



Supplementary material

# In Vitro Comparative Study of Solid Lipid and PLGA Nanoparticles Designed to Facilitate Nose-to-Brain Delivery of Insulin

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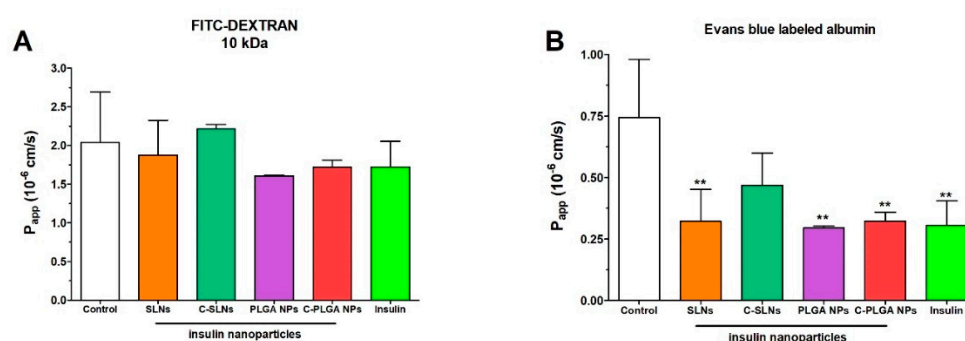
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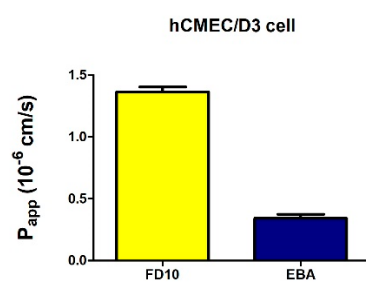
## Permeability of Paracellular Marker Molecules on the Barrier Models

To determine the tightness of the nasal epithelial co-culture model two passive permeability marker molecules were tested [88] after the permeability experiment. In the donor compartments 0.5 mL buffer containing fluorescein isothiocyanate (FITC)-labeled dextran (FD10, 100 µg/mL; Mw: 10 kDa) and Evans blue labeled albumin (167.5 µg/mL Evans blue dye and 10 mg/mL bovine serum albumin; MW: 67.5 kDa) was added. The inserts were kept in 12-well plates on a horizontal shaker (120 rpm) for 30 min (Supplementary Figure S1).



**Figure S1.** Apparent permeability coefficients ( $P_{app}$ ) of passive paracellular permeability markers FITC-Dextran (10 kDa) (**A**) and Evans blue labelled albumin (EBA, 67.5 kDa) (**B**) across RPMI 2650 epithelial cell layers after the 1-hour transport study of insulin. Permeability assay lasted for 30 minutes. Values are presented as means  $\pm$  SD,  $n = 3$ . Statistical analysis: ANOVA followed by Dunett's test. \*\*  $p < 0.01$ , compared to the control group.

In the case of brain endothelial cells paracellular permeability for marker molecules was performed parallelly with insulin transport study for 1 hour (Supplementary Figure S2). The concentrations of the marker molecules in the samples from the compartments were determined by a fluorescence multiwell plate reader (Fluostar Optima, BMG Lab-technologies, Germany; FITC: excitation wavelength: 485 nm, emission wavelength: 520 nm; Evans-blue labeled albumin: excitation wavelength: 584 nm, emission wavelength: 680 nm).



**Figure S2.** Apparent permeability coefficients ( $P_{app}$ ) of passive paracellular permeability markers FITC-Dextran (10 kDa) (**A**) and Evans blue labelled albumin (EBA, 67.5 kDa) (**B**) across D3 endothelial cell layers after the 1-hour transport study of insulin. Permeability assay lasted for 30 minutes. Values are presented as means  $\pm$  SD,  $n = 3$ .