Nanostructuration of antimicrobial peptides: strategies and applications

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Table 1. Summary of strategies for nanostructuring AMPs explored in pharmaceutical industry research

Images from: Liu et al. Nat. Nanotechnol. 4, 457–463; Zetterberg et al. J. Control. Release 156, 323–328; Burchell et al. J. Phys. D: Appl. Phys. 32 1719; Tahirov et al. Nature 465, 747–751; Gribskov et al. To be Publ. at <http://www.rcsb.org/pdb/explore.do?structureId=2mlt>

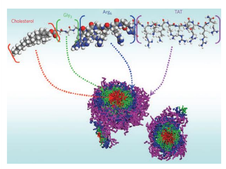
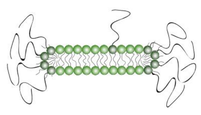
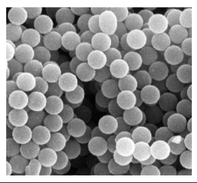
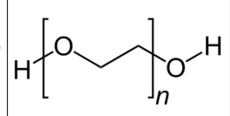
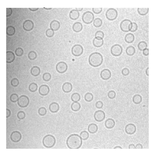
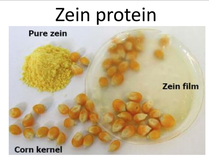
Strategy	AMP	Composition	Synthesis method	Assays	Ref.
 Lipid derived NP 	TAT from VIH virus 	Covalent linked: Cho-GLY_3-ARG_6-TAT	Self-assembly while being dialyzed against deionized water	* Hemolytic assay * Cell culture in vitro: - S. aureus - MRSA * In vivo: - S. aureus infection in mouse and rabbit model	Liu et al. (2009)
	Melittin 	PEG-stabilized lipid disk: - POPC - Cho - Ceramide-PEG5000	Thin film hydration + sonication	* Proteolytic assay * Cell culture in vitro: - E. coli	Zetterberg et al. (2011)
Polymers 	Clavanin A	EUDRAGIT© (methacrylate polymer)	Oil/water emulsion, inducing nanoparticle aggregation by hydrophobicity	* Peptide release assay * Cell culture in vitro: - S. aureus - K. pneumoneae - P. aeruginosa * Immunomodulatory assay * In vivo - Septicemic mice model	Saúde et al. (2014)

Table 1. Summary of strategies for nanostructuring AMPs explored in food industry research

Images from: Hsu et al. Nat.Struct.Mol.Biol. 11, 963; Çağdaş et al. PhD. Diss Ali Demir Sezer (Ed.), ISBN: 978-953-51-1628-8; Xiao,. PhD diss., University of Tennessee (2010).

Strategy	AMP	Composition	Synthesis method	Assays	Ref.
PEGylation 	Nisin 	PEG 5000 Da 	Covalent link to C-ter.	* Cell culture in vitro: - *L. monocytogenes* - *P. aeruginosa* - *S. aureus* - *E. faecalis*	Guiotto et al. (2002)
AMPs-loaded 		Phosphatidylcholine Cholesterol	Thin film hydration + sonication	* Cell culture in vitro: - *L. monocytogenes* - *S. aureus*	Zou et al. (2012)
		Phosphatidylcholine		* Cell culture on milk: - *L. monocytogenes*	Malheiros et al. (2010)
Polymer capsules 		Zein protein 	Spray drying	* In vitro release kinetics * Cell culture in vitro: - *L. monocytogenes*	Dan Xiao (2010)

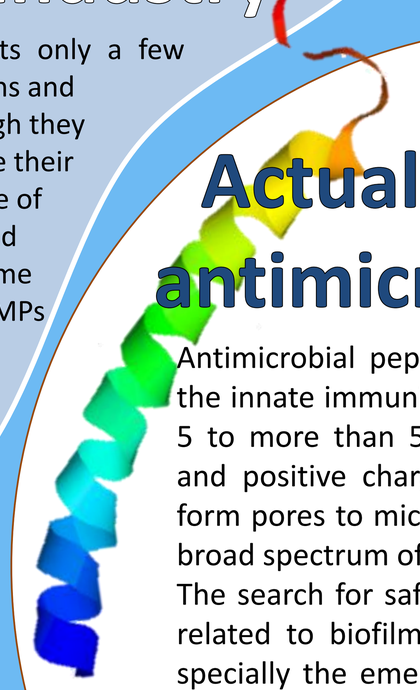
Pharmaceutical industry

Although their potential as therapeutic agents only a few AMPs have really reached the market. Polymyxins and gramicidins are one of those exceptions, although they have been only approved topical application due their hemolytic activity. However, with the emergence of multidrug resistance bacterial infections they had been administrated as in extremis therapy in some patients[2]. Nonetheless is necessary to reduce AMPs cytotoxicity as well as improve their pharmacokinetics and pharmacodynamics in order to have a real substitute of conventional antibiotics.

Food Industry

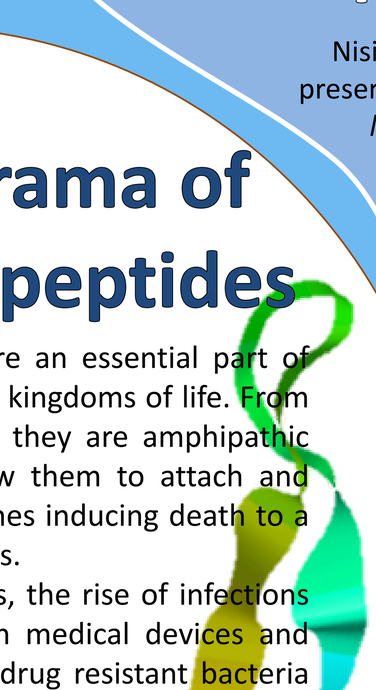
Nisin is the only antimicrobial peptide approved as a food preservative by FDA in 1988. It is synthetized by *Lactococcus lactis* which can generate the peptide directly in situ in fermented products or in bioreactors and adding it afterwards as an additive[3]. However the antimicrobial activity of AMPs can decrease due proteolytic degradation and the interaction between AMPs And food components, limitation that can be overcame nanoencapsulating them.

Actual panorama of antimicrobial peptides



Antimicrobial peptides (AMPs) are an essential part of the innate immunity system of all kingdoms of life. From 5 to more than 50 amino acids, they are amphipathic and positive charged, what allow them to attach and form pores to microbial membranes inducing death to a broad spectrum of microorganisms.

The search for safer preservatives, the rise of infections related to biofilms' formation on medical devices and specially the emergence of multidrug resistant bacteria are challenges that have surpassed traditional antibiotics. For that reason antimicrobial peptides are in the spotlight. However, AMPs have some **limitations** that have truncated their commercial development. The possibility of entrapping them into nanostructures could overcome those hurdles.



Images from:
Wang et al. J. Biol. Chem. 283, 32637–43 (2008);
Hwang et al. Biochemistry 37, 4288–98 (1998)

Antimicrobial peptides as antibiotics[1]

Strengths	limitations
▪ Wide variety of AMPs ▪ Broad spectrum of action, ▪ Few resistance mechanisms reported. ▪ Synergies with conventional antibiotics.	▪ Highly susceptible to be biodegraded. ▪ Not orally available ▪ Cytotoxic at high concentrations due their lose of specificity

Biofilms are matrix-enclosed microbial communities that are adhered to surfaces, including medical devices such as implants and catheters. With cells protected by an extracellular matrix, biofilms are highly tolerant to antimicrobials. Reactive, charged or large antimicrobials are neutralized and diluted to sublethal concentrations before they can reach individual bacterial cells. Moreover, as most of all bacteria are into a dormant physiological state, those antibiotics whose target is related to rapid cell division are not effective[4]. Since AMPs form pores they can be more effective against dormant cells than conventional antibiotics. Due to AMPs can interact with the matrix, instead of treating stablished biofilms it seems more interesting to functionalize surfaces in order to prevent film formation[5].

Biofilms eradication

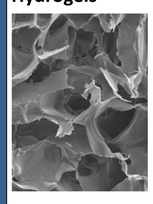
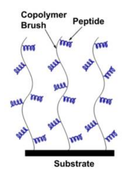
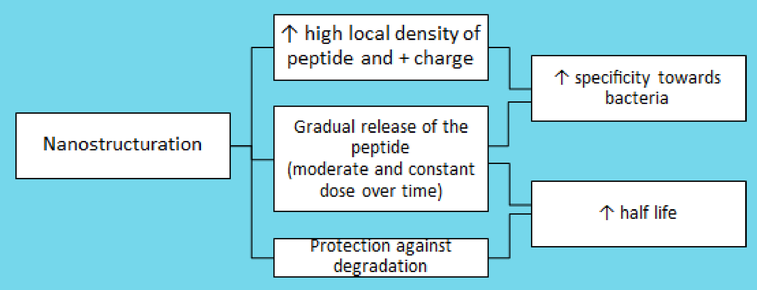
Strategy	Peptide	Composition	Synthesis method	Assays	Ref.
Hydrogels 	Maximin-4 + synthetic AMPs 	Poly(HEMA) hydrogels	HEMA monomers were crosslinked with EGDMA + peptide added by electrostatic interaction or entrapping during synthesis	* Peptide release assay * Cell adhesion: - *S. epidermis*	Laverty et al. (2012)
Polymeric brushes 	Tet-20Cys + other synthetic peptides	DMA and APMA polymer	1) Ti surface immobilization of ATRP initiator. 2) Chain elongation with DMA and APMA 3) Peptide functionalization trough S covalent link.	* Cell adhesion: - S. epidermis * Cytotoxicity test with osteoblast-like cells. * In vivo rat infection model - *S. aureus*	Gao et al. (2011)

Table 1. Summary of strategies for nanostructuring AMPs explored in biofilms eradication research

Images from: Savina et al. Soft Matter, 3(9), 1176; Gao, G. et al.. Biomaterials 32, 3899–3909

To sum up

- Strategies used for entrapping AMPs into nanostructures are different depending on their final purpose, as each application has its own requirements.



- Nanostructuration of AMPs is not excluded from other investigation lines like researching on new synthetic AMPs or the synergic administration of AMPs with conventional antibiotics.

References:
[1] Gordon, Y. J., Romanowski, E. G. & McDermott, A. M. A review of antimicrobial peptides and their therapeutic potential as anti-infective drugs. Curr. Eye Res. 30, 505–515 (2005)
[2] Li, J. et al. Colistin : the re-emerging antibiotic for multidrug-resistant Gram-negative bacterial infections. (2006).
[3] De Arauz, L. J., Jozala, A. F., Mazzola, P. G. & Vessoni Penna, T. C. Nisin biotechnological production and application: a review. Trends Food Sci. Technol. 20, 146–154 (2009)
[4] Hall-Stoodley, L., Costerton, J. W. & Stoodley, P. Bacterial biofilms: from the natural environment to infectious diseases. Nat. Rev. Microbiol. 2, 95–108 (2004).
[5] Bahar, A. A. & Ren, D. Antimicrobial peptides. Pharmaceuticals 6, 1543–1575 (2013).