Glucose and transferrin liganded PLGA nanoparticles internalization in Non-small cell lung cancer cells

Category: Undergraduate students
Discipline: Medical, Biomedical and Health sciences

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Background

- **Multi functional core shell (CS) nanoparticles (NPs)** are among the most efficient **drug delivery systems (DDS)** for active targeting; their **hydrophilic corona** improves tumor homing and targeting ligands result in more efficient internalization (Figure (1)).
- These NPs represent promising new DDS for cancer targeting of overly-expressed **glucose (Glu)** and **Transferrin (Tf)** receptors at the surface of NSCLC cells.
- Having this background, we hypothesized that the **internalization rate (IR)** will be higher for Glu or Tf decorated NPs compared to hydrophilic CS PLGA NPs.
- Therefore, the aim of our study was to design hydrophilic **CS PLGA NPs** decorated with Glu or Tf and to compare their efficacy of internalization to the non-liganded hydrophilic CS PLGA NPs.

Study Objectives

- To design NPs with improved **IR** by decorating the PLGA NPs with **Glu** and **Tf** ligands.
- To characterize the prepared nanoparticles in terms of **particle size**, **zeta potential**, **particle size distribution**, **surface morphology**, **physicochemical interactions** and **cytotoxicity**.
- To compare the cellular uptake of the prepared **Glu** and **Tf PLGA-NP** to that of non-liganded PLGA-NP in **A549** NSCLC cells.

Materials

- **Poly(D,L-lactide-co-glycolide), lactide:glycolide** 50:50, Mw 38 kDa, **PLGA-Glu** ,Mw: 38 kDa, (**PolySciTech** (Akina Inc., West Lafayette, IN, USA))
- **5DTAF** and **transferrin human (Sigma Aldrich)**
- **PEO-PPO-PEO, Lutrol®F127** (**BASF**, Germany)
- **A549** cell culture line (**ATCC**, USA)
- **MTT** (**BoosterBio**, USA)

Methods

- **PLGA and Glu PLGA NPs** preparation by ultrasound assisted **nanoprecipitation**

Dissolution of the polymer in tetrahydrofuran (THF)

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Dropwise addition of polymer THF solution to the Lutrol®F127 water solution and sonication (Sonica Q; amplitude 50%, power 40-43 W and energy 10.000 – 10.800 J)

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Removal of THF at 25 C under gentle agitation

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Washing of the excess Lutrol®F127 in 3 washing cycles with µQ H2O using ultrafiltration

↓

Dispersion in µQ water and Lyophilization

- **Transferrin** was conjugated to PLGA NPs using **EDC/NHS** coupling.
- **Zeta potential**, **Z-average hydrodynamic diameter** and **polydispersity index** were then analyzed using **Zetasizer Nano ZS** (Malvern Instruments, UK).
- **SEM** was done to characterize the morphology of the prepared NPs using benchtop SEM JCM 6000-JEOL.
- **FTIR (** PerkinElmer spectrum 400) studies were recorded from 4000 to 400cm-1 in order to evaluate the interactions in nano-systems.
- **Internalization studies** were done using A549 cells, fluorescently labeled NPs and a plate reader Victor X4 (Perkin Elmer, MA, USA) .
- **Growth inhibition studies (MTT test)** were performed during 24 and 48h using A549 NSCLC cells.

Results and Discussion

Scanning electron microscopy (SEM)

- The micrographs show that the NPs have a **spherical shape** and **low polydispersity**.

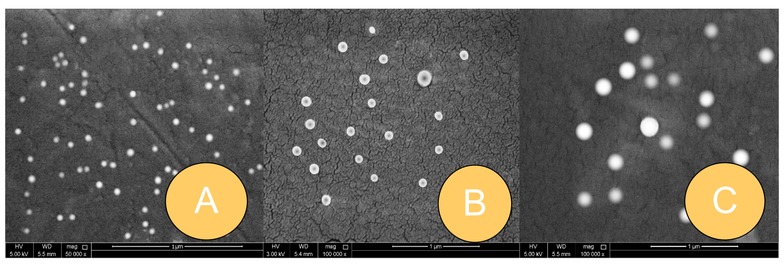


Figure 1: Scanning electron microscopy (SEM) Images of (A) PLGA NPs, (B) Glu-PLGA NPs and (C) Tf-PLGA NPs

Particle Size and Zeta Potential Analysis

- **Particle size**, **zeta potential** and **polydispersity index** of the PLGA-NPs, Glu-PLGA NPs and Tf-PLGA NPs are presented in Table 1.
- NPs size was from 120 to 130nm, they were uniform with **PDI less than 0.025,** their Z potential was negative.
- Uniformity of dosage units is important for the consistency of **nano-bio interactions**, **internalization**, subcellular localization, **efficacy** and **safety** of nanomedicines.
- No significant differences in Z-average Dh and Zeta Potential were noticed with time, which confirms the **stability against aggregation** in biological media.

Table (1): Z-average hydrodynamic diameter, polydispersity index and zeta potential of the NPs in a phosphate buffer pH 7.4

Sample	z-average Dh(nm)±SD	PDI±SD	Z potential (mV) ±SD
Glu-PLGA NPs	122.9nm±3.4	0.019±0.0001	-24.9mV±2.8
Tf-PLGA NPs	137.2nm±2.4	0.013±0.002	-15.1mV±3.4
PLGA-NPs	132.3nm±3.1	0.023±0.003	-31.9mV±4.8

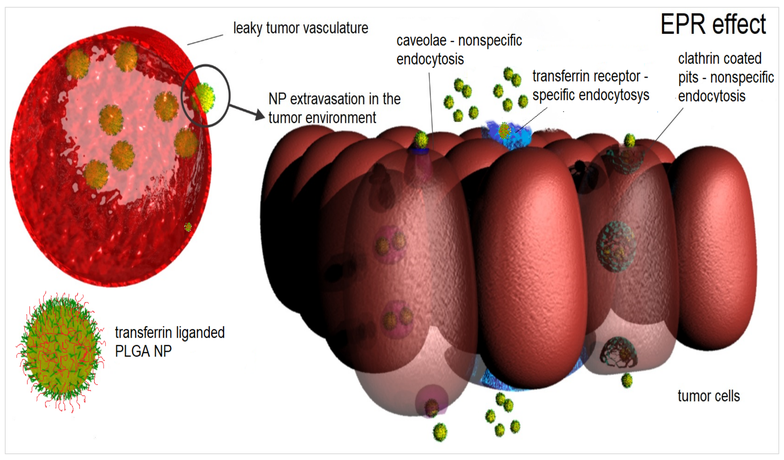
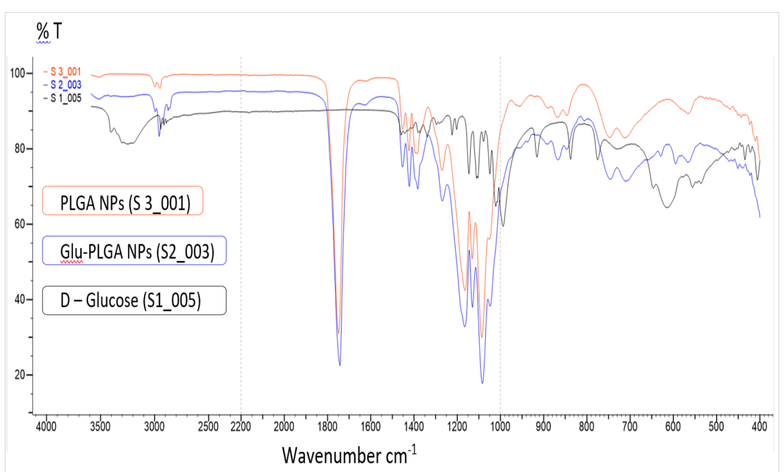


Figure (1): Ligand-receptor interaction and internalization of Tf liganded PLGA NPs after their tumor localization

FTIR Studies

- Glu-PLGA spectrum did not show any characteristic peaks related to Glu. Slight differences were noticed in the **C=O ester band** (shifted to 1743 nm); **C-H stretches** region was richer and there was a new **CH_2** vibration at 613 cm^{-1}.
- **Tf-PLGA NPs** spectrum showed peaks of **amide I** and **amide II** (at 1648 cm-1 and 1543 cm-1, for amide I and amide II vibrations, respectively), coming from the protein adsorbed at the surface of the NPs.



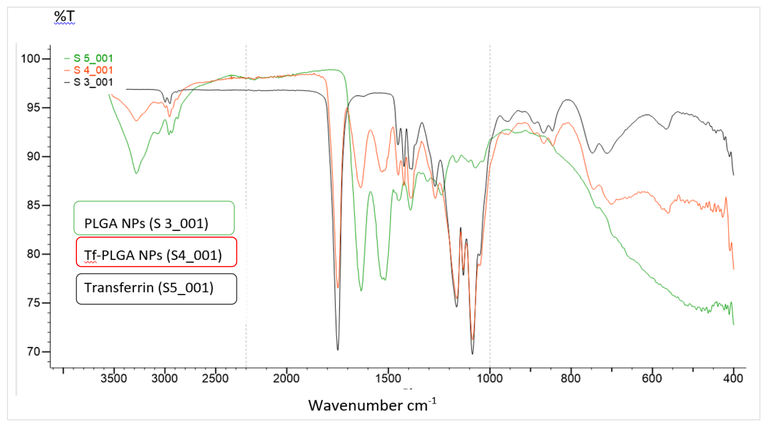


Figure 2: FTIR spectra of (A) PLGA NPs, Glu-PLGA NPs and D-Glucose and (B) PLGA NPs, Tf-PLGA NPs and Transferrin

Growth Inhibition Studies (MTT test)

- Cells mitochondrial activity and NPs biocompatibility were confirmed using the MTT test. Results show that, in the evaluated concentration range, PLGA NPs, Glu modified and Tf modified NPs show **negligible growth inhibition** effect on A549 cells.

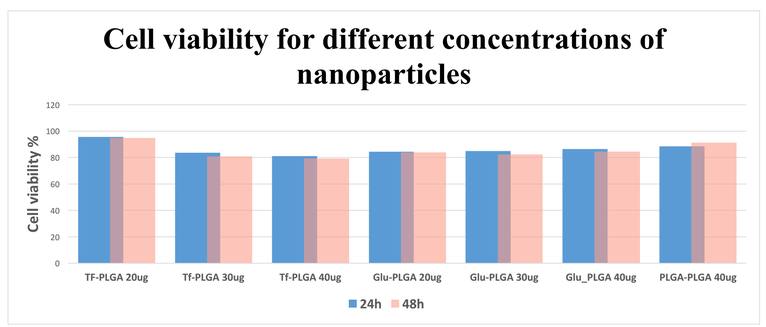


Figure 3: MTT test results for Glu-PLGA NPs, Tf-PLGA NPs and PLGA NPs

Internalization Studies

- The highest % of internalized NPs was noticed for **Glu PLGA NPs**.
- IR was affected by the addition of **FBS** due to **protein binding** to the NPs and the presence of **Tf**.

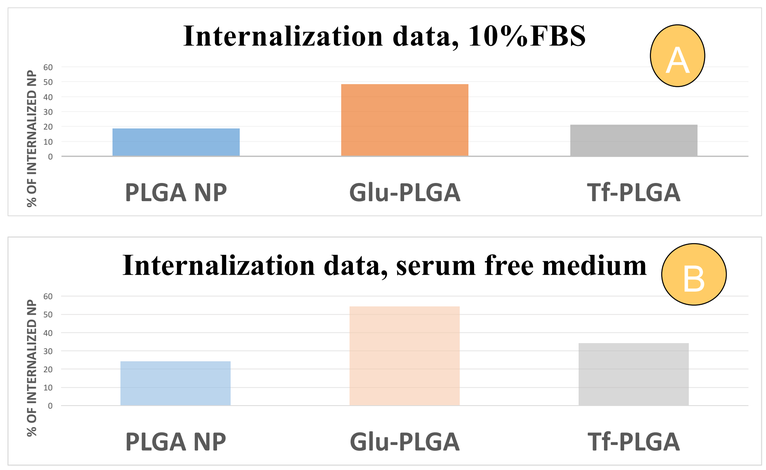


Figure 4: Uptake of particles suspended in (A) DMEM + 10% FBS and (B) in serum free DMEM by A549 cells

Conclusion

- Receptor specific NPs offer enhanced **targetability** and **increased IR**.
- Glu or Tf PLGA NPs were found to have l**ow cytotoxicity** and a **higher internalization rate** compared to non-liganded PLGA NPs. These are important characteristics that can determine the safety of their use as a DDS .
- These findings can be useful in designing future therapeutic alternatives for patients with NSCLC

Acknowledgment

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References

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