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TAT conjugated, FITC doped silica nanoparticles for bioimaging applications†

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Water-in-oil (w/o) microemulsion synthesis of 70 nm size monodisperse TAT (a cell penetrating peptide, CPP) conjugated, FITC (fluorescein isothiocyanate) doped silica nanoparticles (TAT-FSNPs) is reported; human lung adenocarcinoma (A549) cells (*in vitro*) and rat brain tissue (*in vivo*) were successfully labeled using TAT-FSNPs.

The intracellular translocation of HIV-coded TAT regulatory protein was first discovered in 1988.[1] The TAT regulatory protein is an 86-amino-acid-long nuclear protein, of which the 48–57 amino-acid residues (Gly-Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg), named as HIV-TAT peptide, are responsible for translocating across the cell plasma membrane. Like other natural CPPs, such as the third helix of the homeodomain of Antennapedia (Ant),[2] transportant[3] and VP22 herpes virus proteins,[4] TAT provides a new way of delivering various cargo molecules that include anti-tetanus F(ab′)2 fragments,[5] proteins[6] oligonucleotide (20 mer),[7] phosphopeptide (10 mer),[8] magnetite nanoparticles,[9,10] liposomes,[11] imaging and radiotherapeutic agents,[12] and many more.[13] The feasibility of CPP mediated delivery of nanoparticles has also been proven recently.[9,10] With the advancement of nanoparticle technology, CPP mediated transport of nanoparticles would have tremendous potential for delivering imaging and therapeutic agents to treat various diseases.

Dye-doped nanoparticle (NP) probes have shown potential for bioimaging in recent years.[14,15] There are several advantages of using NP probes. First, each NP can carry many fluorescent molecules which would greatly enhance the detection sensitivity. Second, dye molecules are encapsulated within the particle matrix that protects them from photobleaching.[14] Third, NPs are much smaller (about two to three orders of magnitude) than cells, which make them suitable for cellular and other biological applications. Fourth, dye molecules are protected from the adverse biological environment.

In this paper, we report an effective labeling of cells and brain tissue using a TAT peptide conjugated fluorescent nanoparticle probe. The advantage of a fluorescent nanoparticle-based sensitive detection system has been combined with an efficient TAT peptide mediated delivery system for bioimaging application. This strategy opens a new possibility of using CPP-nanoparticle delivery systems for biomedical research and therapy.

Monodisperse FITC doped silica nanoparticles were synthesized using a cyclohexane/Triton X-100/*n*-hexanol/water water-in-oil (w/o) microemulsion system.[14,16] First, a silane precursor of FITC was prepared by reacting FITC with 3-(aminopropyl)triethoxysilane (APTS) in absolute ethanol. For FITC-APTS conjugate synthesis, an excess amount of APTS (molar ratio of APTS to FITC = 23) was used. The hydrolysis and co-condensation reaction of tetraethylorthosilicate (TEOS), FITC-APTS conjugate and a water-dispersible silane agent, 3-(trihydroxysilyl)propylmethylphosphonate (THPMP)[17] in the presence of ammonium hydroxide were reacted to yield FSNPs. The purpose of using an excess amount of APTS to synthesize FITC-APTS conjugate was two-fold; (i) to be sure that all FITC molecules have been reacted and (ii) to directly obtain a coating of primary amine groups on the nanoparticle surface to enable covalent conjugation of TAT peptide. The resulting fluorescein-doped silica NPs (FSNPs) were uniform in size with an average particle size of 70 nm, as determined by transmission electron microscopy (TEM, Fig. 1). UV-Vis absorption, fluorescence excitation (recorded at 515 nm emission) and fluorescence emission spectra (recorded at 490 nm excitation) of FSNPs were measured in aqueous medium. Spectroscopic results confirmed the successful doping of FITC molecules in FSNPs. Since FITC was covalently linked to the silica matrix, no dye leaking was observed in an aqueous suspension.

The purpose of adding THPMP is explained below. Pure silica nanoparticles were reasonably water dispersible. When measured at pH 7.4, silica nanoparticles exhibited a negative zeta potential (ξ) value of $-$ 50 mV. This was due to the presence of deprotonated silanol groups (Si–O^-) on the silica surface (pK_a = 7.0). The co-presence of protonated amine groups (pK_a = 9.0) reduced the ξ value close to the isoelectric point ($\xi \sim -4.0$ mV) at pH 7.4 resulting in particle aggregation. The addition of THPMP (pK_a = 2.0) made negatively charged chemically inert methylphosphonate groups available on the FSNP surface thereby increasing the ξ value to about -40 mV. As a result, FSNPs were observed to be well dispersed in aqueous medium.

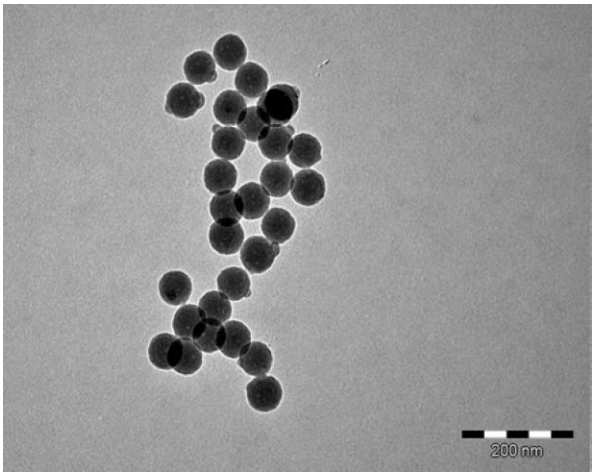

Fig. 1 TEM micrograph of fluorescein-doped silica nanoparticles. Nanoparticles were synthesized using a cyclohexane/Triton X-100/*n*-hexanol/water w/o microemulsion system. The average particle size was 70 nm.

† Electronic supplementary information (ESI) available: Experimental section. See http://www.rsc.org/suppdata/cc/b4/b411916a/

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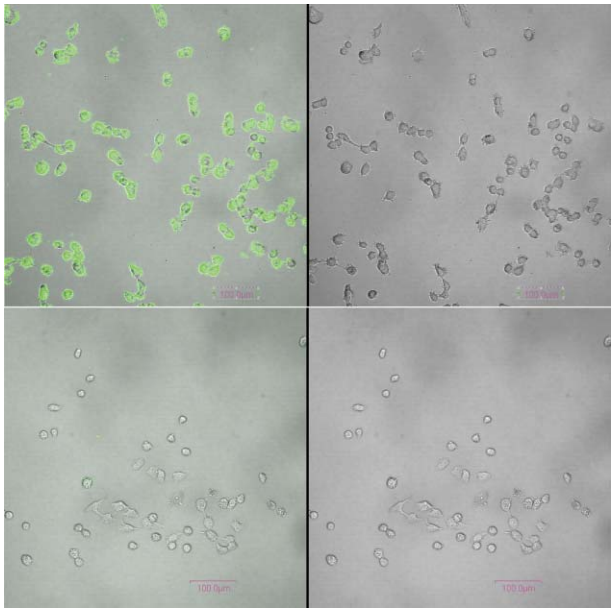

Fig. 2 Laser scanning confocal images (right: transmission image, left: transmission plus fluorescence images) of human lung adenocarcinoma (A-549) cells labeled with TAT-FSNPs (top image) and with FSNPs (bottom image) as control nanoparticles. From the top image it was clear that TAT-FSNPs successfully labeled A-549 cells.

TAT-conjugated FSNPs were synthesized by using *N*-succinimidyl 3-(2-pyridyldithio)propionate (SPDP) coupling chemistry as described by Zhao *et al.*[10] TAT-FSNPs were soluble in phosphate buffer saline (PBS, pH 7.4). To demonstrate the biolabeling capability, A-549 cells were incubated with TAT-FSNPs for 2 h using a nanoparticle concentration of 250 μg ml^{-1}. After washing 6–7 times with cold PBS, cells were imaged with a laser scanning confocal microscope. As shown in Fig. 2, TAT–FSNPs labeled the A-549 cells, thus validating the TAT mediated delivery mechanism. Control experiments were performed with only FSNPs which showed no effective labeling. A-549 cells are known to naturally phagocytos particulate systems that are exposed to the lung. To avoid such a possible artifact, TAT-FSNPs were also targeted to human squamous adenocarcinoma (SCC-9 cells). We observed similar cell labeling with TAT-FSNPs (images not shown).

To demonstrate *in vivo* bioimaging capability, TAT-FSNPs in PBS (pH 7.4) were administered intra-arterially through the right common carotid artery (CCA) that supplies blood to the right part of the brain of a Sprague–Dawley rat. After completing the procedure, the whole brain was sliced into four pieces and imaged with a fluorescence microscope. Fig. 3 confirmed labeling of branches of the right MCA (middle cerebral artery, a distal branch of the internal carotid artery) in the brain and confirmed the efficacy of the TAT-FSNP based *in vivo* bioimaging, which again validated the TAT mediated delivery mechanism. No labeling occurred on the left brain hemisphere (control experiment).

The purpose of this study was to selectively label brain blood vessels using an endovascular approach. It is well understood that the blood-brain-barrier (BBB, a tight junction of endothelial cells) protects the brain from toxic substances present in the circulating blood from passing into the brain parenchyma. However, it has been shown in this study that by using a TAT-mediated delivery system, it is possible to deliver diagnostic and therapeutic agents to the brain without compromising the BBB. *In vivo* experiments clearly showed that TAT conjugated NPs stained the brain blood vessels.

In conclusion, application of TAT-FSNP based bioimaging both *in vitro* and *in vivo* for bioimaging has been demonstrated in the present study. Using this nanoparticle-based CPP mediated imaging strategy, diagnostic and therapeutic agents can be delivered to the biological target of interest.

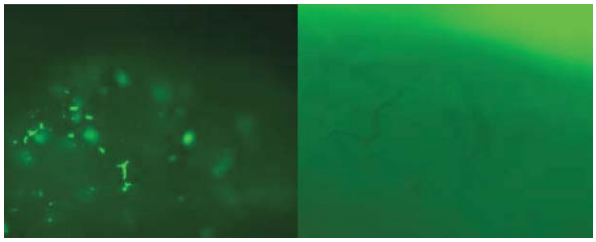

Fig. 3 Branches of right middle cerebral artery (MCA): optical (right) and fluorescence (left) images of a cross-section of a gross specimen of a rat brain (magnification 10×). 0.75 ml (10 mg ml^{-1}) of TAT-FSNPs were administered intra-arterially over a 3 min time period. The fluorescence image clearly showed successful labeling. Green dots appearing in the fluorescence image are out-of-focus branches of right MCA.

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Notes and references

1 (*a*) M. Green and P. M. Loewenstein, *Cell*, 1988, **55**, 1179; (*b*) A. D. Frankel and C. O. Pabo, *Cell*, 1988, **55**, 1189.
2 D. Derossi, S. Calvet, A. Trembleau, A. Brunissen, G. Chassaing and A. Prochiantz, *J. Biol. Chem.*, 1996, **271**, 18188.
3 U. Soomets, M. Lindgren, X. Gallet, M. Hallbrink, A. Elmquist, L. Balaspiri, M. Zorko, M. Pooga, R. Brasseur and U. Langel, *BBA-Biomembranes*, 2000, **1467**, 165.
4 (*a*) G. Elliott and P. O'Hare, *Cell*, 1997, **88**, 223; (*b*) A. Phelan, G. Elliott and P. O'Hare, *Nat. Biotechnol.*, 1998, **16**, 440.
5 S. Stein, A. Weiss, K. Adermann, P. Lazarovici, J. Hochman and H. Wellhoner, *FEBS Lett.*, 1999, **458**, 383.
6 (*a*) M. Becker-Hapak, S. S. McAllister and S. F. Dowdy, *Methods*, 2001, **24**, 247; (*b*) S. R. Schwarze, A. Ho, A. Vocero-Akbani and S. F. Dowdy, *Science*, 1999, **285**, 1569.
7 (*a*) A. Astriab-Fisher, D. Sergueev, M. Fisher, B. R. Shaw and R. L. Juliano, *Pharm. Res.*, 2002, **19**, 744; (*b*) A. Astriab-Fisher, D. S. Sergueev, M. Fisher, B. R. Shaw and R. L. Juliano, *Biochem. Pharmacol.*, 2000, **60**, 83.
8 C. T. Taylor, G. T. Furuta, K. Synnestvedt and S. P. Colgan, *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 12091.
9 (*a*) M. Lewin, N. Carlesso, C. H. Tung, X. W. Tang, D. Cory, D. T. Scadden and R. Weissleder, *Nat. Biotechnol.*, 2000, **18**, 410; (*b*) M. Zhao and R. Weissleder, *Med. Res. Rev.*, 2004, **24**, 1.
10 M. Zhao, M. F. Kircher, L. Josephson and R. Weissleder, *Bioconjugate Chem.*, 2002, **13**, 840.
11 (*a*) T. S. Levchenko, R. Rammohan, N. Volodina and V. P. Torchilin, *Liposomes, Part B*, 2003, **372**; (*b*) V. P. Torchilin and T. S. Levchenko, *Curr. Protein Pept. Sci.*, 2003, **4**, 133.
12 V. Polyakov, V. Sharma, J. L. Dahlheimer, C. M. Pica, G. D. Luker and D. Piwnica-Worms, *Bioconjugate Chem.*, 2000, **11**, 762.
13 P. Jarver and U. Langel, *Drug Discovery Today*, 2004, **9**, 395.
14 (*a*) S. Santra, P. Zhang, K. M. Wang, R. Tapec and W. H. Tan, *Anal. Chem.*, 2001, **73**, 4988; (*b*) S. Santra, K. M. Wang, R. Tapec and W. H. Tan, *J. Biomed. Opt.*, 2001, **6**, 160.
15 (*a*) X. X. He, K. M. Wang, W. H. Tan, J. Li, X. H. Yang, S. S. Huang, D. Li and D. Xiao, *J. Nanosci. Nanotechnol.*, 2002, **2**, 317; (*b*) M. K. Bhalgat, R. P. Haugland, J. S. Pollack and S. Swan, *J. Immunol. Methods*, 1998, **219**, 57; (*c*) D. M. Moss, J. M. Montgomery, S. V. Newland, J. W. Priest and P. J. Lammie, *J. Parasitol.*, 2004, **90**, 397; (*d*) M. Hall, I. Kazakova and Y. M. Yao, *Anal. Biochem.*, 1999, **272**, 165.
16 (*a*) F. J. Arriagada and K. Osseo-Asare, *J. Colloid Interface Sci.*, 1999, **211**, 210; (*b*) S. Santra, R. Tapec, N. Theodoropoulou, J. Dobson, A. Hebard and W. H. Tan, *Langmuir*, 2001, **17**, 2900.
17 A. Schroedter and H. Weller, *Angew. Chem., Int. Ed.*, 2002, **41**, 3218.