Cecropin-mellitin modified surfaces exhibit high antimicrobial activity and low cytotoxicity against human cells

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Statement of Purpose:
In recent years, antimicrobial peptides (AMPs) have been chemically immobilized on surfaces of medical devices to render them with antimicrobial properties.[1-4] However, the demonstration that the activity of immobilized AMP can be maintained in the presence of serum remains elusive. Surfaces having immobilized cationic peptides are susceptible to be adsorbed by plasma proteins with the subsequent loss of antimicrobial activity. Furthermore, with the exception of very few studies that have determined the cytotoxicity of surfaces in mammalian cells[3, 5], although in conditions that is unclear whether they maintain their antimicrobial activity, the effect of the immobilized AMP on human cells is relatively unknown. Here we report a coating based on *cecropin-mellitin* peptide (CM) that maintains its activity in the presence of serum and has relatively low cytotoxicity against human cells.

Methods:
The coating consists on the covalent immobilization of the CM, having a terminal cysteine group (C-terminal), on gold (Au) nanoparticles (NPs) immobilized on surfaces and functionalized with a mixture of poly(ethylene glycol) with terminal amine and carboxylic groups. Antimicrobial activity of immobilized CM peptides was tested against 10^5 CFU/mL Gram positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria in PBS and serum containing PBS, incubated at 37 °C for 4 h. The biocompatibility of CM attached surfaces was evaluated on human vein endothelial cells (HUVEC) and normal dermal fibroblasts (NDHF) cells cultured on top of the surfaces for 24 h. Cell metabolism (MTT and ATP analysis), cell morphology and attachment (SEM analysis), membrane cell integrity (LDH analysis) and membrane cell potential ($DioOC_5(3)$) were evaluated.

Results:
The concentration of AMP immobilized in our surface (1.02 mg/cm^2) is higher than most of the values previously reported (below 10 $\mu g/cm^2$)[3] in the literature. Our surfaces showed excellent antimicrobial activity against both *S. aureus* and *E. coli* in the presence of 20 % (v/v) human serum in PBS within 2 h incubation time. The antimicrobial activity of CM peptide was preserved due to zwitterionic property of thiol-PEG-amine and thiol-PEG-acid, which prevented the adsorption of serum on the surface. The mechanism of antimicrobial activity was studied by atomic force microscopy (AFM). The cross-sectional analysis shows that the cell wall of *E. coli* collapsed after bacteria exposure to CM peptide immobilized on Au-PEG-NH_2 surface. Next, we evaluate the cytotoxic effect of CM immobilized on surfaces by culturing HUVECs and NDHF on top of CM-immobilized on Au-PEG surfaces. Based on metabolic results, the IC50 of soluble CM in HUVECs and NDHF is approximately 5 and 60 μg/mL, respectively. However, no statistical difference ($P<0.05$, $n=4$) on cell viability, cell metabolism and cell integrity was observed for cells cultured on surfaces having CM immobilized and the control group. We evaluated also the effect of surfaces with immobilized CM in the membrane potential of HUVECs and fibroblasts after 24 h. Our results show no significant differences in both cell membrane potentials after 24 h exposure to the surface.

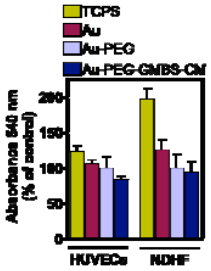

Figure 1. Cell metabolic activity of HUVECs and fibroblasts cultured on top of CM-functionalized surfaces. (TCPS, tissue culture polystyrene; Au, gold; Au-PEG, gold-grafted with polyethylene glycol molecule; and, Au-PEG-GMBS-CM, gold grafted with *cecropin mellitin*).

Conclusions:
We have developed an antimicrobial coating based on AMPs that is active against bacteria in medium with serum and is relatively non-cytotoxic against human cells. This coating technique can be very useful to render medical devices with antimicrobial properties.

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References:
[1] M. Bagheri, M. Beyermann, M. Dathe, Antimicrob Agents Chemother 2009, 53, 1132.
[2] G. Gao, K. Yu, J. Kindrachuk, D. E. Brooks, R. E. Hancock, J. N. Kizhakkedathu, Biomacromolecules 2011, 12, 3715.
[3] G. Z. Gao, D. Lange, K. Hilpert, J. Kindrachuk, Y. Q. Zou, J. T. J. Cheng, M. Kazemzadeh-Narbat, K. Yu, R. Z. Wang, S. K. Straus, D. E. Brooks, B. H. Chew, R. E. W. Hancock, J. N. Kizhakkedathu, Biomaterials 2011, 32, 3899.
[4] V. Humblot, J. F. Yala, P. Thebault, K. Boukerma, A. Hequet, J. M. Berjeaud, C. M. Pradier, Biomaterials 2009, 30, 3503.
[5] M. Nakamura, T. Iwasaki, S. Tokino, A. Asaoka, M. Yamakawa, J. Ishibashi, Biomacromolecules 2011, 12, 1540.