Functionalization of Gold Nanostars Sensors through Engineered Peptides for Bacterial Detection

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Statement of Purpose: Pathogenic contamination and resistant 'superbug' infections are critical concerns all over the world, due to extremely low minimum infective doses (MID) for many bacteria and the lack of inexpensive and portable methods to detect bacteria at these limits. Currently available methods for the detection of microbiological threats utilize specific enrichment media to separate, identify and count bacterial cells. There are some other detection techniques such as PCR and DNA-based nanobarcode detection, which have proven to be fast and highly sensitive. However, such methods require extra steps including pretreatment of cells to extract their DNA [1]. Here, we propose to use peptide functionalized gold nanostars for the detection of bacteria and to prevent bacterial infection. For this, we utilized material binding peptides recognition capability on gold surfaces, particularly gold binding peptides (AuBP2) [2]. To detect bacteria and to prevent bacterial infection, antimicrobial peptides (AMP) were used as natural antibiotics (due to their broad spectrum activity and infection prophylaxis). Dual functional peptides were designed to decorate the gold nanostars and gold nanospheres.

Methods:

Synthesis and Characterization of Gold Nanostars: Gold nanostars were prepared at room temperature, at a pH of 7.4 using a seedless, green chemistry method in which $HAuCl_4$ was reduced by HEPES.

Dual-Functional Peptide Design and Antimicrobial Activity: The peptide was designed in conjugation with AuBP2 and AMP via a GGG flexible linker. The dual-functional peptide was tested for their antimicrobial activity against *S. aureus and P. aeruginosa* in solution. All experiments were run in triplicate and repeated at least three times.

Results:

Characterization of Gold Nanostars: In this study, the synthesized gold nanostars and commercial nanospheres were first characterized under Transmission Electron Microscopy (TEM) to determine their shape and size properties (Figures 1a and b and Figure 2 a). Their physical properties were confirmed via adsorption spectra (Figure 2b).

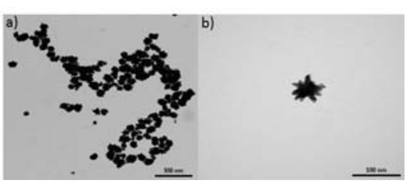

Figure 1a: TEM images of Gold Nanostars: a) shows the 2D distribution of Gold Nanostars with their branches and b) shows the 2D view of a single Gold Nanostars with 12 to 14 branches.

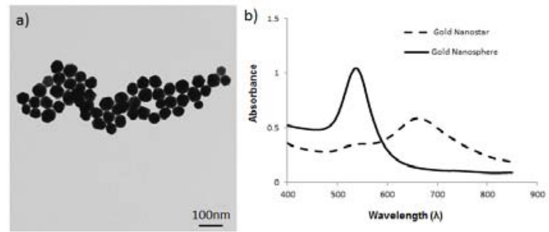

Figure 2: a) TEM images of gold nanospheres and b) Adsorption spectra of gold nanostar and gold nanospheres.

Determination of Minimal Inhibitory Concentration (MIC) for AMP and AuBP2-AMP

The dual functional peptide had remarkable antimicrobial activity against two types of bacteria (*S. aureus and P. aeruginosa*) at a 50µM peptide concentration after 8 hours (Fig. 3). This may have a significant impact on the prevention of pre-implant infections.

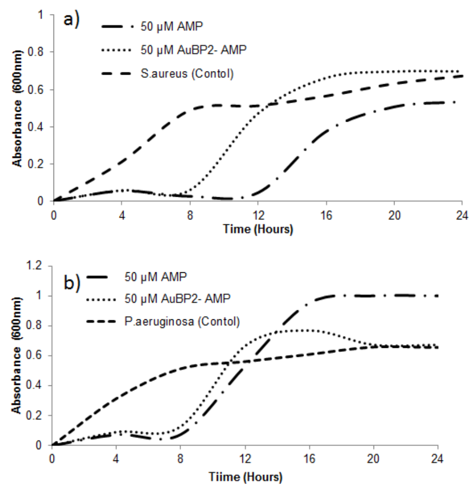

Figure 3: Minimum growth inhibition concentration of AMP and AuBP2-GGG-AMP against a) *Staphylococcus aureus* and b) *Pseudomonas aeruginosa.*

Conclusions: Material binding peptides are promising biomolecules for surface functionalization of nanoparticles. Due to their high affinity, they provide a robust coating on implant surfaces. They are also able to conjugate with antimicrobial agents such as antimicrobial peptides for the detection of bacteria in a nanosensor application.

References:

[1] Mannoor, M.S., Nature Communications, 2012; 3:763
[2] Hnilova, M., Langmuir, 2008; 24:21,12440-45

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