated with aminated dextran-MNP-NH2 reacts with SPDP then bind with peptides PRPQIHNDGDFEEIPEEYLQ-GGSS-LVSVLDLEPLDAAW (Figure.1A). in the early stage of neuroinflammation, endothelial cells will be activated and release P selectin. These MNP-PBP can detect P selectin. This kind of nanoparticles can increase their number at the occlusion sites in a fast speed and can be removed from normal parts very quick [13].

Tang *et al.* developed a neutrophil-mimetic magnetic nanoprobe (NMNPs) which helps imaging neuroinflammation via targeting the activated endothelial cells. The inner shell composition of the nanoprobes is PLGA nanoparticles combined with superparamagnetic iron oxide while the outer shell employs neutrophil membranes (Figure. 1C). A temporary middle cerebral artery occlusion (tMCAO) animal model is used in this experiment. Same nanoparticles with red blood cell membrane modified (RMNPs) are used as control group. After 24 hours of finished setting of tMCAO model, NMNPS or

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RMNPs are injected into the mice. The model mice will have MRI 2 hours later. Result analysis will depend on the number of dark voxels [14].

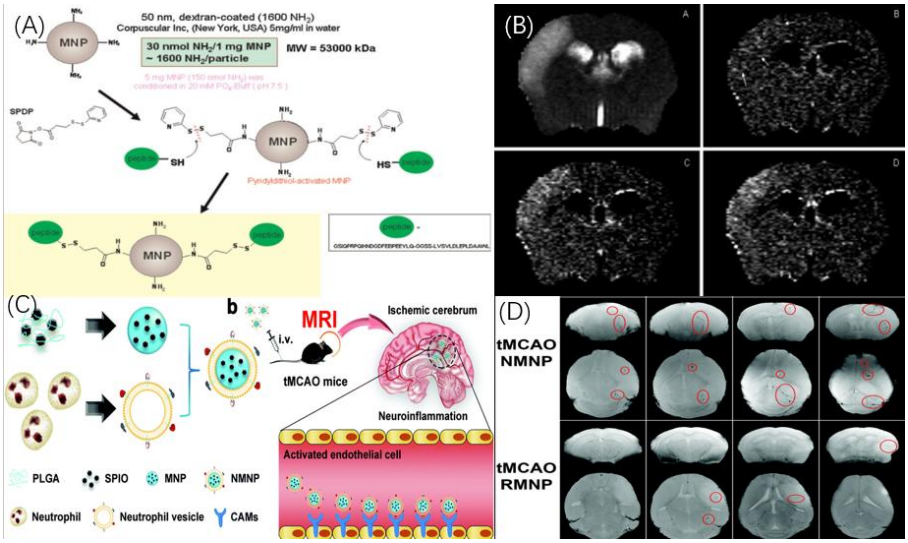


Figure 1. (A): Synthesis route of MNP-PBP from iron oxide nanoparticles [13]. (B): MRI results of MNP-PBP nanoparticles [13]. (C): Synthesis of NMNPs from PLGA nanoparticles, neutrophils, and superparamagnetic iron oxide as well as the targeting process of NMPs [14]. (D): Comparison of MRI results between tMCAO mice injected with NMNPs or RMNPs [14].

2.2. Nanoparticles for microwave imaging

Microwave imaging (MI) is a method using non-ionizing electromagnetic signals from 100 megahertz (MHz) to 10 gigahertz (GHz). A study shows there is slight difference between diseased and normal glandular tissues in breast when investigating their dielectric properties [15]. This means there may be confusion when telling the differences between them, so a strong contrast agent is needed. Super paramagnetic iron oxide nanoparticles can interact with microwave radiation which is useful to improve the MI by affecting the di-electric properties of different tissues. Injection of this kind of nanoparticles from intravenous (IV) way can maximize the efficiency of the nanoparticles while using a small dose because the particles may not spread everywhere in the brain part at once. This injection method can show which part has ischemic stroke by telling if there is more contrast or not [16].

2.3. Nanoparticles for fluorescence imaging

Reactive oxygen species (ROS), molecules with high reactivity formed from O2 [17], will produce in a large and abnormal quantity after ischemic stroke. Gold nanoparticles combined with near-infrared fluorescence (NIFR) dye labeled hyaluronic acid (HA) are able to detect ROS (Figure 2A). These gold nanoparticles proceed the detection via nanoparticle surface energy transfer (NSET), an effective method which absorb less background noise and can detect light in wider range of wavelength compared to fluorescence resonance energy transfer (FRET) [18]. The connection between fluorescent dye labeled HA and the nanoparticles when they meet HAdase in ROS region. The fluorescent dyes can be only detected when the cleavage process happens. In the experiment, some rats are in middle cerebral artery occlusion (MCAO) while some are without the MCAO condition. The specific modified gold nanoparticles are injected into the brain directly (near the bregma). A dose of nanoparticles will take 10 minutes to be injected into the rats. The nanoparticles will be injected at different time depending on whether rats are with MCAO or not. After specific time, the brains of rats are cut into slices and stained with 2,3,5-Triphenyl tetrazolium chloride. Then the image of the brains can be captured, and ROS active sites can be found (Figure 2B) [19].

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A study in 2009 shows: PLGA nanoparticles encapsulated with fluorescein-isothiocyanate (FITC) are produced using emulsion solvent diffusion method and are coated to balloon-expandable stents as electrodeposition material. The FITC modified nanoparticles can be detected via transmission electron microscopy after entering the human smooth muscle cells. It proves that if the PLGA nanoparticles loaded with FITC or other hydrophilic agent together with fluorescent, PLGA nanoparticles will be a good detector for stroke [20].

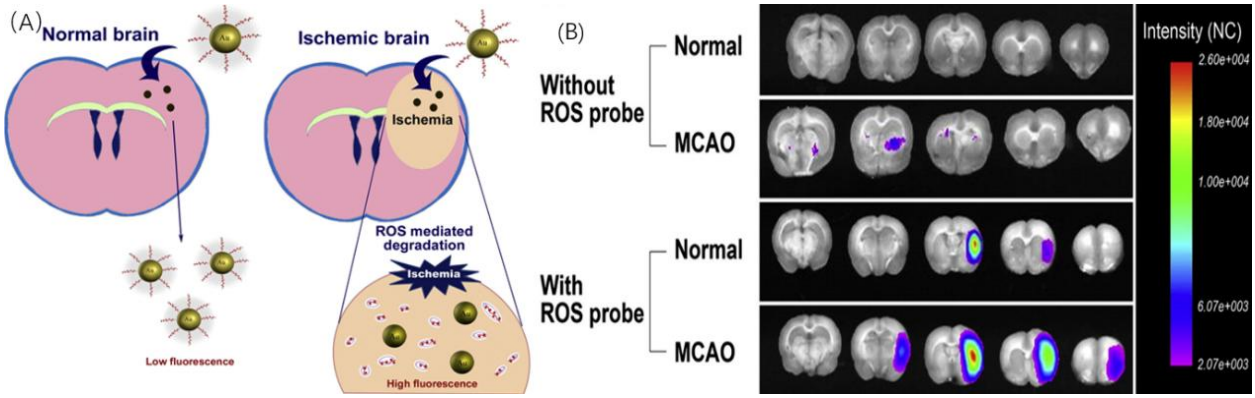


Figure 2. (A): The structure of modified gold nanoparticles. The mechanism of nanoparticles emits light at ROS rich sites but not at low ROS sites [19]. (B): Comparison of fluorescence images of gold nanoparticles with or without ROS probes in stroke models [19].

3. Nanomaterials for treatment of stroke

Treatment will consist of two parts: the first one is thrombolysis including PLGA and PFC nanoparticles, the second stem cell transplantation includes iron nanoparticles.

3.1. Nanoparticles for thrombolysis

In thrombolysis treatment of stroke, PEG (polyethylene glycol) coated to PLGA nanoparticles using carbodiimide/N-hydroxysuccinimide chemistry. The tPA (a necessary protein used in thrombolysis) is entrapped into the PEG-PLGA nanoparticles via single emulsion solvent diffusion/evaporation technique. During the experiment, Zamanlu *et al.* found the PEG-PLGA nanoparticles holding tPA will be more efficient than just injecting tPA without any cover into the body [21].

PFC nanoparticles modified with anti-fibrin monoclonal antibody and urokinase. This kind of PFC nanoparticles can go to the blood clot sites and dissolve the fibrin clots in ischemic stroke. 100-400 enzymes per nanoparticle can maximize the dissolution process but the number of antibodies per nanoparticle does not improve the efficiency [22]. Urokinase will be a good substance in treating stroke in human body because it will cause less immune response [23].

Marsh *et al.* used one kind of Streptokinase-modified PFC nanoparticles to dissolve the blood clots. Streptokinase is chosen in this experiment rather than the tPA is because tPA only process its function when they directly bind to fibrin in blood clots which means tPA must be presented on the nanoparticle surface which is difficult to achieve. Acoustic microscopy was used to scan the samples and the radiofrequency data were analyzed. The streptokinase-modified PFC nanoparticles will dissolve the blood clots in a faster pace than injecting streptokinase directly into samples. However, this experiment was done outside of human body which means the streptokinase-modified PFC nanoparticles needs to be further modified by ligand and enzymes [22].

3.2. Nanoparticles for stem cell transplantation

Stem cells, cells which can differentiate to several cell types, are proved useful in treatment of brain injury parts due to ischemic stroke [24].

Iron nanoparticles are very helpful to check if the transplantation of the stem cells is successful and if there are some problems. MRI is a preferrable way to check this because of its high resolution and

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low danger to hurt people. The high biocompatibility and high sensitivity and low risk of hurting human bodies enable super paramagnetic iron oxide nanoparticles to become one famous contrast agent when using the MRI. The unmodified iron oxide nanoparticles cannot be easily uptaken by stem cells. The iron oxide nanoparticles as well as fluorescent nile red can combine with cationic polymeric micelles designed by Lu et al. (polymer of poly(aspartic acid-dimethylethanediamine) (PAsp(DMA)) (hydrophilic) connected to cholic acid (CA) (hydrophobic) under the help of lysine). The cationic poly meric micelles have high efficiency, no harmful effect to body and also property of biodegradation (Fig. 3A and 3B) [25]. Another kind of modification of iron nanoparticles can also achieve the same goal. The ultrasmall superparamagnetic iron nanoparticles with amylose cationized with spermine (ASP-SPIONs) to detect the positions of stem cells modified with green fluorescent protein (GFP) gene. This kind of nanoparticles can reach a high detecting efficiency with low concentration of nanoparticles as well as a short period of time (Figure. 3C and 3D) [26].

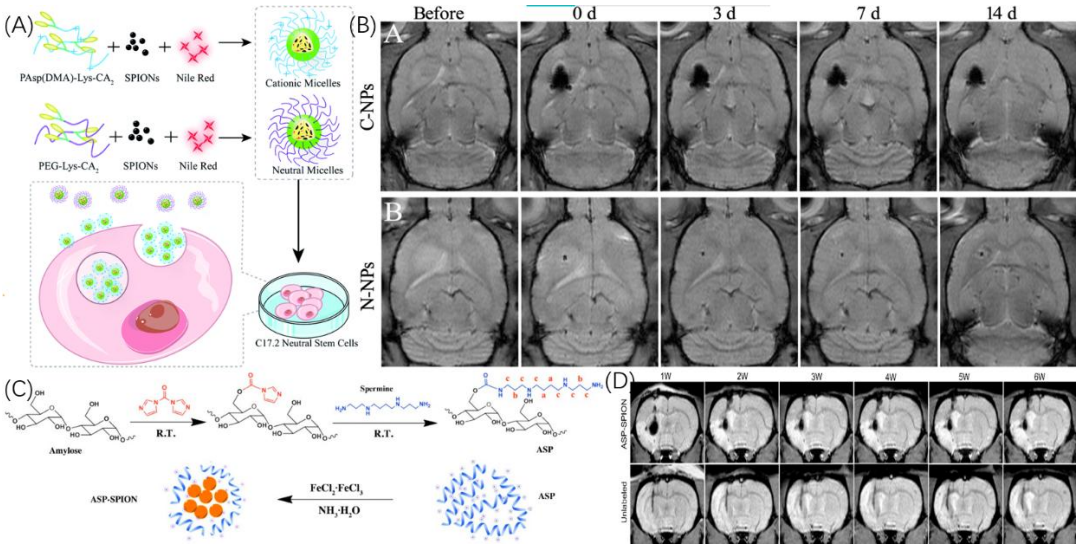


Figure 3. (A): Diagram of synthesis of cationic polymeric micelles and the labeling process of stem cells [25] (B): Comparison of MRI results between C-NPs and N-NPs [25]. (C): Diagram of synthesis of ASP-SPIONs [26]. (D): MRI results of ASP-SPION labeled cells and unlabeled cells [26].

4. Conclusion

For diagnosis, this paper focuses on MRI, MI and fluorescence imaging. MRI employs PFC nanoparticles with Gadolinium, MNP-PBP and NMNPs as contrast agents. MNP-PBP and NMNPs targets the activated endothelial cells in neuroinflammation. The second MI diagnosis uses super paramagnetic iron oxide nanoparticles to distinguish between two similar cell types. The fluorescence imaging is slightly different with MI and MRI. NIFR dye labeled HA gold nanoparticles target the ROS rich site and only emits fluorescent light at binding sites. The treatment part is divided into thrombolysis and stem cell transplantation. In the section titled “Thrombolysis”, PLGA nanoparticles and PFC nanoparticles are coated with various thrombolysis factors, such as tPA, anti-fibrin monoclonal antibody plus urokinase and streptokinase in order to dissolve the blood clots at ischemic stroke sites. Modified iron nanoparticles are used in checking if the transplanted stem cells work well. Nanotechnology operating in ischemic stroke is limited by blood cut at stroke sites which means nanoparticles injected into blood stream may be unable to reach exact positions. This must be solved out in the future study to improve the diagnosis and treatment in patients. The other limitation is that although these nanoparticles have all been proven effective in laboratory, they may face difficulties when operating in patients’ bodies [20].

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