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Penumbra-targeted CircOGDH siRNA-loaded nanoparticles alleviate neuronal apoptosis in focal brain ischaemia

Yanfang Liu,[1,2,3] Tianyuan Zhang,[1,2,3] Xing Zou,[1,2,3] Zhongwen Yuan,[1,2] Yufeng Li,[1,2,3] Jiankun Zang,[1,2,3] Niu He,[1,2,3] Lizhen He,[1,2] Anding Xu ,[1,2,3] Dan Lu[1,2,3]

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YL, TZ and XZ contributed equally.

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[1]Department of Neurology and Stroke Center, Jinan University First Affiliated Hospital, Guangzhou, Guangdong, China
[2]Department of Clinical Neuroscience Institute, The First Affiliated Hospital of Jinan University, Guangzhou, Guangdong, China
[3]Key Lab of Guangzhou Basic and Translational Research of Pan-vascular Diseases, The First Affiliated Hospital of Jinan University, Guangzhou, China

Correspondence to
Dr Dan Lu, Guangzhou, China; ludan@jnu.edu.cn

Dr Anding Xu; tlil@jnu.edu.cn

ABSTRACT

Background Nanoparticles (NPs) are a class of substances that can be loaded with therapeutic agents delivered to specific areas. In our earlier research, we identified a neuron-derived circular RNA (circRNA), circular oxoglutarate dehydrogenase (CircOGDH), as a promising therapeutic target for acute ischaemic stroke. This study dedicated to explore a prospective preliminary strategy of CircOGDH-based NP delivered to the ischaemic penumbra region in middle cerebral artery occlusion/reperfusion (MCAO/R) mice.

Methods Immunofluorescence in primary cortex neurons and in vivo fluorescence imaging revealed endocytosis of Poly(lactide-co-glycolide) (PLGA) poly amidoamine(PAMAM)@CircOGDH small interfering RNA (siRNA) NPs. Western blotting analysis and CCK8 assay were performed to evaluate the apoptotic level in ischaemic neurons treated with PLGA–PAMAM@CircOGDH siRNA NPs. Quantitative reverse transcription PCR experiments, mice behaviour test, T2 MRI analysis, Nissl and TdT-mediated dUTP nick end labeling (TUNEL) co-staining were performed to evaluate the apoptosis level of ischaemic penumbra neurons in MCAO/R mice. Biosafety evaluation of NPs in MCAO/R mice was detected by blood routine examination, liver and kidney function examination and HE staining.

Results PLGA–PAMAM@CircOGDH siRNA NPs were successfully assembled. Endocytosis of PLGA–PAMAM@CircOGDH siRNA NPs in ischaemic neurons alleviated neuronal apoptotic level in vitro and in vivo. Furthermore, mice behaviour test showed that the neurological defects of MCAO/R mice were significantly alleviated after the tail injection of PLGA–PAMAM@CircOGDH siRNA NPs, and no toxic effects were observed.

Conclusion In conclusion, our results suggest that PLGA–PAMAM@CircOGDH siRNA NPs can be delivered to the ischaemic penumbra region and alleviate neuron apoptosis in MCAO/R mice and in ischaemic neurons; therefore, our study provides a desirable approach for using circRNA-based NPs for the treatment of ischaemic stroke.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Nanoparticles (NPs) are reported to be effective vehicles to transport therapeutic agents, including drugs, proteins, vaccines, small interfering RNA (siRNA) and DNA, to the brain. In our previous research, we identified a circular RNA (circRNA), circular oxoglutarate dehydrogenase (CircOGDH), which serve as a penumbra biomarker of patients with acute ischaemic stroke, adenovirus-mediated CircOGDH knockdown ameliorated neuronal apoptosis in permanent middle cerebral artery occlusion (MCAO) mice. In this study, we present a promising nanotherapeutic strategy for CircOGDH-targeting agents during ischaemic reperfusion in ischaemic stroke.

WHAT THIS STUDY ADDS

⇒ Our study indicated that NP delivery is a promising strategy for targeting circRNA during ischaemic reperfusion in ischaemic stroke. We demonstrated that poly(lactide-co-glycolide) poly amidoamine (PAMAM)-mediated in vivo delivery of CircOGDH siRNA improved penumbra neuron survival and neurological function in MCAO/middle cerebral artery reperfusion mice.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Our study provides a promising nanotherapeutic strategy for targeting circRNAs during ischaemic reperfusion in ischaemic stroke.

INTRODUCTION

Ischaemic stroke is a leading cause of disability and mortality worldwide and China faces a great challenge.[1] For optimal treatment results after acute ischaemic stroke (AIS), the ischaemic penumbra tissue must be rescued in a stringent time window. Although the current guidelines for the clinical treatment of AIS recommends recombinant tissue plasminogen activator and endovascular thrombectomy (EVT) to improve functional recovery, the limited therapeutic time window and the serviceable remedy remain a question.[2] Moreover, during the ischaemic reperfusion process in EVT, complicated pathophysiological responses can lead to neural loss and blood–brain barrier breakdown.[3] Thus, initiatives in new neuroprotective drugs delivered to the brain still need to explore.

Data from the past decades have revealed that nanoparticles (NPs) can be effective vehicles to transport therapeutic agents, including drugs, proteins, vaccines, small

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interfering RNA (siRNA) and DNA, to the brain.[4] The rapid development of nanotechnology has yielded various types of NPs, such as polymer NPs, polymer micelles, liposomes and inorganic NPs.[4] Poly(lactide-co-glycolide) (PLGA) is an FDA-approved polymer that has been widely used as a drug carrier because of its excellent biocompatibility, biosafety and biodegradability.[4] PAMAM dendrimers are excellent candidate polymers because of their positive charge, which allows them to form highly stable complexes with negatively charged siRNA; thus, they are commonly used as gene delivery systems.[5] But an emerging consensus is that beyond their gene delivery effects, PAMAM-type dendrimers (<10 nm) also exerts several toxicological actions such as haemolysis and cell death, and removes easily through the blood circulation.[6 7] Thus PLGA–PAMAM NPs were assemblied to minimise the usage amount of PAMAM in the core, and also decreased the strongly positive charge of PAMAM. Research has shown that PLGA biomimetic nanocarriers loaded with human fat extract can increase neurobehavioral recovery in ischaemic stroke,[8] and PAMAM dendrimer NPs can be taken up by neurons after focal brain ischemia.[5] Actually, there are series of NPs for RNA therapeutics delivery, NP-based targeting and delivery approaches are becoming promising strategy for effective treatments in ischaemic stroke.[9]

Circular RNAs (circRNAs) are a type of non-coding RNAs that are found in various species and are more stable than linear RNA.[10] They can interact with microRNAs and RNA-binding proteins that affect axon growth, neuronal apoptosis and autophagy—processes that are critical in the pathogenesis of ischaemic stroke.[11–16] In our previous research, we observed that a circRNA derived from oxoglutarate dehydrogenase (OGDH), CircOGDH, was significantly upregulated in the plasma of AIS patients and penumbra neurons of middle cerebral artery occlusion (MCAO) mice. Furthermore, adenovirus-mediated CircOGDH knockdown ameliorated neuronal apoptosis in MCAO mice and ischaemic neurons. These results indicate that CircOGDH is a potential therapeutic target in patients with AIS.[11] Moreover, studies have reported that gold NPs conjugated with CircDNMT1 siRNA are a potential treatment for breast cancer.[17] Poly(β-amino ester) NPs loaded with CircMDK siRNA were reported as a therapeutic strategy to treat hepatocellular carcinoma.[18] These studies indicate that NP delivery is a promising strategy for targeting circRNAs.

In this study, we assembled PLGA–PAMAM@CircOGDH siRNA NPs and demonstrated that PLGA–PAMAM-mediated in vivo delivery of CircOGDH siRNA improved penumbra neuron survival and neurological function in middle cerebral artery occlusion/reperfusion (MCAO/R) mice. Our study provides a promising nanotherapeutic strategy for circRNA-targeting agents during ischaemic reperfusion in ischaemic stroke.

MATERIALS AND METHODS

Detailed experimental procedures were provided in the online supplemental file.

RESULTS

Design, assembly and characterisation of PLGA–PAMAM@CircOGDH siRNA NPs

PLGA–PAMAM and PLGA–PAMAM@CircOGDH siRNA NPs were assembled as illustrated in figure 1A. The PAMAM dendrimers displayed a positive charge and formed high-stability complexes with the negatively charged siRNA via electrostatic interaction; therefore, a Zetasizer Nano ZS particle analyzer was used to examine the zeta potential of the PLGA, PAMAM, PLGA–PAMAM and PLGA–PAMAM@CircOGDH siRNA NPs. The zeta potential of the PLGA–PAMAM@CircOGDH siRNA NPs was significantly lower compared with that of PLGA–PAMAM NPs (figure 1B), indicating that PLGA–PAMAM@CircOGDH siRNA NPs were successfully assembled. The particle size distribution demonstrated a similar range of sizes for PLGA–PAMAM (165±2.8 nm) and PLGA–PAMAM@CircOGDH siRNA NPs (163±10.3 nm) (figure 1C). In addition, the morphology of PLGA–PAMAM and PLGA–PAMAM@CircOGDH siRNA NPs was characterised by transmission Electron Microscope (TEM). The TEM images (figure 1D) revealed that the two types of NPs had a different morphology, and element mapping showed that C, O, N and P are present (online supplemental figure 1A), indicating that PLGA–PAMAM@CircOGDH siRNA NPs were successfully assembled. Agarose gel electrophoresis was used to analyse the binding capability of NPs when combined with siRNA. As shown in figure 1E, the migration of the PLGA–PAMAM@CircOGDH siRNA NPs was completely inhibited, whereas that of CircOGDH siRNA alone was not. Furthermore, as NP stability is an important parameter for siRNA delivery, first, the size changes were detected in Fetal bovine serum (FBS) solution, which mimics a human physiological environment, and we found PLGA–PAMAM@CircOGDH siRNA NPs maintained at similar size (online supplemental figure 1B,C). Next, NPs were assembled using Cy3-labelled CircOGDH siRNA to form PLGA–PAMAM@Cy3-CircOGDH siRNA NPs. The extension time of PLGA–PAMAM@Cy3-CircOGDH siRNA NPs had no effect on the ultraviolet–visible (UV–Vis) (figure 1F) or fluorescence (figure 1G) spectra at different time points.

PLGA–PAMAM@CircOGDH siRNA NPs ameliorates neuronal apoptosis in vitro

Subsequently, the cellular uptake of PLGA–PAMAM@CircOGDH siRNA NPs was evaluated. Cy3 labelling revealed that PLGA–PAMAM@cy3-CircOGDH siRNA NPs were taken up by cortical primary neurons in vitro (figure 2A). Previous studies have reported that endocytosis is a key pathway through which NPs enter cells.[19] Thus, the mechanism by which NPs entered neurons was investigated. Lysotracker (cherry) and cy3 (red) labelling

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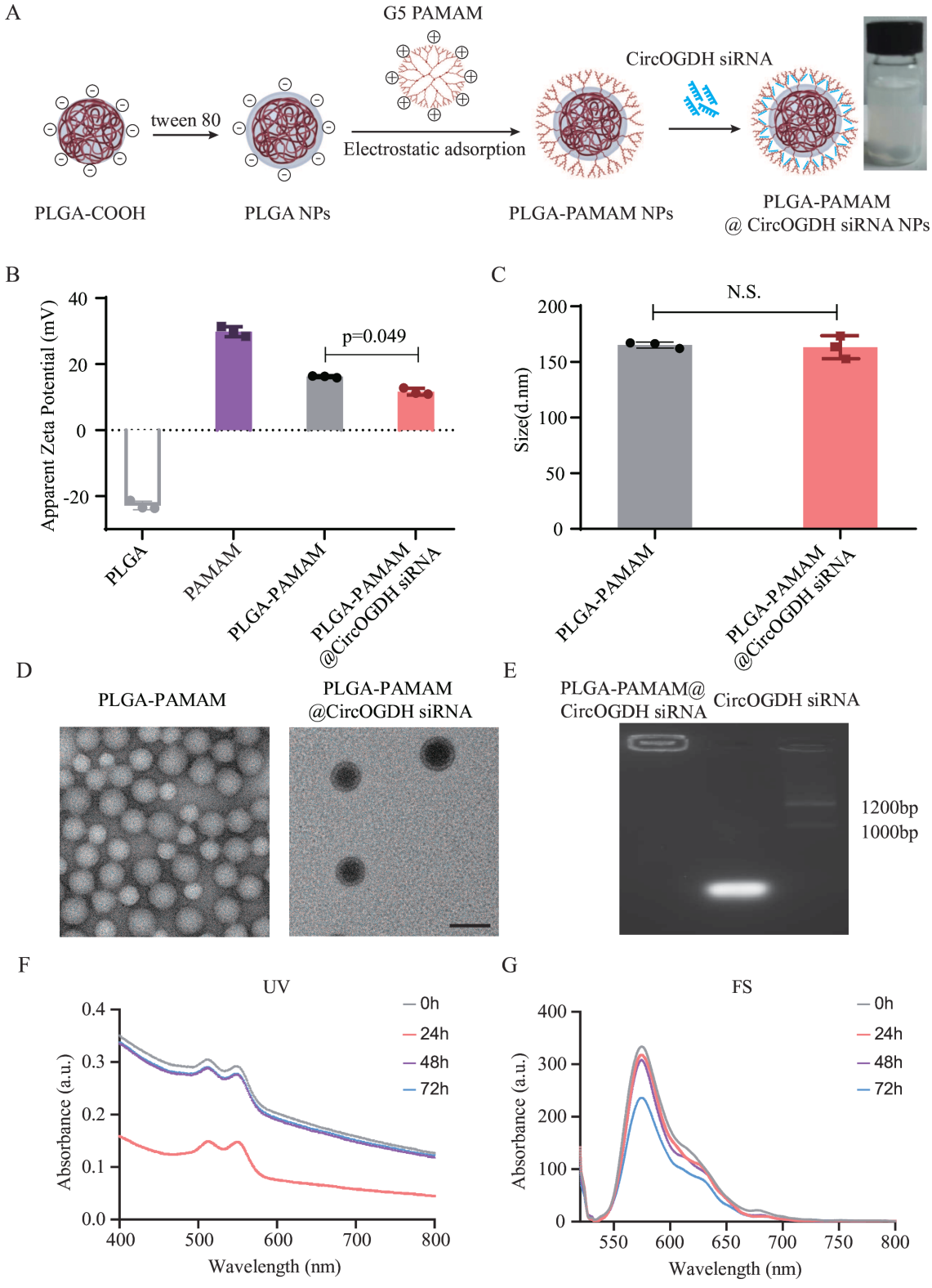


Figure 1 Characterisation of PLGA–PAMAM@CircOGDH siRNA NPs. (A) Schematic illustrations of the assembly of PLGA–PAMAM@CircOGDH siRNA NPs. (B) Zeta potential of PLGA, PAMAM, PLGA–PAMAM and PLGA–PAMAM@CircOGDH siRNA NPs. Data are presented as means±SD; n=3 (Mann-Whitney U test). (C) Size distribution of PLGA–PAMAM and PLGA–PAMAM@CircOGDH siRNA NPs. Data are presented as means±SD; n=3 (Mann-Whitney U test). (D) TEM images of PLGA–PAMAM and PLGA–PAMAM@CircOGDH siRNA NPs. Scale bar=200 nm. (E) Electrophoretic mobility of siRNA and PLGA–PAMAM@CircOGDH siRNA NPs on agarose gel. Marker: 1000 bp DNA ladder. Extension time of PLGA–PAMAM@Cy3-CircOGDH siRNA NPs at 0 hour, 24 hours, 48 hours and 72 hours on the UV–Vis (F) and fluorescence spectra (G). CircOGDH, circular RNAs derived from oxoglutarate dehydrogenase; NPs, nanoparticles; PLGA, poly(lactide-co-glycolide); siRNA, small interfering RNA; UV–Vis, ultraviolet–visible; PAMAM, poly amidoamine); TEM, transmission Electron Microscope .

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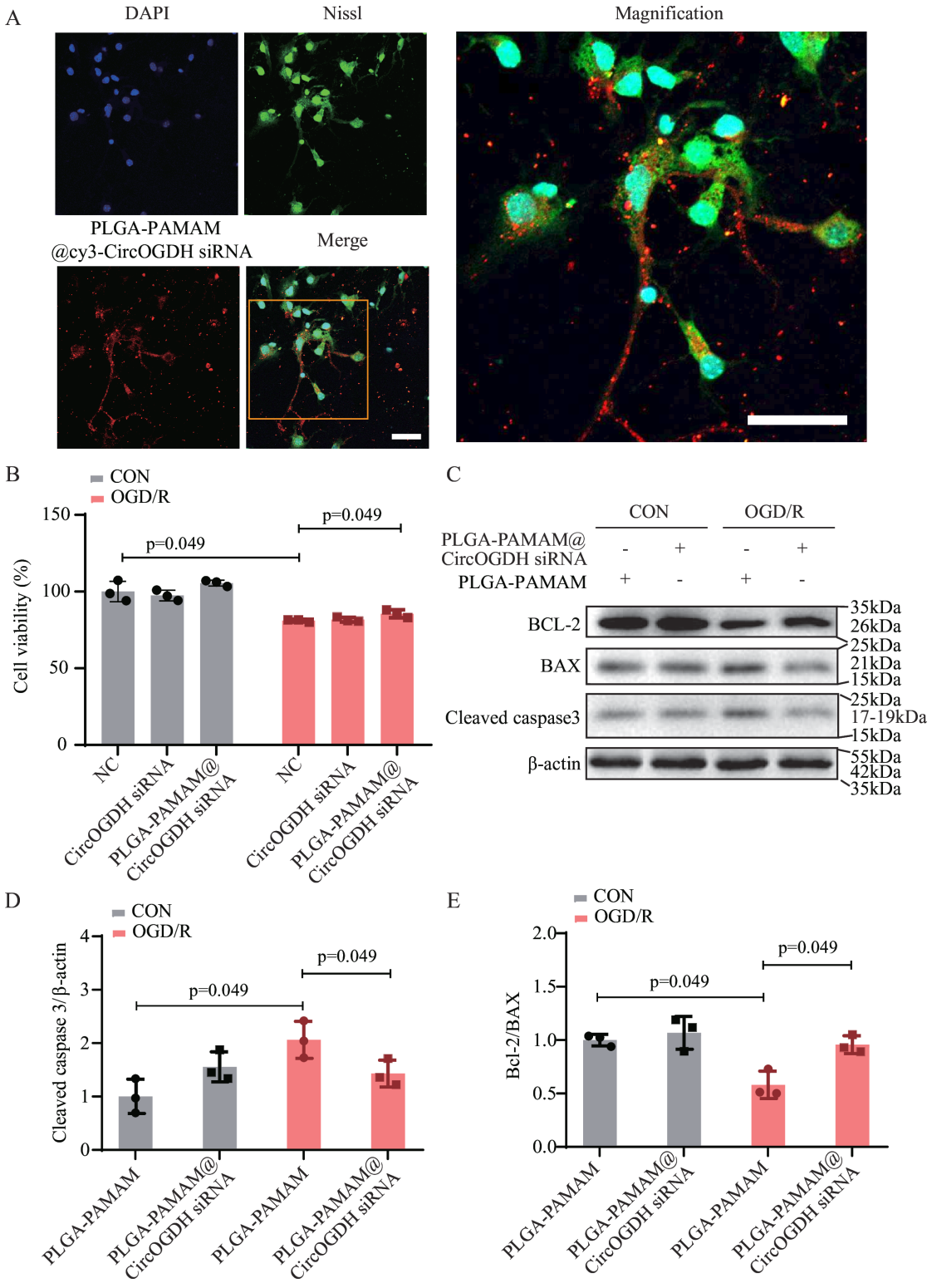


Figure 2 Cellular uptake of PLGA–PAMAM@CircOGDH siRNA NPs. (A) Representative fluorescent microscope images showing the transfection efficiency of PLGA–PAMAM@Cy3-CircOGDH siRNA NPs (red) in primary cortex neurons at 24 hours. Neurons were stained with Nissl (green), and nuclei were stained with DAPI (blue). Scale bar=50 µm. (B) Cell viability was determined in primary cortical neurons treated with NC, CircOGDH siRNA and PLGA–PAMAM@CircOGDH siRNA NPs. Data were presented as mean ± SD; n=3, Mann-Whitney U test. (C–E) Western blot analysis of BAX, BCL2 and cleaved caspase 3 expression levels in CON and ischaemic-reperfusion (OGD/R) neurons treated with PLGA–PAMAM and PLGA–PAMAM@CircOGDH siRNA NPs. Data were presented as mean±SD; n=3, Mann-Whitney U test. CircOGDH, circular RNAs derived from circular oxoglutarate dehydrogenase; CON, control; NC, normal control; NPs, nanoparticles; OGD/R, oxygen and glucose deprivation reperfusion; PLGA, poly(lactide-co-glycolide); siRNA, small interfering RNA; PAMAM, poly amidoamine; DAPI, 4',6-Diamidino-2'-phenylindole.

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were chosen to monitor the intracellular localisation of PLGA–PAMAM@CircOGDH siRNA NPs in neurons. The results indicated that PLGA–PAMAM@cy3-CircOGDH siRNA NPs entered neurons via endocytosis beginning at 3 hours after administration and reaching a peak at 9 hours (online supplemental figure 2A). Moreover, we performed flow cytometry analysis in SH-SY5Y cells treated with PLGA–PAMAM@cy3-CircOGDH siRNA NPs and found it can be uptake by SH-SY5Y cells (online supplemental figure 3). These results demonstrate that the assembled PLGA–PAMAM@CircOGDH siRNA NPs can be taken up by cortical primary neurons and SH-SY5Y cells. To further explore the pathophysiological function of PLGA–PAMAM@CircOGDH siRNA NPs in neurons, we established oxygen and glucose deprivation reperfusion model (OGD/R) of neurons. After confirming that PLGA–PAMAM had no effect on neurons (online supplemental figure 2B), we conducted the CCK8 experiment and found that PLGA–PAMAM@CircOGDH siRNA NPs significantly ameliorated neuronal death (figure 2B). And we found that PLGA–PAMAM@CircOGDH siRNA NPs caused an increase of BCL-2/BAX ratio and a decrease of cleaved caspase-3 expression in OGD/R neurons (figure 2C–E). Our previous research indicated that knockdown of CircOGDH ameliorates neuronal apoptosis via targeting miR-5112/COL4A4 mRNA axis, next we detected COL4A4 protein expression level neurons treated with PLGA–PAMAM@CircOGDH siRNA NPs and we found that it caused an decrease of COL4A4 protein expression in OGD/R neurons (online supplemental figure 4), which was consistent with the decrease of COL4A4 mRNA expression in our previous research. Taken together, these results suggest that PLGA–PAMAM@CircOGDH siRNA NPs ameliorate neuronal apoptosis under ischaemic condition in vitro.

PLGA–PAMAM@CircOGDH siRNA NPs downregulate CircOGDH expression levels in MCAO/R mice

After confirming that the PLGA–PAMAM@CircOGDH siRNA NPs could be taken up by cortical primary neurons in vitro, we focused on their delivery in MCAO mice. Mice underwent MCAO[20] for 40 min and reperfusion for 3 days; NPs were administered by tail vein injection every 24 hours during reperfusion (figure 3A). Cerebral blood flow measurement confirmed the success of the MCAO and MCAO/R models (figure 3B). We observed increased uptake of NPs in the penumbra neurons (figure 3C,D), while only a few were localised in microglia and astrocytes (online supplemental figures 5 and 6A), compared with the contralateral tissue of MCAO/R mice after PLGA–PAMAM@Cy3-CircOGDH siRNA NP injection. Furthermore, using Cy5.5-labelled PLGA–PAMAM NPs, we evaluated the biodistribution of PLGA–PAMAM NPs in vivo after tail vein injection in MCAO mice by performing fluorescence imaging. The results demonstrated that Cy5.5-labelled PLGA–PAMAM NPs mainly accumulated in the liver and kidneys, but they were also observed in the brain, within an extension time of 0.5 hour, 1.5 hours, 2.5 hours and day 3 (figure 3E, online supplemental figure 6B). Furthermore, quantitative reverse transcription PCR analysis showed that PLGA–PAMAM@CircOGDH siRNA NPs significantly downregulated the CircOGDH expression level in the penumbra tissue of MCAO/R mice (figure 3F). Taken together, these results demonstrate that PLGA–PAMAM@CircOGDH siRNA NPs can be delivered to the brain and significantly downregulate the CircOGDH expression level in the penumbra tissues of MCAO/R mice.

PLGA–PAMAM@CircOGDH siRNA NPs improve neuron survival and neurologic function in MCAO/R mice

Next, we evaluated the neuroprotective effect of PLGA–PAMAM@CircOGDH siRNA NPs in MCAO/R mice. Mice underwent neurological behavioural experiments on day 3 after reperfusion, and their brain tissues were harvested for analysis. After confirmed that PLGA–PAMAM exerted no effect in MCAO/R mice (online supplemental figure 7), we conducted the neurological behavioural experiments and demonstrated that MCAO/R mice injected with PLGA–PAMAM@CircOGDH siRNA NPs exhibited a decreased total time to complete the adhesive removal somatosensory test (figure 4A), a lower ratio of foot faults in the grid-walking test (figure 4B) and a lower ratio of right-biased counts in the cylinder test (figure 4C) compared with the mice in the MCAO/R+PLGA–PAMAM group. Furthermore, we showed that PLGA–PAMAM@CircOGDH siRNA NPs significantly decreased brain infarct size as revealed by T2 MRI (online supplemental figure 8A,B). Nissl staining of brain sections indicated that MCAO/R mice injected with PLGA–PAMAM@CircOGDH siRNA NPs had decreased neuron loss in the penumbra tissue compared with mice in the MCAO/R+PLGA–PAMAM group (figure 4D,E and online supplemental figure 8C). The TUNEL and Nissl co-staining assay of brain sections revealed similar results; MCAO/R mice injected with PLGA–PAMAM@CircOGDH siRNA NPs showed decreased neuron apoptosis in the penumbra tissue compared with mice in the MCAO/R+PLGA–PAMAM group (figure 4F–H). Thus, our results demonstrated that PLGA–PAMAM@CircOGDH siRNA NPs improved neuron survival and neurologic function in MCAO/R mice.

PLGA–PAMAM@CircOGDH siRNA NPs exert no significant toxicity in MCAO/R mice

We next performed haematological and pathological analyses to test the toxicity of these NPs in MCAO/R mice. The haematological analysis showed that the red blood cell (RBC), white blood cell (WBC) and platelet (PLT) levels fluctuated within the normal range in MCAO/R mice injected with PLGA–PAMAM@CircOGDH siRNA NPs or PLGA–PAMAM NPs (online supplemental figure 9). Biochemical analysis of the main organs was conducted by aspartate transaminase, alanine transaminase and creatinine examination, which indicated no obvious injury in MCAO/R mice injected with PLGA–PAMAM@CircOGDH

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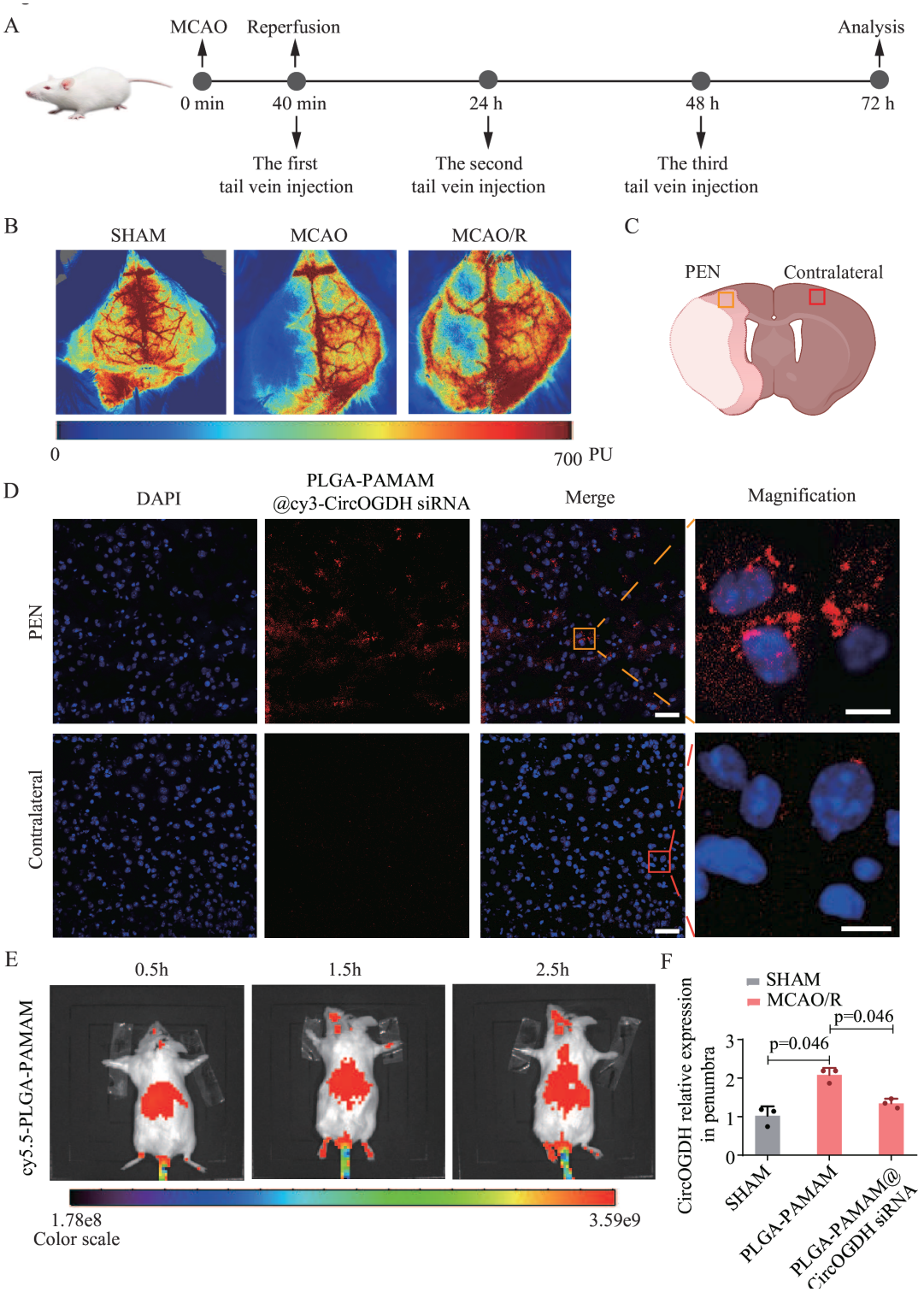


Figure 3 PLGA–PAMAM@CircOGDH siRNA NPs downregulated CircOGDH expression level in MCAO/R mice. (A) Illustration of the in vivo experimental design. (B) Representative images showing the CBF of mice in the SHAM, MCAO and MCAO/R groups. Units of the colour scale: PUs. (C) and (D) Representative fluorescence imaging showing the cellular uptake of PLGA–PAMAM@Cy3-CircOGDH siRNA NPs (red) in the penumbra tissues of MCAO/R mice 3 days after tail injection. Nuclei were stained with DAPI (blue). Scale bar=50 µm (left), scale bar=10 µm (right). (E) In vivo fluorescence imaging of Cy5.5-labelled PLGA–PAMAM NPs in MCAO mice at 0.5 hour 1.5 hours and 2.5 hours. Units of the colour scale: PUs. (F) RT-qPCR analysis of the CircOGDH expression level in the penumbra tissues from SHAM and MCAO/R mice after tail injection of PLGA–PAMAM and PLGA–PAMAM@CircOGDH siRNA NPs on day 1. Data are presented as means±SD; n=3, Mann-Whitney U test. CBF, Cerebral blood flow; CircOGDH, circular RNAs derived from circular oxoglutarate dehydrogenase; MCAO/R, middle cerebral artery occlusion/reperfusion; NPs, nanoparticles; PLGA, poly(lactide-co-glycolide); PUs, perfusion units; RT-qPCR, quantitative reverse transcription PCR; siRNA, small interfering RNA; PAMAM, poly amidoamine ; DAPI, 4',6-Diamidino-2'-phenylindole .

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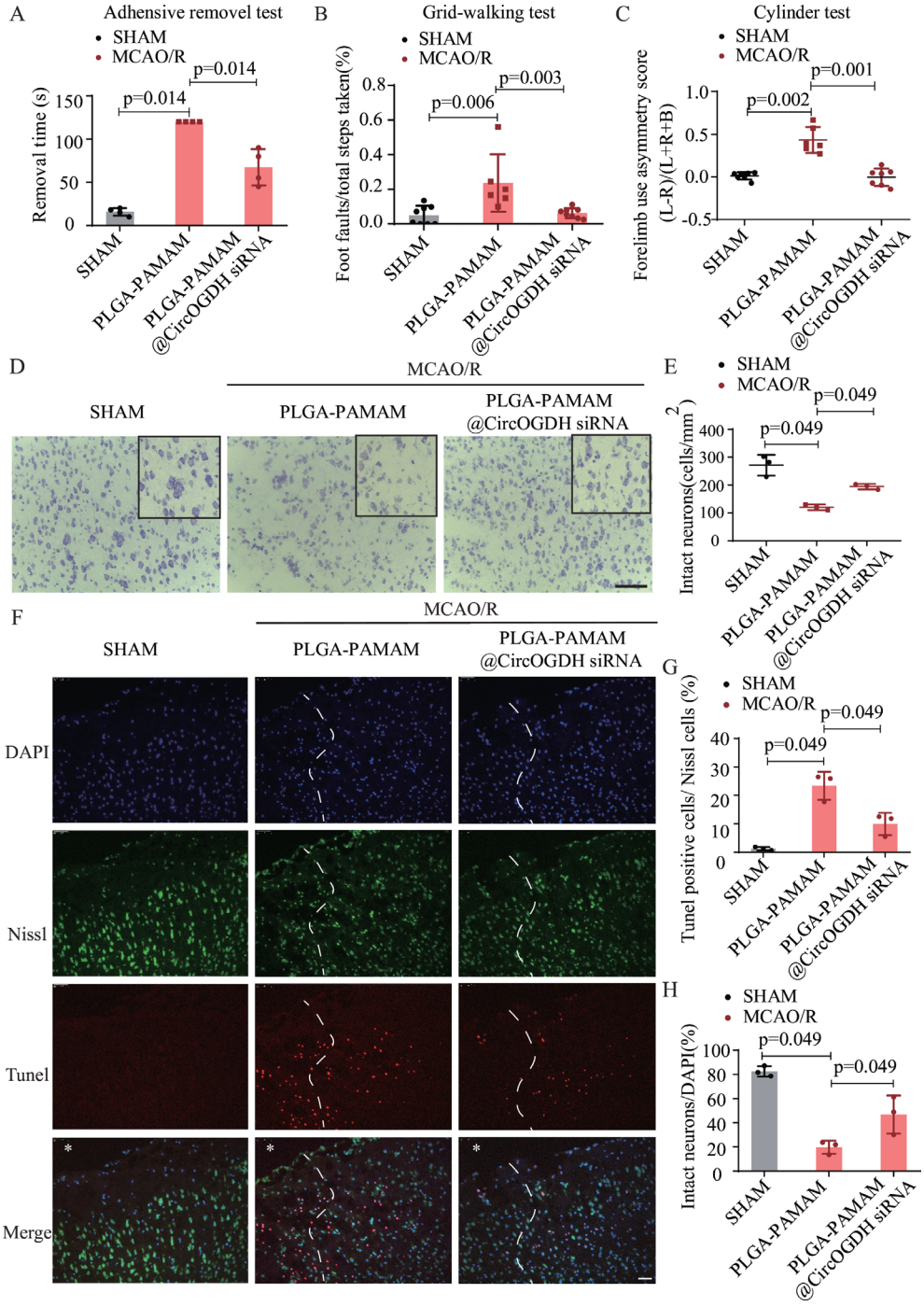


Figure 4 PLGA–PAMAM@CircOGDH siRNA NPs alleviated neuronal apoptosis in MCAO/R mice. PLGA–PAMAM@CircOGDH siRNA NPs improved behavioural recovery in MCAO/R mice 3 days after tail injection, as demonstrated by the adhesive removal test (A), grid-walking test (B) and cylinder test (C). Data are presented as means±SD adhesive removal test: n=4 in each group (Mann-Whitney U test); grid-walking test: n=8 in the SHAM group and the MCAO/R+PLGA–PAMAM@CircOGDH siRNA group, n=6 for MCAO/R+PLGA–PAMAM group (Mann-Whitney U test); cylinder test: n=7 in the SHAM group and the MCAO/R+PLGA–PAMAM@CircOGDH siRNA group, n=6 for the MCAO/R+PLGA–PAMAM group (one-way ANOVA followed by Tamhane T2 test). (D) and (E) Nissl staining showing the number of neurons in the SHAM, MCAO/R+PLGA–PAMAM and MCAO/R+PLGA–PAMAM@CircOGDH siRNA groups 3 days after tail injection. Scale bar=100 µm. For the quantification of results in (E), data were presented as mean±SD; n=3, Mann-Whitney U test. **(F)–(H)** TUNEL and Nissl staining of brain sections showing TUNEL-positive and intact neurons in the brains of mice in the MCAO/R+PLGA–PAMAM and MCAO/R+PLGA–PAMAM@CircOGDH siRNA groups 3 days after tail injection. White dashed lines (left) indicate potential infarct regions; yellow dashed lines (right) indicate potential penumbra regions. Scale bar=50 µm. Data were presented as mean±SD; n=3, Mann-Whitney U test. CircOGDH, circular RNAs derived from circular oxoglutarate dehydrogenase; MCAO/R, middle cerebral artery occlusion/reperfusion; NPs, nanoparticles; PLGA, poly(lactide-co-glycolide); siRNA, small interfering RNA; PAMAM, poly amidoamine; ANOVA, analysis of Variance; TUNEL, TdT-mediated dUTP nick end labeling.

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siRNA NPs or PLGA–PAMAM NPs (figure 5A–C). Finally, H&E staining revealed that the main organs (the heart, liver, spleen, lungs and kidneys) displayed no observable pathological injuries on day 3 after treatment with PLGA–PAMAM@CircOGDH siRNA NPs or PLGA–PAMAM NPs in MCAO/R mice (figure 5D). Thus, our results indicated that PLGA–PAMAM@CircOGDH siRNA NPs improved penumbra neuron survival and exerted no significant toxicity in MCAO/R mice.

DISCUSSION

The pathological process of ischaemic stroke is complicated and involves inflammation, oxidative stress and excitotoxicity, which lead to neuronal death.[21] The management of ischaemic stroke largely depends on intervention within a narrow time window, and rescuing the maximum number of ischaemic penumbra neurons is crucial for optimal AIS treatment.[22] Mechanical thrombectomy is a well-established treatment used in patients with AIS for proximal large vessel occlusion within a stringent time window.[23–25] Though the brain blood flow is restored, secondary damage will occur during the ischaemic reperfusion process in ischaemic region after mechanical thrombectomy.[9] Our previous studies identified and screened a neuron-derived CircOGDH, which was identified as a potential penumbra therapeutic target in AIS. Furthermore, we found that the binding of CircOGDH to microRNA-5112 regulated the downstream COL4A4 expression level, thus to alleviate neuronal apoptotic level under ischaemic conditions in a permanent 3-hour MCAO mouse model without ischaemic reperfusion.[11] As numerous neuroprotective agents have failed to show benefit in a Phase III clinical trial of AIS, effective and new treatment strategies that combine reperfusion raised attention.[26] In the present study, we assembled PLGA–PAMAM NPs loaded with CircOGDH siRNA and demonstrated that they exerted a potential protective effect in OGD/R primary cortical neurons and MCAO/R mice using a series of experiments.

Neuronal apoptosis is widely recognised as crucial factors during the pathological process of ischaemic stroke. circRNAs are a type of noncoding RNA expressed in tissue-specific, cell-specific and developmental stage-specific patterns, they are abundant in the brain and are more stable than linear RNA.[10] Increasing evidence identified that circRNAs have been implicated in the pathological process of ischaemic stroke,[27–29] and circRNA-based therapeutics emerge as a potential therapy for various therapeutic areas, such as prophylactic vaccines.[30] Our previous studies identified a neuron-derived CircOGDH, which could aggravating neuronal apoptosis and was identified as a potential penumbra biomarker in AIS.[11] However, still many challenges remain regarding targeted delivery of circRNAs.[31] siRNA is one of the meaningful gene silencing tool that has been widely used to control target gene expression in vivo and in vitro. Still many challenges remain regarding siRNA delivery because of the molecule's rapid enzymatic degradation.[32 33] Previous studies have reported that NPs loaded with siRNA improve therapeutic efficacy in ischaemic stroke when administered intravenously.[33 34] In line with these findings, our results indicate that PLGA–PAMAM@CircOGDH siRNA NPs can protect CircOGDH siRNA from enzymatic degradation for 72 hours (according to UV–Vis and fluorescence spectra analysis). PLGA–PAMAM@CircOGDH siRNA NPs can be taken up by ischaemic neurons in vitro and penumbra neurons in MCAO/R mice. We found PLGA–PAMAM@CircOGDH siRNA NPs preferably accumulated in the penumbra area of MCAO/R mice, thus significantly downregulated CircOGDH expression levels in penumbra tissues of MCAO/R mice. Additionally, we demonstrated that PLGA–PAMAM@CircOGDH siRNA NPs alleviated neuronal apoptotic level in ischaemic neurons, and tail injection of PLGA–PAMAM@CircOGDH siRNA NPs significantly decreased brain infarct size and alleviated neurologic function injury and apoptotic level of ischaemic penumbra neurons in MCAO/R mice.

Polymers, lipids, inorganic materials and exosomes can be used in nanostructured gene delivery systems.[35] PLGA is an FDA-approved polymer commonly employed in gene and drug delivery systems for clinical use.[36] Despite its excellent biocompatibility and biodegradability, PLGA is difficult to load with nucleic acids because it is negatively charged.[4] Thus, our study assembled PLGA–PAMAM NPs. PAMAM dendrimers are widely used as a nonviral gene carrier because they exhibit positively charged amine groups on their surface and can be internalised into cells by endocytosis; however, the cytotoxic activity of PAMAM dendrimers requires careful attention,[6 37] which was a limitation of our study. To address this limitation, haematological and pathological analyses were conducted. The results revealed that the biomarkers evaluated in these analyses remained within a normal range in MCAO/R mice injected with PLGA–PAMAM@CircOGDH siRNA NPs or PLGA–PAMAM NPs. Studies have reported that biomimetic NPs exert high blood compatibility and long circulation times[8 35 38]; and many different nanoplatforms have been used as nanotherapies to provide neuroprotection in brain injury.[39] Therefore, after confirming the protective effect of PLGA–PAMAM@CircOGDH siRNA NPs in MCAO/R mice, our future work will be dedicated to optimise our NPs to minimise the toxicity and improve the delivery efficiency targeting the neurons in the brain.

CONCLUSION

In summary, our data showed that we successfully assembled the PLGA–PAMAM@CircOGDH siRNA NPs and found that they could exert protective effects and preserve the integrity of CircOGDH siRNA by preventing potential degradation. We revealed that PLGA–PAMAM@CircOGDH siRNA NPs alleviated neuronal apoptotic level in ischaemic neurons, and tail injection of PLGA–PAMAM@CircOGDH siRNA NPs significantly decreased brain infarct size and alleviated neurologic function injury

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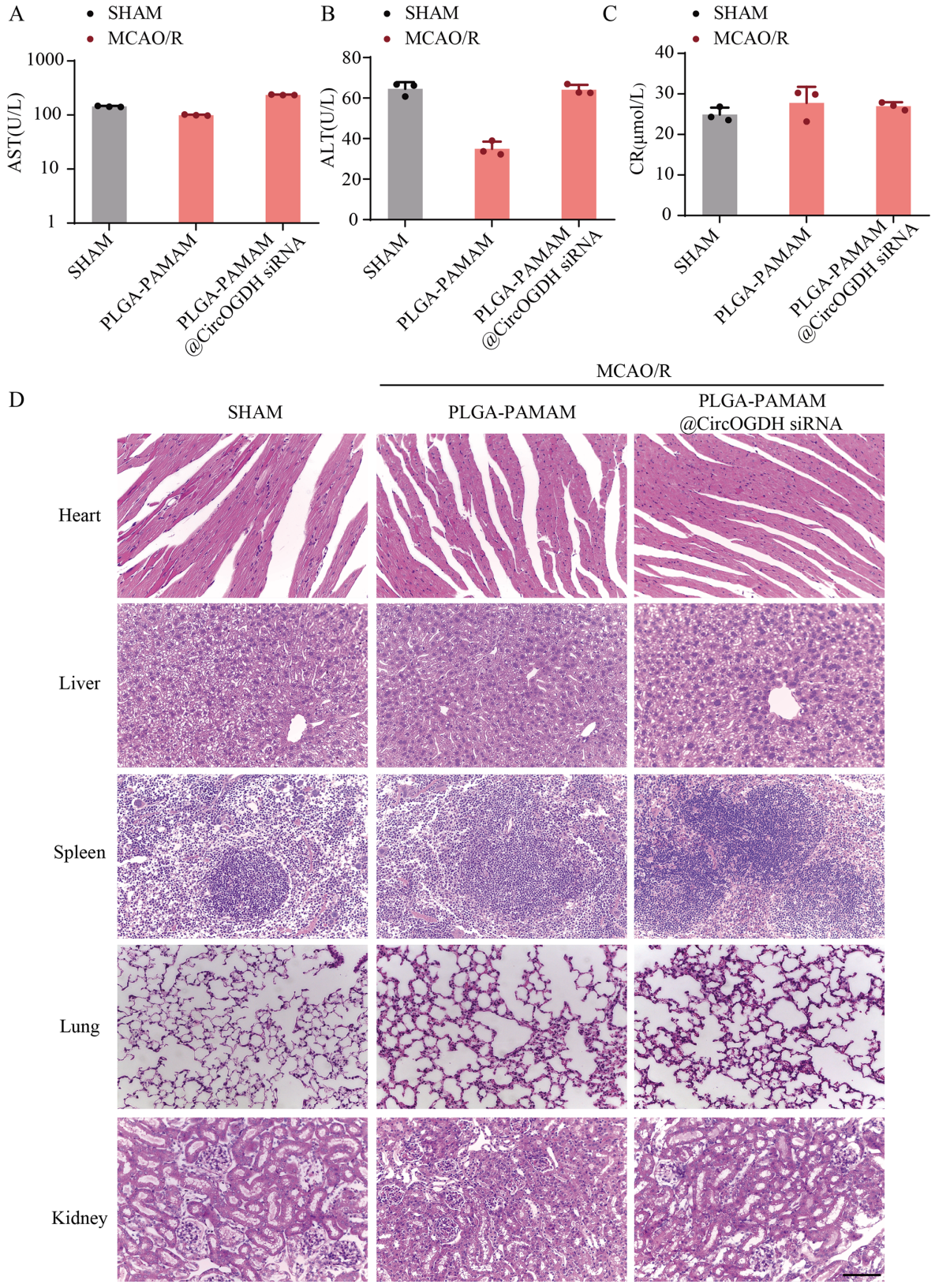


Figure 5 PLGA–PAMAM@CircOGDH siRNA NPs exert no significant toxicity in MCAO/R mice. Haematological analysis of AST (A), ALT (B) and CR (C) were evaluated in the SHAM, MCAO+PLGA–PAMAM and MCAO+PLGA–PAMAM@CircOGDH siRNA groups 3 days after tail injection. Data were presented as mean±SD; n=3, Mann-Whitney U test. (D) H&E staining of the heart, liver, spleen, lungs and kidneys in SHAM, MCAO+PLGA–PAMAM and MCAO+PLGA–PAMAM@CircOGDH siRNA mice 3 days after tail injection. Scale bar=100 μm. ALT, alanine transaminase; AST, aspartate transaminase; CircOGDH, circular RNAs derived from circular oxoglutarate dehydrogenase; CR, creatinine; MCAO/R, middle cerebral artery occlusion/reperfusion; NPs, nanoparticles; PLGA, poly(lactide-co-glycolide); siRNA, small interfering RNA; PAMAM, poly amidoamine.

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and apoptotic level of ischaemic penumbra neurons in MCAO/R mice. Thus, our study provides a nanotherapeutic strategy for targeting CircOGDH in ischaemic stroke, which may promote the potential clinical transformation of multifunctional nanodrugs targeting circRNA (online supplemental file 2).

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Contributors YLiu performed experiments, prepared figures, analysed data, wrote, edited and proofread the manuscript. TZ performed experiments and the animal data analysis, prepared figures, wrote, edited and proofread the manuscript. XZ performed experiments and the cell data analysis and prepared figures, wrote, edited and proofread the manuscript. ZY and NH help performed experiments on nanoparticles. YLi and JZ assisted the animal experiments. LH edited and proofread the manuscript. DL and AX conceptualised and directed the overall project. DL and AX are responsible for the overall content as guarantors. The guarantor accepts full responsibility for the finished work and the conduct of the study, had access to the data, and controlled the decision to publish.

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ORCID iD

Anding Xu http://orcid.org/0000-0003-3154-0985

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