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Toxicity and cellular trafficking of functionalized nanoparticles for drug delivery through the blood-brain barrier

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1. Introduction

- Nanoparticle-mediated delivery of therapeutics is a promising method for treating central nervous system (CNS) disorders.[1]
- To be effective, nanocarriers must cross the blood-brain barrier (BBB) while protecting the potency of the carried therapeutics and minimizing potential toxicities to the BBB.
- Understanding the mechanisms by which nanocarriers are internalized, transported within cells and released into the CNS is crucial.

Aim:

➢ **We engineered silica nanoparticles (SNPs) to target the BBB and investigate their toxicity and cellular trafficking in the human brain microvascular endothelial cells.**

2. Materials & methods

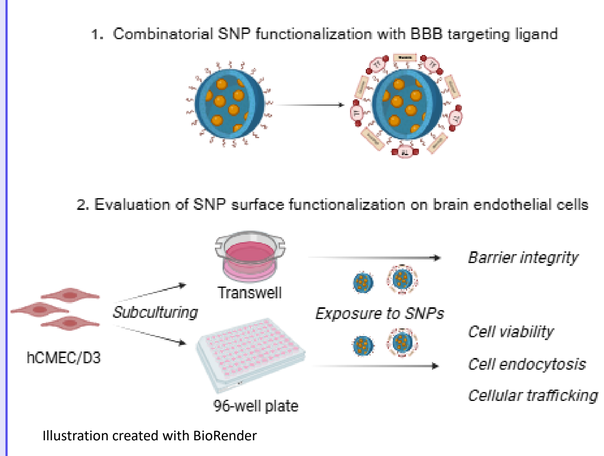

Figure 1. Schematic illustration of the approach adopted. SNPs are functionalized via combinatorial chemistry with transferrin (Tf) and glucose to promote particle internalization via transferrin receptor (TfR) and/or the glucose transporter 1 (GLUT1). The influence of SNP surface functionalization on barrier integrity, cell viability and different endocytic pathways is investigated using the human brain microvascular endothelial cell line, hCMEC/D3, grown either on the apical side of transwells or in a 96-well plate. Mechanisms influencing transcytosis are addressed using endocytosis inhibitors (chloroquine, MCBD and BAY 876) and uptake competitors (free transferrin).

3. Results

3.1 Cell characterization and functional evaluation

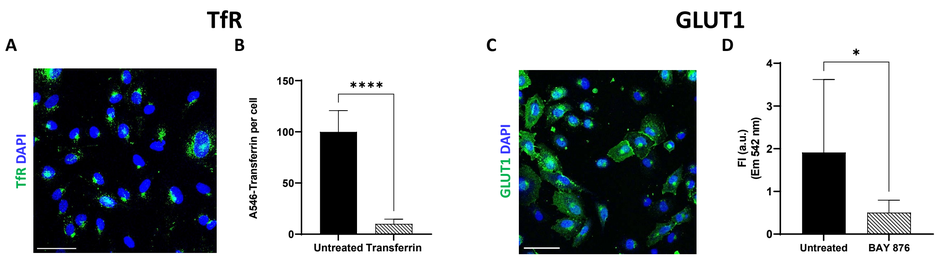

Figure 2. The expression of BBB-specific markers, such as (A) TfR and (C) GLUT1, was assessed by immunostaining (green fluorescence). Cell nuclei were stained with DAPI (blue), scale bar: 50 µM.
(B) To assess the activity of TfR, cells were incubated with fluorescently labeled transferrin without or with excess free transferrin. Results expressed as mean ± SD and data normalized to untreated cells, used as control. (D) To assess the activity of GLUT1, cells were incubated with 2-NBDG without and with inhibitor, BAY 876. Results expressed as mean ± SD.

3.2 Cytotoxicity assessment and barrier integrity evaluation

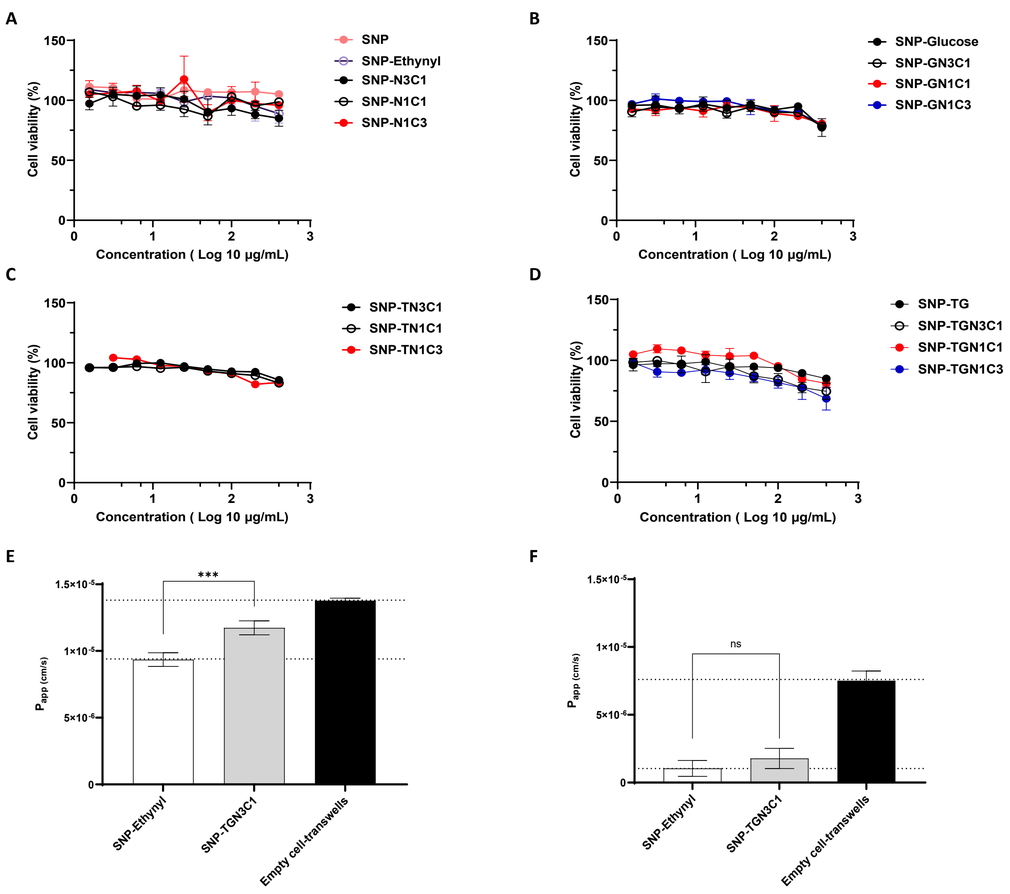

Figure 3. Cells were exposed to SNPs modulated with (A) carboxyl- and amino-functional groups, or further functionalized with (B) glucose, (C) transferrin and (D) transferrin and glucose at concentrations between 0-400 µg/mL. Cell viability was evaluated by measuring intracellular ATP. Results expressed as mean ± SD and data normalized to untreated cells, used as control.
Barrier integrity was assessed in the transwells system. Cells were treated simultaneously with (E) SNPs and (F) Dextran Texas Red (40 kDa). Permeability is demontrated using the apparent permeability coefficient (P_{app}). Results expressed as mean ± SD.

3.3 Transporter- or Receptor-mediated endocytosis of SNPs

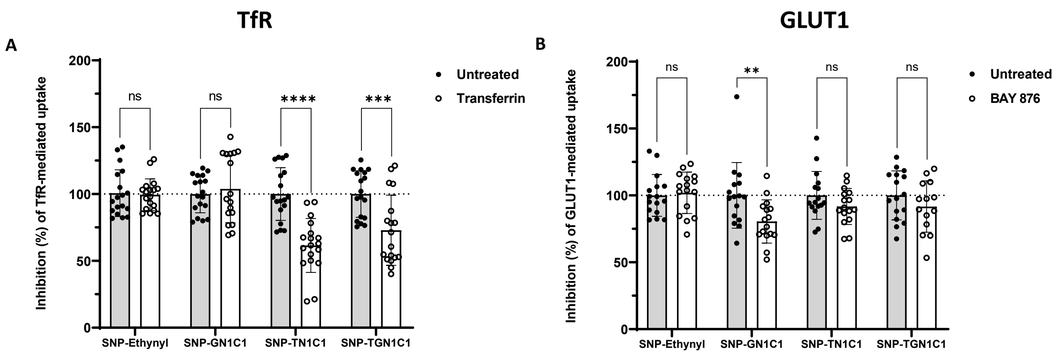

Figure 3. Cells were treated with (A) free transferrin or (B) BAY 876 followed by incubation with SNPs differently functionalized. Image analysis of intracellular quantification of SNPs (SNP Area normalized to nuclei count). Results expressed as mean ± SD and data normalized to untreated cells, used as control.

3.4 Effect of Chloroquine and MBCD on SNPs internalization

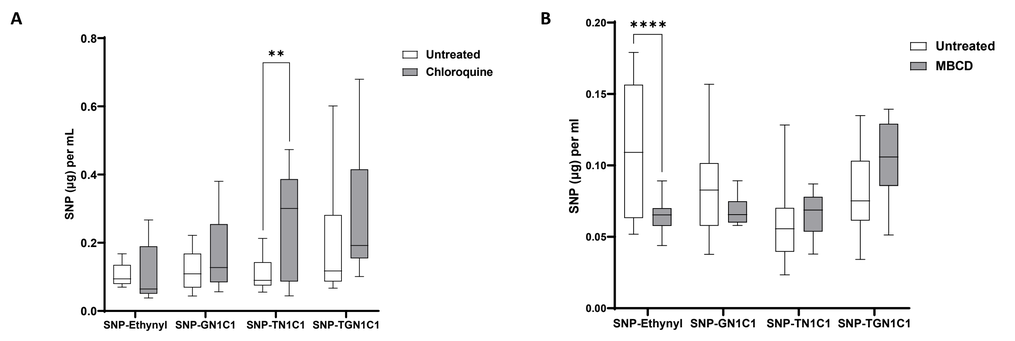

Figure 4. Cells were treated with (A) chloroquine or (B) MBCD followed by incubation with SNPs differently functionalized. The intracellular concentration of SNPs was quantified by measuring fluorescently labeled SNPs with the spectrofluorometer and by interpolating the values with a calibration curve created for each type of SNP. Results expressed as box and whisker plots, min to max.

3.5 Colocalization of SNPs and lysosomes to assess lysosomal escape

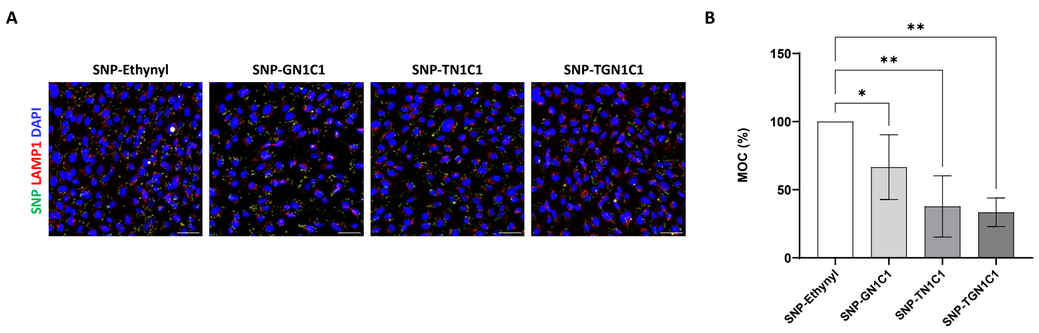

Figure 5. (A) Representative fluorescence images of cells incubated with SNPs differently functionalized. Lysosomes stained against LAMP1 (red), SNPs (green) and DAPI nuclei stain (blue), scale bar: 50 µM. (B) Plotted bars represent the Mander's overlap coefficient (MOC) values of functionalized SNPs. Results expressed as mean ± SD and data normalized to SNP-Ethynyl, used as control.

4. Conclusions

- Produced SNPs are not toxic to the cells and do not affect their barrier tightness. The attachment of transferrin and glucose promote SNPs uptake through clathrin-mediated endocytosis. On the contrary, non-functionalized SNPs (SNP-Ethynyl) are internalized via caveolae and clathrin-independent endocytosis as confirmed by their decreased uptake in cells pretreated with MBCD. The attachment of transferrin and glucose results in SNPs accumulation in cells pretreated with chloroquine. Moreover, it reduces the lysosomal trapping of SNPs, an essential requirement for successful transcytosis.

➢ **In summary, combinatorial chemistry is an effective approach to generate and evaluate several SNPs surface modifications. Nanocarrier optimization enhances cellular uptake, advancing the delivery of therapeutic agents to the target CNS area. Moreover, in vitro assessment can help evaluate any potential adverse effects on the BBB.**

Reference: Ahlawat, Jyoti et al. "Nanocarriers as Potential Drug Delivery Candidates for Overcoming the Blood-Brain Barrier: Challenges and Possibilities." ACS omega vol. 5,22 12583-12595. 1 Jun. 2020, doi:10.1021/acsomega.0c01592

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SST annual meeting, 14th November 2024, Basel