Rapid and effective labeling of brain tissue using TAT-conjugated CdS:Mn/ZnS quantum dots†

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TAT (a cell penetrating peptide)-conjugated CdS:Mn/ZnS quantum dots (Qdots), intra-arterially delivered to a rat brain, rapidly (within a few minutes) labeled the brain tissue without manipulating the blood-brain-barrier (BBB). Qdot loading was sufficiently high that it allowed a gross fluorescent visualization of the whole rat brain using a low power hand-held UV lamp. Histological data clearly showed that TAT-conjugated Qdots migrated beyond the endothelial cell line and reached the brain parenchyma. Qdots without TAT did not label the brain tissue confirming the fact that TAT peptide was necessary to overcome the BBB. The present study clearly demonstrated the possibility of delivering a large amount of Qdot-based imaging agents to the brain tissue.

Delivery of imaging and therapeutic agents to the brain is highly important for diagnosis and therapy of many brain diseases such as brain tumors. However, the delivery to these agents to the brain is often restricted by the BBB, a tight junction of endothelial cells that regulates the exchange of substances between brain and blood. Cell membrane is another natural barrier that also can restrict transport of these agents. A method to overcome the cellular membrane barrier is provided by the use of membrane translocation peptides such as TAT peptide.[1,2] This method has been successfully used to internalize various substances such as proteins,[3,4] oligonucleotides,[5] plasmid DNA[6] and even nanoparticles.[7–9] Recently, we have shown that it is possible to deliver various nanoparticle-based imaging agents into cells using TAT peptide-mediated delivery system.[8,9]

For delivery of imaging/therapeutic agents to the brain at high concentration, microcatheter based intra-arterial administration approach can be more attractive over the conventional intravenous administration approach.[10] Through intravenous administration, agents are evenly distributed throughout the body. As a result, the availability of agents in the target site would be diluted by the loss of agent to non-targeted sites. However, using a microcatheter, agents can be administered intra-arterially to distal selective cerebral arteries in a human, at high concentration to a pre-selected area of the brain, permitting direct interaction with the target brain tissue.

In the present study, we will take the advantage of intra-arterial approach to administer highly sensitive and photostable Qdots to the brain tissue. This approach results in high Qdot concentration at the target site. This technique, if eventually applied to humans, could help neurosurgeons to clearly see target brain tissues, such as brain tumors, during a surgical procedure. However, just having a suitable approach of administering Qdots may not help in brain imaging unless the following critical challenges are addressed. First, one must find a way to overcome the BBB to transport Qdots to the brain tissue. Second, Qdots must be rapidly transported from the blood vessels to the capillary endothelial cells to take the advantage of high local dosing of Qdots at the target site. To address these challenges, we present a robust TAT peptide-mediated Qdot delivery technique that allows rapid and high dosing of Qdots in the brain tissue. To our knowledge, we demonstrate for the first time the application of TAT-Qdots for gross fluorescence visualization of a pre-selected rat brain tissue using a low power handheld light source. This technique could help doctors to clearly see pathological tissues such as brain tumors during a surgical procedure.

We have previously reported the synthesis and characterization of CdS:Mn/ZnS Qdots over-coated with a amine functionalized silica layer. Typically, Qdots were synthesized in a water-in-oil (sodium dioctyl sulfosuccinate–heptane–water) microemulsion system where water droplets served as a nano-reactor. Amine functionalized silica overcoating, by which Qdots become highly water-soluble, was accomplished by the hydrolysis and co-condensation reaction of tetraethyl orthosilicate, 3-(aminopropyl) triethoxysilane and 3-(trihydroxysilyl)propyl methylphosphonate in the presence of ammonium hydroxide base.[11] TAT peptide (the italicized amino acids in *Gly-Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg*-Gly-Tyr-Lys-Cys-NH_2) contained a cysteine (Cys) residue. The thiol (–SH) group of the cysteine was covalently attached to the amine group on the particle surface using a thiol-amine coupling agent, *N*-succinimidyl 3-(2-pyridyldithio)propionate.[11] TAT-conjugated Qdots were completely dispersed in phosphate buffer saline (PBS) at pH 7.4.

Sprague-Dawley rats (250–300 g)[12] were used for *in vivo* experiments. The infusion catheter was placed in a proximal, non-selective, cervical carotid artery in the rat, rather than a distal, selective, cerebral artery that would be used if performed in humans. This type of distal selective cerebral artery catheterization is routinely performed by physicians in humans, but is not possible in animal models because of the small size of their cerebral arteries. Rats were anesthetized with a Ketamine/Xylazine mixture (0.1 ml per 100 g) administered intraperitoneally. The necks were shaved and prepped with chlorhexidine scrub. A 2–3 cm long incision was made on the neck. The right common carotid artery (CCA) was located and isolated from the surrounding tissue. A 4-0 silk suture

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was used to ligate the CCA proximally. Another length of suture was wrapped around the external carotid artery (ECA) and occipital artery to temporarily stop blood flow during injection of the substance into the internal carotid artery (ICA). A small catheter was placed in the right CCA and secured in place. Fig. 1 shows the schematic representation of the surgical procedure.

About 0.75 ml (10 mg ml^{-1}) of Qdot suspension in PBS was loaded into a syringe and placed into an infusion pump. The syringe was attached to the sheath and the pump was started. The infusion pump injected Qdots over a period of 5 minutes. At the same injection rate, PBS (pH 7.4) was then injected for 15 minutes to remove residual Qdots, which were not uptaken by the endothelial cells. The ECA was then opened and collateral blood flow allowed for 3 minutes. The rat was then euthanized with an overdose of sodium pentobarbital. A craniotomy was performed on the rat after euthanasia and the brain was removed. The whole brain was then immediately placed under a handheld 366 nm multi-band UV source (Spectroline, model UV-4B) and photographed using a standard digital camera as shown in Fig. 2 (a) and (b). The appearance of pink color in Fig. 2 is due to Qdot fluorescence and the blue color originates from the background without Qdot labeling. Note that the presence of fluorescence on the medial aspect of the left hemisphere (Fig. 2 (a,c)) is secondary to the proximal, non-selective position of the catheter. This catheter position allows the Qdots to travel through the arterial communication between the medial hemispheres called the anterior communicating artery, which is a component of the Circle of Willis (connection of proximal cerebral arteries at the base of the brain). This would not occur in humans because the microcatheter would be placed far distal to the Circle of Willis. The time that it takes to stain brain tissues depends on the velocity of the cerebral blood flow, and is not significantly different from a clinical perspective. The brain was then sliced into four 5 mm sections (one of the brain slices is shown in Fig. 2 (c)) and placed into 10% buffered formalin. The tissue samples were then processed, embedded in paraffin, and

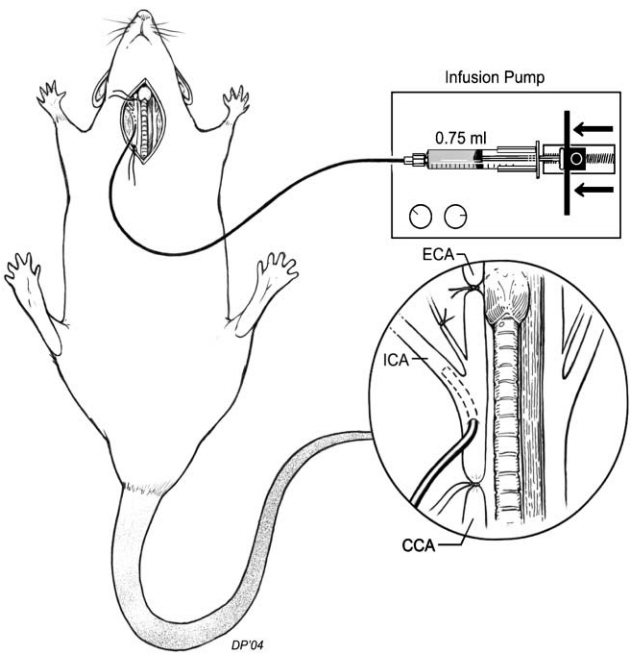

Fig. 1 Schematic representation of the surgical procedure.

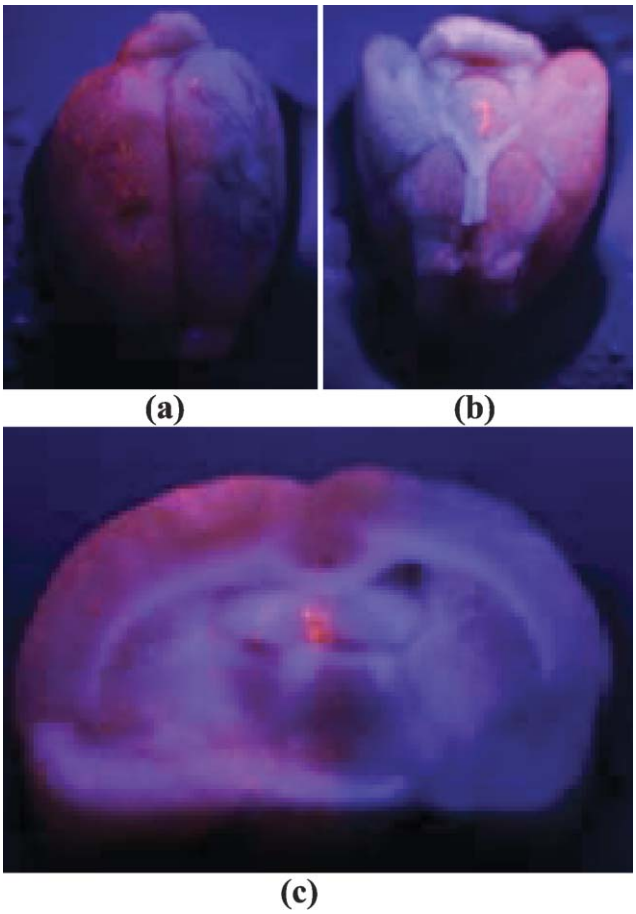

Fig. 2 Gross views of a rat brain labeled with TAT-conjugated Qdots; (a) and (b) represent dorsal views and (c) represents coronal section. Pink color (left side in (a,c) and right side in (b)) originates primarily from Qdot fluorescence and background blue color (right side in (a,c) and left side in (b)) is due to the combination of UV excitation, autofluorescence, and scattering lights. No filters were used for gross visualization of rat brain.

two unstained slides were made from each section of brain for histological analysis. In addition, amine functionalized Qdots without TAT-conjugation were also tested as a control. However, no fluorescence was observed, confirming that these Qdots did not exhibit a labeling capability.

Gross visualization of the whole rat brain does not provide information at the cellular level. It is important to understand whether TAT-conjugated Qdots penetrated the endothelial cells. We were also interested in whether Qdots were localized in the endothelial cell line or migrated further to reach brain parenchyma. Histological analysis, as shown in Fig. 3, was performed to obtain this information. Fig. 3 (a) and (b) showed transmission and fluorescence images of a cross section of a fixed brain tissue. Blood capillaries (distant branches of middle cerebral arteries) were shown with white arrows in Fig. 3 (a). The corresponding fluorescence image of Qdot labeled blood capillaries and surrounding brain cells in the brain parenchyma were clearly seen by Qdot fluorescence (Fig. 3 (b)). It is also seen that TAT conjugated Qdots reached the nucleus of brain cells (as shown by green-circled brown spots in Fig. 3 (b)).

It is known that TAT peptide can rapidly translocate through the plasma membrane and accumulate in the cell nucleus.[13] Our

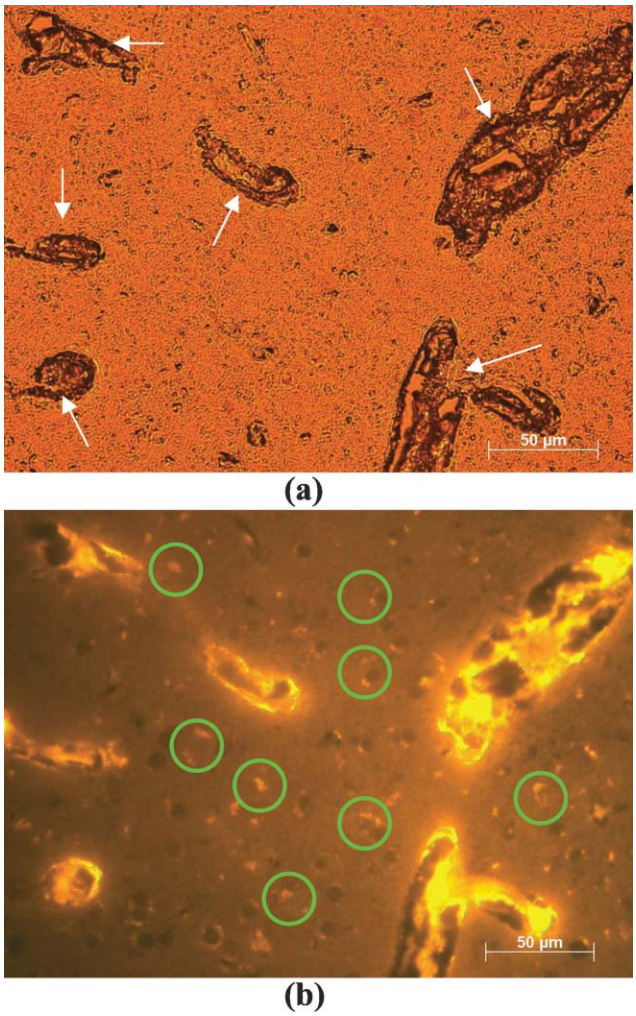

Fig. 3 Transmission (**a**) and fluorescence (**b**) microscope images (magnification was 40 X) of a cross-section of a fixed brain tissue, showing blood capillaries (distant branches of the middle cerebral artery as pointed with white arrows in image **a**) and the surrounding brain parenchyma (green circled brown dots around blood capillaries as shown in image **b**, representing the nucleus of brain cells). Fluorescence images were taken using excitation bandpass 360/40, 400 dichroic longpass and emission bandpass 600/50 filters (obtained from Chroma Technology Corporation, Rockingham, VT). Image **b** clearly showed that TAT conjugated Qdots extensively labeled blood capillaries, crossed BBB and reached brain parenchyma.

histological analysis of the brain tissue indeed supported the fact that TAT-Qdots crossed the BBB, further migrated to brain parenchyma and reached cell nuclei. As expected, endothelial cells in blood capillaries were highly loaded with Qdots and as a result they appeared bright yellow in Fig. 3 (b).

Here, we demonstrated a rapid intra-arterial loading of yellow-emitting Qdots in a pre-selected brain tissue of a rat. Our experimental data clearly showed that Qdot-loaded brain tissue could be visualized grossly (by a handheld UV excitation) as well as microscopically using a fluorescence microscope, confirming the fact that TAT peptide is responsible for a significant biological effect in labeling brain tissue. Like other tumor labeling approaches with Qdots,[14] the present approach could be useful for the visualization of tumors during the surgical procedure. Currently, a great deal of research effort has been placed to understand the transport mechanism of TAT conjugated Qdots to the brain tissue.

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