

Antimicrobial peptide permanently immobilized on surfaces with high activity in the presence of serum and low cytotoxicity against human cells

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Abstract

Antimicrobial peptides (AMPs) are a class of molecules that are present in large number in nature and are very effective against several strains of bacteria, fungi and viruses, preventing by several mechanisms infections and contaminations.^{1, 2} Because they quickly kill, and target the organisms's membrane nonspecifically, they have been shown to be less likely to develop resistant bacteria than traditional antibiotics.¹ In recent years, AMPs have been chemically immobilized on surfaces of medical devices to render them with antimicrobial properties.³⁻⁸ However, the impact of immobilized AMPs in human cells remains elusive.

Here we report an AMP coating that can be applied to various surfaces while maintaining its antimicrobial activity in the presence of human serum while being non-cytotoxic against human cells. The coating consists on the covalent immobilization of the antimicrobial peptide *cecropin-mellitin* (CM) on gold (Au) nanoparticles (NPs) immobilized on surfaces. CM peptide is a hybrid antimicrobial peptide with 15 amino acids, containing sequences from cecropin-A and mellitin antimicrobial peptides. We show that we can immobilize larger concentrations of AMP peptide per centimeter square (1.02 mg/cm²) than by methods reported in the literature^{5, 7, 9, 10}. The CM peptide immobilized on the Au NP-coated titanium surfaces maintains antimicrobial activity in the presence of human serum with an excellent reusability for five cycles. We further show that the surfaces having immobilized CM have little impact in cell viability, cell metabolism, membrane cell integrity and membrane cell potential as measured by different tests, and thus can be considered as relatively not cytotoxic.

References

- [1] Hancock RE, Sahl HG, *Nat Biotechnol* **24** (2006) 1551.
- [2] Zasloff M, *Nature* **415** (2002) 389.
- [3] Bagheri M, Beyermann M, Dathe M, *Antimicrob Agents Chemother* **53** (2009) 1132.
- [4] Ferreira L Zumbuehl, A, *J Mater Chem* **19** (2009) 7796.

- [5] Gabriel M, Nazmi K, Veerman EC, Nieuw Amerongen AV, Zentner A. *Bioconjug Chem* **17** (2006) 548.
- [6] Gao G et al. *Biomacromolecules* **12** (2011) 3715.
- [7] Gao GZ et al. *Biomaterials* **32** (2011) 3899.
- [8] Humblot V et al. *Biomaterials* **30** (2009) 3503.
- [9] Kazemzadeh-Narbat M. et al. *Biomaterials* **31** (2010) 9519.
- [10] Chen CP, Wickstrom E. *Bioconjug Chem* **21** (2010) 1978.

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