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The antimicrobial peptides delivery strategies based on nanotechnology to treat infectious diseases

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INTRODUCTION

The increasing problem of resistance to traditional antibiotics

Antimicrobial peptides (AMPs) have a huge potential as new therapeutics

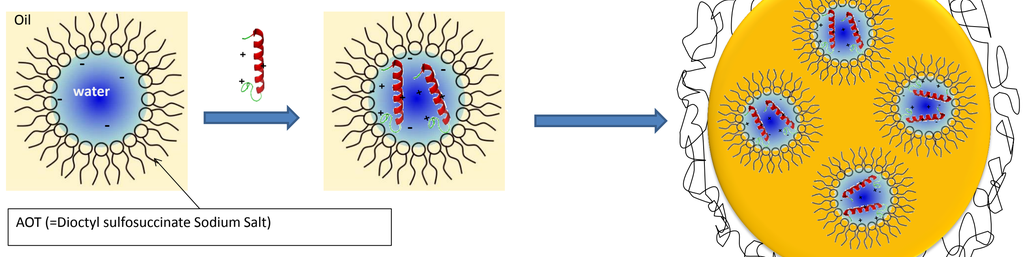
The delivery strategies based on nanotechnology has the potential to improve the efficiency and stability of AMPs in clinical development

Promising for peptide delivery, controlled release strategies and technologies against proteolytic degradation of peptides?

Lipid nanocapsules (LNCs): a new generation of biomimetic nanocarriers and were used to deliver the peptide

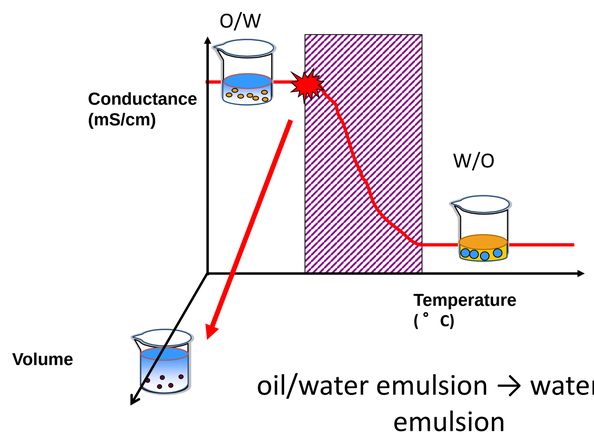
RM-LNCs

Structure



Phase inversion process

- ✓ Low energy process
- ✓ Adapted to scale up
- ✓ Without the use of toxic solvent

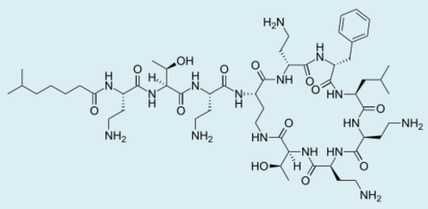


oil/water emulsion → water/oil emulsion

PolymyxinB-RM-LNCs

PMB: a peptide model to evaluate feasibility of AMPs-loaded LNCs.

Avantages of PMB:
- Well-know antibiotic peptide
- Cheap
- Cationic



Composition and properties of PMB-loaded LNCs.

LNCs	mass of peptide (mg)	Theoretical PMB concentration (µg/ml)	Experimental / theoretical PMB concentration (%)	Percentage of encapsulated PMB (%) Method 1	Percentage of encapsulated PMB (%) Method 2	Size (nm)	PDI	Zeta potential (mV)
AP3	6	453.6	87.9±4.6	92.0	74.8	60.8±0.6	0.035±0.003	-29.3±1.2
AP4	8	605.4	87.8±9.6	92.0	90.7	61.9±2.4	0.084±0.067	-29.7±2.4
AP3 dil	6	289.4	99.0±8.1	94.0	88.2	60.9±1.6	0.056±0.001	-31.9±1.1
AP4 dil	8	386.1	96.1±7.4	93.0	97.3	58.6±0.7	0.050±0.013	-29.1±1.7

Two methods were selected for LNCs-RM: the combined diafiltration-centrifugation technique (2*20min, 3220rcf, Amicon Ultra filters with MWCO of 100kDa) = method 1, and dialysis (100kDa tubing, 24H) = method 2

Properties stability of "AP4 dil" LNCs at 4°C.

	Experimental / theoretical PMB concentration (%)	Percentage of encapsulated PMB (%) Method 1	Percentage of encapsulated PMB (%) Method 2	Size (nm)	PDI	Zeta potential (mV)
Day 0	101.0±1.3	99.0	89.2	59.1±0.1	0.042±0.006	-34.4±2.75
+ 14 days	95.0±1.8	90.0	105.4	61.6±1.9	0.071±0.032	-37.5±4.6

MIC of PMB-RM-LNC (in µg of PMB/ml) with BHI used as a diluent.

Sample name	Pseudomonas aeruginosa (reference strains)	Pseudomonas aeruginosa (clinical strain)	Escherichia coli (reference strain)	Acinetobacter baumannii AYE (reference strain)
PMB solution	0.5	0.25	0.25	0.25
AP4 dil	[0.125-0.25]	0.065-0.125	0.125	0.125

Polymyxin B was efficiency encapsulated in LNCs using reverse micelles and the antimicrobial activity was intact.

The study shows that LNCs are an excellent candidate to deliver AMPs.

AMP3-RM-LNCs

Properties of AMP3.

Size	Secondary structure	Origin	Spectrum	Main administration route
40 aa	B sheet	*Pseudoplectania nigrella* defensin	Gram+ bacteria	Pulmonary (Pneumonia)

AMP3-RM composition: AMP3 (8mg/g) +1g blank RM (AOT/Labrafac 1:10)+ water (<4% W/W)

AMP3 loaded RM-LNCs: add 1mL AMP3-RM before fast the cooling-dilution

Properties of AMP3-loaded LNCs.

Size (nm)	PDI	Zeta Potential (mV)	Experimental / theoretical AMP3 concentration (%)	Drug loading (µg AMP3/g)	Percentage of encapsulated AMP3 (%) (Dialysis)
62.9±0.6	0.059±0.003	-25.6±0.9	74.0±1.0	285.5±3.9	97.8±1.2

Minimum inhibitory concentration (MIC) of AMP3-RM-LNC (in µg of AMP3/mL) with BHI used as a diluent.

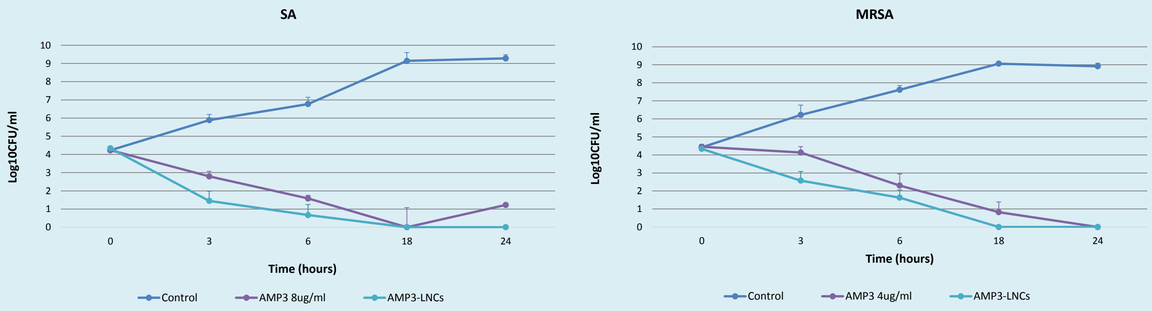
MIC via a broth microdilution method with BHI used as a diluent

Incubate microtiter plate at 37°C for 24 hours

Sample name	Staphylococcus aureus (SA)	Methicillin-resistant Staphylococcus aureus (MRSA)
AMP3 solution	4	2
AMP3-LNCS	4	1

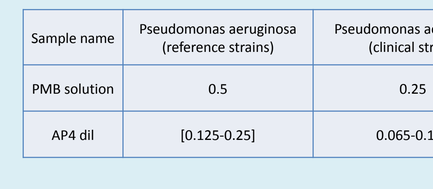
Time kill kinetics of AMP3-RM-LNC.

Time kill kinetics were determined against SA and MRSA at 8 and 4µg/mL respectively, corresponding at twice the MIC of AMP3 solution.
At each time, bacterial suspensions were plated on Columbia agar with sheep blood and counted.



- ➢ Keeping of AMP3 bactericidal activity

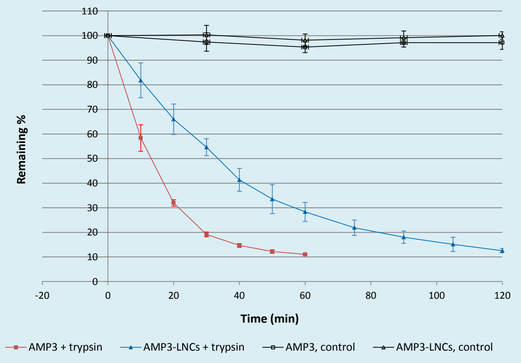
AMP3 degradation by trypsin.



PBS pH 7.4
+ LNC or AMP3 solution
+ Trypsin
Peptide: trypsin (2:1)

Every 10min during incubation:
- Concentration of AMP3 (HPLC-UV)

after incubation:
- Size
- PDI
- zeta potential

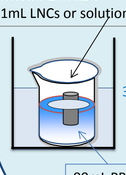


	Size (nm)	PDI	Zeta Potential (mV)
Before test	62.9±0.6	0.059±0.003	-25.6±0.9
After test: control	64.7±0.9	0.042±0.006	-35.0±2.2
After test: trypsin	65.5±0.2	0.040±0.007	-31.0±1.0

- ➢ LNCs protect AMP3 against trypsin attack

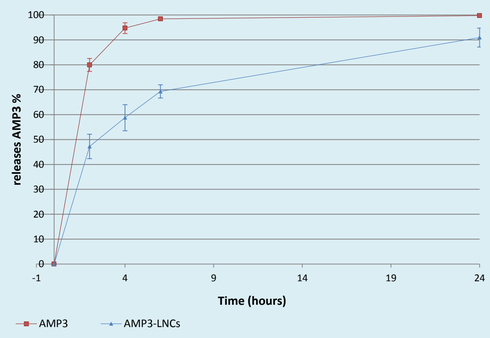
AMP3 release.

Spectra-Por® Float-A-Lyzer® G2
MWCO 100 kDa
1mL LNCs or solution



Samples at 0/2h/4h/6h/24h
- Concentration of AMP3 (HPLC-UV)

after incubation:
- Size
- PDI
- zeta potential



	Size (nm)	PDI	Zeta Potential (mV)
Before test	62.9±0.6	0.059±0.003	-25.6±0.9
After release:	72.7±1.4	0.054±0.007	-40.2±6.2

- ➢ Fast release at 37°C

Conclusion

RM-LNCS are promining AMPs delivery systems .
- ➢ Suitable properties (size, PDI, encapsulation efficiency, stability)
- ➢ Preservation of the anti-bacterial activity of the native peptide
- ➢ Protection against protease

References - Acknowledgements

Heurtault B et al. Pharm Res. 2002, Vonarbourg A. et al. Biomaterials 2009, Vrignaud S. et al. Eur. J. Pharm. Biopharm 2011

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