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Polymeric nanoparticles for targeted gene delivery for CMT disease

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Abstract: The synthesis of PVA-modified PLGA nanoparticles containing plasmid DNA of medical importance was studied in our Laboratory, as PLGA is one of the most commonly and effectively used biodegradable biopolymer in the development of nanocarriers targeting CMT disease. Specifically, plasmid pDNA was encapsulated in PLGA nanoparticles and the latter were conjugated to a peptide used as a "guiding rod" for recognition by a specific receptor on the surface of a neuronal cell. This receptor is TMPRSS5 (Transmembrane protease serine 5), a protein which is expressed preferentially in Schwann cells compared to different tissue cells.[1,2] Following the design and assembly of the nanoparticle, physicochemical characterization was pursued using UV-Visible spectroscopy for plasmid encapsulation, Fluorescence, Fourier Transform Infrared Spectroscopy (FT-IR), Dynamic Light Scattering (DLS), particle size and shape analysis , and SEM.

Introduction

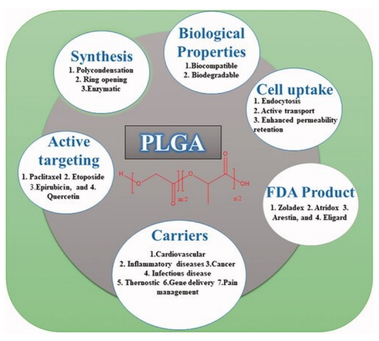


Fig. 1: PLGA applications[4]

In recent years, nanoparticles (NPs) have become an extremely attractive choice in the fields of biology and medicine as shown in **Fig. 1**. Their main advantage is the encapsulation and targeted delivery of various drugs and macromolecules, which collectively make it suitable for Charcot–Marie–Tooth (CMT) disease therapeutics. Taking into consideration the nature of the therapeutic plasmid, an appropriately PVA-modified PLGA (poly(lactic-co-glycolic acid)) NP could meet all necessary requirements and exhibit bioactivity. PLGA is one of the most commonly and effectively used biodegradable polymers for the development of nanocarriers. PLGA NP atoxicity is attributed to the hydrolysis of lactic and glycolic acid components in the human body, easily metabolized via the Krebs cycle.[3] The goal of constructing well-defined PLGA NPs for transport and delivery of the genetic material to the target neurons is shown in **Fig. 2**.

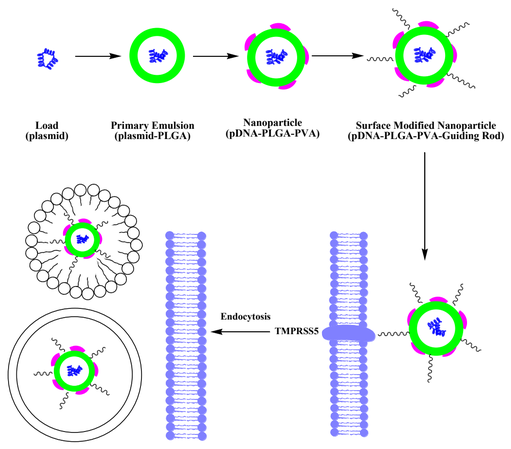
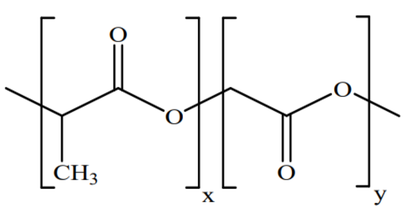


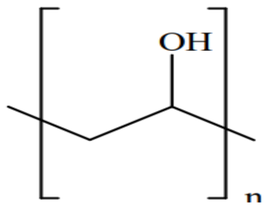
Fig. 2: Synthesis of NPs and pDNA delivery

Materials and methods

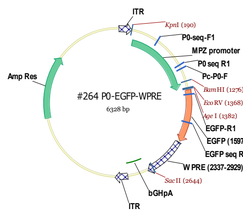
Materials: PLGA, PVA, pDNA (264-PO-EGFP-WPRE), EDC/NHS, Gln-Ala-Arg-AFC-TFA salt
Preparation technique: Double emulsification, solvent evaporation, 25 °C and 3 h.
Physicochemical Characterization: Fourier Transform Infrared Spectroscopy (FT-IR), UV-Visible, Scanning Electron Microscope (SEM), Fluorescence, Dynamic light scattering (DLS)



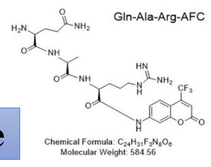
PLGA



PVA

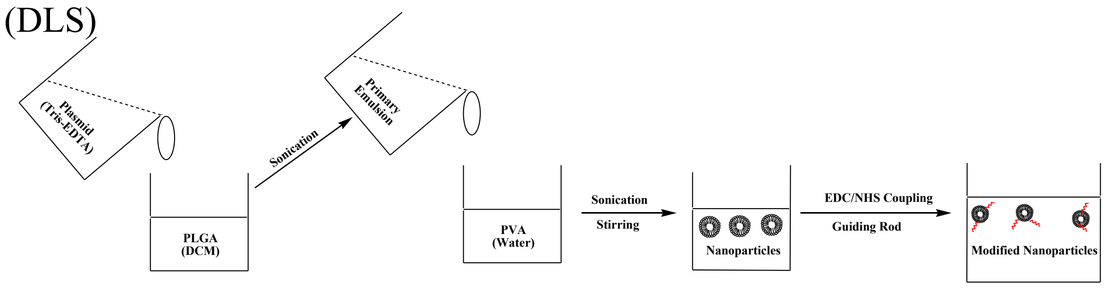


pDNA



Peptide

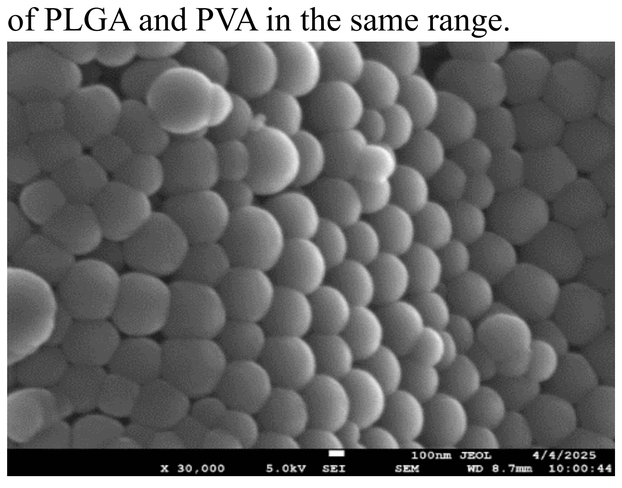
Synthesis of PLGA NPs

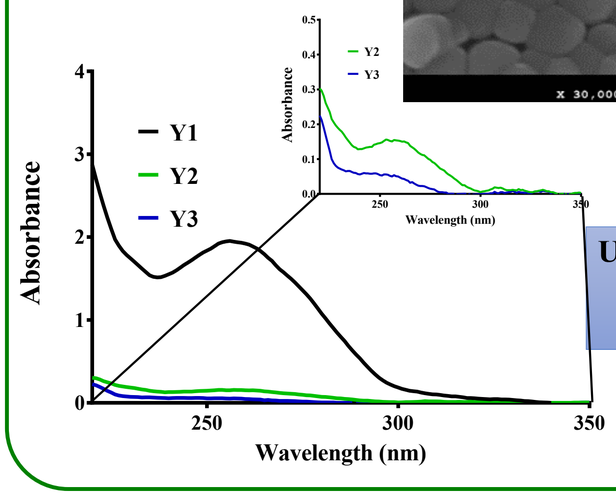


Results and discussion

The encapsulation efficiency of PLGA nanoparticles was examined through UV-Visible spectrophotometry and showed an encapsulation of ~30-40%. From fluorescent measurements, it is evident that the peptide conjugate is ~66% on the surface of the nanoparticles. Also, PLGA nanoparticles with no peptide and pDNA were examined through DLS, with the median hydrodynamic dimension ±SD (Standard Deviation) being 302±3 nm. Surface potential and mobility were measured using ζ-Potential. The mean mobility of the particles ±SD is –0.59±0.04 (μ/s)/(V/cm), with the mean surface charge being –7.53±0.45 mV. Also, the final nanoparticle morphology and surface properties in the solid-state were evaluated using SEM microscopy. The NPs appear to be smooth and spherical, with a size below 400 nm. The PLGA particle FT-IR spectrum shows characteristics peaks of PLGA and PVA in the same range.

SEM of Final NPs





UV-Visible spectrum from the supernatants of the NPs after three washes

FT-IR

DLS and ζ-potential of the PLGA NPs

Conclusions

- PVA-modified PLGA nanoparticles with conjugated peptide and encapsulated plasmid DNA have been synthesized through a double emulsification solvent evaporation method and characterized in our Lab.
- A medium encapsulation efficiency of PDNA was achieved, along with a good peptide conjugation.
- The produced nanoparticles appear to be completely uniform and spherical, their surface is smooth without any irregularities and porous structure, and they do not appear to exhibit aggregation.
- The physicochemical properties of the PLGA NPs support their bioprofile in CMT disease therapeutics.

Literature

[1]. H. Wang et al., ACTN 7(1) 69-82.
[2]. N. Yamaguchi et al., JBC 277(9) (2002) 6806-6812.
[3]. F.S. T. Mirakabad et al., APJCP 15(2) (2014) 517-535.
[4]. S.R Pardeshi et al, Inter. J. Polym. Mat. and Polymer. Biomat. 72(1) (2021) 49-78.