Supporting information

Would colloidal gold nanocarriers present an effective diagnosis or treatment for ischemic stroke?

Hamed Amani[a,b,1], Ebrahim Mostafavi[c,1], Mahmoud Reza Alebouyeh[d,1] , Hamidreza Arzaghi[e], Abolfazl Akbarzadeh [f], , Hamidreza Pazoki-Toroudi[g *], and Thomas J. Webster[c **]

a Department of Medical Nanotechnology, Faculty of Advanced Technologies in Medicine, Iran University of Medical Science, Tehran, Iran
b Physiology Research Center, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran
c Department of Chemical Engineering, Northeastern University, Boston, MA, USA
d Anesthesia Department, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran
e Department of Medical Biotechnology, Faculty of Allied Medical Sciences, Iran University of Medical Sciences, Tehran, Iran
f Department of Medical Nanotechnology, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, University Street, Tabriz, Iran
g Physiology Research Center and Department of Physiology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran

[1] These authors contributed equally to this work.

*Correspondence to: Thomas J. Webster, The Art Zafiropoulo Chair and Professor, Chair, Department of Chemical Engineering, Northeastern University, Email: websterthomas02@gmail.com, th.webster@northeastern.edu
Hamidreza Pazoki-Toroudi, Physiology Research Center and Department of Physiology, Iran University of Medical Sciences, P.O. Box 14515-763, Tehran, Iran. Fax: +98-21-88675760. Email: pazoki.h@iums.ac.ir, pazoki49@gmail.com,

In supplementary file: Number of figures 5; Number of tables 2

Supplementary methods

1. MCAO model

In brief, a midline neck incision was made to carefully isolate the right common carotid artery (CCA), the external carotid and the internal carotid under sterile conditions. The external carotid artery was tied and a monofilament nylon suture (0.24 mm in diameter) gently inserted from the right CCA into the internal carotid to occlude the origin of the right middle cerebral artery. Then, the right CCA was tied permanently. After 45 min, the monofilament was gently removed to allow recirculation/reperfusion of cerebral blood flow. A rectal probe was used to monitor and maintain body temperature at 37°C during the whole surgical procedure.

2. Assessment of cell membrane damage and necrosis

The LDH release was measured by monitoring the rate of decrease in NADH (at 340nm). This method is based on the conversion of pyruvate into lactate in a buffer containing 0.1 mM NADH, 1mM Na-pyruvate (pH 7.4), and 0.1M Tris–HCl. The LDH unit was presented as the amount of the enzyme that leads to oxidation of 1μmol of NADH per minute (IU).

3. Quantitation of OX26 antibody conjugated to GNPs

The number of immobilized OX26 antibody molecules onto the surface of GNPs was determined using the Bradford assay. Briefly, standard solutions of the OX26 antibody (1, 5 and 10 μg/mL) were prepared in 2 mM borate buffer (pH 8.0). The samples (standard solutions and the OX26 coated GNPs) were then centrifuged at 5000g for 5 minutes. Then, 90 μL of the supernatant or the standard solutions were transferred to a 96-well plate. The samples were diluted with 70 μL of 2 mM borate buffer and then 40 μL of the Bio-Rad reagent was added to each well.

Afterwards, the samples were incubated at room temperature for 15 minutes. To quantify the OX26 antibody in the supernatant, UV−visible absorption was determined at 595 nm. Ultimately, the total number of immobilized OX26 antibody molecules onto the surface of GNPs was determined using the following formula:

X= (the number of OX26 antibody molecules added to the GNP suspension) – (antibody remaining in the supernatant).

Supplementary Results

1. **Characterization of PEGylated GNPs**

Figure S1. Changes in the zeta potential after PEGylation

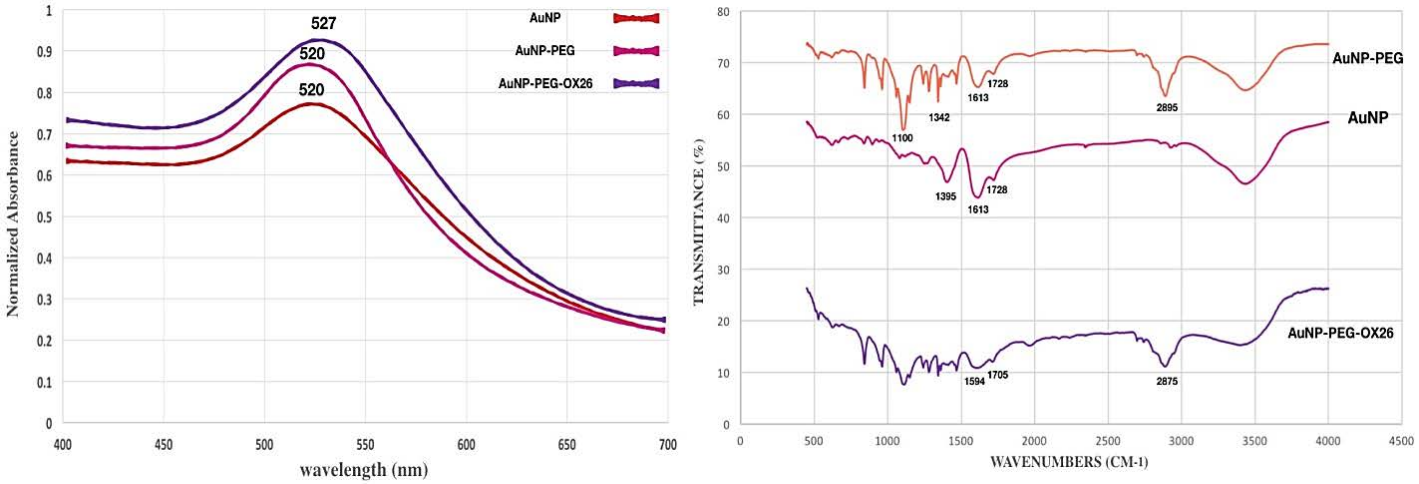

Figure S2. Ultraviolet–visible spectrophotometry (UV–Vis) and FTIR spectra of AuNP, AuNP-PEG and AuNP-PEG-OX26.

2. Evaluation of the effect of intravenous injection of OX26@GNPs on infarct volume

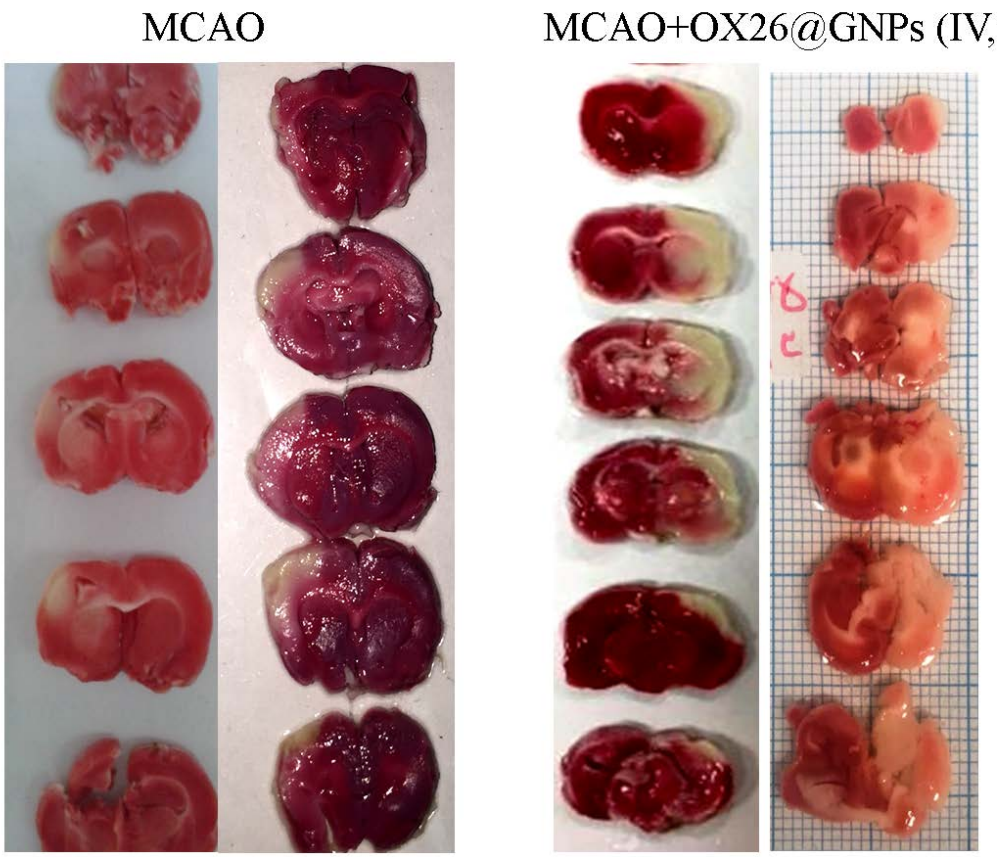

Figure S3. The effect of intravenous injection of OX26@GNPs on infarct size

3. Evaluation of myelin damage and neutrophil recruitment

We investigated whether the targeted delivery of GNPs to brain tissue alters brain function through myelin damage. Neutrophil recruitment and myelin loss induced by MCAO was significantly increased when OX26-PEG coated GNPs were administered intravenously (**Figure S4 a, b and c**).

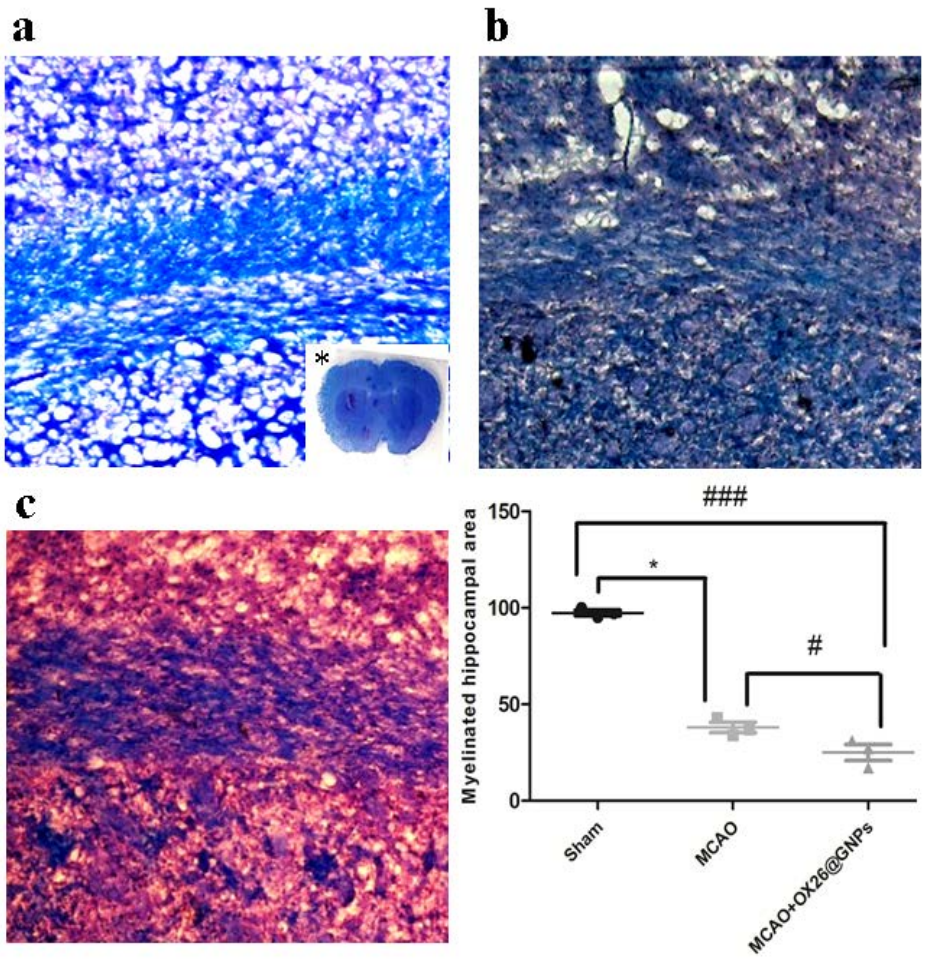

Figure S4. The Luxol fast blue (LFB) staining of the CA1 subregion of the hippocampus 72 h post-MCAO at a 500 µg/ml dose of OX26@GNPs (magnification 200×). * macro picture demonstrates the region where the assessments were done. a) Sham, b) MCAO, and c) MCAO+ OX26@GNPs. The graph demonstrates myelinated hippocampal area cells in the hippocampal CA1subregion (Myelinated fibers

(blue), neutrophils (pink) and nerve cells (purple); *P<0.05, #P<0.05, ### P<0.001 between indicated groups).

2. Evaluation of axonal loss

The number of axons in the hippocampus region was significantly decreased at 72 h after reperfusion. Surprisingly, OX26@GNPs–treated rats had a remarkably higher increase in axonal loss (**Figure S5 a, b and c**).

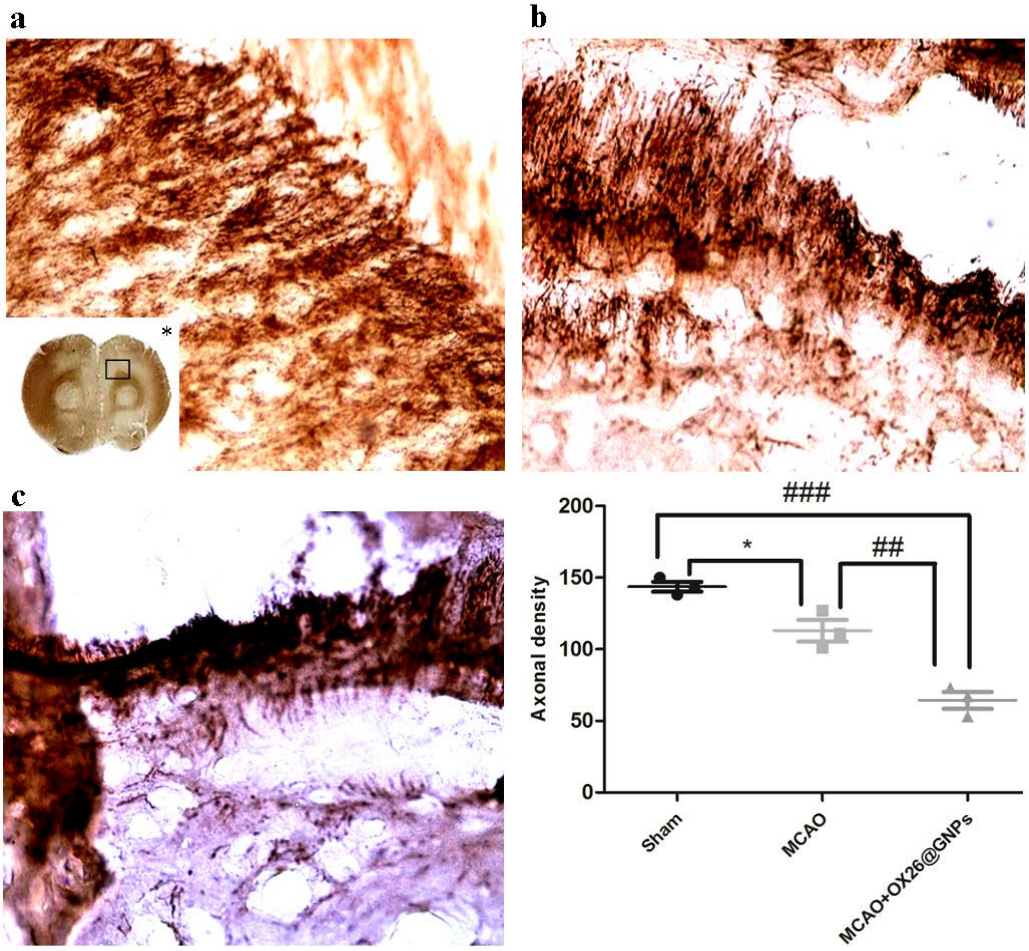

Figure S5. Bielschowsky's silver staining of CA1subregion of hippocampus 72 h post-MCAO at 500 µg/ml dose of OX26@GNPs (magnification 200×). * Macro picture demonstrates the region where the assessments were done. a) Sham, b) MCAO, and c) MCAO+ OX26@GNPs. Axons: black or brown strands (*P<0.05, ##P<0.01, ### P<0.001 between indicated groups)

Table S1. Quantitation of the immobilized OX26 antibody onto the surface of GNPs

Added antibody concentration to PEGylated GNPs (µg/mL)	Corrected absorbance*	Concentration of particle in Supernatant (µg/mL)#	Concentration of adsorbed antibody (µg/mL) $	Average of adsorbed antibody molecules onto the particle surface**
1	0.0435±0.0014	0.69±0.05	0.31±0.05	43±7
5	0.1652±0.0039	3.42±0.41	1.58±0.41	197±35
10	0.2646±0.0041	8.47±0.49	1.53±0.49	179± 51

* Correction of absorbance was calculated via subtraction of absorbance for the negative control (0 µg/mL antibody) per Bio-Rad protocol.

This was based on the amount of antibody remaining in the supernatant.

$ This was calculated as the difference in added antibody concentration and antibody remaining in the supernatant.

** This was based on dividing of adsorbed antibody (converted to antibody/mL, using antibody MW of 150 K mol. wt) by the concentration of GNPs (2×10^{10} GNP/mL).

Table S2. The number of GNPs/mL for different concentrations

	GNPs Concentration	Number of Particles $*10^9$
1	10	7.936507937
2	100	79.36507937
3	250	198.4126984
4	500	396.8253968
5	1000	793.6507937