Development of visceral smooth muscle

Optimization of pDNA delivery by WRAP cell–penetrating–peptide

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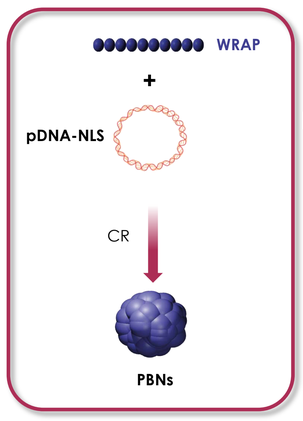
https://doi.org/10.17952/37EPS.2024.P2178

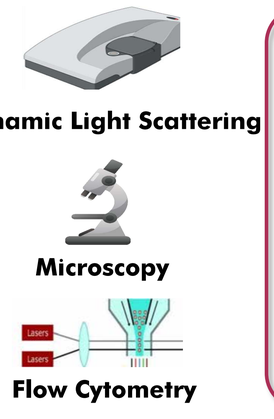
Introduction

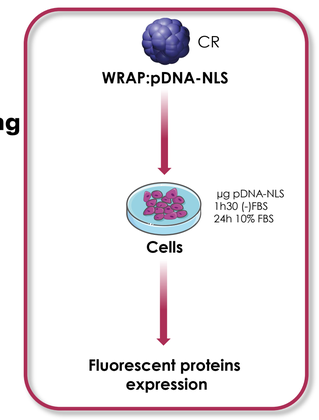
WRAP peptides are short tryptophan and arginine-rich amphipathic cell-penetrating peptides (CPPs) first dedicated to the delivery of short interfering RNA (siRNA) [1]. By a non-covalent strategy, the peptide and the siRNA form spontaneously peptide-based nanoparticles (PBNs) with a size of around 100 nm and able to silence efficiently the targeted protein in numerous cell lines but also *in vivo* [1,2]. Transfection that introduces DNA plasmid (pDNA) in cells represents a promising tool for fundamental, translational and therapeutic fields. The difference in size and flexibility between small, rigid siRNA and larger, more flexible pDNA poses a challenge for WRAP-based PBNs in improving cellular transfection and protein expression.

In the present study, we optimize formulation and internalization conditions for pDNA to obtain effective transfection with WRAP peptides. This fine-tuning between PBN formation and delivery implies the definition of an optimal charge of ratio and the final quantity applied on cells to avoid cytotoxicity. By employing a model plasmid that expresses and addresses fluorophores exclusively in the nuclei trough a Nuclear Localization Sequence (NLS), we successfully demonstrated significant GFP and mCHERRY expression levels in HEK293 and HELA cell lines through flow cytometry experiments and confocal microscopy. In order to obtain protein expression even in non-dividing cells, WRAP peptides were functionalized with a NLS sequence to obtain PBNs able to cross the nuclear envelope. This conducts to the efficient delivery and expression of GFP into non-dividing cells [3].

CCP	Sequence	Length	Charge	Nb W	Nb R	Nb L
WRAP1 (W1)	LLWRLWRLLWRLWRLL	16	5	4	4	8
WRAP5 (W5)	LLRLLRWWWRLLRLL	15	5	3	4	8
NLS-WRAP1 (WIN)	KKKRKV-LLWRLWRLLWRLWRLL	22	10	3	5	8

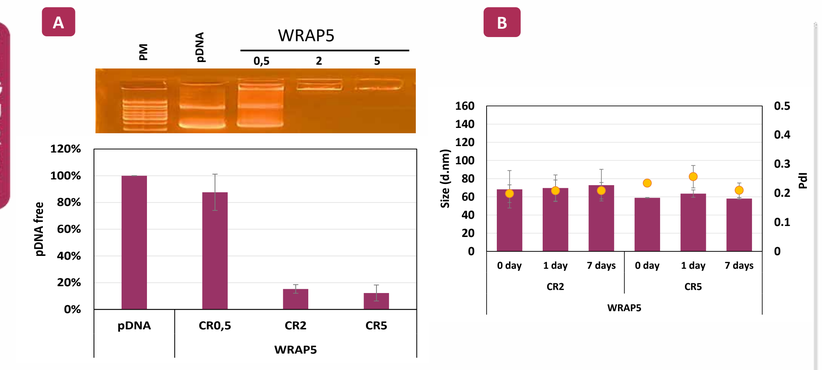






1. WRAP5 for Plasmid Delivery

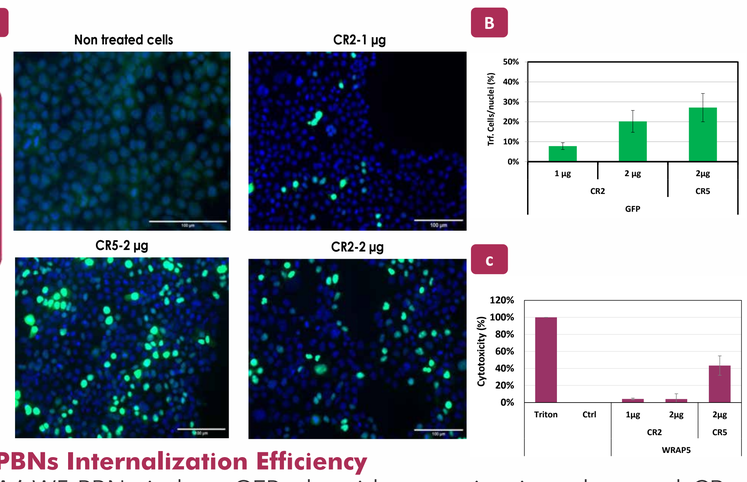
FIGURE 1



PBNs Formation

A/ By gel shift assay we determined a fully complexation of the plasmids from CR2 with WRAP5. B/ W5-PBNs are characterized as homogeneous nanoparticles around 60 nm and stable until 7 days by DLS.

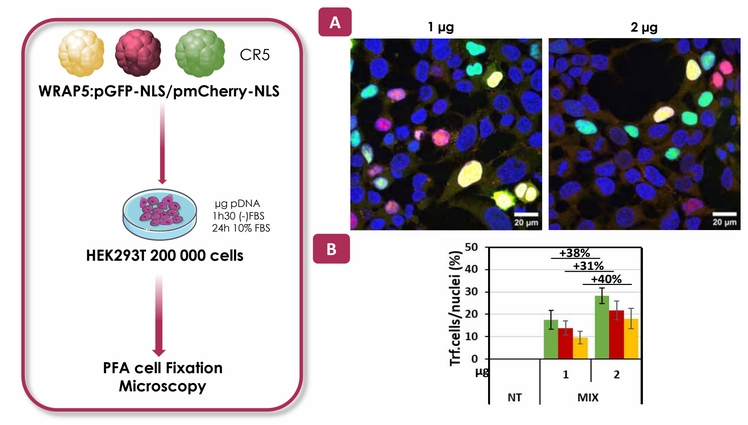
FIGURE 2



PBNs Internalization Efficiency

A/ W5-PBNs induce GFP plasmid expression in a dose and CR-dependent manner in HEK293 cells. B/ Quantification of GFP-expressing cells (ratio between GFP-expressing cells and total number of nuclei stained by DAPI - approximately 30% of the cells expressed GFP. C/ LDH cytotoxicity test (W5-PBNs toxicity reached 40% for the highest CR and dose applied).

FIGURE 3



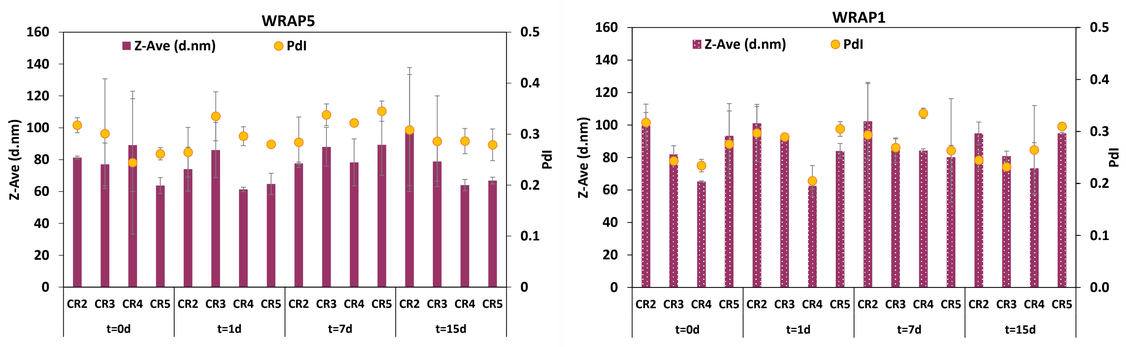
PBNs Encapsulating pDNA–NLS for Multi–Protein Expression

A/ W5 can internalize both plasmids in one PBN that conducts the co-expression of GFP and mCherry in the same nuclei in HEK293 cells.

B/ Quantification reveals a dose-dependent expression of GFP, mCherry, or both proteins (approximately 30% of the cells expressed the GFP).

2. Ajusting of parameters to optimize efficiency and to reduce cytotoxicity

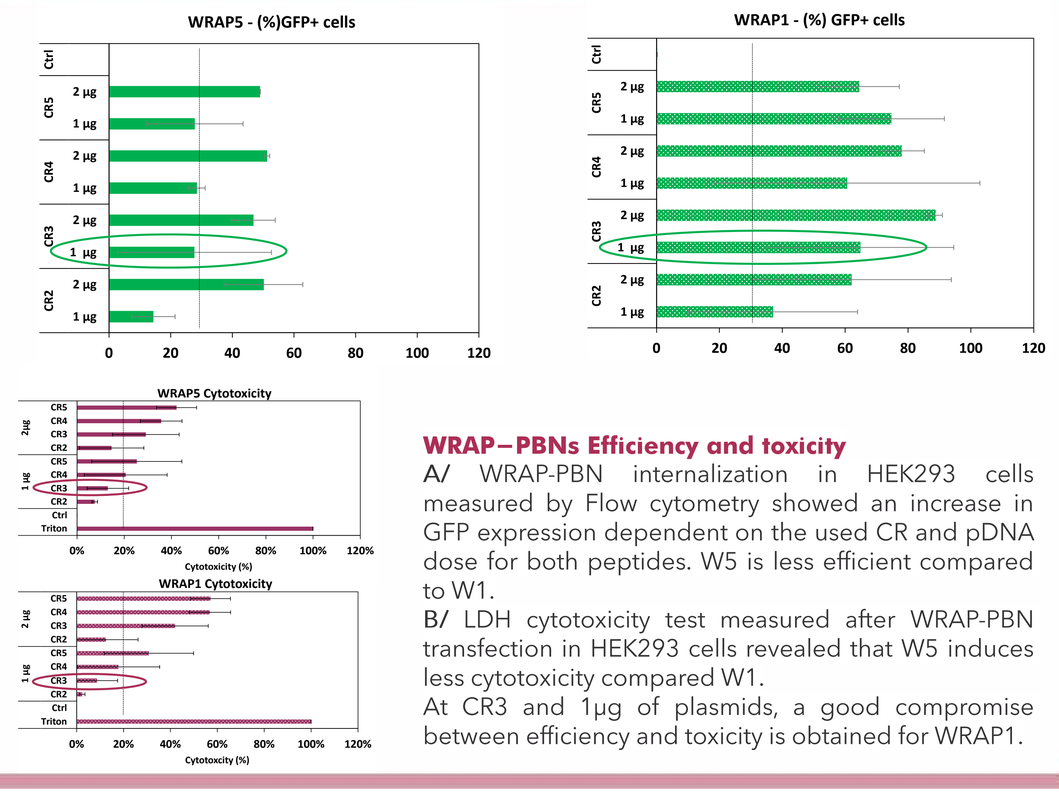
FIGURE 4



Comparison of WRAP5 and WRAP1 PBN Formation at different charge ratios (CR)

PBNs formation was compared at CR of 2, 3, 4 and 5 for W5 and W1. Both harbor constant size, homogeneous, and stable over time nanoparticles whatever CR of formulation. Globally sizes are under 100 nm.

FIGURE 5



WRAP–PBNs Efficiency and toxicity

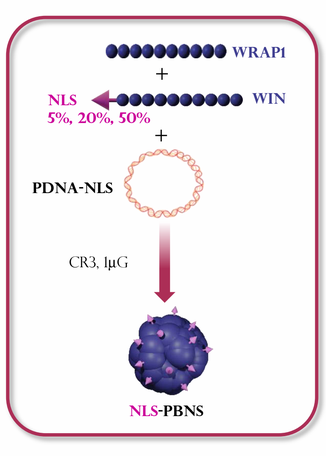
A/ WRAP-PBN internalization in HEK293 cells measured by Flow cytometry showed an increase in GFP expression dependent on the used CR and pDNA dose for both peptides. W5 is less efficient compared to W1.

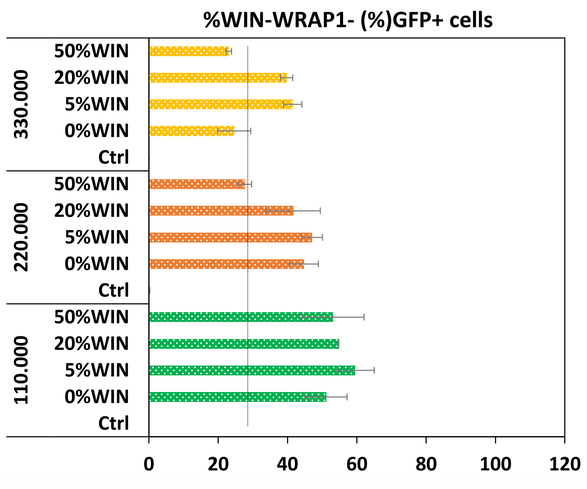
B/ LDH cytotoxicity test measured after WRAP-PBN transfection in HEK293 cells revealed that W5 induces less cytotoxicity compared W1.

At CR3 and 1µg of plasmids, a good compromise between efficiency and toxicity is obtained for WRAP1.

3. Internalization in confluent cell condition

FIGURE 6





WRAP1 functionalized PBNs on HELA cells

W1- PBNs functionalized with 0%, 5%, 20%, or 50% of WIN-peptide were applied on HeLa cells at different cell confluence (110 000 cells = ~70% confluence compared to 220 000 cells = ~85% confluence, and 330 000 cells = full confluence / non-divinding cells).

CONCLUSION

- WRAP peptides can fully complexed plasmids at CR ≥2
- WRAP1 and WRAP5 form homogeneous and stable nanoparticles (<100 nm)
- WRAP-PBNs are able to internalize two plasmids for multi protein expression
- WRAP-PBNs internalize plasmid for gene expression in a CR and dose-dependent manner
- Efficient delivery with lowest toxiciy are obtained for PBNs at CR3 using 1 µg of plasmid
- Functionalization of WRAP1 with NLS allows efficient delivery in non-dividing cells

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[3] Guerin A. *et al.* LIX1-mediated changes in mitochondrial metabolism control the fate of digestive mesenchyme-derived cells. *Redox Biol*. **2022** Oct,56:102431