DOI 10.5162/Ultrasonic2025/B1-c3

UTMD-Mediated Nano-Stem Cells for the Treatment of Ischemic Stroke

Mingxuan Li[1], *Fan Li*[2], *and Lianfang Du*[1]
[1]*Department of Medical Ultrasound, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, 201620, China*
[2]*Department of Medical Ultrasound, Shanghai Chest Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, 200030, China*
medicineli@163.com

Abstract: Ischemic stroke, a leading cause of global disability and death, affects about 15 million annually[.] Current therapies face limitations from narrow treatment windows and the blood-brain barrier (BBB), which restricts drug delivery[1]. Nanotechnology offers promising solutions: copper-selenium nanoparticles (Cu2-xSe) combine biocompatibility with antioxidant/anti-inflammatory effects, scavenging ROS and modulating ferroptosis[2,3]. Selenium upregulates GPX4 via TFAP2c/Sp1, inhibiting lipid peroxidation[4]. Similar Cu-based nanozymes have shown anti-inflammatory effects in osteoarthritis by modulating macrophage polarization[5]. Stem cell therapy, particularly BMSCs, promotes neurorepair through differentiation and neurotrophic factor secretion[6]. Our innovation combines UTMD technology - which transiently opens the BBB via microbubble cavitation[7,8] - with engineered Cu2-xSe@BMSCs. This multimodal strategy simultaneously addresses BBB penetration, oxidative stress, inflammation, apoptosis, and neural regeneration.

Keywords: ischemic stroke, copper selenium nanoparticles, mesenchymal stem cells, blood-brain barrier, ultrasound therapy

Cu2-xSe Nanoparticle Synthesis and Characterization

Cu2-xSe nanoparticles (NPs) were successfully synthesized using a modified method based on previous reports. TEM analysis revealed that Cu2-xSe NPs exhibited a uniform spherical morphology with distinct copper and selenium element distribution, confirming the successful formation of Cu2-xSe NPs.

In Vitro Hypoxia Protection Study

The cytotoxicity of Cu2-xSe NPs was evaluated using the CCK-8 assay. At concentrations up to 10 μg/mL, Cu2-xSe NPs showed low cytotoxicity. In CoCl2-induced hypoxic models, Cu2-xSe NPs significantly improved cell survival rates and reduced oxidative stress. Fluorescence staining revealed that cells treated with Cu2-xSe NPs exhibited stronger green fluorescence (indicating live cells) compared to untreated hypoxic cells, highlighting their protective effect against hypoxic injury. Furthermore, Cu2-xSe NPs effectively reduced reactive oxygen species (ROS) accumulation, as evidenced by DCFH-DA staining. Lipid peroxidation levels, assessed by C11-BOdipy 581/591 staining, were significantly reduced in the Cu2-xSe-treated group, particularly at a concentration of 4 μg/mL. These findings suggest that Cu2-xSe NPs attenuate oxidative damage and lipid peroxidation in hypoxic cells.

Iron Death Inhibition

Western blot analysis showed that Cu2-xSe NPs restored the expression of GPX4 and FTH, which were downregulated in the CoCl2-induced hypoxia group. Simultaneously, the expression of ACSL4, a marker of ferroptosis, was decreased. These results indicate that Cu2-xSe NPs could protect against ferroptosis by modulating oxidative stress and iron homeostasis under hypoxic conditions.

In Vivo Neuroprotective Effect

The neuroprotective efficacy of Cu2-xSe nanoparticles (NPs) was systematically evaluated in a transient middle cerebral artery occlusion (tMCAO) mouse model. Behavioral assessments revealed that combination therapy with UTMD and BMSC-loaded Cu2-xSe NPs significantly improved neurological functional recovery compared to model controls. Treated animals demonstrated markedly enhanced performance in adhesive removal, water maze, and rotarod tests, indicating substantial improvements in sensory-motor function, spatial learning, and motor coordination. Neuroimaging and histopathological analyses provided compelling evidence for the therapeutic effects, showing dramatic reductions in cerebral infarct volume and neuronal apoptosis. These findings collectively demonstrate

that Cu2-xSe NPs confer comprehensive neuroprotection through multiple mechanisms, including oxidative stress mitigation and neuronal survival promotion, highlighting their potential as a novel therapeutic agent for ischemic stroke.

References

1.Campbell BCV, De Silva DA, Macleod MR, Coutts SB, Schwamm LH, Davis SM, et al. Ischaemic stroke. Nat Rev Dis Primers. 2019;5(1):70.
2.Baldinotti R, Fronza MG, Fetter J, Silva L, Bender CB, Lüdtke DS, et al. Protective effects of octylseleno-xylofuranoside in a streptozotocin-induced mouse model of Alzheimer's disease. Eur J Pharmacol. 2021;910:174499.
3.Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE, et al. Ferroptosis: an iron-dependent form of nonapoptotic cell death. Cell. 2012;149(5):1060-72.
4.Alim I, Caulfield JT, Chen Y, Swarup V, Geschwind DH, Ivanova E, et al. Selenium Drives a Transcriptional Adaptive Program to Block Ferroptosis and Treat Stroke. Cell. 2019;177(5):1262-79.e25.
5.Yu B, Sun W, Lin J, Fan C, Wang C, Zhang Z, et al. Using Cu-Based Metal-Organic Framework as a Comprehensive and Powerful Antioxidant Nanozyme for Efficient Osteoarthritis Treatment. Adv Sci (Weinh). 2024;11(13):e2307798.
6.Tang W, Zhao K, Li X, Zhou X, Liao P. Bone Marrow Mesenchymal Stem Cell-Derived Exosomes Promote the Recovery of Spinal Cord Injury and Inhibit Ferroptosis by Inactivating IL-17 Pathway. J Mol Neurosci. 2024;74(2):33.
7.Jin LF, Li F, Wang HP, Wei F, Qin P, Du LF. Ultrasound targeted microbubble destruction stimulates cellular endocytosis in facilitation of adeno-associated virus delivery. Int J Mol Sci. 2013;14(5):9737-50.
8.Bai Y, Du Y, Yang Y, Wälchli T, Constanthin PE, Li F. Ultrasound-Targeted Microbubble Destruction Increases BBB Permeability and Promotes Stem Cell-Induced Regeneration of Stroke by Downregulating MMP8. Cell Transplant. 2024;33:9636897231223293.

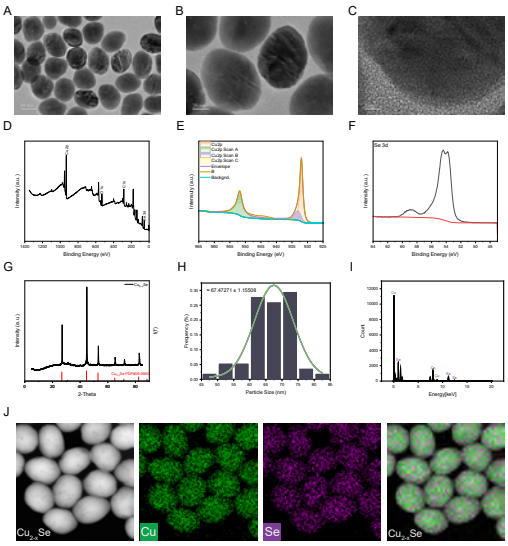

*Fig. 1: Synthesis and functional characterization of ferroptosis-targeting Cu2-xSe nanoparticles (NPs). (A–C) Transmission electron microscopy (TEM) images; (D–F) X-ray photoelectron spectroscopy (XPS); (G) X-ray diffraction (XRD): Peaks indexed to cubic Cu2-xSe (JCPDS 06-0680) phase, with no detectable impurities. (H) Size distribution histogram: Dynamic light scattering (DLS) / TEM-derived average diameter: 67.47 ± 1.16 nm (mean ± SD, *n* = 60). (I) Energy-dispersive X-ray spectroscopy (EDS): Spot analysis verifying Cu:Se atomic ratio (~2:1) and trace elements. (J) EDS elemental mapping: Homogeneous spatial distribution of Cu (red) and Se (green) in NPs.*

DOI 10.5162/Ultrasonic2025/B1-c3

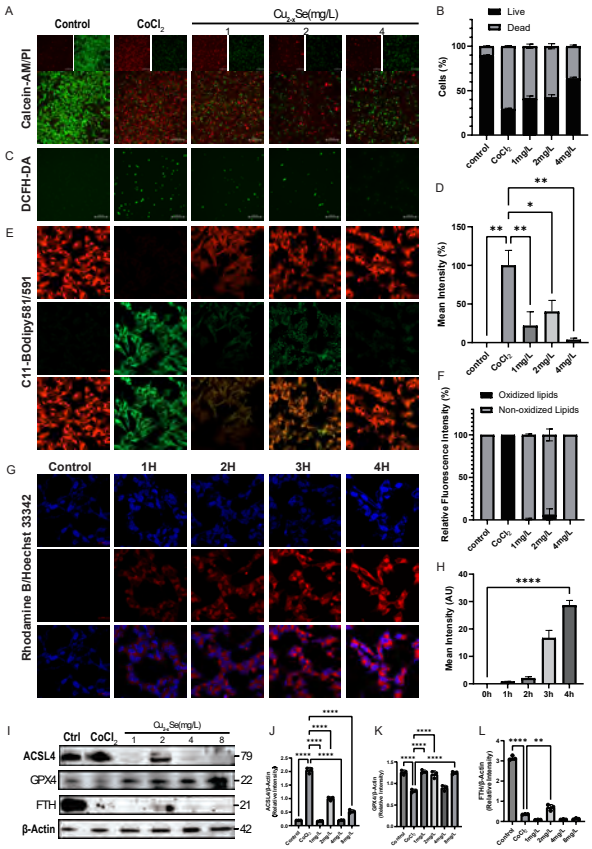

Fig. 2: Cytoprotective effects of Cu2-xSe NPs against CoCl2-induced hypoxia in SH-SY5Y cells (A,B) Cell viability rescue Live/dead staining (AMPI); (C,D) Oxidative stress mitigation; (E,F)Lipid peroxidation (C11-BODIPY); (G,H) Rhodamine B/Hoechst staining; (I-L) Ferroptosis-related protein modulation (Western blot).

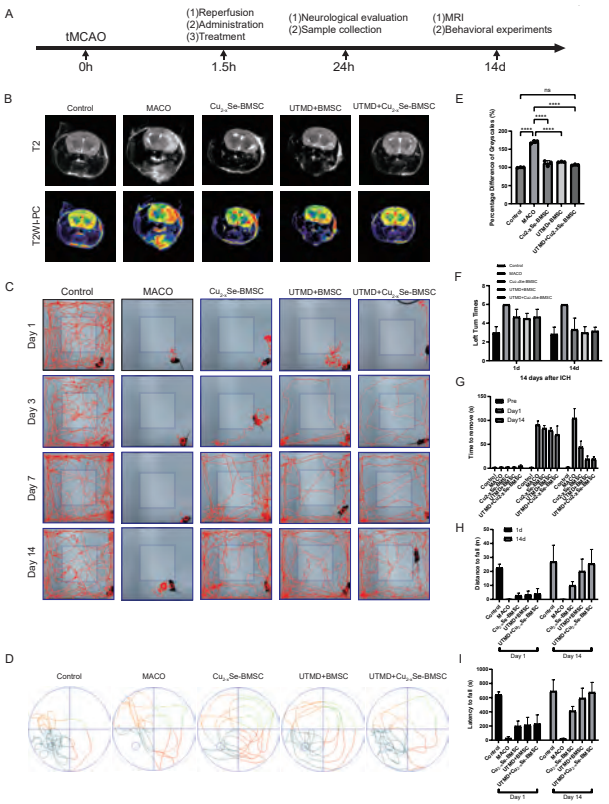

Fig. 3: Therapeutic workflow and functional outcomes of UTMD-enhanced Cu2-xSe NPs-BMSC therapy in tMCAO mice (A) Therapeutic workflow: tMCAO induction: 90-min middle cerebral artery occlusion. Treatment groups: Sham, Model (tMCAO), NPs-BMSC, UTMD-BMSC, UTMD+NPs-BMSC (n=6/group). UTMD delivery: Ultrasound-targeted microbubble destruction (1 MHz, 0.5 W/cm², 30s) to enhance NP-BMSC homing. (B,E) MRI assessment (T2-weighted). (C,F,G,H) Results of Open-Field experiments; (D,I)Results of Water maze experiment.