Corona of biologically synthesized nanoparticle provide better interface to trap monomeric α-synuclein in non-cytotoxic amorphous aggregate structure

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Abstract

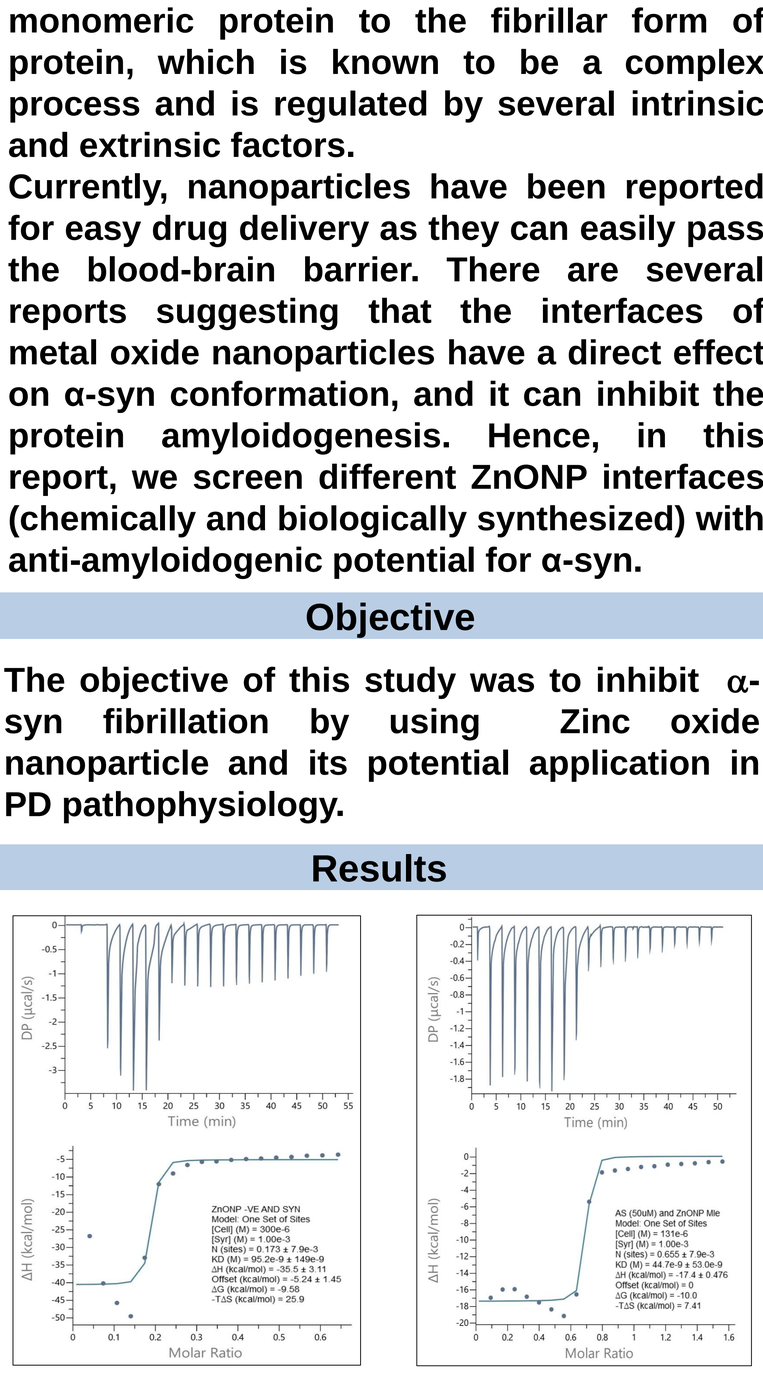
Parkinson's disease (PD) is a progressive neurodegenerative disorder, characterized by the degeneration of dopaminergic neurons in the substantia nigra and the abnormal accumulation of Alpha-synuclein (α-syn) aggregates or Lewy bodies, leading to a major loss of motor function. α-Syn is an intrinsically disordered protein, involved in vesicular trafficking and neurotransmitter release. The aggregation of α-syn involves a cascade of structural transition from monomeric protein to the fibrillar form of protein, which is known to be a complex process and is regulated by several intrinsic and extrinsic factors.

Currently, nanoparticles have been reported for easy drug delivery as they can easily pass the blood-brain barrier. There are several reports suggesting that the interfaces of metal oxide nanoparticles have a direct effect on α-syn conformation, and it can inhibit the protein amyloidogenesis. Hence, in this report, we screen different ZnONP interfaces (chemically and biologically synthesized) with anti-amyloidogenic potential for α-syn.

Objective

The objective of this study was to inhibit α-syn fibrillation by using Zinc oxide nanoparticle and its potential application in PD pathophysiology.

Results

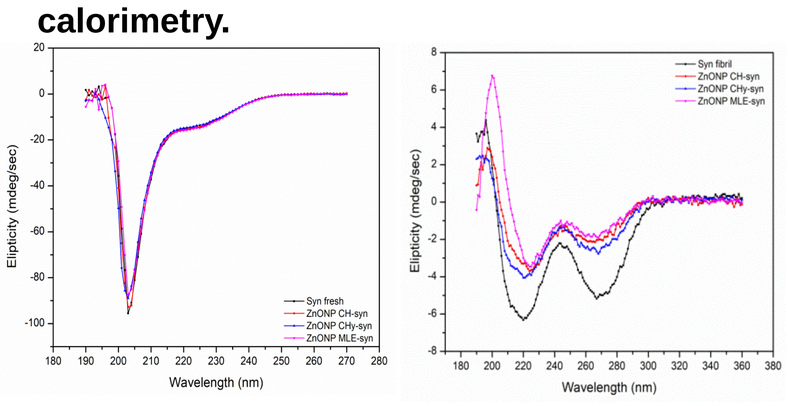


(a) (b)

Figure 1 Thermograms showing isothermal titration curves for 32µg/mL ZnONPs (synthesized a. chemically and b. biologically) titrated with 50 µM a-syn at 25^{0}C.

Parameters	ZnONP MLE	ZnONP CH
Enthalpy change (ΔH), Cal/mol	-35.5	-17.4
Entropy change (-TΔS), Cal/mol/deg	25.9	7.41
Free energy change (ΔG), Cal/mol	-9.58	-10

Table 1. Thermodynamic parameters of α-synuclein and ZnONPs interaction as analyzed by isothermal titration calorimetry.



(a) (b)

Figure 2. Far-UV spectra of α-synuclein incubated with ZnONP (ZnONP CH, ZnONP CHy, ZnONP MLE), (a) freshly prepared sample, (b) 5 days incubated sample.

Results cont....

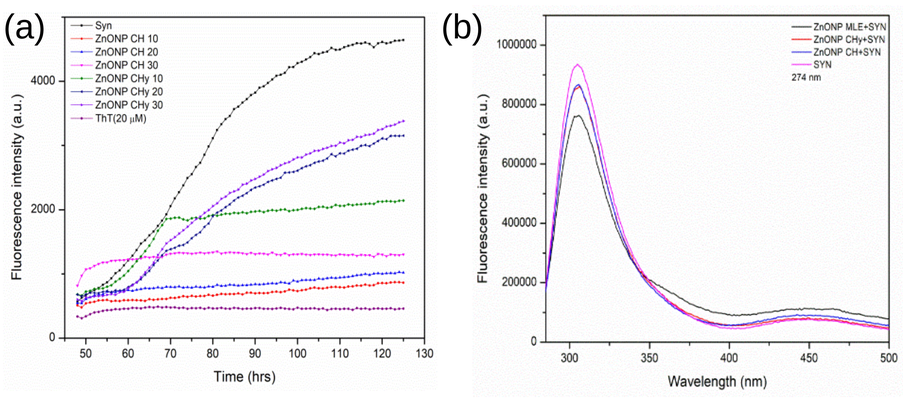
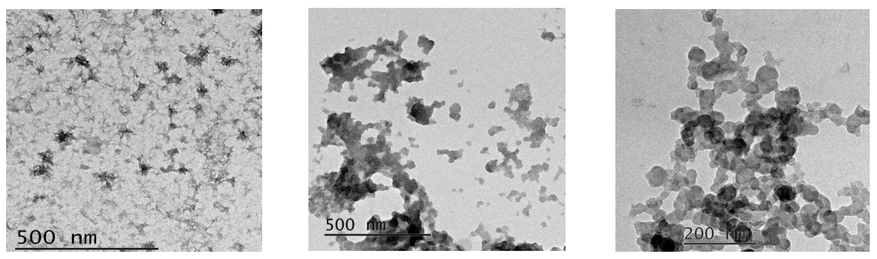
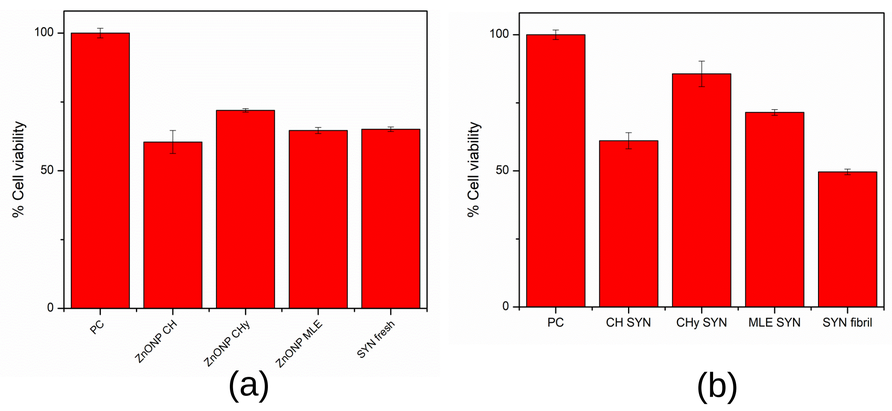


Figure 3. ThT binding assay profile of (a) 50 µM α-synuclein in absence and presence of increasing concentration of ZnONPs, ThT was excited at 440 nm, and the emission was collected at 490 nm for a period of 50–120 h, (b) Intrinsic fluorescence spectra of α-synuclein incubated with 30 µg/mL ZnONPs at 274 nm.



(a) (b) (c)

Figure 4. TEM micrograph of (a) 50 µM α-synuclein (b) 50µM α-synuclein in presence of 30 µg/mL ZnONP (c) only ZnONPs. All the samples were incubated for 120 hrs.



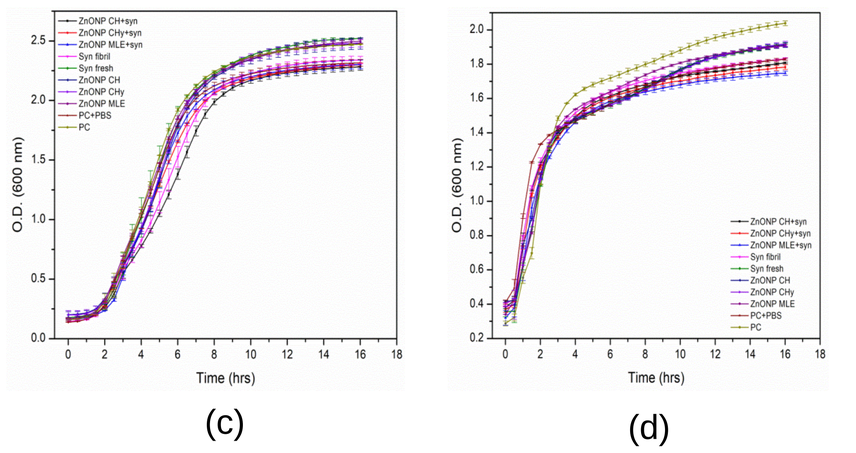


Figure 5. Alamar Blue dye reduction assay of SHSY-5Y, neuroblastoma cell line treated with (a) ZnONPs (CH, CHy, MLE), α-syn monomer, (b) ZnONPs and α-syn (50 µM) agglomerates and α-syn fibril, (c, d) growth kinetics of *L. plantarum* and *E. coli* in presence of α-syn and ZnONPs agglomerates respectively.

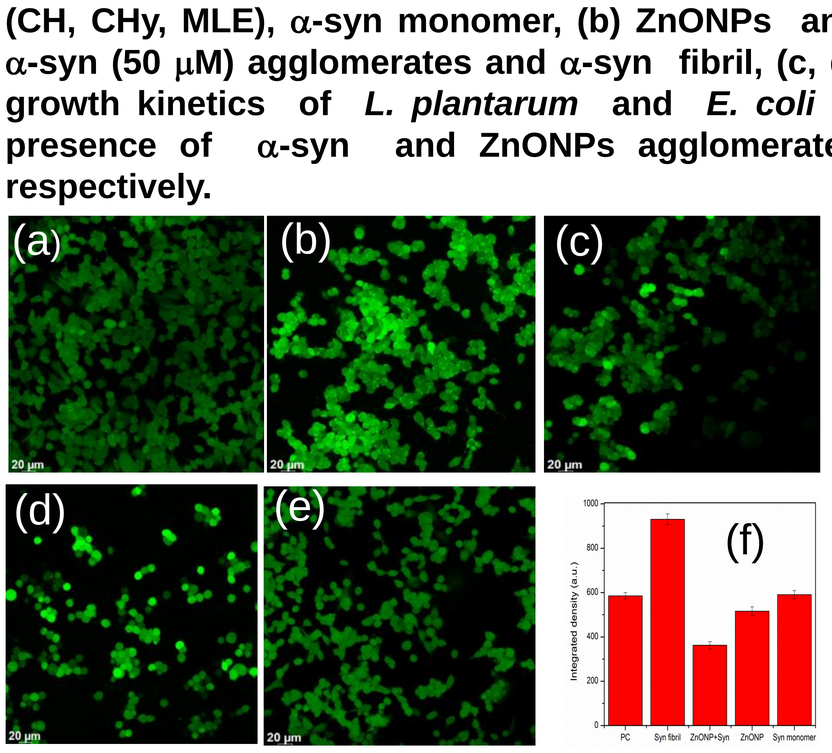


Figure 6. Confocal imaging of SHSY-5Y cell line treated with (a) control, (b) α-syn fibril, (c) α-syn (50µM)+ ZnONP (30 µg/mL), (d) ZnONP (30 µg/mL), (e) α-syn monomer, (f) statistical representation of all the images.

Discussion

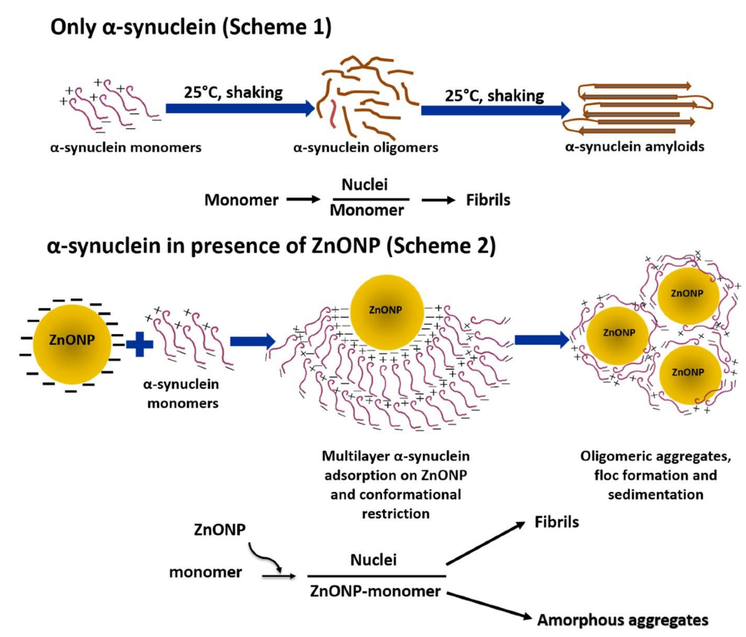


Figure 7. Schematic representation of α-synuclein adsorbed onto ZnONP interface and kinetically trapped into amorphous aggregates, which did not convert into amyloid fibrils within the studied time frame.

Inferences

- **This study shows how effectively ZnONPs retard aggregation and fibrillation of α-syn.**
- **Biophysical studies confirmed that multi-layered adsorption of α-syn monomer onto the surface of the ZnONPs leads to formation of amorphous agglomerate-like structure.**
- **Biologically synthesized ZnONPs provide better interface to trap monomeric α-syn into flocs like structure as compared to the bare ZnONPs.**
- **Altogether, this amorphous agglomerates is relatively cytocompatible to the neuronal cell as compared to the α-syn fibril.**

Reference

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