
بسم الله الرحمن الرحيم

Gene Therapy for Drug-Resistant Glioblastoma via Lipid-Polymer Hybrid Nanoparticles Combined with Focused Ultrasound

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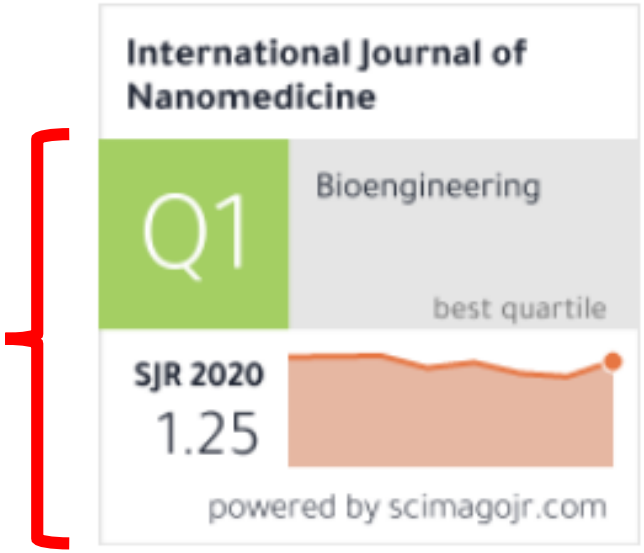


Abstract

FUS-assisted MBs-LPHNspCas9/MGMT-cRGD + TMZ → for drug(TMZ)-resistant glioblastoma treatment

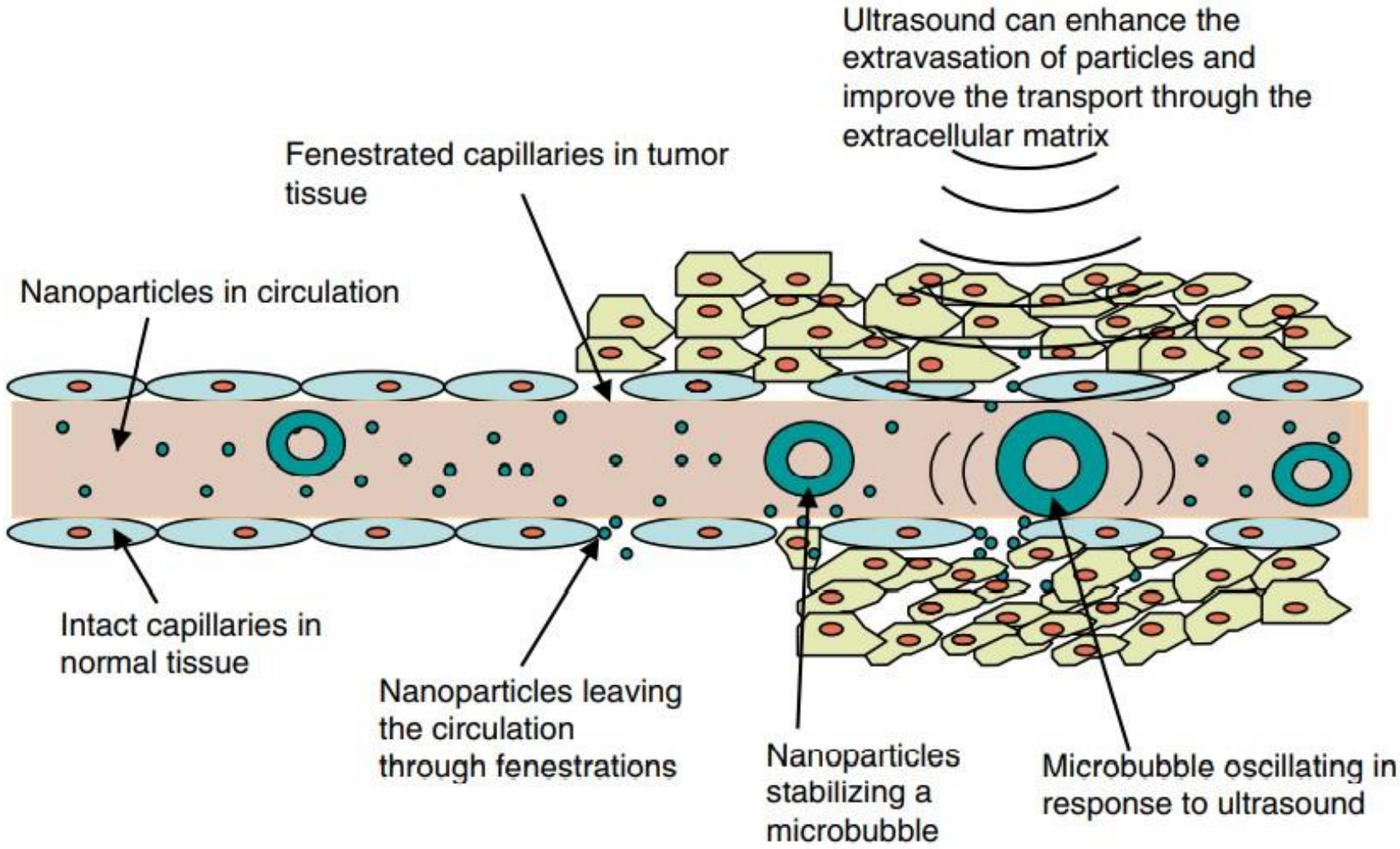


Image source: https://doi.org/10.1016/j.jconrel.2014.05.020

introduction

- Glioblastoma (GBM) is the most common and lethal primary brain tumor in adults.
- The mean survival of patients is only 12–14 months (even after treatment with comprehensive therapy).
- Temozolomide (TMZ), is the main drug therapy for glioblastoma.

1. easily cross the blood-brain barrier (BBB) and inhibit GBM cell proliferation
2. low levels of systematic toxicity

- the therapeutic efficacy of TMZ is often limited by O6-methylguanine-DNA methyltransferase (MGMT)
- Downregulating the expression of MGMT can reduce chemoresistance
- CRISPR/Cas9 has been considered one of the most significant gene editing technologies.
- This system shows tremendous potential for various cancer treatments.

- the Cas9 endonuclease, under the direction of guide RNA (gRNA), can recognize and cleave specific DNA sequences.
- generating double stranded breaks (DSBs)
- leading to insertions, deletions or mutations at target genomic locations

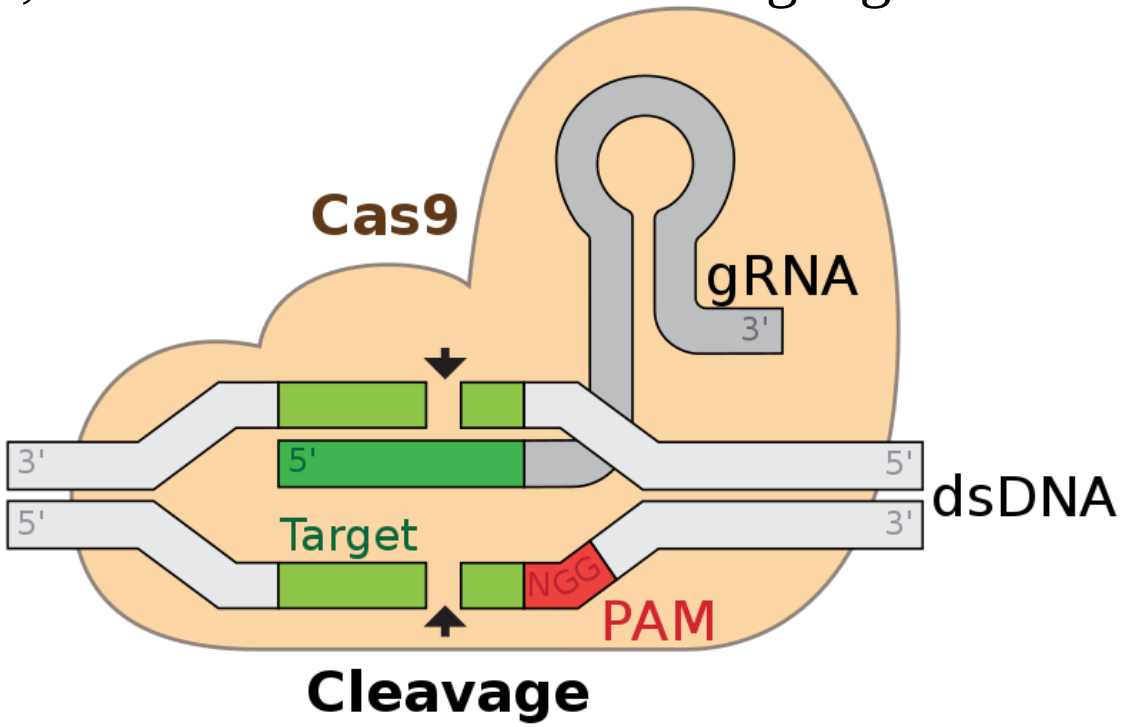


Image source: https://en.wikipedia.org/

- current delivery systems for CRISPR/Cas9 are mostly viral vectors.
  - lentiviruses (LVs)
  - adenoviruses (AVs)

→ ✗ immunogenic and carcinogenic, especially in vivo

- non-viral vectors have emerged as important alternatives for gene delivery

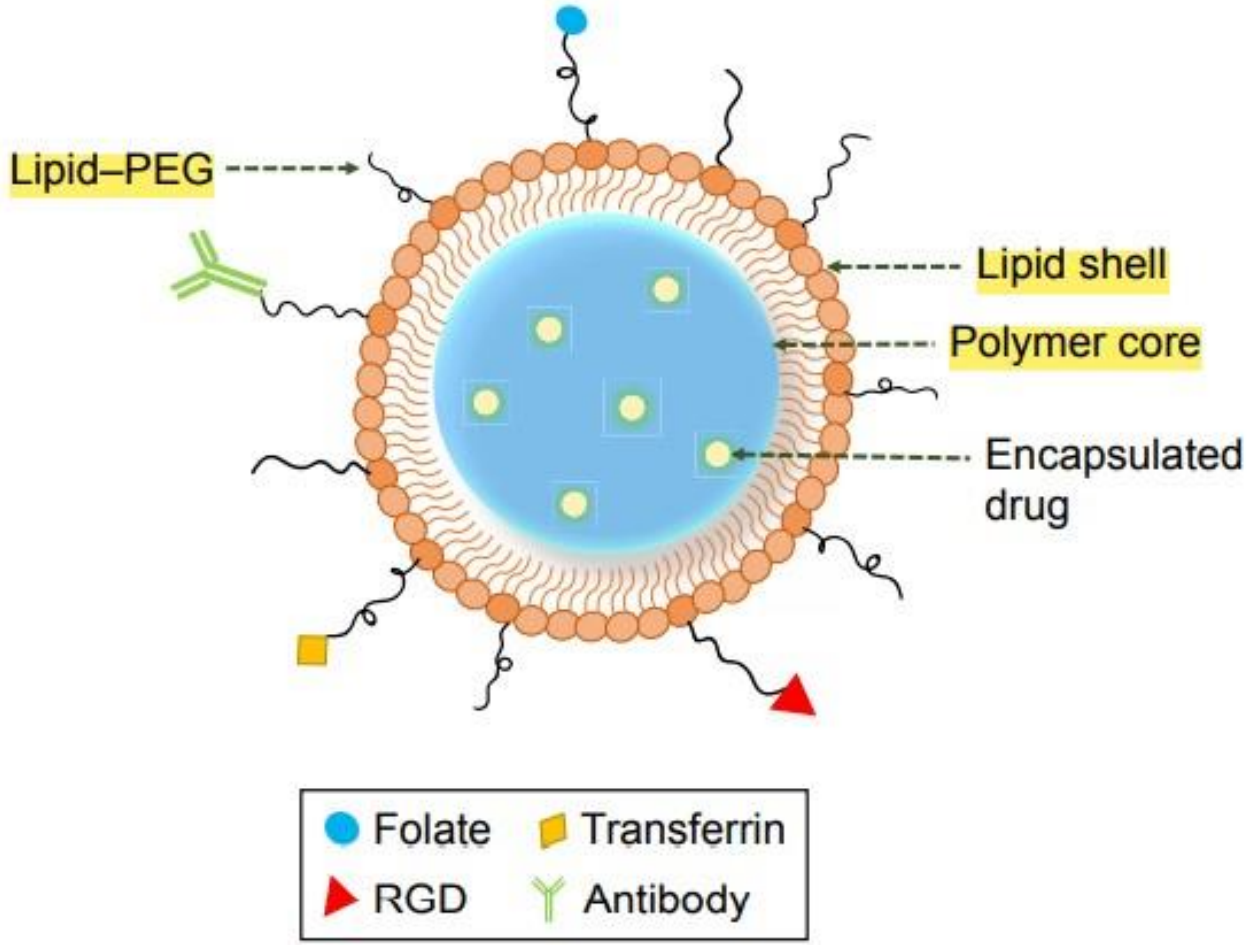
✓
- good biocompatibility
- low immunogenicity
- easy modification

- Nanoparticles, such as lipid- and polymer-based systems, have made significant progress in targeting drug delivery over the past decades.
- lipid-polymer hybrid nanoparticles (LPHNs) were constructed for efficient and targeting delivery.
- combine the complementary advantages of lipids and polymer nanoparticles.

lipids	high biological compatibility	low toxicity	ease of surface modification
polymers	high stability	controlled-release profile	high drug-loading efficiency

1. a polymer core encapsulating therapeutic agents
2. an inner lipid layer surrounding the polymer core, which can confer biocompatibility and retain therapeutic agents inside the polymer core
3. an outer lipid-PEG layer coating the lipid layer to extend the blood circulation time and enhance steric stabilization
4. PEG chains can be further modified with various bioactive molecules to meet different needs

Structure of a lipid–polymer hybrid nanoparticle (LPHNP)



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- The BBB blocks more than 98% of material

  - prevent toxic substances from entering the central nervous system (CNS)
  - maintain brain homeostasis.

- important alternative strategy to permeabilize the BBB:

*Focused ultrasound (FUS) combined with microbubbles (MBs)

- Noninvasive, reversible, local and site-specific
- through the cavitation effect
- without causing damage to the surrounding brain tissue.

- (LPHNspCas9/MGMT-cRGD) developed and evaluated the gene editing efficiency in vitro.

* (cRGD peptide targets the overexpressed integrin αvβ3 receptors in tumor cells)

- Subsequently,(MBsLPHNspCas9/MGMT-cRGD) complexes constructed for BBB-opening and targeting gene delivery in vivo under FUS irradiation.

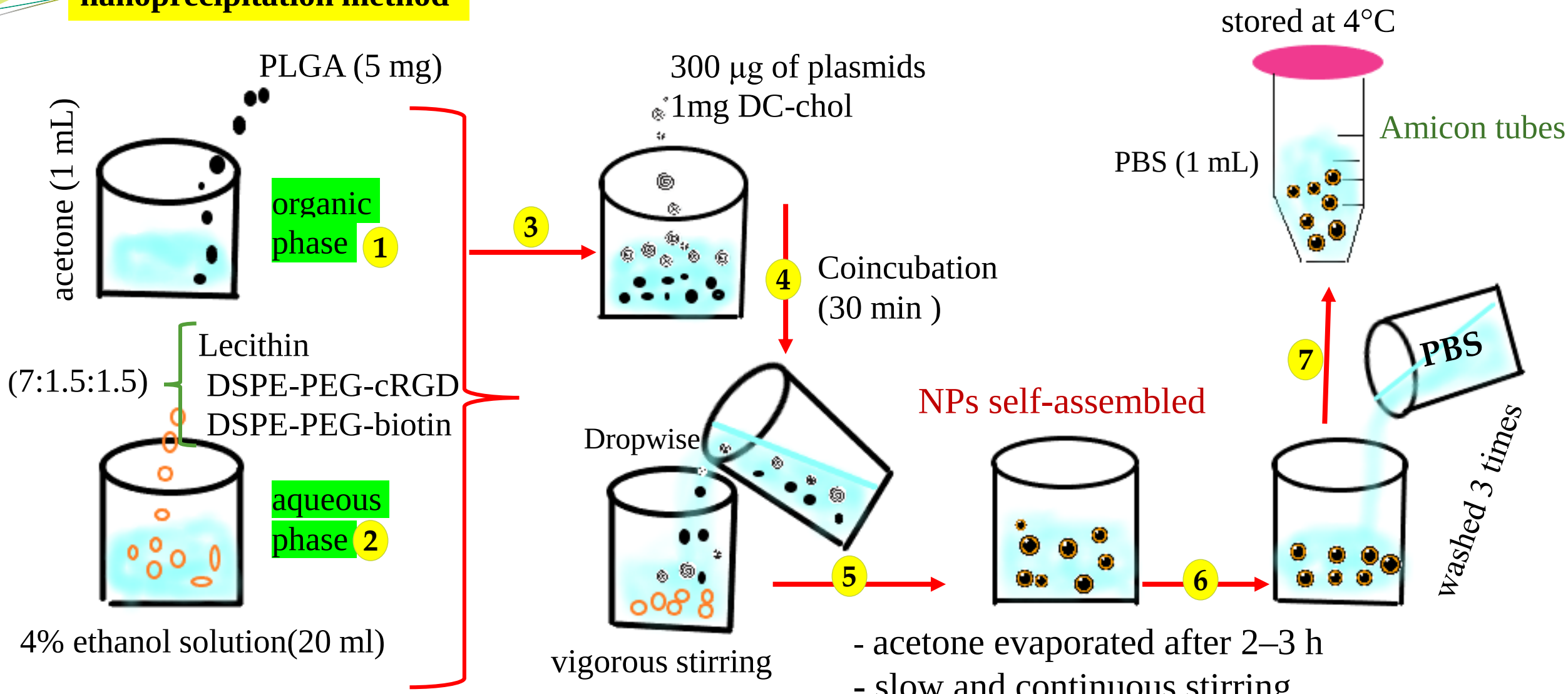
➢ **Resulting in:**

- a safe, efficient and multi-functional complex (with a high level of biosafety and biocompatibility)
- effective gene delivery (targeted delivery of CRISPR/Cas9)
- restore the sensitivity of GBM cells to TMZ,(treatment of TMZ-resistant glioblastoma)


MATERIALS &
METHODS

Preparation and Characterization of Lipid-Polymer-Hybrid PEG Nanoparticles (LPHNs-cRGD)

nanoprecipitation method



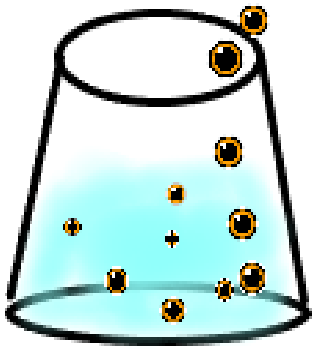
- The particle size, zeta potential and polydispersity index (PDI): Zetasizer Nano ZS instrument
- The morphology and structure of the LPHNs-cRGD were observed via transmission electron microscopy(TEM)
- Stability of nanoparticles was examined in MEM with 10% FBS (v/v) at 37°C
- Fluorescence microscope was used to observe the DiI-labeled LPHNs-cRGD
- The encapsulation efficiency of the pCas9/MGMT in LPHNs-cRGD was determined from the ratio of the amount of encapsulated pCas9/MGMT to the amount of pCas9/MGMT initially added using PicoGreen dsDNA Quantitation Kit
- The structure of the plasmid in nanoparticles was observed through agarose electrophoresis after extracted from nanoparticles

Release of pCas9/MGMT in vitro

carried out in PBS at 37°C

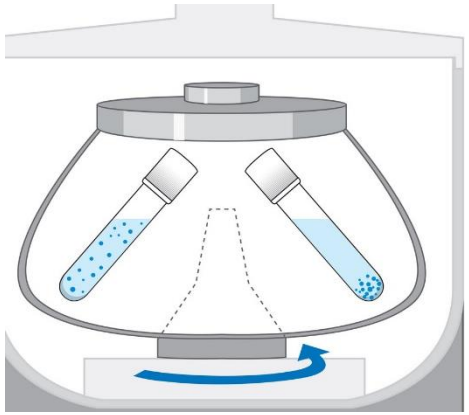
pCas9/MGMT-loaded NPs (5 mg)

1 mL PBS



shaken slowly at 37°C.

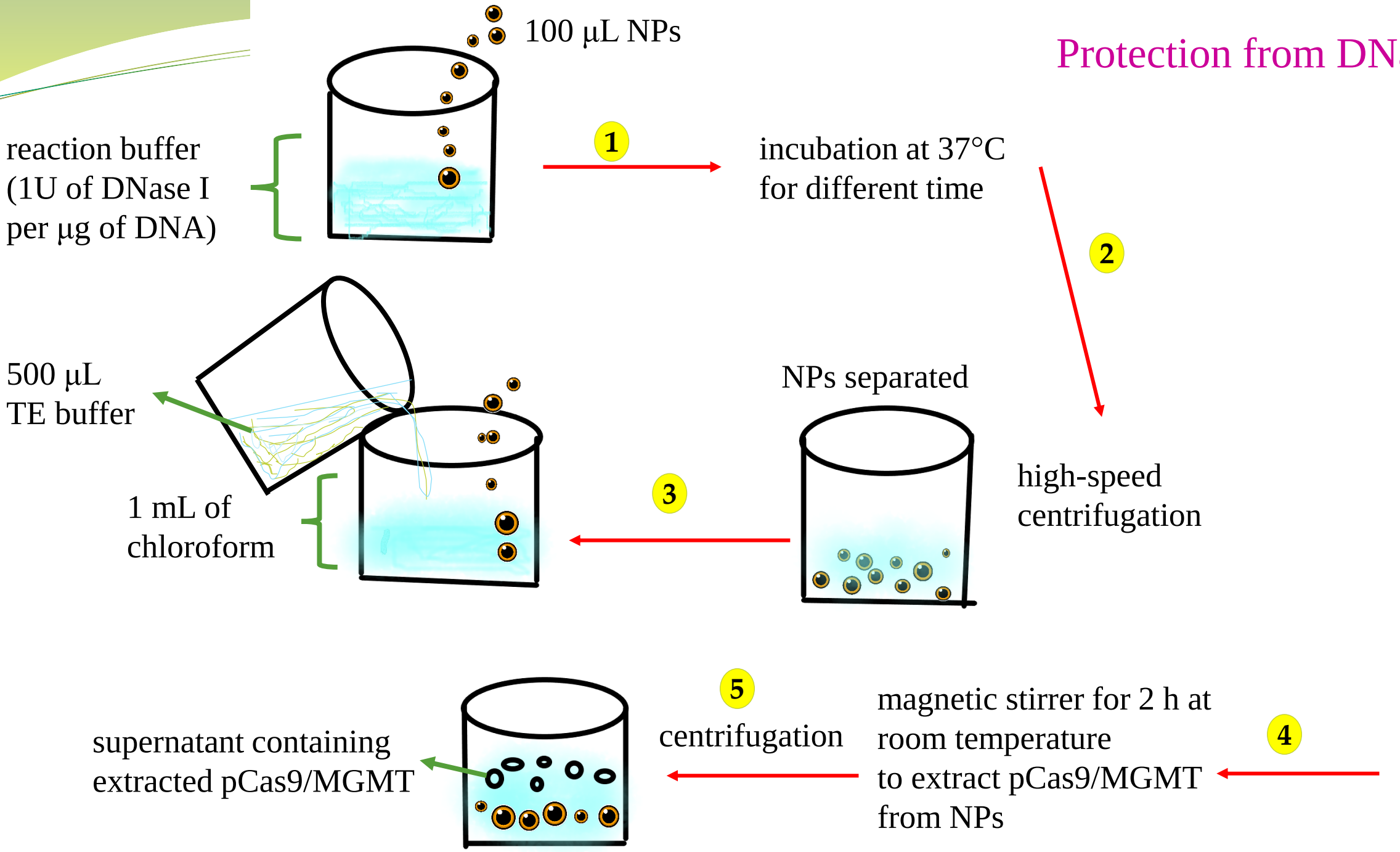
the solution was centrifuged



supernatants were collected to measure the released pCas9/MGMT using the Picogreen assay

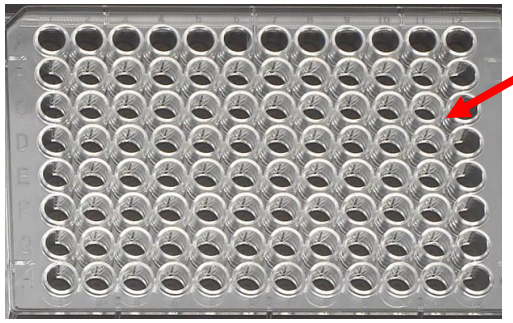
Nanoparticles were then immediately resuspended in fresh PBS and shaken in the incubator at 37°C.

Protection from DNase I



- samples + DNA loading buffer, applied to a 0.8% agarose gel in 0.5 x TAE buffer containing gold-view for 40 min at 70 mV.
- images were observed using Bio-Rad imaging system
- As a control ,50 µL naked pCas9/MGMT + DNase I → incubation(3 min at 37°C)

Cytotoxicity and Targeting Ability of LPHNs-cRGD in vitro

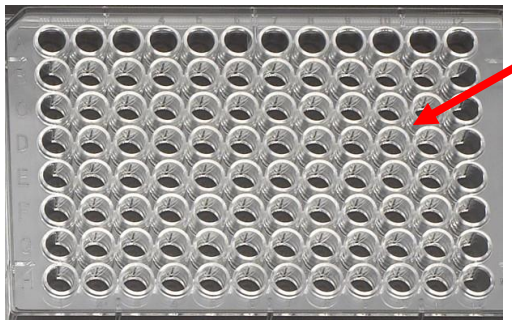


96-well plates

5 × 103 T98G cells per well

1

incubated overnight



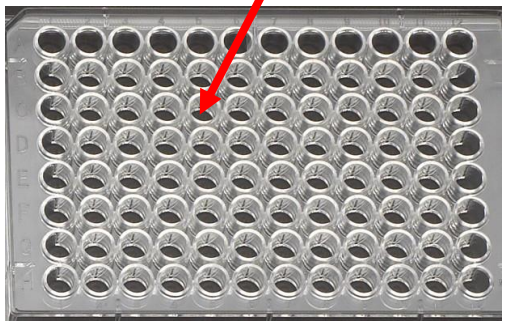
cells were added with different concentrations of LPHNs or LPHNs-cRGD

2

incubated for 48 h

*Untreated cells in culture medium were used as the negative control groups

cells were added with 10μL of CCK-8 solution



3

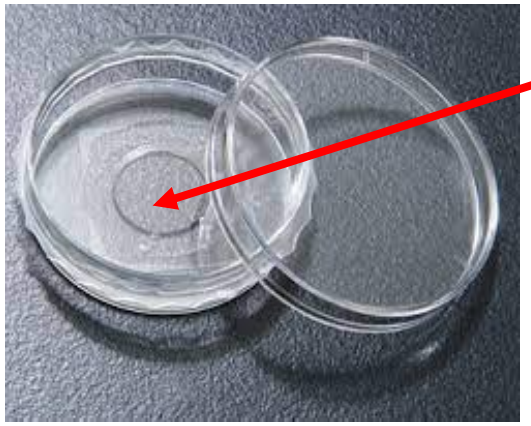
incubated for 1–2 h

Then the absorbance of solution in each well was measured at 450 nm using a microplate reader, The cell viability (%) was calculated according to the instructions of the CCK-8 assay

targeting ability of the LPHNs-cRGD toward T98G cells

Laser scanning confocal microscope (LSCM)

flow cytometry (FCM)



confocal culture dishes
(density of 1.0 × 105 cells per dish)

T98G cells

1

incubated overnight

control group / LPHNs group / LPHNs-cRGD group

cells mixed with different nanoparticles

24 h of incubation

2

LPHNs group + 10 μL DiI-labeled LPHNs emulsions

LPHNs-cRGD group +10 μL DiI-labeled LPHNs-cRGD emulsions.

4 h of coincubation

3

- cells in the dishes were rinsed 3 times with PBS
- fixed with 4% paraformaldehyde for 15 min at room temperature
- then stained with DAPI for 5 min & rinsing again
- the fixed cells were imaged using LSCM

For FCM

6-well plates
(density of 2.0 × 105 cells per dish)

T98G cells

incubated overnight

The previous steps repeated

4 h of co-incubation

- the cells were washed with PBS + digestion with trypsin
- Centrifugation
- suspension in 500 μL PBS
- fluorescence intensity of the cellular uptake was detected via FCM

Transfection Efficiency and Gene Editing with LPHNs-cRGD in vitro

evaluate the transfection efficiency → Fluorescence microscopy

evaluate the transfection efficiency → FCM

6-well plates

T98G cells

incubated overnight & treated with

- naked pCas9/MGMT
- LPHNspCas9/MGMT
- LPHNspCas9/MGMT -cRDG
- Lip3000 (positive control)

*amount of pCas9/MGMT: 5 μg

incubation for 48 h → EGFP expression was observed with a fluorescence microscope, and then cells were collected for FCM

Genome Editing

to test the expression of MGMT protein after transfection with different nanoparticle → Western blotting

6-well plates

T98G cells

incubated overnight & treated with

- LPHNspCas9/MGMT
- LPHNspCas9/MGMT-cRGD
- *amount of pCas9/MGMT: 5 μg

incubation for 48 h (with PBS)

- Untreated cells
- PBS
- LPHNspctrl-cRGD

negative controls

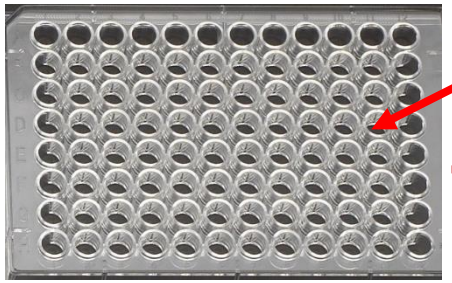
Whole-cell protein was isolated using RIPA lysis buffer with phenylmethylsulfonyl fluoride and quantified by a BCA assay

- Isolated protein was separated on SDS-PAGE gel
- transferred to polyvinylidene fluoride membranes
- The membranes were incubated with the appropriate antibodies against MGMT
- subsequently incubated with secondary anti-rabbit IgG-HRP antibodies
- Images were obtained using the BioRad imaging system

CCK-8 and Apoptosis Assays for Chemosensitivity Testing

Evaluate the sensitivity of the GBM cells to TMZ after treatment with LPHNspCas9/MGMT in vitro: CCK8 and apoptosis assays

T98G cells



96-well plates

incubated overnight

cells divided into five groups

negative control	LPHNspCas9/MGMT-cRGD	TMZ	LPHNspCas9/MGMT (for 48 h) + TMZ (150 μM)	LPHNspCas9/MGMT-cRGD (for 48 h) + TMZ (150 μM)

*amount of pCas9/MGMT: 0.2 μg

incubation for 36 h

cell viability was evaluated using the CCK-8 assay

apoptosis assay

T98G cells

6-well plates

incubated overnight

cells divided into five groups

negative control	LPHNspCas9/MGMT-cRGD	TMZ	LPHNspCas9/MGMT (for 48 h) + TMZ (150 μM)	LPHNspCas9/MGMT-cRGD (for 48 h) + TMZ (150 μM)

*amount of pCas9/MGMT:5 μg

incubation

the cells were washed three times with PBS, digested, centrifuged, and resuspended in 500 μL of PBS. Finally, cells were subjected to flow cytometry analysis

Preparation of Microbubbles (MBs) and MBs-LPHNs-cRGD

lipid film hydration method.

DPPC
DSPE-PEG-biotin
cholesterol (at a mass ratio of 5:2:1)

Chloroform (5 mL)

round-bottomed flask

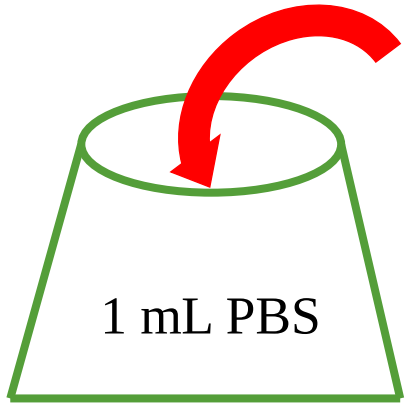
processed in a rotatory evaporator in a water bath at 50°C to form lipid film

dried lipid film was rehydrated

0.5 mL of PBS
glycerine solution (at a volume ratio of 9:1) perfluoropropane (C3F8)

The mixture was rapidly mechanically vibrated for 50 s. the biotinylated MBs were purified by centrifugation and stored at 4°C for further use.

The MBs and LPHNs-cRGD were bound together via the biotin-avidin linkage.



MBs (10 μL)
avidin

30 min of incubation
at room temperature

the mixture solution was washed
to remove unreacted agents

biotin-avidin MBs +
LPHNs-cRGD emulsions

incubated for 1 h

MBs-LPHNs-cRGD obtained

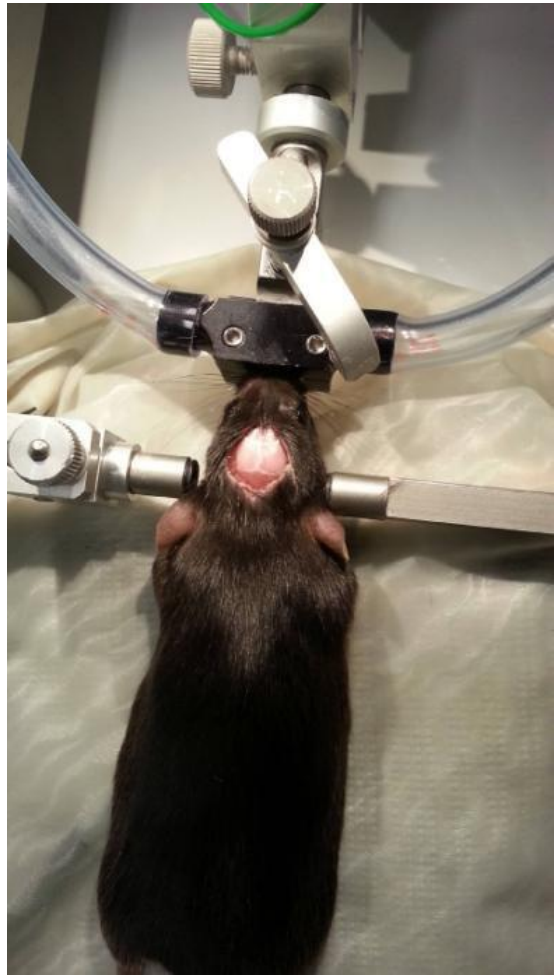
to confirm the connection of the two nanoparticles:

DiO-labeled LPHNscRGD
DiI-labeled MBs

observed by fluorescence microscope

Opening the Blood Brain Barrier

- Mice (NOD-SCID mice (4–6 weeks)) were anesthetized with pentobarbital and immobilized on a stereotaxic device.
- The hair on top of the heads was shaved
- The focused ultrasound probe was placed above the heads of mice and vertically fixed on the stereotactic frame
- The mice were randomly divided into different groups according to the irradiation times and amounts of MBs-LPHNs-cRGD
- injected with MBs-LPHNs-cRGD through the tail vein,
  exposed to FUS irradiation immediately +
  injected with 2% Evans blue dye (EB) → 1 h later



- ✓ the mice were sacrificed and perfused transcardially with saline
- ✓ the brain tissue was removed
- ✓ and coronally incised for digital photography and H&E staining

Orthotopic Xenograft Model

- NOD-SCID mice were anesthetized with pentobarbital
- and immobilized on a stereotaxic device
- The T98G cells (2 × 105) were intracranially injected into the striatum of the mice
- Tumor growth was monitored by a 7.0T MRI scanner seven days after implantation.

Targeting Ability, Distribution and Biosafety in vivo

mice were divided into 4 groups

DiR-labeled LPHNs-cRGD

DiR-labeled MBs-LPHNs-cRGD.

DiR-labeled MBs-LPHNs +FUS irradiation.

DiR-labeled MBs-LPHNscRGD +FUS irradiation.

2 h and 24 h later → brains and major organs were collected for ex vivo imaging

Using fluorescence imaging system → The fluorescence intensity was calculated

mice were divided into 2 groups

- Control (intravenously injected Saline)
- MBs-LPHNs-cRGD

- The mice were sacrificed seven days after injection.
- The major organs (liver, spleen, kidneys, heart, and lungs) were harvested
- and fixed via polyoxymethylene for H&E staining

Therapeutic Effect in vivo

- Mice with orthotopic T98G glioblastoma were divided into five groups

control (was treated with saline)
TMZ alone
MBs-LPHNspCas9/MGMT-cRGD + TMZ
MBs-LPHNspCas9/MGMT + FUS + TMZ
MBs-LPHNspCas9/MGMT-cRGD +FUS +TMZ

Nanocomplexes injected intravenously then the mice were exposed to FUS irradiation immediately or not.
Two days later, TMZ (50 mg/kg) was administered via daily intraperitoneal injection for 5 consecutive days.

- Three mice in each group were scanned with a 7.0T MRI to monitor tumor volume every seven days
- then all mice were administered with the next cycle of treatment
- The mice were sacrificed when they demonstrated severe neurological symptoms.

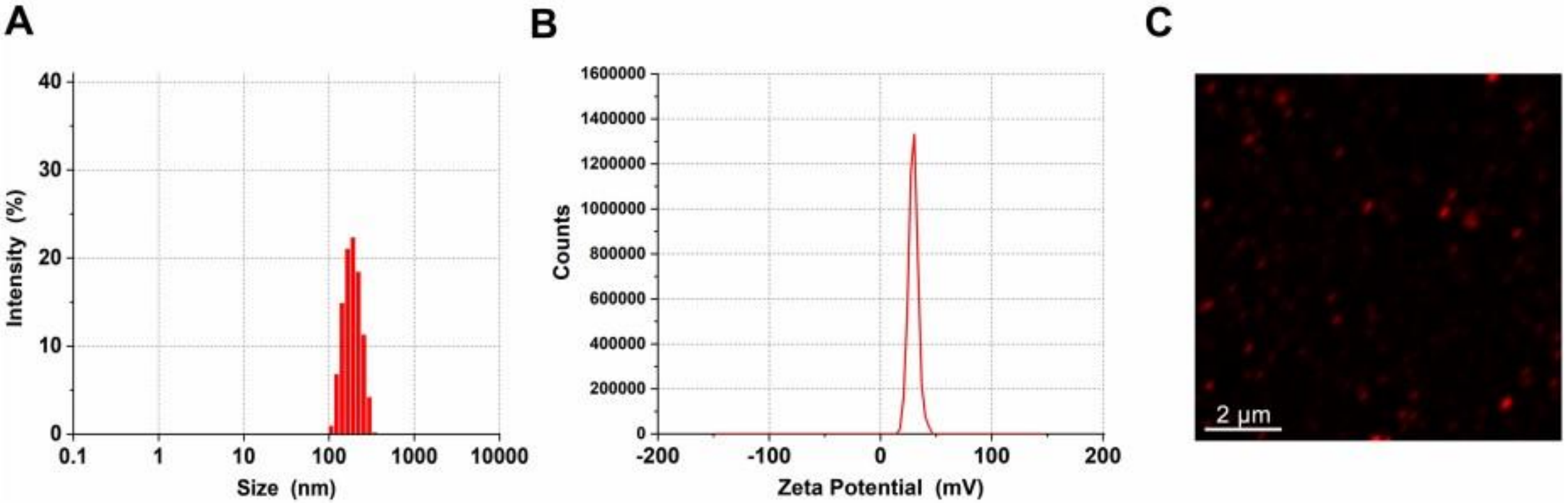
- Tumor volume was calculated as follows:
- volume = [length (max layer) × width (max layer)] × layers × 0.8/2 (0.8 mean the slices interval)
- The survival of each mouse was recorded and the median survival for each group was calculated

Statistical Analysis

- All experiments were performed in triplicate
- analysis was performed using SPSS software (SPSS version 23.0) and GraphPad software (GraphPad Prism 8.0).
- Data are presented as the mean ± standard deviation (SD). $p < 0.05$ were considered to be statistically significant


RESULTS

Formulation and Characterization of the LPHNs-cRGD

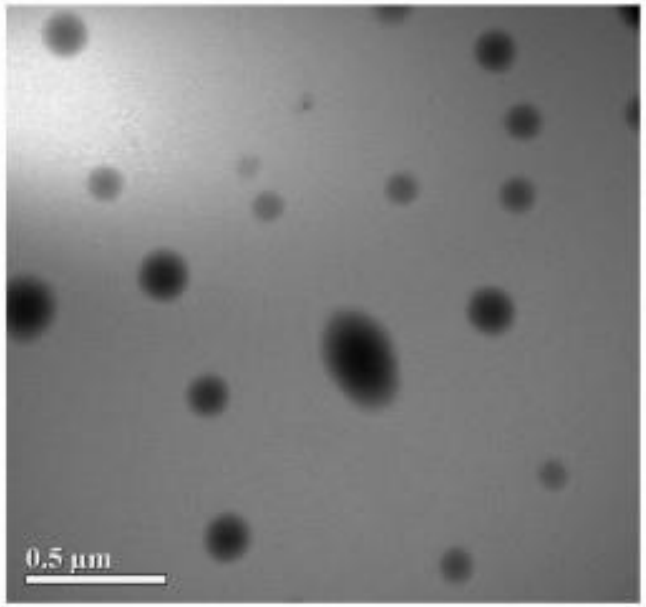


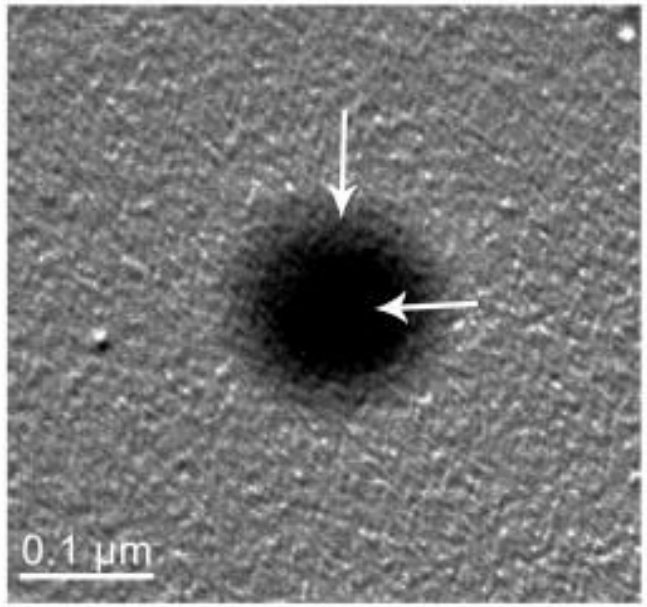
diameters and zeta potentials of the LPHNs-cRGD :

179.6 ± 44.82 nm and 29.6 ± 4.33 mv, respectively,

with a small PDI (0.048)

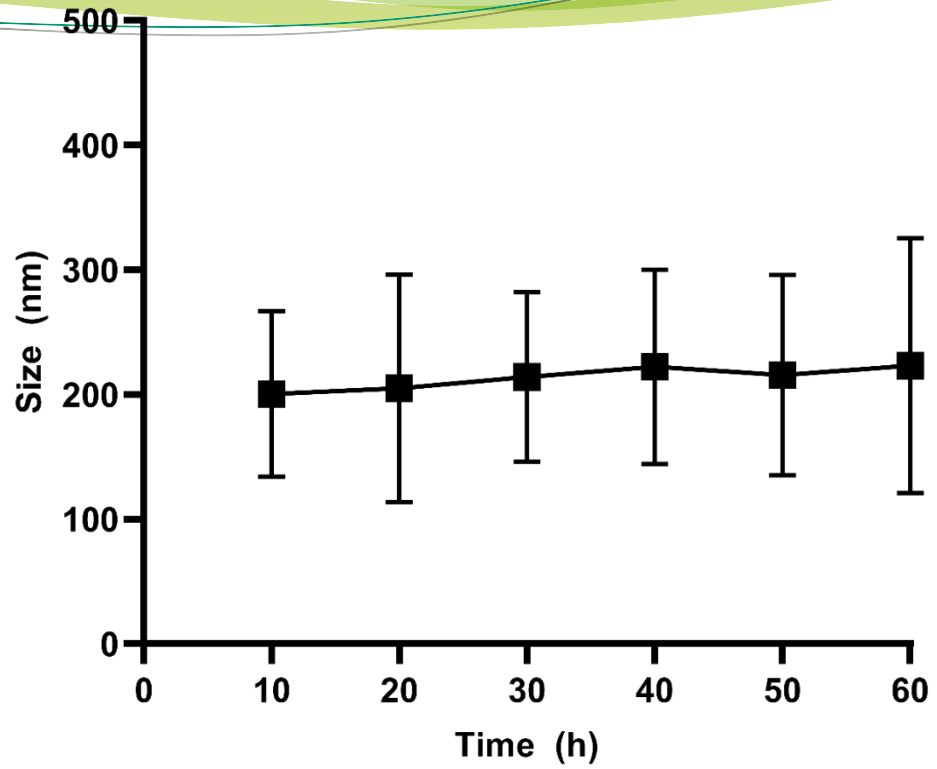
staining by DiI :
the LPHNs-cRGD exhibited uniform red fluorescence signal





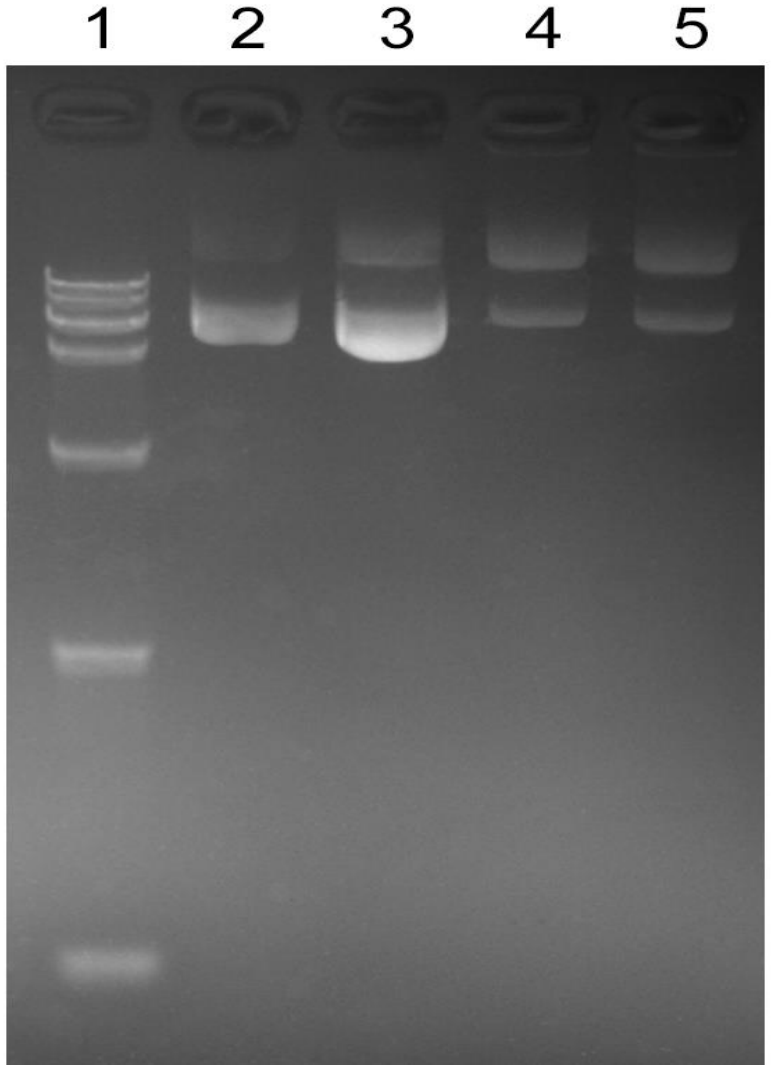
TEM showed that the LPHNs-cRGD had uniform spherical shape and typical core-shell structures

*the encapsulation efficiency was 76.5 ± 7.2%



Size stability of LPHNs-cRGD under physiological conditions in MEM containing 10% FBS.

Gel electrophoresis for testing the structural integrity of pCas9/MGMT in nanoparticles

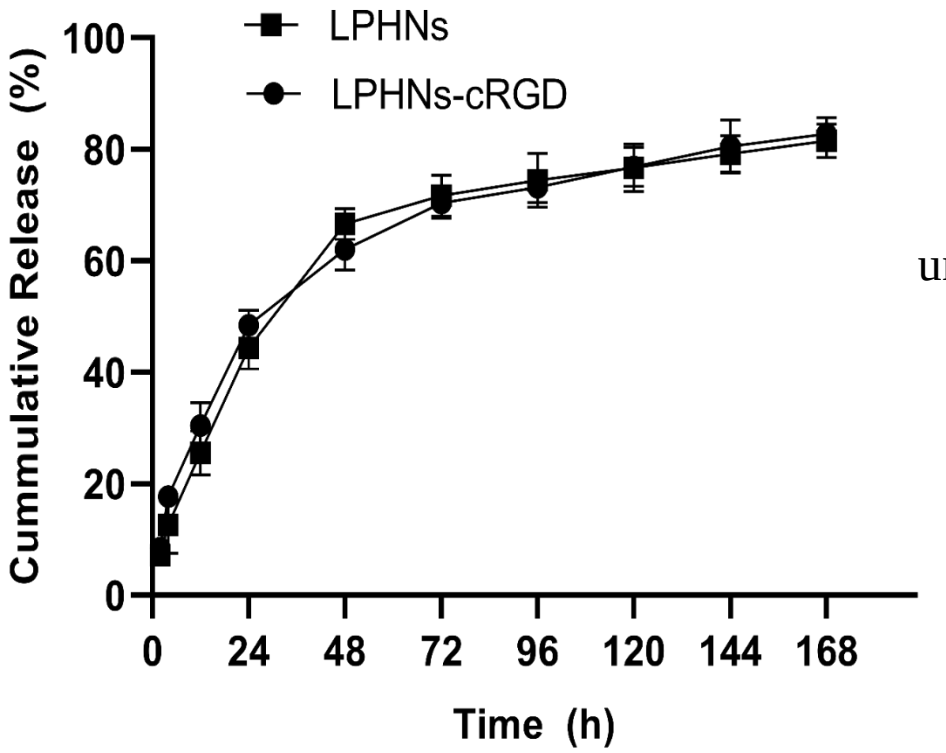


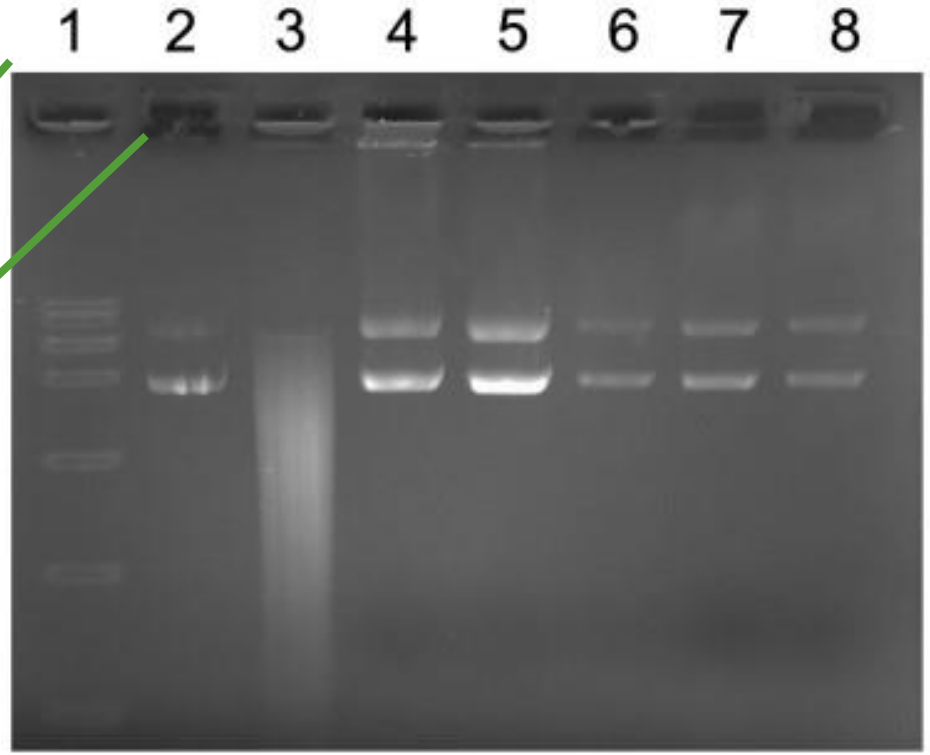
During the preparation process, the structure of the pCas9/ MGMT was unaffected and remained intact

Lane 1, DNA marke
Lane 2 and 3, untreated pCas9
Lane 4 and 5, extracted pCas9/MGMT from LPHNs and LPHNs-cRGD, respectively

Release of Plasmids in vitro and Protection from DNase I

In PBS solution



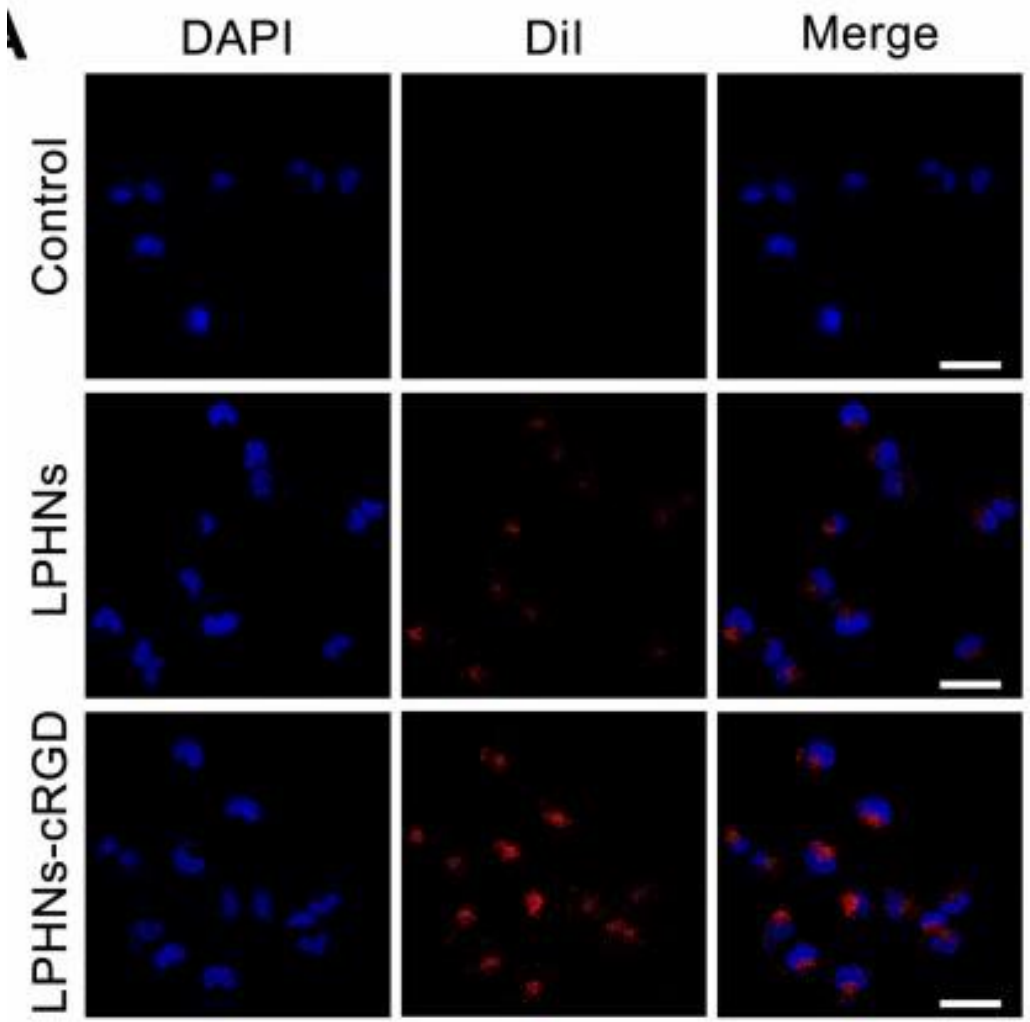


Lane 3, naked pCas9/MGMT after incubation with DNase I for 3min

Lanes 4–8,the extracted pCas9/MGMT from LPHNs-cRGD after incubation with DNase I for 3 min, 15 min, 30 min, 1 h and 2 h, respectively.

Targeting Ability and Biosafety Study in vitro

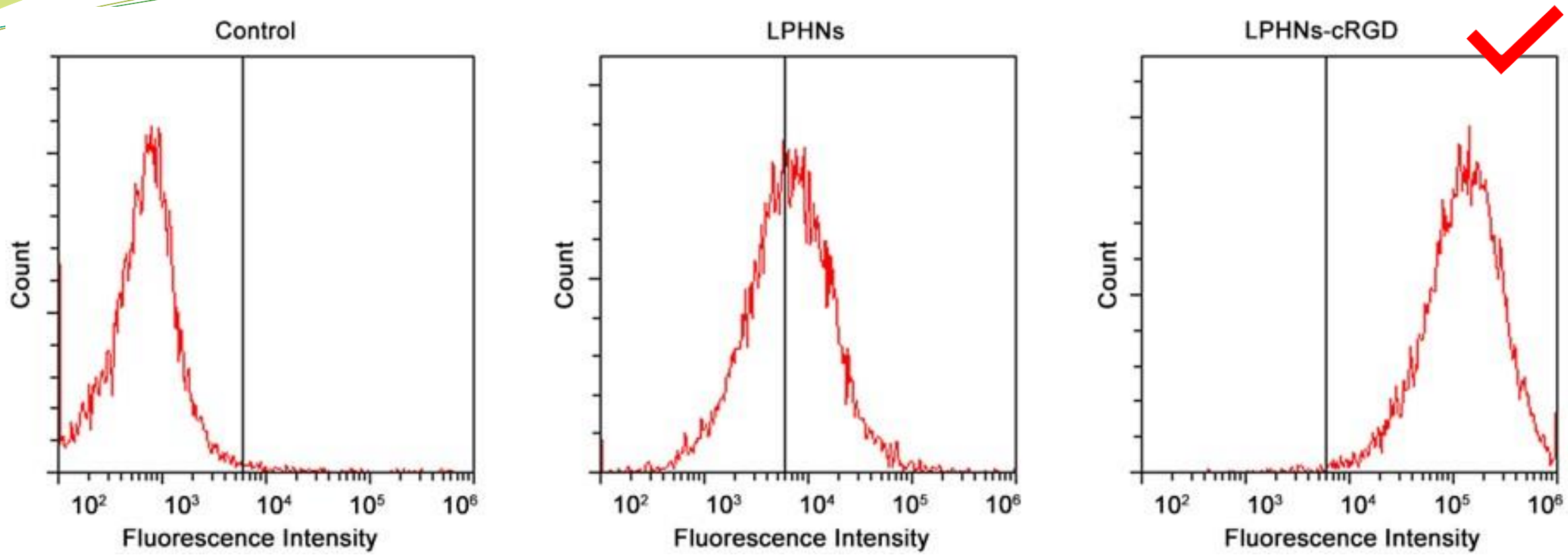
LSCM & FCM



strong red fluorescence in the T98G cells treated with the LPHNs-cRGD, which was much stronger than that in the LPHNs group

The LSCM images of T98G cells with different treatment

FCM results of T98G cells after different treatments



nanoparticles with cRGD peptides were more readily taken up than the nanoparticles without cRGD

in vitro biosafety of the nanoparticles

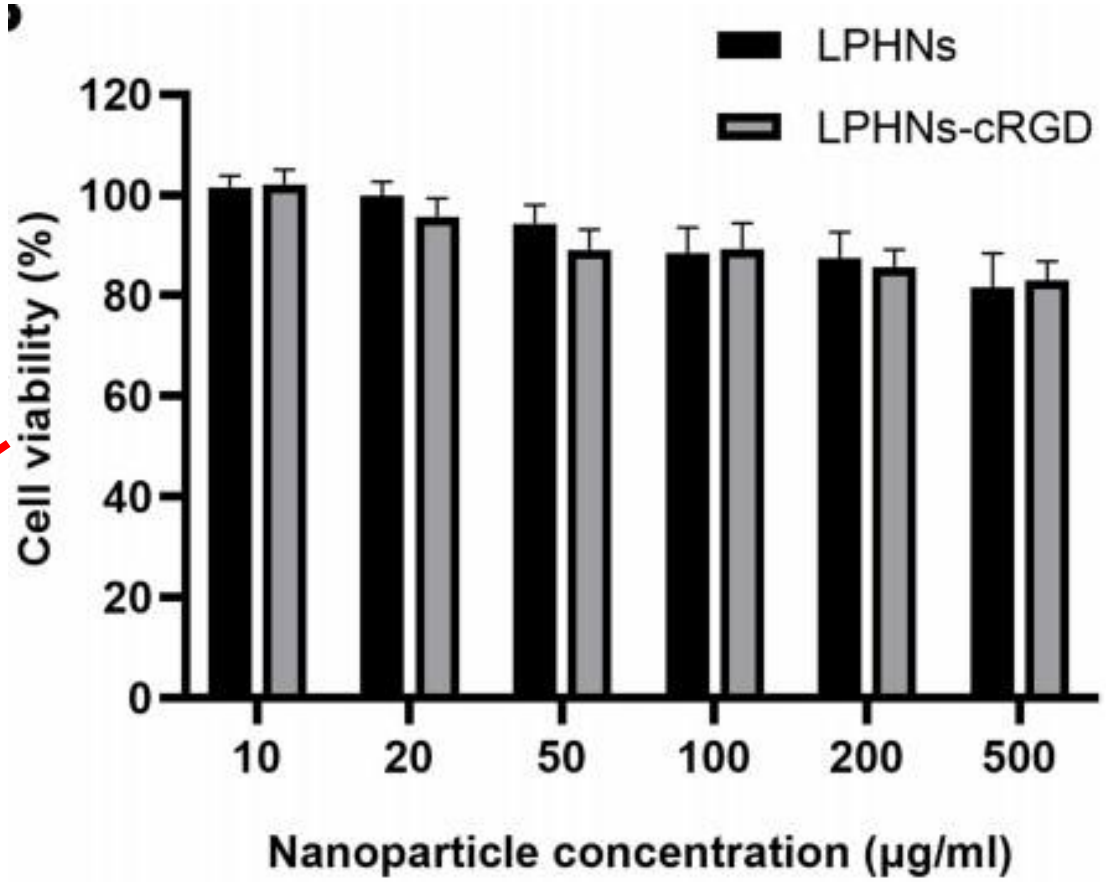
using a CCK-8 assay

to verify that the nanoparticles were biocompatible and non-toxic to cells.

cell viability >80%

nanoparticles were safe for cells

Cell viability at various concentrations of LPHNs and LPHNs-cRGD in T98G cells after 48 h (n=3).

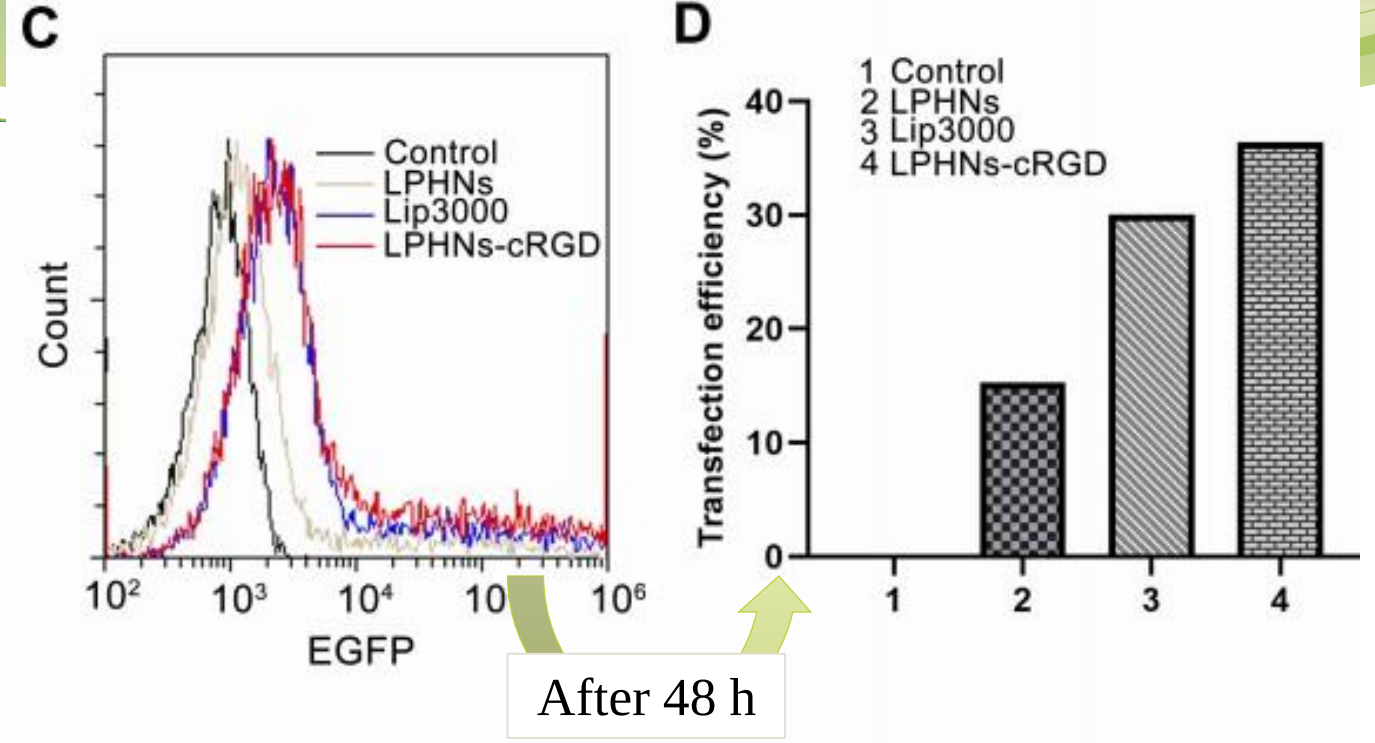


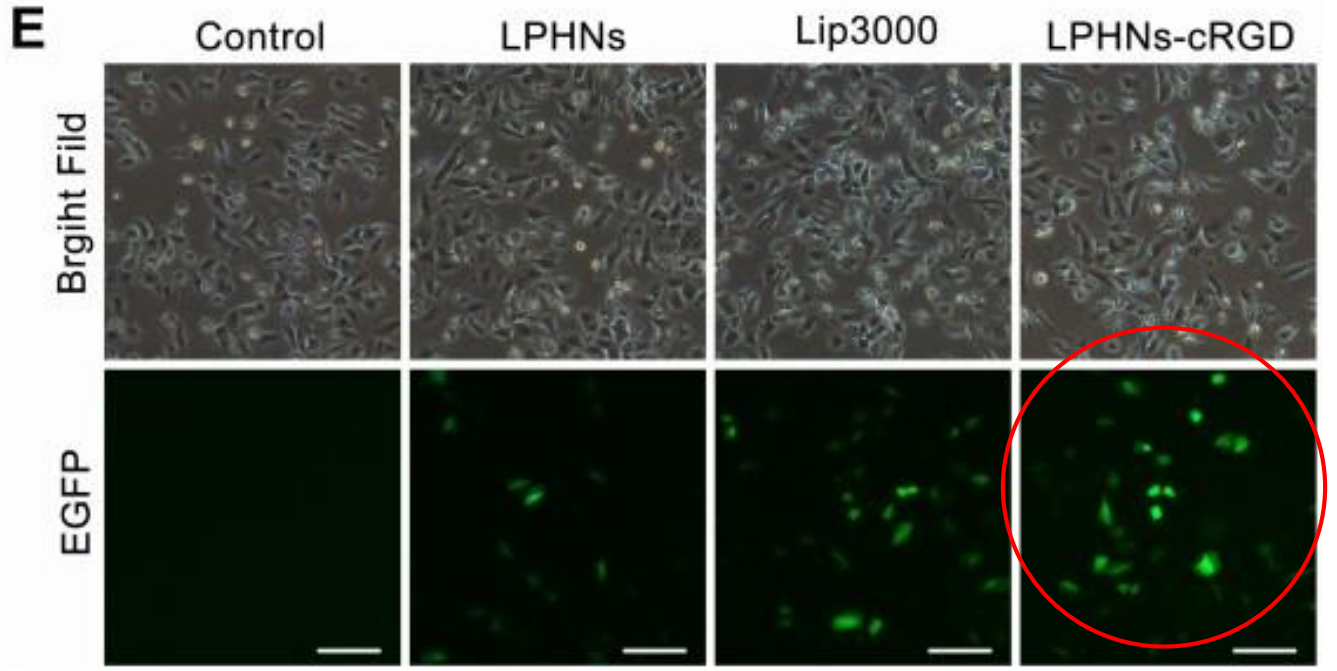
Transfection Efficiency

FCM and fluorescence microscopy

(C) and (D) FCM and statistical analysis of EGFP-positive T98G cells

(E) Microscopy images of EGFP expression in T98G cells at 48 h after transfection



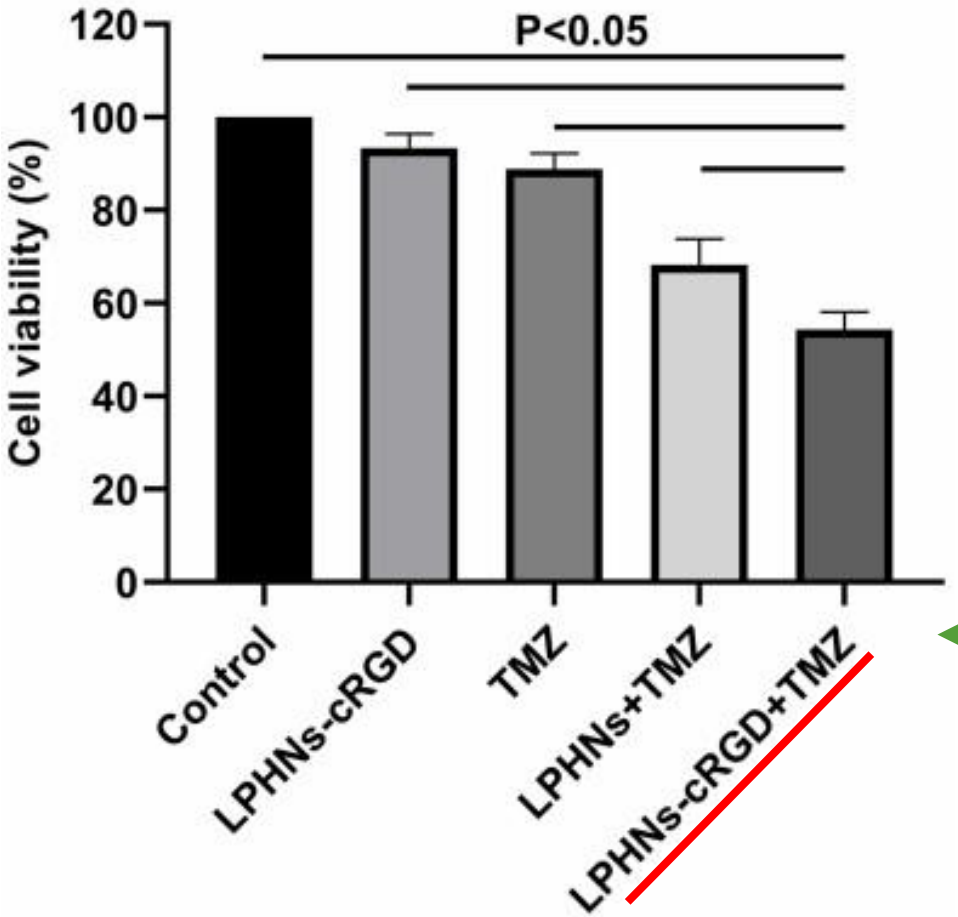


✔ pCas9/MGMT encapsulated in the LPHNs-cRGD were successfully introduced into the T98G cells.

Genome Editing and Chemosensitivity Testing in vitro

to determine the MGMT protein expression :

Western blotting assays

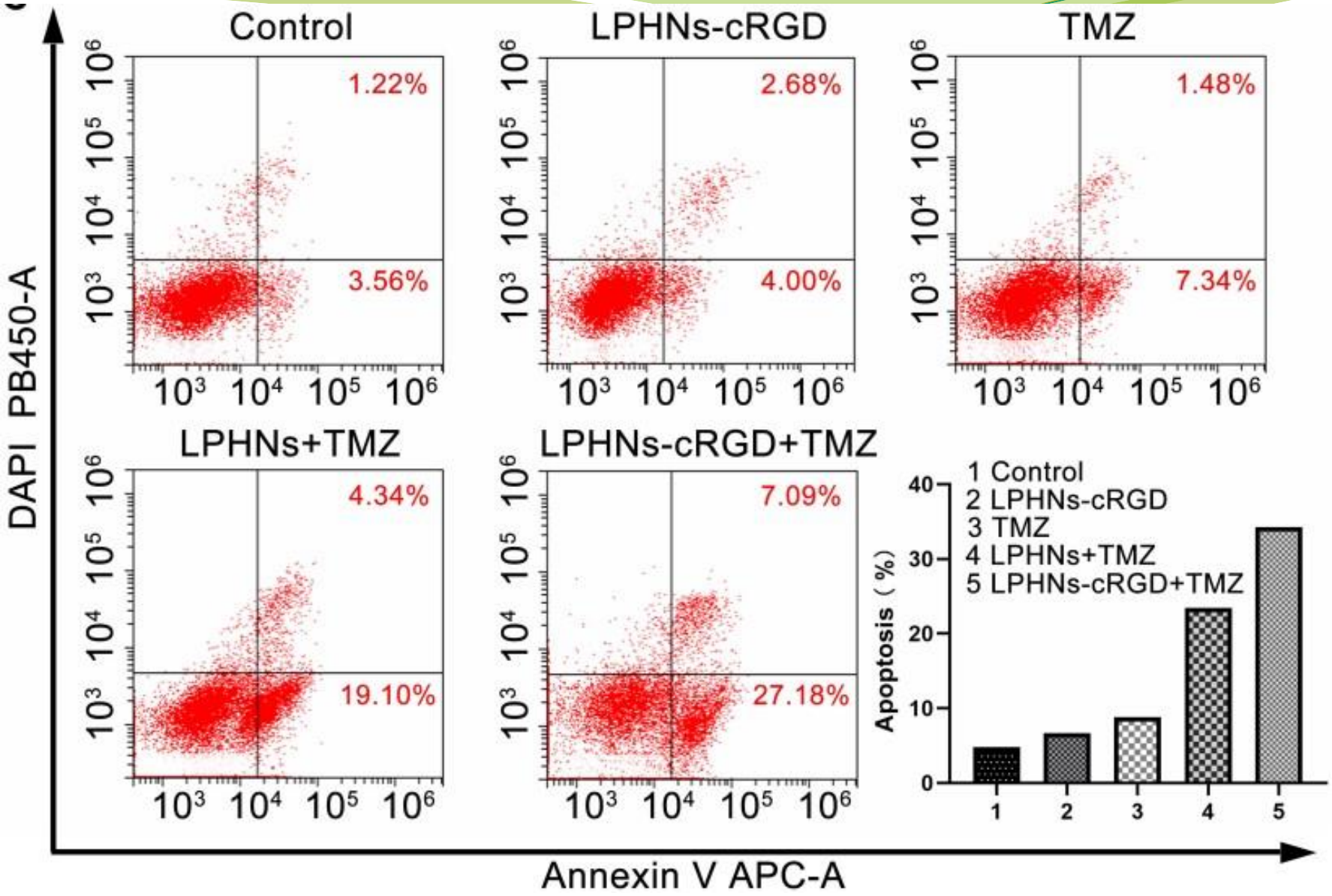


Cell viability of T98G cells after different treatments:

Combination treatment resulted in significantly decreased cell viability, especially in the LPHNspCas9/MGMT-cRGD + TMZ group

The percent of apoptotic cells was significantly higher in the combination treatment group

LPHNspCas9/MGMT-cRGD + TMZ treatment induced more apoptosis compared with LPHNspCas9/MGMT + TMZ due to the targeting ability of the cRGD (23.44% vs 34.27%)



FCM analysis of apoptosis after different treatments.

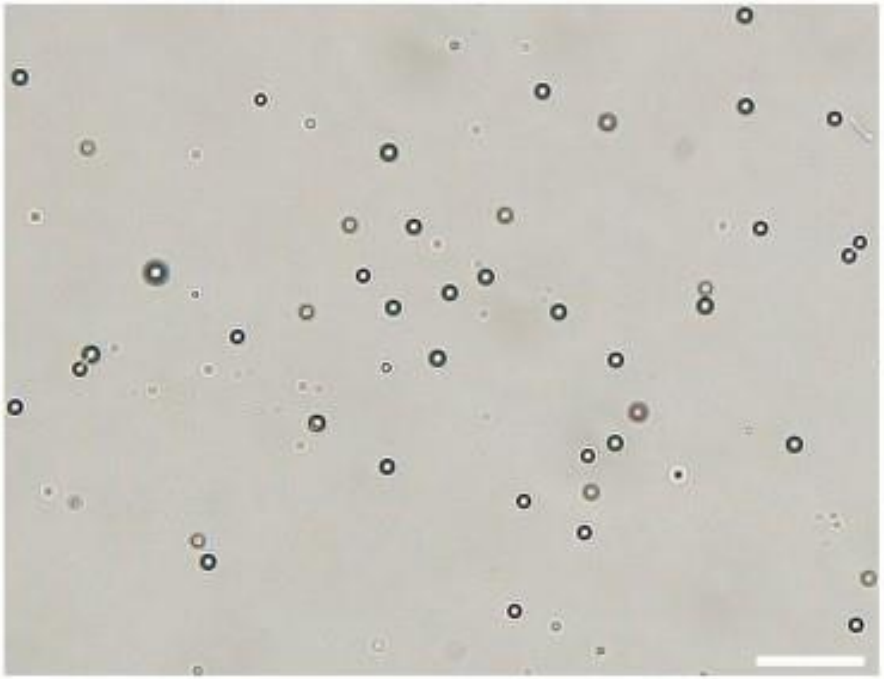
Local BBB Disruption

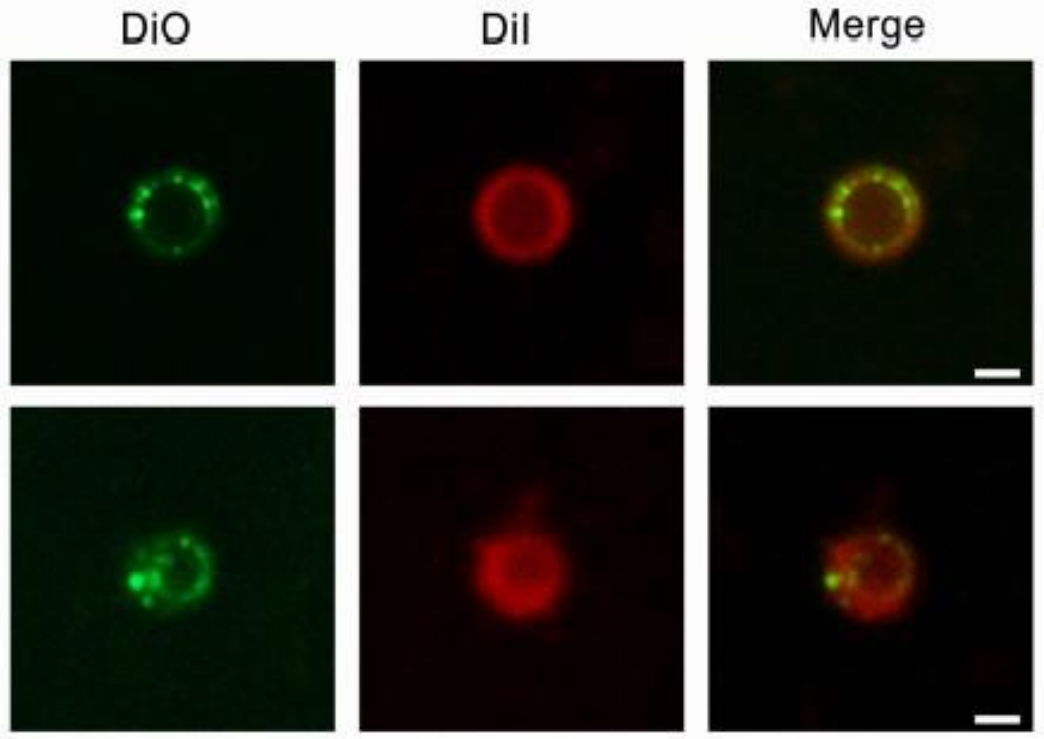
microscopy image of the MBs:

Density: $3.95 \pm 0.89 \times 10^9$ /mL,

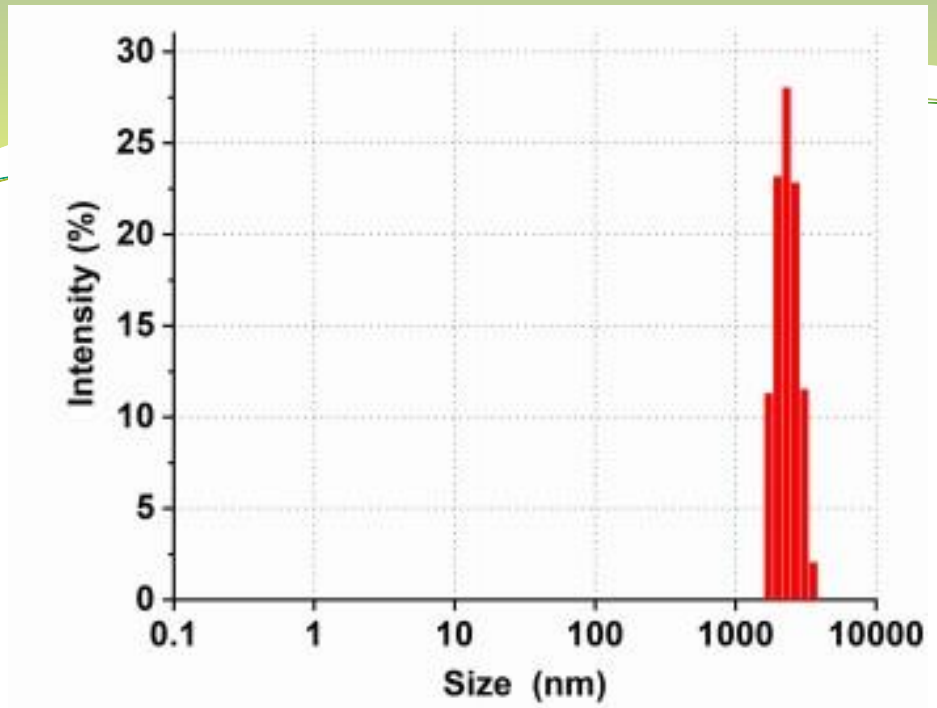
Diameter: 1845.2 ± 366.7 nm

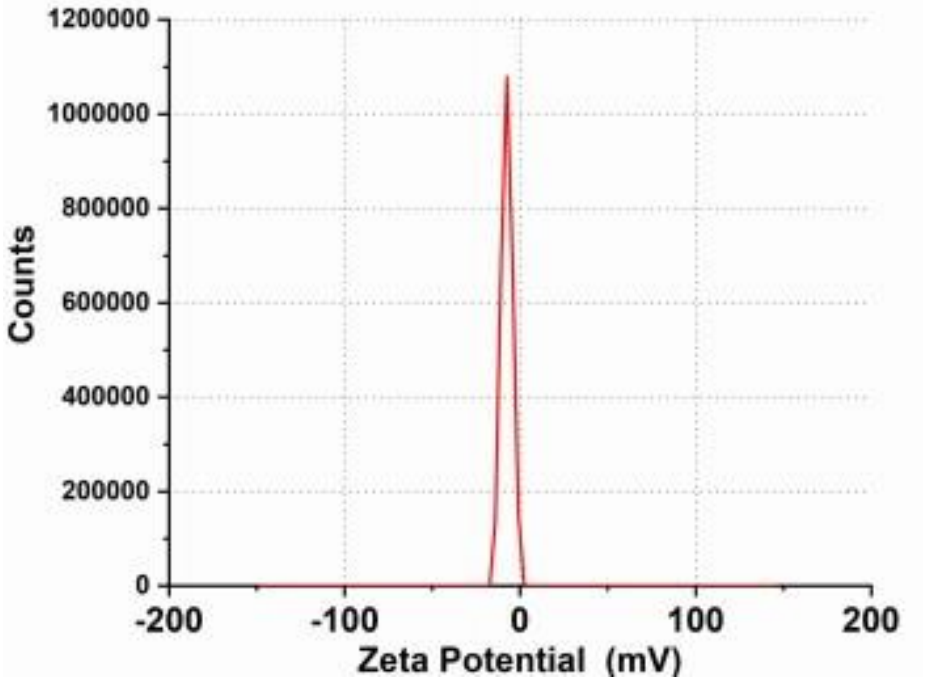
zeta potential: -16.2 ± 6.82 mV





Fluorescence image of MBs-LPHNs-cRGD complexes: illustrating that the LPHNs-cRGD adhered to the MBs' surface.





Particle size and zeta potential of MBs-LPHNs-cRGD complexes:

Diameter: 2197.35 ± 448.7 nm

zeta potential: -7.49± 3.14 mV

BBB-opening experiment in vivo : focused ultrasound intensity of 1.84 W

EB dye staining : to investigate the permeability of the BBB

H&E staining: to observe whether there were any damages to brain tissue

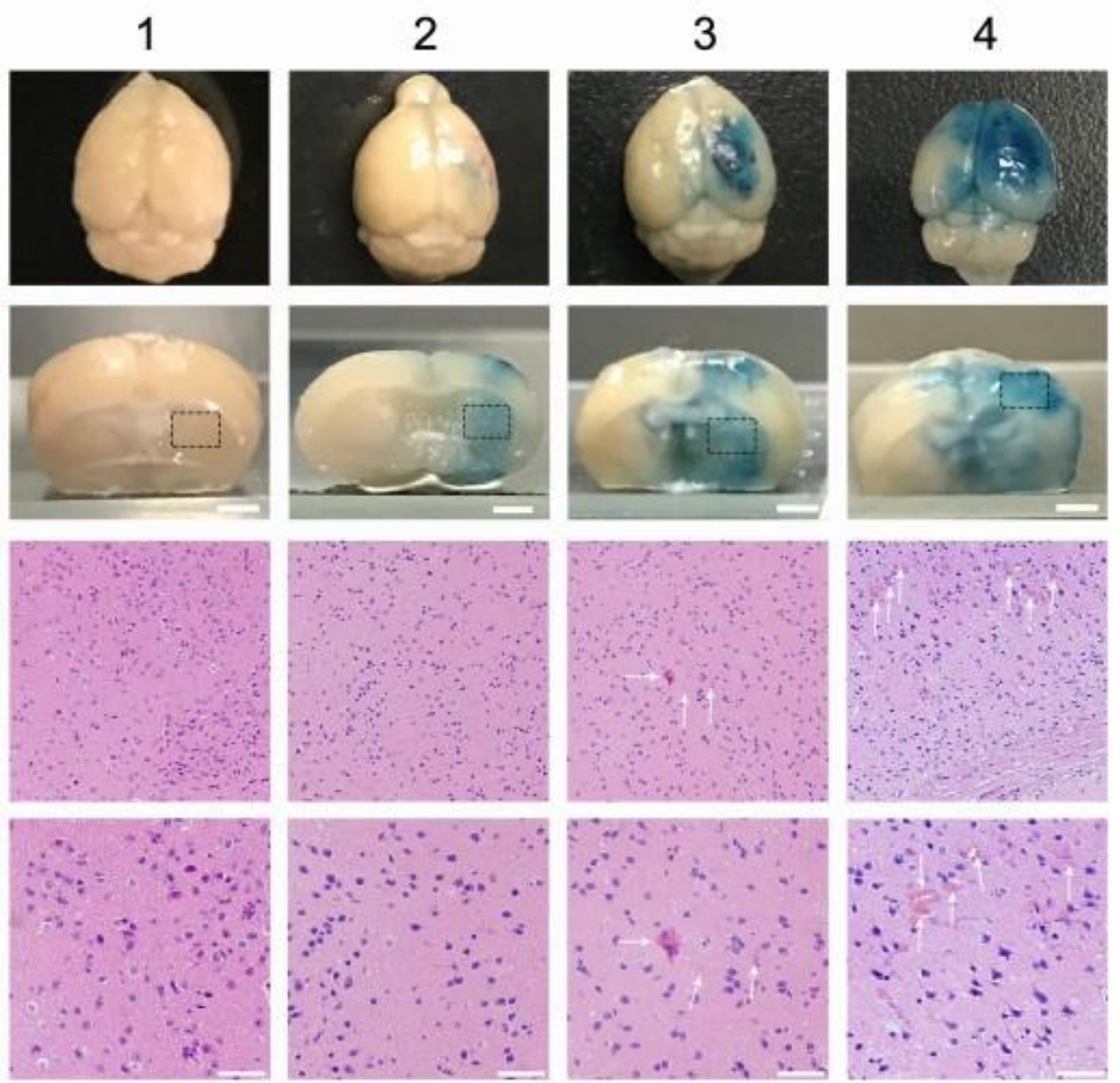
The FUS radiation time and amounts of MBs-LPHNs-cRGD:

3 min and 2 x 10^6

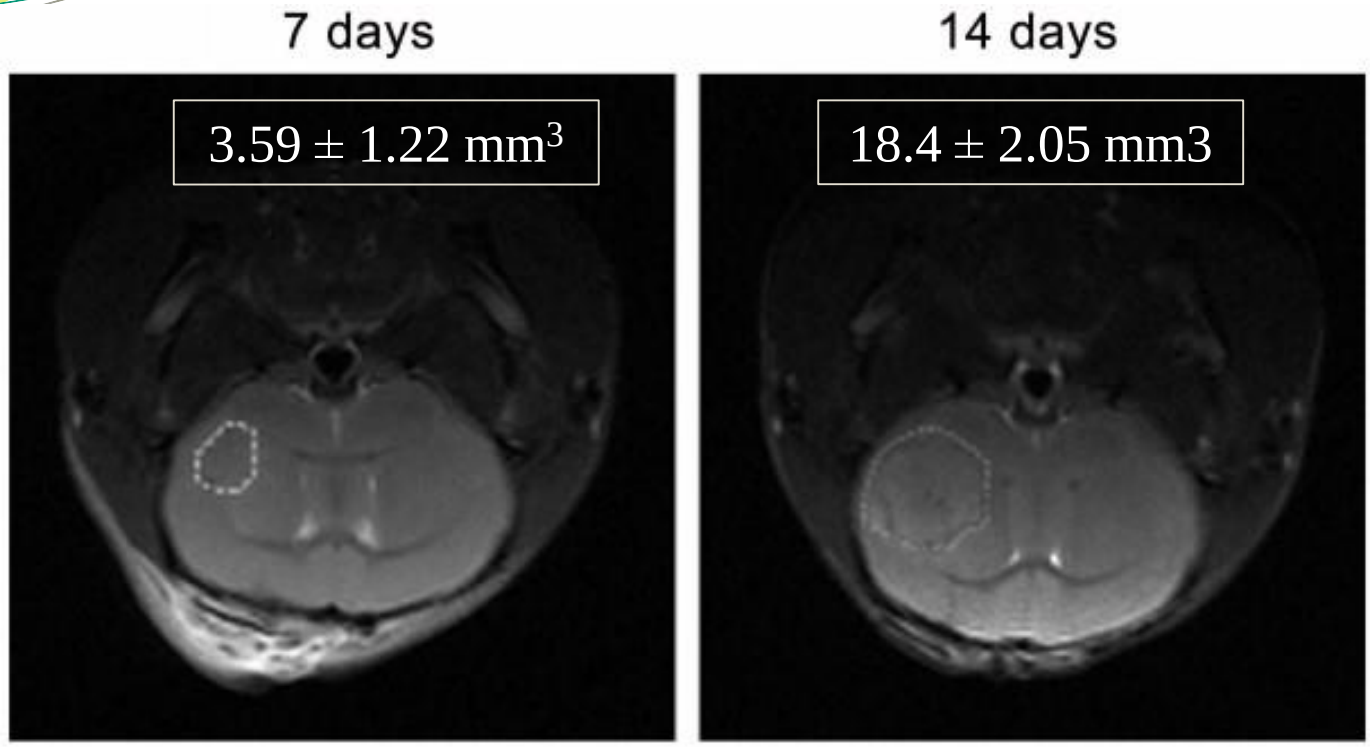
3 min and 4 x 10^6

5 min and 4 x 10^6

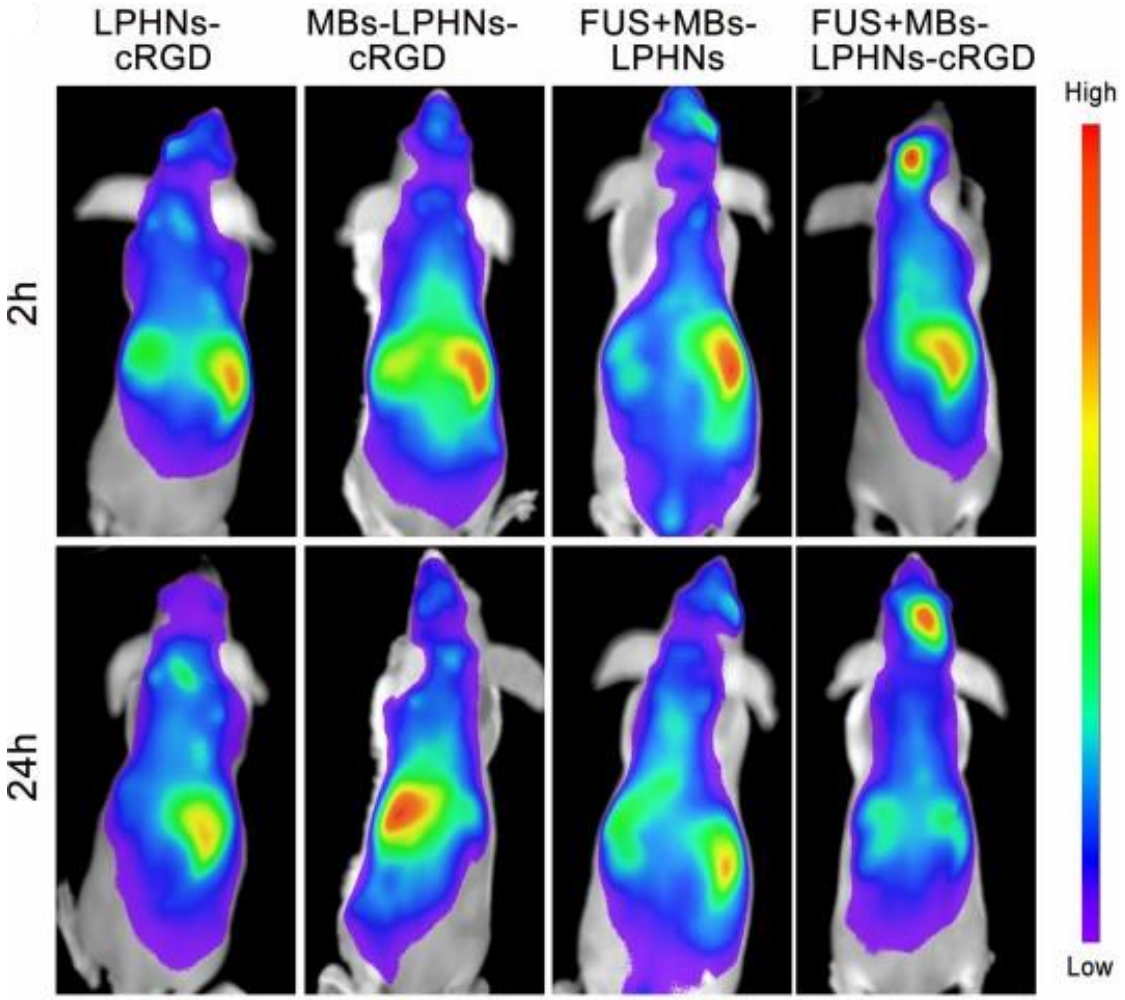
3 min and 8 x 10^6



Targeting Ability, Biodistribution, and Biosafety in vivo

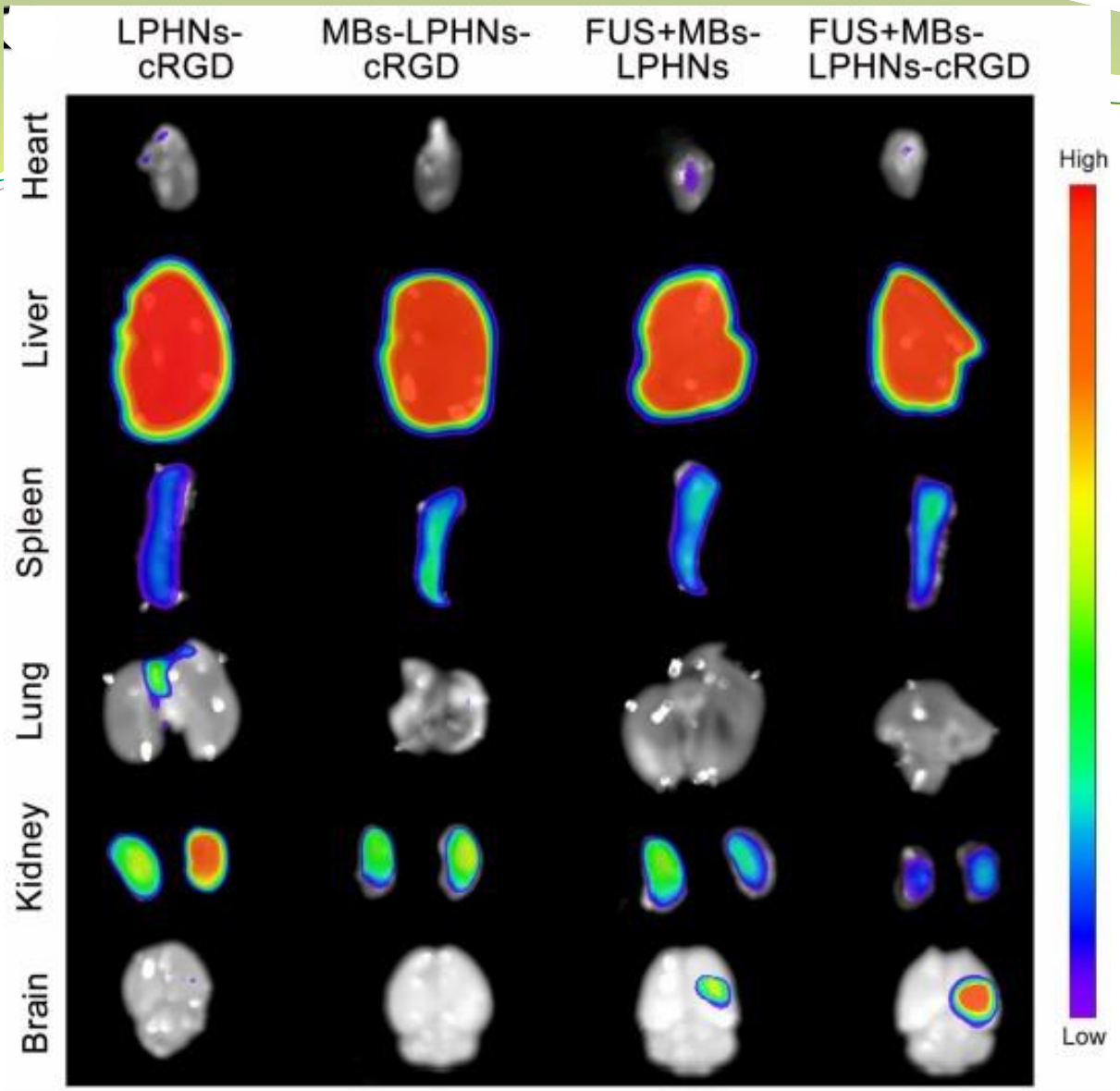


MRI images of orthotopic tumor
after implantation of T98G cells



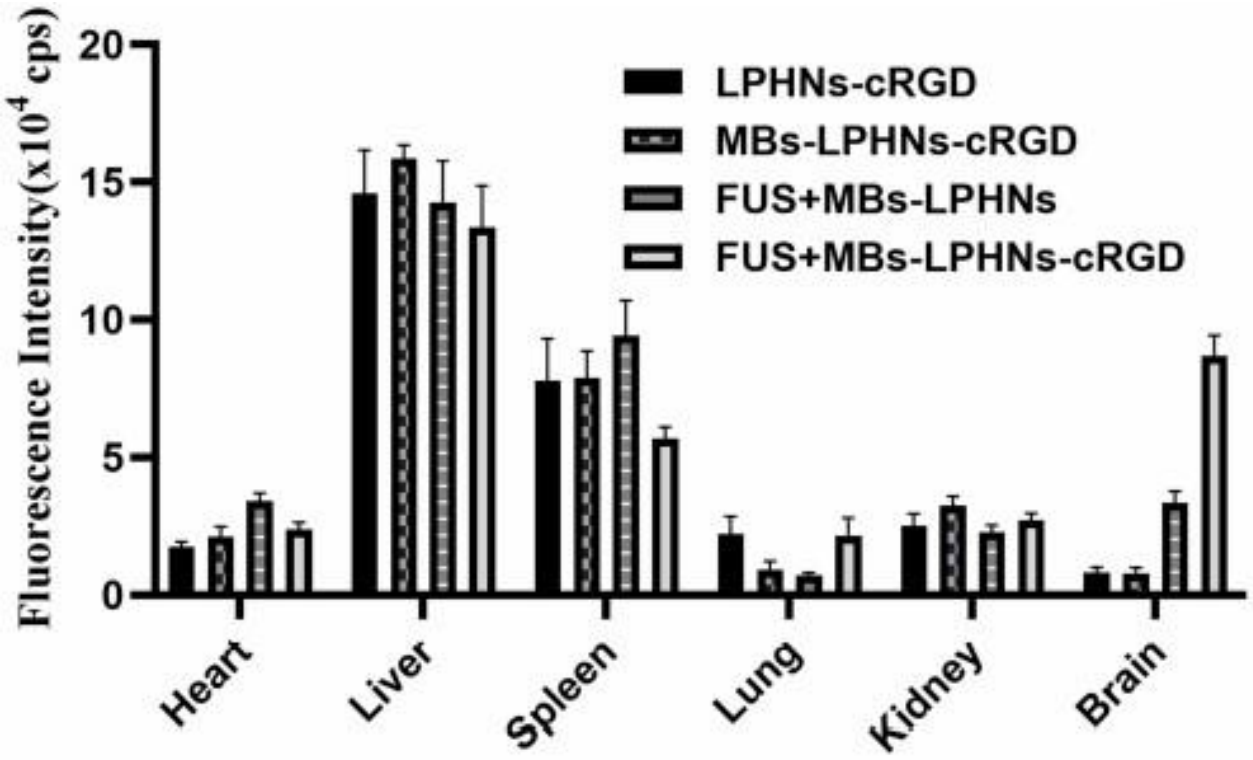
in vivo fluorescence imaging

significant fluorescence signal was observed in brain after treatment with the FUS-assisted MBs-LPHNs-cRGD complexes



ex vivo fluorescence images of major organs and brain of mice after 24 h of treatments

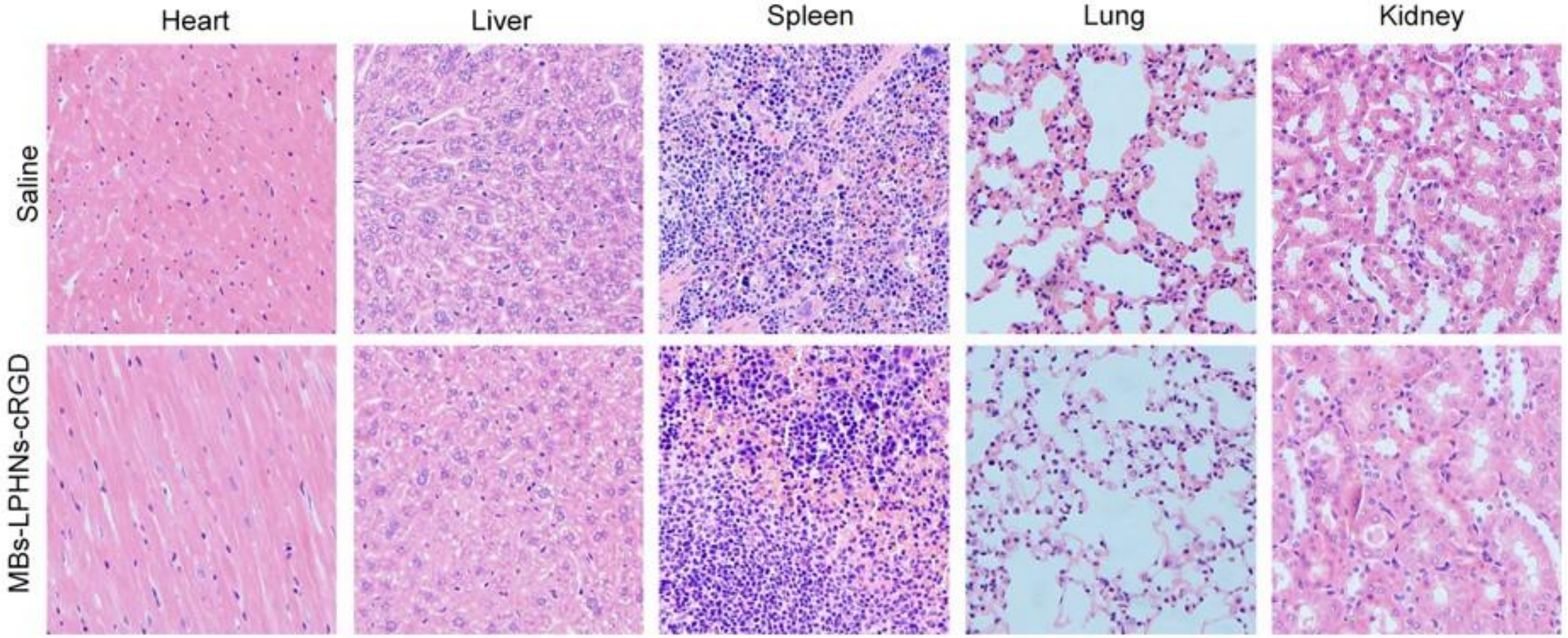
Corresponding quantitative analysis of fluorescence intensity in major organs (n=3)



FUS in combination with MBs can significantly promote nanoparticle delivery to the brain

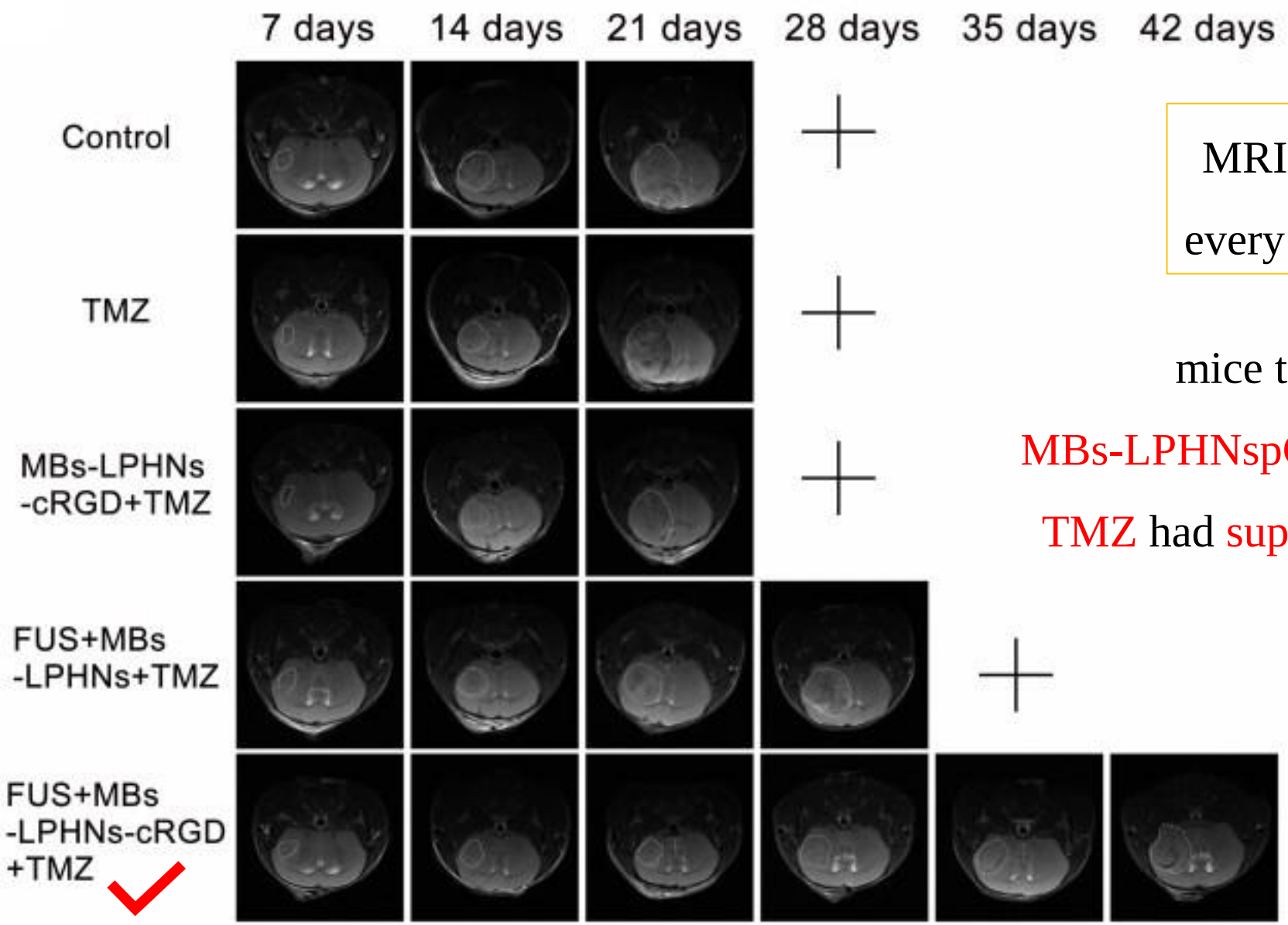
LPHNscRGD can exert an active targeting effect to further improve nanoparticle delivery in the tumor region in vivo

H&E staining of the major organs of mice 7 days after injection of MBs-LPHNs-cRGD and saline



no significant histological abnormalities between the two groups, indicating the high histocompatibility and biosafety of the nanoparticles in vivo

Therapeutic Effects of FUS-Assisted MBs-LPHNs-cRGD Complexes in vivo



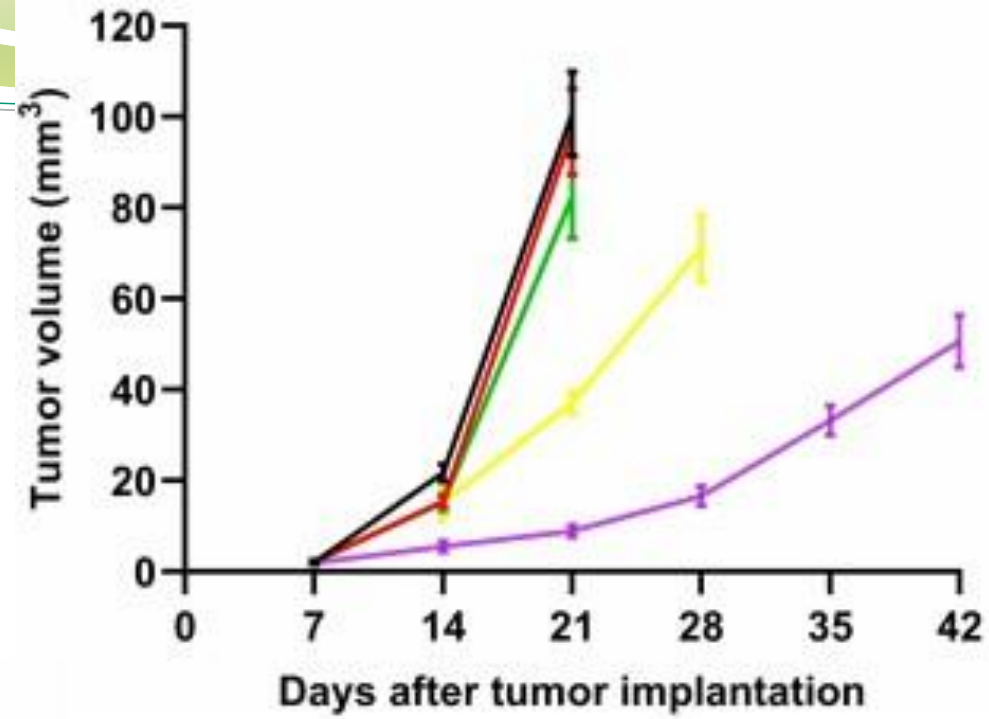
MRI images of tumors monitored every 7 days of different treatments

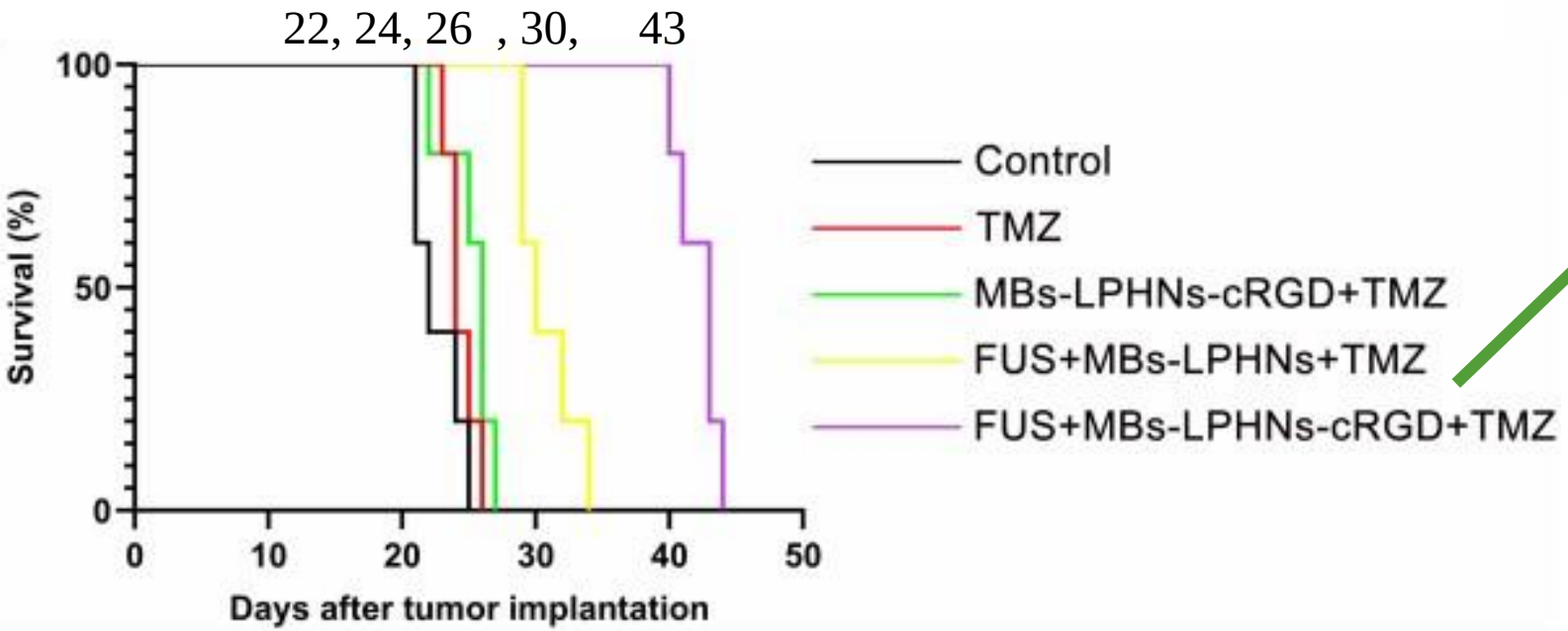
mice treated with FUS-assisted MBs-LPHNspCas9/MGMT-cRGD complexes + TMZ had suppressed tumor growth compared with other groups

FUS-assisted MBs-LPHNspCas9/MGMT-cRGD + TMZ:

tumor volume was smaller

At the time of death, the final tumor volume was also smaller





enhanced the therapeutic effect of TMZ in glioblastoma, inhibited the growth of glioblastoma, and prolonged survival in tumor bearing mice compared with other treatments

Discussion

- MGMT protein is an important factor in the resistance of tumor cells to alkylating agents (such as TMZ)
- TMZ induces the O6 -methylguanine (O6 -MeG) lesion in DNA,

→ resulting in cell cycle arrest and, eventually, apoptosis.

- MGMT can remove the methyl adducts from DNA and transfer it to the cysteine residue of the MGMT molecule

→ leading to the repair of DNA damage and irreversible inactivation of MGMT.

- Many studies have shown that downregulation of MGMT expression can re-sensitize GBM cells to TMZ.

In previous studies

- signaling pathway interference
- siRNA technology

✗ instability and short working times

In CRISPR/Cas9 system → Used dual-gRNA sequencesz → can recognize two adjacent regions of the target gene and remove large fragments of nucleotides between the two sites

we used the "all-in-one" plasmid system

- Cas9 expression cassette
- dual-gRNA expression cassettes

→ instead of Cas9 mRNA or the Cas9 protein/ gRNA ribonucleoprotein complexes (Cas9 RNPs). (mRNA- or RNP-based delivery method)

plasmid DNA: more stable and cost-effective, and has a more sustained gene expression.

✗ large size of the plasmid system: transfection efficiency and editing efficiency may be relatively low

LPHNs nanoparticles → powerful drug delivery vehicle → high structural integrity and stability, controlled drug release, high biocompatibility and bioavailability

In this study synthesized by nanoprecipitation → an easier and more effective alternative to emulsification-solvent-evaporation (ESE) and two-step methods.

PLGA, an FDA-approved polymer → used as the polymer core

DC-chol, a type of cationic lipid → used to adsorb the pCas9/MGMT by electrostatic action and encapsulate it within the polymer core

PEG chains conjugated to the lipid (DSPE) → enhance the steric stability

PEG chains conjugated to the lipid (DSPE) → prolong circulation time in vivo

cRGD peptide + DSPE-PEG → targets integrin αvβ3 receptors → To endow the delivery platform with active targeting ability

LPHNs-cRGD

- have relatively small particle size (179 nm)
- typical core-shell structure.
- preparation process does not affect the plasmid structure
- protect the plasmid against enzyme degradation
- ✗ But, the nanoparticles have a relatively wide size distribution

some strategies have been used to improve the size homogeneity of the LPHNs
- optimizing formulation parameter
- microfluidic nanoprecipitation process

FUS + MBs → plays an important role in drug delivery in the CNS.

LPHNs-cRGD + MBs → to obtain effective nanoparticle delivery

FUS irradiation → triggers MBs' vibrations → (BBB-opening) →

separation of LPHNs-cRGD from MBs → enter the brain and target tumor cells via cRGD-αvβ3 reaction

FUS-MBs → the duration, burst length, pulse frequency of FUS, and the composition, size, concentration, dose of MBs and so on → should be carefully adjusted to obtain safe and effective BBB-opening

Tumors in the treatment group were not completely eliminated during the course of treatment. → possibly because the CRISPR/Cas9 system cannot effectively edit genes in all GBM cells → due to the relatively low transfection & editing efficiency

the presence of glioma stem cells (GSCs) and genotype heterogeneity → could protect part of the GBM population from treatment →

These residual tumor cells can continue to proliferate, leading to tumor recurrence

TMZ-resistance in glioblastoma → complicated process → whose mechanism has not been fully elucidated

- MGMT: one of the many important factors
- excision repair (BER)
- DNA mismatch repair (MMR) system

In the future study

CRISPR/Cas9 system can be optimized to achieve an enhanced gene editing efficiency

several gRNAs that target key proteins in the TMZ-resistance pathway can be co-encapsulated in LPHNs-cRGD

We will also explore the potential off-target effects of CRISPR/Cas9 → one of the main concerns in the clinical application of this system

Recently, many efforts have been made to improve the specificity and reduce the off-target effects of the CRISPR/Cas9 system

↓

dCas9-FokI, SpCas9-HF1 and truncated guide RNAs

With these modified strategies, we hope that the FUS-assisted MBs-LPHNs-cRGD delivery system can significantly improve the specificity and reduce the off-target effects of CRISPR/Cas9 in glioblastoma treatment.


Conclusion

- developed a multi-functional (MBsLPHNs-cRGD complexes) CRISPR/Cas9 delivery system
- demonstrated the inhibition effect on the tumor growth in an orthotopic model of glioblastoma with FUS-assisted BBB-opening.
- LPHNs-cRGD
  - could efficiently deliver CRISPR/Cas9 plasmids into GBM cells
  - and downregulate the expression of MGMT
  - resulting in an increased sensitivity of the cells to TMZ
- FUS-MBs parameters were evaluated for effective BBB-opening to ensure biosafety
- FUS-assisted MBs-LPHNspCas9/MGMT-cRGD + TMZ treatment: efficiently inhibited tumor growth and prolonged survival in tumor-bearing mice.

- biotoxicity study { delivery platform had high biocompatibility and biosafety
  which indicates its potential use in further clinical applications
- the synergistic targeting ability of the physically site-specific characteristics of FUS and the biologically active targeting ability of cRGD peptide significantly improve nanoparticle delivery into the CNS
- This study provides a promising strategy for drug-resistant glioblastoma treatment via a safe, effective and targeted CRISPR/Cas9 delivery system
- further illustrates the therapeutic applications of FUS-assisted BBB-opening in CNS diseases


THANK YOU
FOR YOUR
ATTENTION