P-0381-ORAL INSULIN DELIVERY, the challenge to increase insulin bioavailability : influence of surface charge in nanoparticle system

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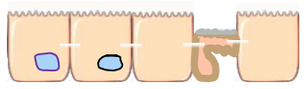
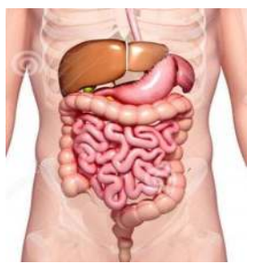
Background and aims

Since many years, oral insulin is a real challenge mainly due to a low protein bioavailability caused by degradation in gastro intestinal tract. Moreover, insulin present a poor epithelial permeability leading to a low bioavailability following oral administration. To increase this bioavailability, we have patented (CEED/CNRS: FR 0304976) a unique technology based on the double encapsulation approach (combination of nanoparticles (NPs) with a gastroresistant capsule). It has observed that physicochemical factors such as particle size, stability and surface charge may affect particle absorption. The objective of our work is to enhance nanoparticle bioavailability through surface charge modulation. To this aim, experiences were conducted to evaluate the absorption of NPs *in vitro* and the biofunctionality of the whole delivery *in vivo*.

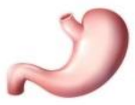
GI tractus

Enzymes, acid pH, intestinal epithelium

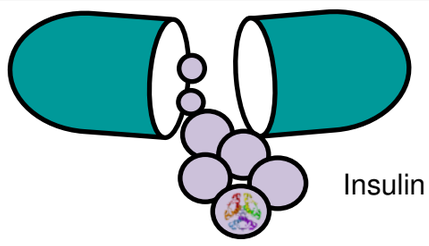




Double encapsulation system

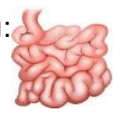


1st encapsulation: gastro-resistant vector



Insulin

2nd encapsulation: PLGA NPs



Material & methods

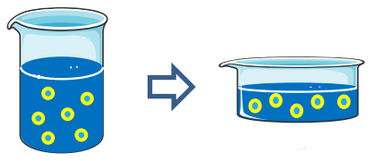
NP formulation

Insulin

PLGA (50:50) + Pluronic® F68 in ethyl acetate

Surfactants

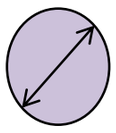
w/o emulsion



w/o/w emulsion

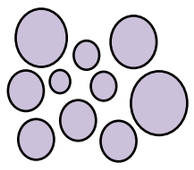
Solvant evaporation

Physicochemical parameters

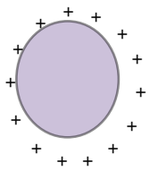


Hydrodynamic diameter

Size

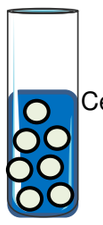


Size distribution = polydispersity index (PdI)



Surface charge

Encapsulation efficacy

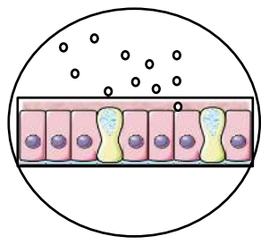


Centrifugation: 20000g 1h

Free Insulin quantification by HPLC

SEM characterization

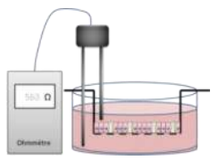
Cell line culture



Co-culture: Enterocytes (caco-2), 75% with mucus production (HT29), 25%

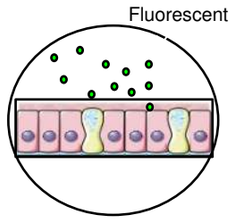
Nps incubation

Transepithelial electric resistance



Flow cytometry

Fluorescent NPs



Uptake quantification

***In vivo* validation: diabetic rat model with streptozotocine (100mg/kg)**

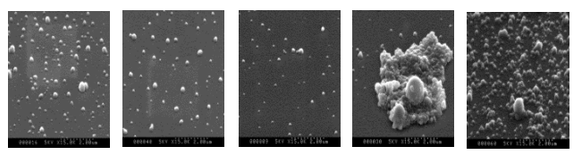
- Glycaemia
- Insulinemia

Results

NPs and vector characterization

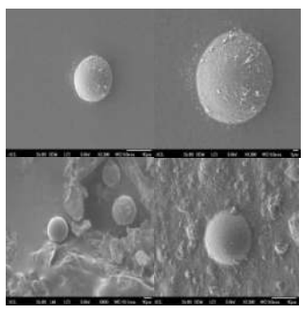
- Size <200nmn (Fig.1A)
- PdI< 0.3
- Positive surface charge with chitosan coating and negative surface charge for -PVA and SDS NPs
- Encapsulation efficacy >80% except with chitosan NPs
- Round shape for NPs (Fig.1B)
- Size of 10µm for gastroresistant vector (Fig.1C)

B



+ PVA | - PVA | SDS | Chitosan | SDS-Chitosan

C



A

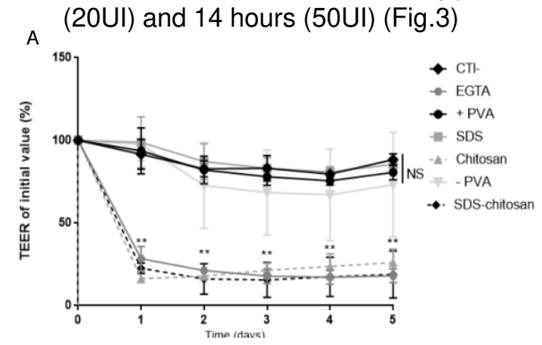
	+ PVA NPs	- PVA NPs	SDS NPs	Chitosan coating NPs	SDS NPs with chitosan coating
Size (nm)	188 ± 4	167 ± 25	150 ± 13	162 ± 11	185 ± 12
PdI	0,16 ± 0,03	0,23 ± 0,07	0,19 ± 0,05	0,27± 0,03	0,15± 0,02
Zêta potential (mV)	-1 ± 1	-22 ± 2	-42± 3	56 ± 5	40 ± 3
EE (%)	100 ± 0	100 ± 0	86 ± 6	34 ± 11	92 ± 10

Figure 1:
A:Size, polydispersity index, zêta potential and encapsulation efficacy of insulin NPs
B:SEM images of NPs
C:SEM images of gastroresistant capsule

***In vitro* and *in vivo* validation**

- A long-term toxicity of chitosan coating NPs due to a significant decrease of TEER (Fig.2A)
- Significant increase of NP uptake in cell with negatively charged NPs (SDS) compared to positive or uncharged NPs (Fig.2B)
- SDS-NPs 20 and 50UI reduced glycaemia faster than other conditions from 12 hours (20UI) and 14 hours (50UI) (Fig.3)

A



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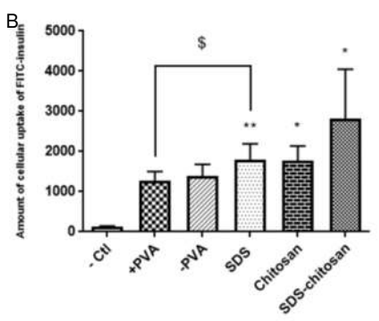

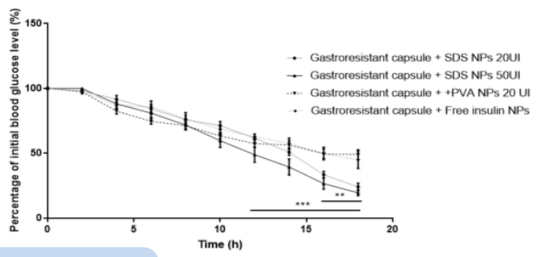

Figure 2:
A:Transepithelial electric resistance of Caco-2
B:Amount of cellular uptake of Caco-2

Figure 3:
Pourcentage of glycaemia after oral administration of insulin NPs

Conclusion

Negative charge contribution is a good approach to improve the bioavailability of encapsulated insulin in PLGA nanoparticle system. Indeed, negatively charged NPs are the most efficient both *in vitro* and *in vivo*, and represent a promising formulation for oral insulin delivery.