Preparation of Chitosan-based Nanoparticles for Bioactive agent Delivery in Nerve Tissue

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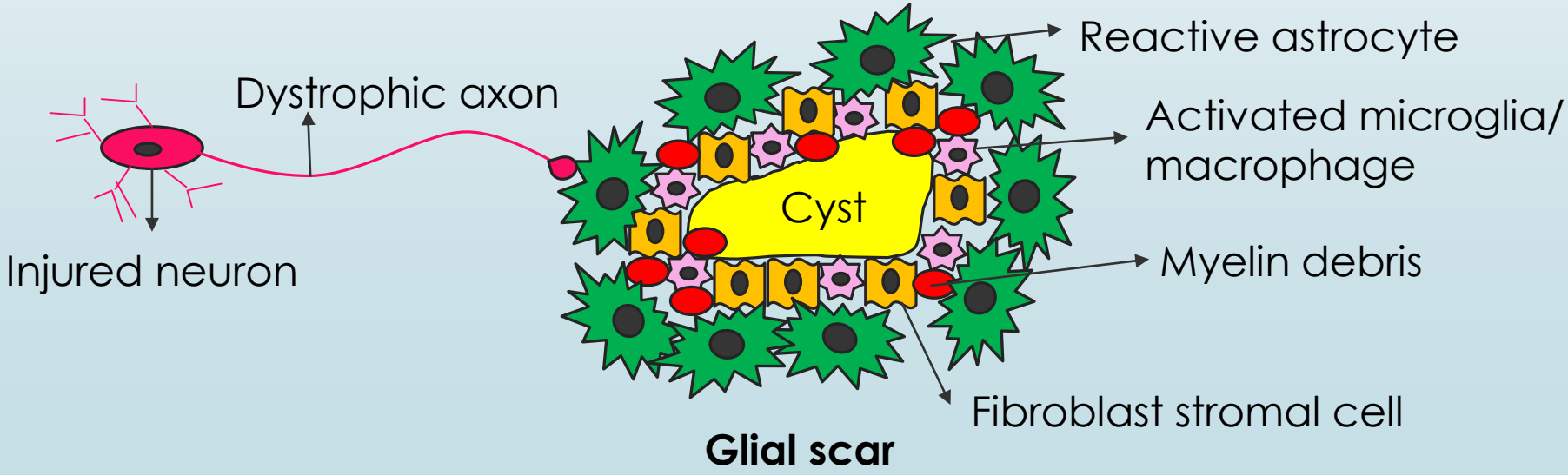
Outlines

- Introduction
- Significance of research
- Materials and methods
- Characterization
- Results and discussion
- Conclusion
- Future works

Introduction

- Central Nervous System (CNS) Injury
  - Damage to the nerve fibers
  - Activation of neurons and glia cells
  - Changing the morphology and molecular expression
  - Producing inhibitors of axonal regeneration
  - Glial scar formation



Significance of research

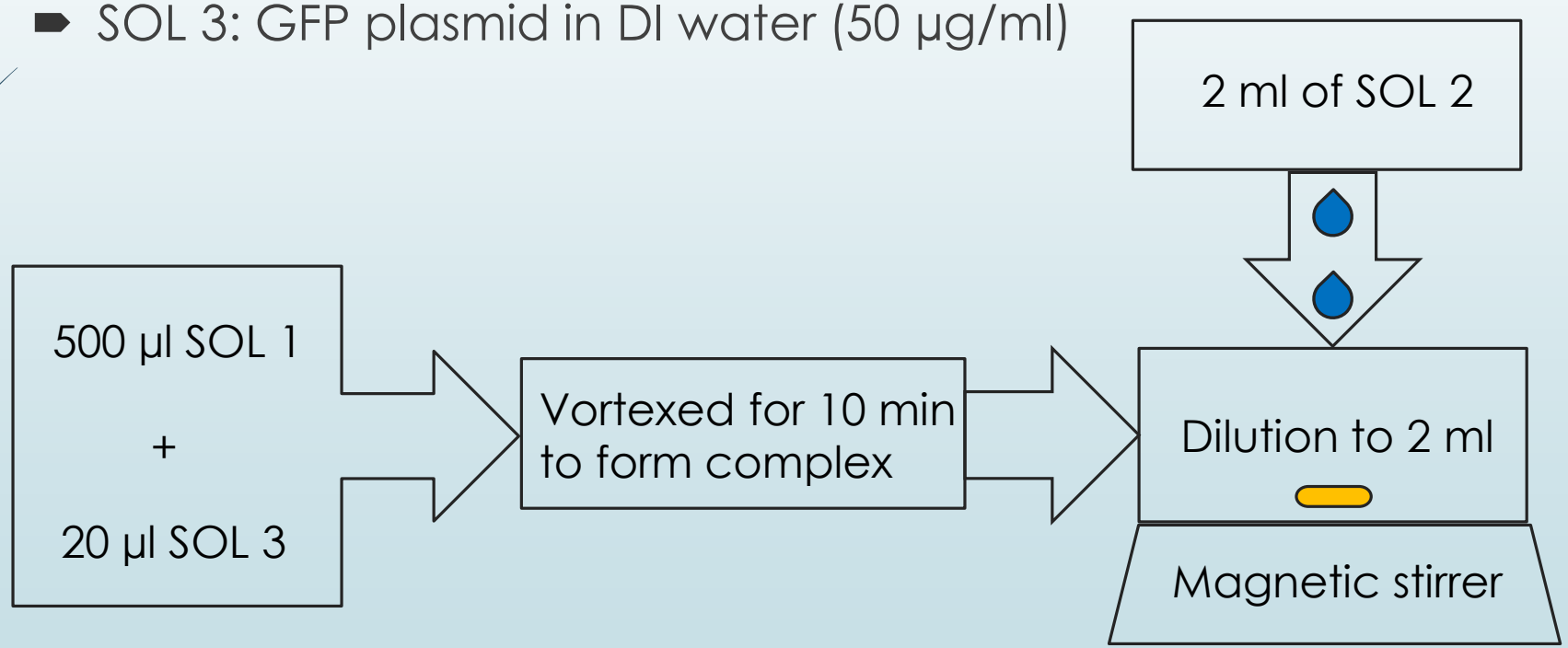
- Therapeutic approaches after CNS injury
  - Surgery
  - Cell therapy
  - Biomaterial scaffolds
  - **Molecular therapy**
    - Naked bioactive agents
    - **Loaded bioactive agents**
      - Viral vectors
      - **Polymeric nanoparticles**
      - liposomes

Materials

- Medium molecular weight chitosan (MMW CS)
  - MW: 390 kDa
  - DD: 75-85%
- Polyacrylic acid (PAA)
  - MW: 450 kDa
  - Purity: 99.5%
- Hyaluronic acid (HA)
  - MW: 750 KDa
- Green fluorescent protein (GFP) plasmid
  - MW: 26.9 kDa (7829 bp)

Methods

- SOL 1: CS stock solution in acidic media (0.1% w/v, 50°C, pH: 5.5)
- SOL 2: PAA/HA stock solution in deionized (DI) water (0.1, 1 and 2 mg/ml)
- SOL 3: GFP plasmid in DI water (50 µg/ml)



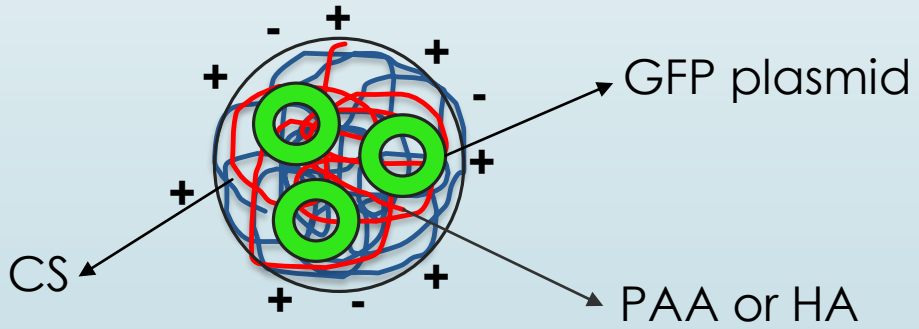
Methods

Formation of ionically gelated nanoparticles

Centrifugation at 12000 rpm under 4°C for 1h

3 times washing with DI water

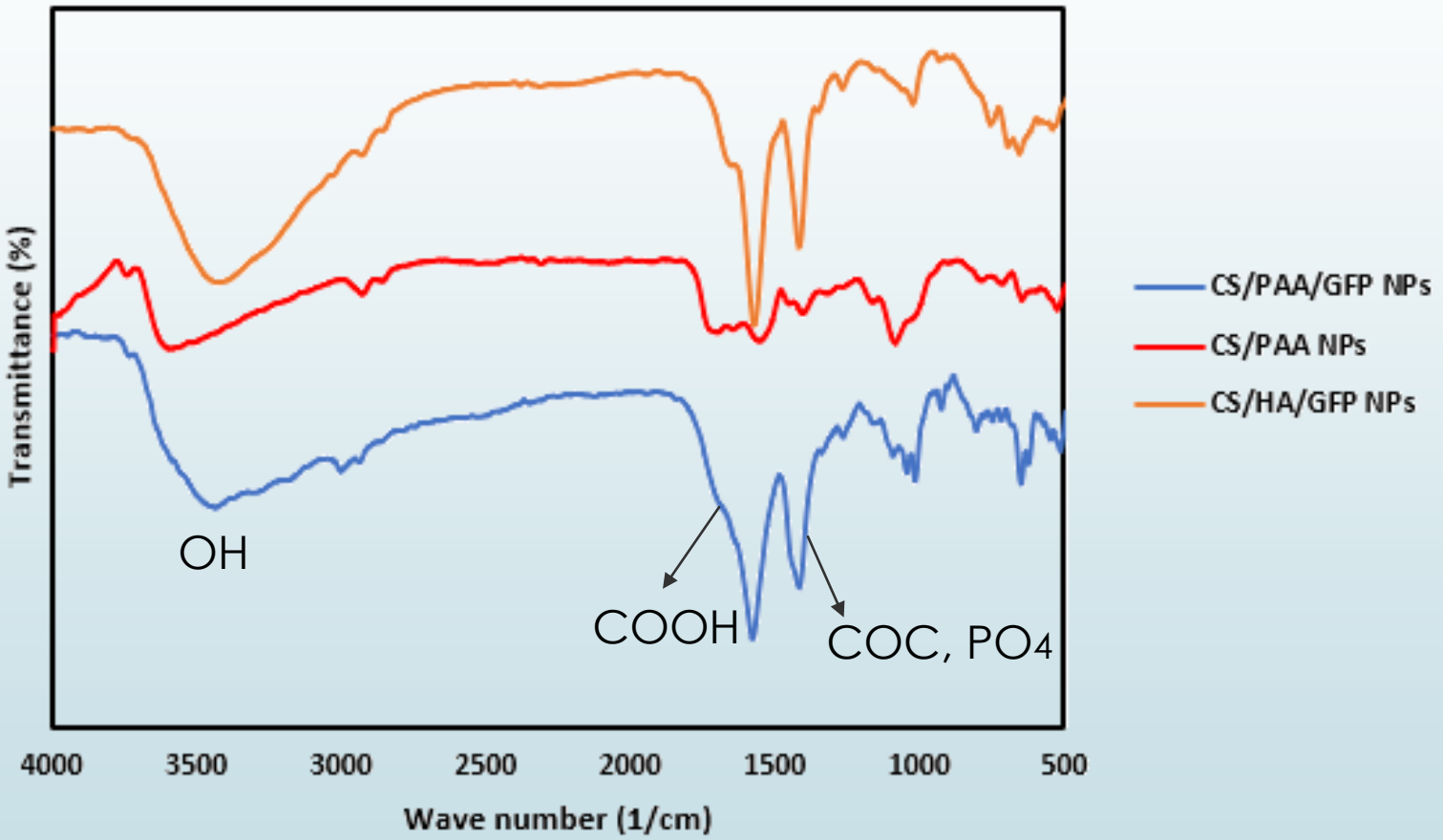
Drying in vacuum oven at 30°C for 24h



Characterization

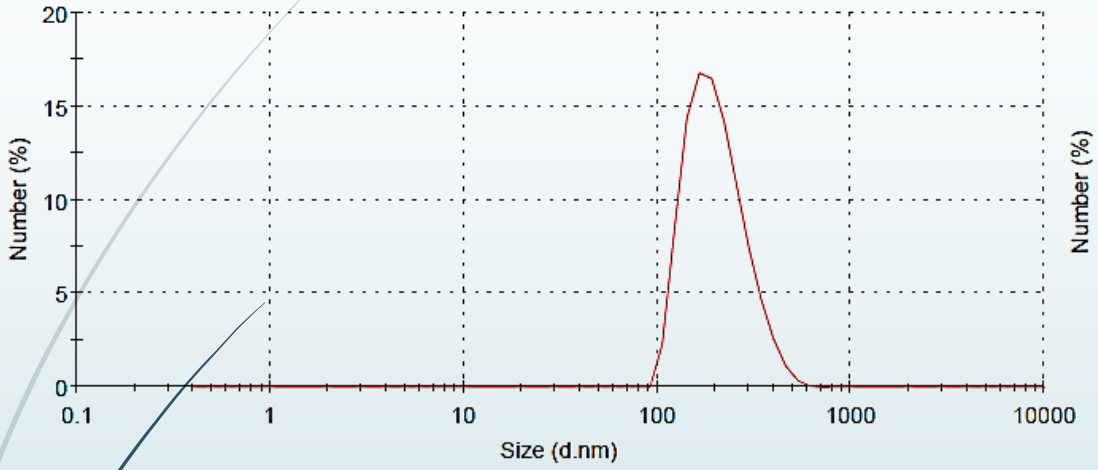
- Fourier-transform infrared (FTIR) spectroscopy
- Dynamic light scattering (DLS)
- Zeta potential
- Biological assays

Results of FTIR spectroscopy

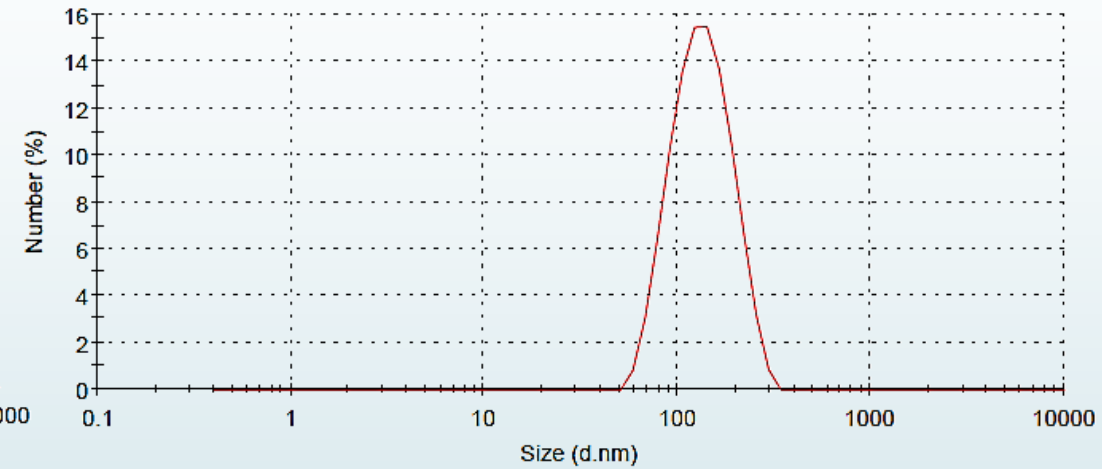


Results of DLS and zeta potential

CS/HA/GFP NPs



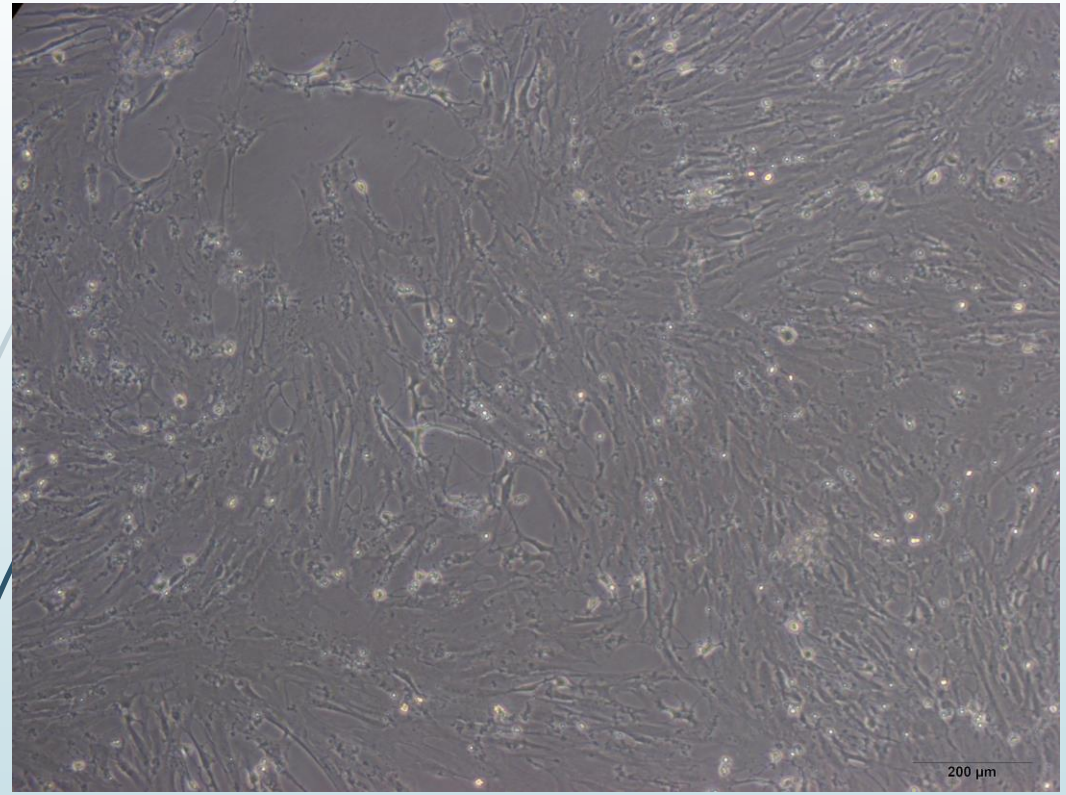
CS/PAA/GFP NPs



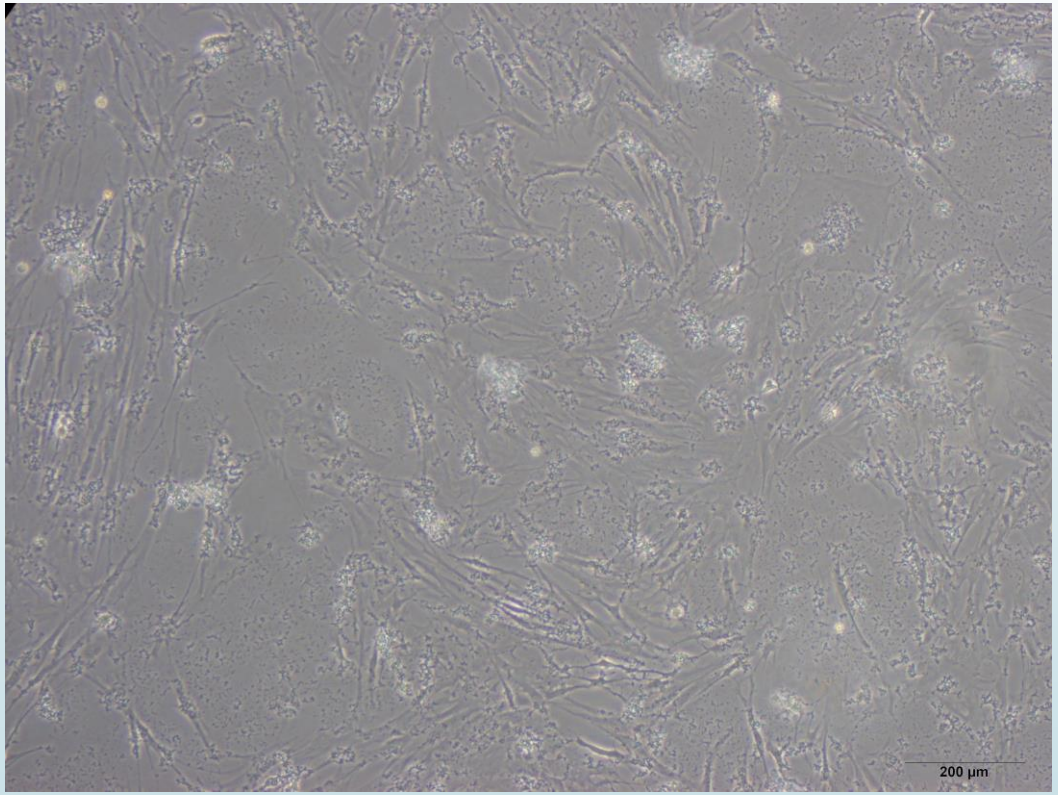
Types of NPs	PAA concentration (mg/ml)	Mean diameter (nm)	PDI	Zeta potential (mV)
CS/PAA	0.1	45.3	0.316	-1.2
CS/PAA	1	74	0.705	NA
CS/PAA	2	84.8	0.210	NA
CS/PAA/GFP	0.1	140	0.238	+26
CS/HA	0.1	182	0.345	NA
CS/HA	1	206	0.169	NA
CS/HA	2	292	0.396	NA
CS/HA/GFP	0.1	208	0.118	+4.4

Biological assay: cell uptake

- Cell culturing: activated cells of rat cerebral cortex



(A)100 µl of CS/PAA/GFP NPs with concentration of 1 µg/ml



(B) 100 µl of CS/HA/GFP NPs with concentration of 1 µg/ml

Conclusions

- Optimized GFP loaded CS/PAA and CS/HA NPs with 140 and 208 nm size and +26 mV surface charge
- Successful transfection of NPs into the activated cells of rat cerebral cortex *in vitro*
- Showing no green fluorescence property due to the low concentration of GFP plasmids
- CS-based NPs as effective carriers for transfection into activated cells in neural injuries

Future works

- Using higher concentrations of GFP plasmid to express higher fluorescence intensity
- Using another cargo for tracking cell uptake of NPs
- Applying the most optimized nanocarriers in animal model of nerve injury
- Employing biocompatible CS-based NPs for further gene delivery applications in nerve injury

Thanks for your kind attention

Any questions?