Supplementary information

Combined Anti-angiogenic and Anti-inflammatory Nanoformulation for Effective Treatment of Ocular Vascular Diseases

Jianguo Sun[1, *], Huiling Nie [2, *], Panpan Pan[3, *], Qin Jiang [2, *], Chang Liu [1], Min Wang [1], Yonghui Deng[3, 4, #], Biao Yan[1, #]

[1] Eye Institute and Department of Ophthalmology, Eye & ENT Hospital, State Key Laboratory of Medical Neurobiology, Fudan University, Shanghai 200031, China

[2] The Affiliated Eye Hospital and The Fourth School of Clinical Medicine, Nanjing Medical University, Nanjing 210003, China

[3] Department of Chemistry, Fudan University, Shanghai 200433, China

[4] Department of Gastroenterology and Hepatology, Zhongshan Hospital, Institute of Biomedical Sciences, Fudan University, Shanghai 200032, China

*These authors contributed equally to this work.

[#]Correspondence authors:

Yonghui Deng, Ph.D. E-mail: yhdeng@fudan.edu.cn

Biao Yan, Ph.D. E-mail: biao.yan@fdeent.org

Preparation and characterization of hMSN nanoparticles

Three kinds of hMSNs with the rambutan-like rough surfaces and the hollow cavities interconnected with a plenty of mesoporous (A7, A12, and A16) were synthesized at 0.7 vol%, 1.2 vol%, and 1.6 vol % TEOS volume fraction from. The morphology of hMSNs had the discrete spheres (Fig. S1a). The average diameters of hMSNs were 252 ± 11 nm (A7), 254 ± 13 nm (A12), and 250 ± 10 nm (A16). Notably, hMSN-A16 had the greatest surface roughness (Fig. S1b). Nitrogen adsorption-desorption measurement revealed that compared with hMSN-A7 or A12, hMSN-A16 had larger pore volume, smaller porn size, and greater specific surface area (Fig. 1c)

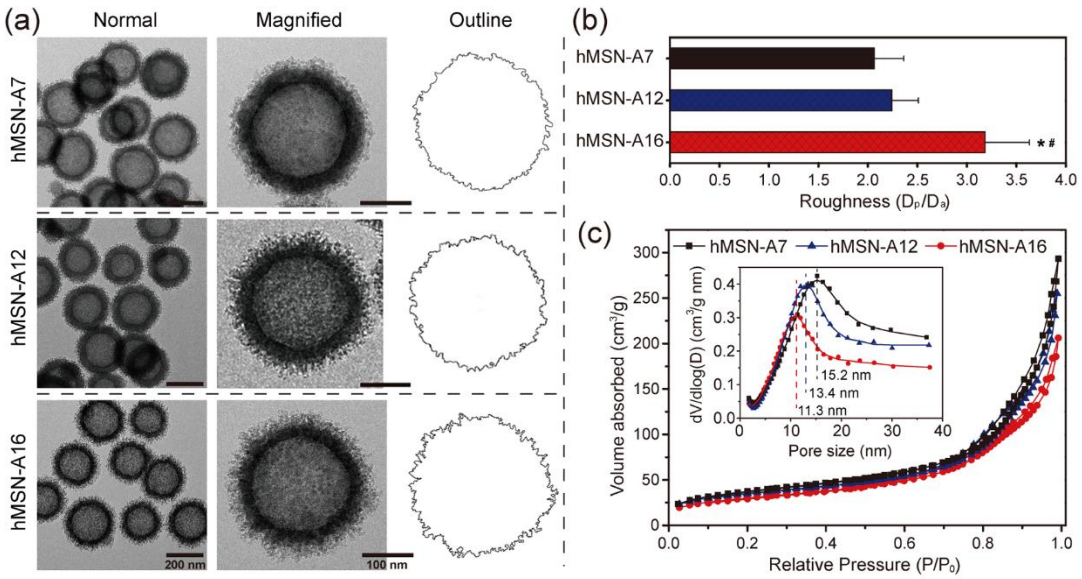

Figure S1: Characterization of hMSN nanoparticles

(a) TEM images, the magnified nanoparticle images, and the outlines of hMSN-A7, A12, and A16. (b) Surface roughness of hMSN-A7, A12, and A16. (c) Nitrogen adsorption-desorption isotherms of hMSN-A7, A12, and A16. The adsorbed volume against relative pressure plot was used to calculate the specific surface area in the relative pressure (P/P0) ranging from 0.04 to 0.1 by BET model, the pore size distribution and pore volume by BJH model, and the total pore volumes (Vt) based on the adsorbed N_2 amount at the P/P0 of 0.995. The pore size against dV/dlog(D) plot

was used to determine the pore size distribution. *$P < 0.05$ *vs*. hMSN-A7; $^{\#}P < 0.05$ *vs*. hMSN-A12.

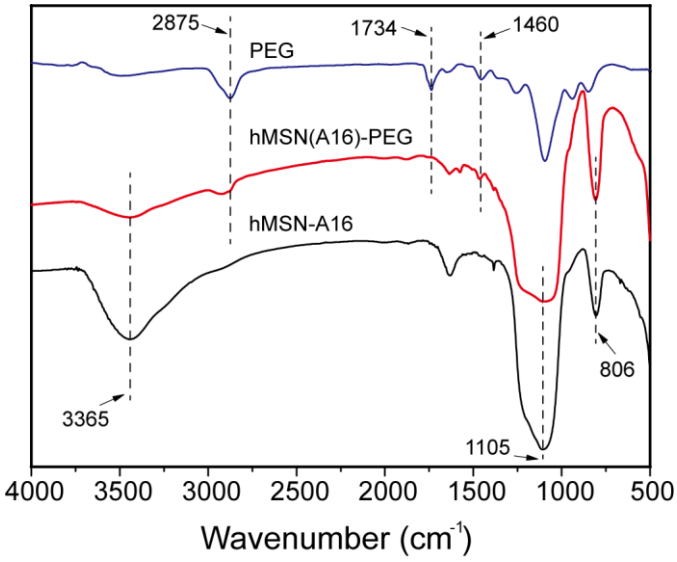

Figure S2: FTIR spectra of hMSN, hMSN(SM) and PEG

The characteristic peaks: 806 cm^{-1} (ν (Si-O)), a broad band at 1050-1200 cm^{-1} with the highest point at 1105 cm^{-1} (δ (Si-O-Si)), 1734 cm^{-1} (δ (C=O), and a broad band at 3000-4000 cm^{-1} with the highest point at 3365 cm^{-1} (ν (OH)).

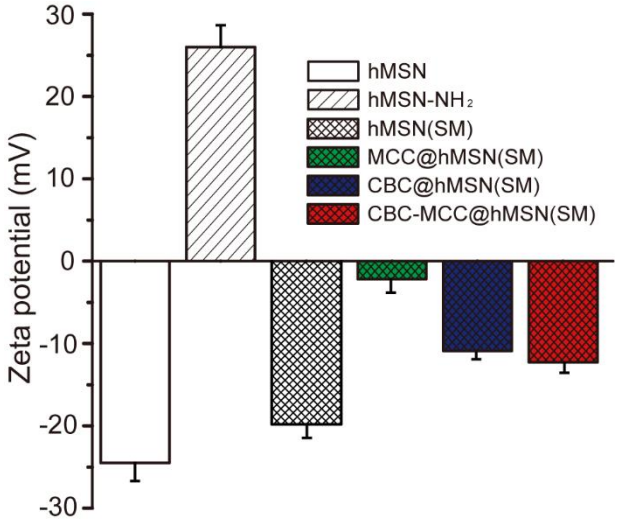

Figure S3: Zeta potentials of hMSN, hMSN-NH2, hMSN(SM), MCC@hMSN(SM), CBC@hMSN(SM) and CBC-MCC@hMSN(SM) complex formulations

Detection of MCC950 amount

The amount of MCC950 was detected by LC-MS/MS method using a AB SCIEX Triple TOF 5600 system equipped with a Waters Atlantis T3 column (2.1 × 50 mm, 5 μm). The mobile phase was used as following: solvent A = 0.1% formic acid/water, solvent B = 0.1% formic acid/acetonitrile. A binary gradient solvent was used with the B being 2% in 0-1 min, 40% in 1-15 min, 80% in 15-21 min, 98% in 21-23 min, 2% in 23-24 min, and washed in 24-32 min. The flow rate was 0.18 mL/min and data were acquired from m/z 70-600 in positive and negative scan mode (ACS Med Chem Lett, 2016, 7: 1034-1038).

Detection of CBC amount

The amounts of CBC in cell samples or ocular tissues were detected by ELISA assays. The capture antibody was the goat anti-human IgG-Fc (2.5 mg/mL; Bethyl, Montgomery, TX) and the detection antibody was the goat anti-human VEGFR2 conjugated with biotin (100 ng/mL; R&D). After incubation with streptavidin-conjugated horseradish peroxidase (HRP, R&D), the tetramethylbenzidine (TMB) substrate was added. The reaction was stopped using the dilute sulfuric acid. The optical density was detected using a microplate reader at 450 nm (J Ophthalmol, 2020, 2020: 2674780).