Targeted cathelicidin nanomedicines as novel glucoregulator for diabetes therapy

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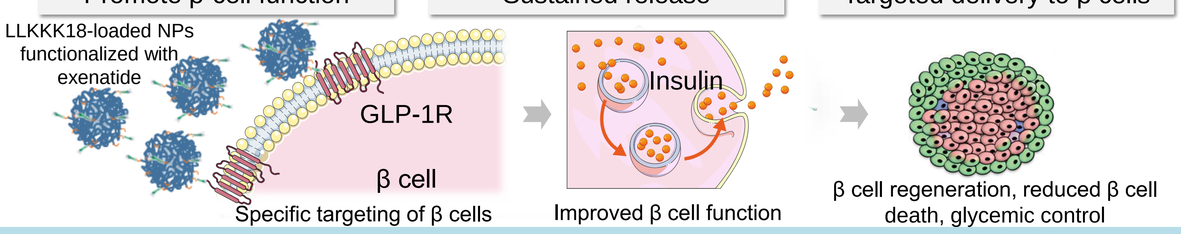
Introduction

Type 1 Diabetes (T1D) incidence has dramatically increased over the past 50 years [1] with still no cure available [2]. Cathelicidin is a human antimicrobial peptide which promotes pancreatic β cell function [3]. In this work, we propose the use of a cathelicidin-derived peptide, LLKKK18, to develop an alternative treatment for T1D. To overcome the premature enzymatic degradation and prevent cytotoxicity, it will be loaded on poly-lactide-co-glycolide acid (PLGA) nanoparticles (NPs) [4]. For the targeting of β cells, the NPs will be functionalized with exenatide, an agonist of the GLP-1 Receptor (GLP-1R), present on β cells.

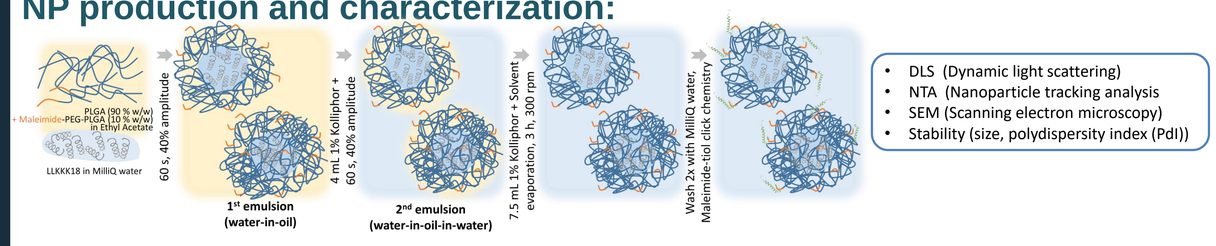
LLKKK18	+	PLGA NPs	+	Cys^{40}exenatide
Promote β-cell function		Sustained release		Targeted delivery to β cells



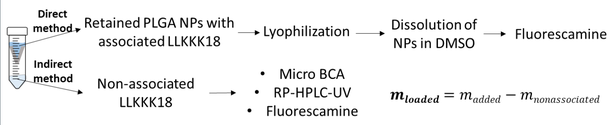
Aims
- Production of β cell-targeting NPs to promote β cell function and proliferation;
- Assess biodistribution and functionality *in vivo* using diabetic mice;
- Conclude on the potential of the formulation to revert T1D.

Methods

NP production and characterization:

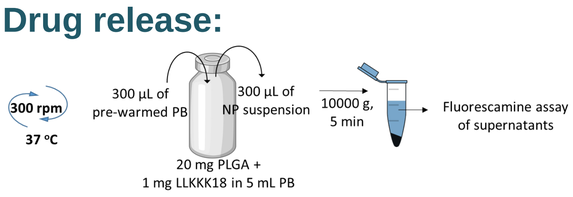


Loading efficiency:

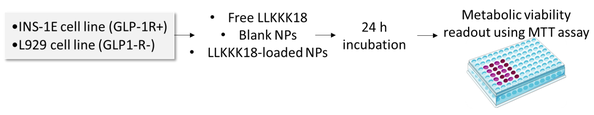


$$m_{loaded} = m_{added} - m_{nonassociated}$$

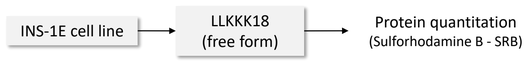
Drug release:



Viability assay:



β cell replication assay



Results

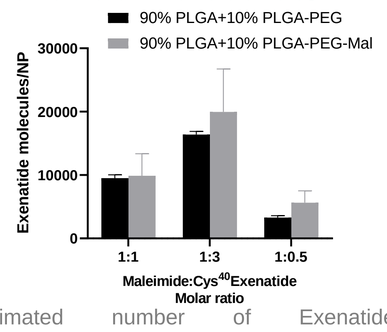
NP characterization

Table 1. Mean Size, PdI and ζ potential (± SD) of PLGA NPs by DLS (Zetasizer Nano ZS) and NTA (Nanosight). Association efficiency (AE) and drug loading (DL) were determined via de fluorescamine assay. Results represent the mean ± SD from 3 independent batches, each subject to 3 measurements.

NPs		Size (nm)	PdI	Zeta (mV)	AE (%) indirect	AE (%) direct	DL (%) indirect	DL (%) indirect
Blank		94.8 ± 1.2	0.11 ± 0.02	-5.4 ± 0.5	-	-	-	-
LLKKK18-loaded		98.3 ± 1.1	0.10 ± 0.02	-3.74 ± 0.5	88.2 ± 0.2	37.0 ± 6.31	0.87 ± 0.0	0.4 ± 0.06
Cys^{40}-Exenatide functionalization	NF	99.8 ± 0.7	0.14 ± 0.02	-5.2 ± 1.0	-			
	F1:1	94.7 ± 1.7	0.15 ± 0.02	-12.6 ± 1.9	71.5 ± 0.3			
	F1:0.5	98.1 ± 1.8	0.16 ± 0.03	-10.3 ± 1.0	78.6 ± 2.8			
	F1:3	93.5 ± 2.0	0.17 ± 0.02	-11.5 ± 1.2	56.3 ± 0.8			

- No change in size after loading or after functionalization;
- Monodisperse distribution;
- Surface charge decreases after functionalization correlated with exenatide negative net charge at physiologic pH;

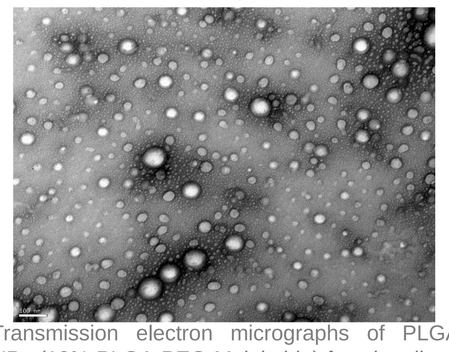
Exenatide molecules/NP



Estimated number of Exenatide molecules/NP taking into account the association efficiency and NP concentration determined using NTA (NanoSight NS500).

Higher amount of peptide on the surface when using the molar ratio maleimide:exenatide 1:3.

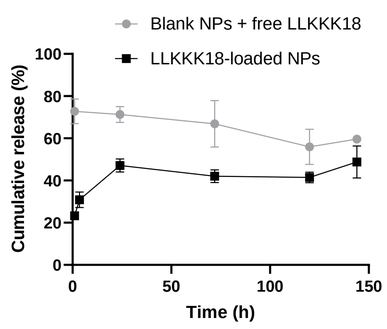
NP morphology



Transmission electron micrographs of PLGA NPs (10% PLGA-PEG-Maleimide) functionalized in the molar ratio 1:3 of maleimide:exenatide.

NPs have a spherical shape with a smooth, non-porous surface. Size comparable to observed in DLS.

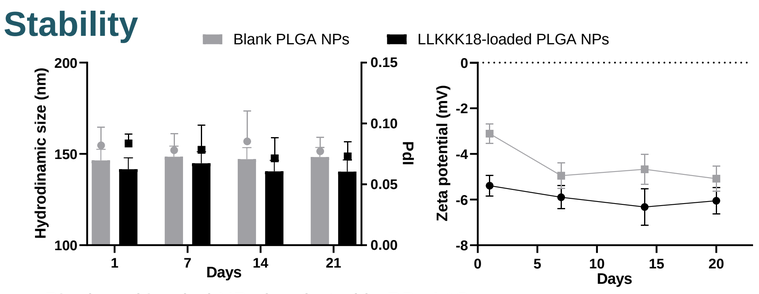
Drug release



In vitro release of LLKKK18 from PLGA NPs, at 37ºC in PB. LLKKK18 was determined using fluorescamine.

Sustained release of LLKKK18 from PLGA NPs up to a week; Adsorption to NPs surface may delay the release.

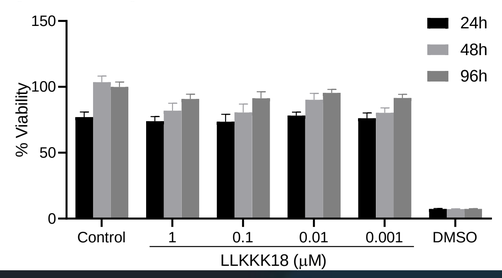
Stability



Blank and loaded NPs incubated in PB, 4 ºC.

Blank and loaded NP size was constant; PdI remained 0.1 (monodisperse); ζ-potential of peptide-loaded NPs decreased.

β cell replication



Effect of LLKKK18 (free form) on INS-1E cell replication analyzed by the SRB assay.

LLKKK18 showed no significant improvement in β cell replication over the time of study.

Viability

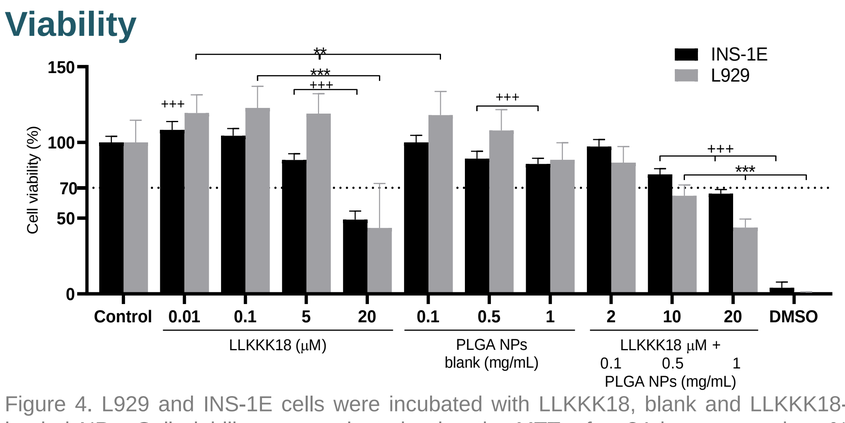


Figure 4. L929 and INS-1E cells were incubated with LLKKK18, blank and LLKKK18-loaded NPs. Cell viability was evaluated using the MTT, after 24 h, expressed as % relative to control. Mean and SD of 3 independent experiments. +++ $p < 0.001$ (INS-1E cells); ** $p < 0.01$ and *** $p < 0.001$ (L929 cells).

- LLKKK18 had no cytotoxic effect, below 5 µM and blank PLGA NPs showed no cytotoxicity up to 1 mg/mL;
- LLKKK18-loaded NPs could be safely used up to 0.5 mg/mL + 10 µM LLKKK18 in INS-1E cells;
- 20 µM LLKKK18-loaded NPs slightly improved the effect on cell viability, relative to the free peptide.

Conclusions

Until now it was possible to obtain LLKKK18-loaded, and exenatide-functionalized PLGA nanoparticles with a monodisperse distribution. LLKKK18 release from PLGA NPs is sustained up to one week. The lowest peptide concentrations had no cytotoxic effect on cells, blank PLGA NPs were not cytotoxic in the used concentrations, however LLKKK18 loading on PLGA NPs had only a slight improvement on cell viability and LLKKK18 did not show the promising effect on improving β cell replication. In the future, the functionalization of NPs with exenatide and the ability of the formulation to promote glucose-mediated insulin release will be addressed.

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References

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