The fate of adhering bacteria on antimicrobial surfaces: transcriptomic analysis of resistance associated genes and macrophage-mediated phagocytosis

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The growing number of biomaterial-associated infections (BAI) has led to the need of developing novel antibacterial coatings. In our previous work [1], an antimicrobial lipopeptide and an enzyme were successfully co-immobilized onto polydimethylsiloxane (PDMS) imparting it with antimicrobial properties. Although results were quite promising, it could not be overlooked that some bacteria managed to adhere to these coatings. Thus, to know the fate of these bacteria on the pathogenesis of BAI became imperative. This study aimed: i) to inspect the susceptibility profiles of these remaining bacteria to lower doses of vancomycin and to the antimicrobials used to functionalize PDMS; ii) to investigate bacterial removal and digestion by macrophages. Results showed that the cells that managed to adhere to both unmodified and modified surfaces were able to grow into a biofilm with metabolic active cells. Vancomycin, used at a MIC, had no effect on the metabolic activity of biofilms formed on these surfaces. Conversely, biofilms formed on functionalized PDMS were more susceptible to vancomycin, suggesting a synergistic effect. To evaluate the development of bacterial resistance towards compounds immobilized onto PDMS, cells of a 72 h – 96 h-old biofilms formed on the functionalized surfaces were continuously recovered and allowed to adhere to new modified surfaces, for a total of 30 days, using PDMS surfaces as a control. Afterwards, the MIC and MBC of the lipopeptide were determined against the recovered cells and the transcript levels of several genes involved in antimicrobial resistance and virulence mechanisms were assessed using quantitative RT-PCR. Results showed that cells adhered to functionalized PDMS exhibited identical susceptibility patterns to those of cells recovered from unmodified surfaces, suggesting no development of resistance. The transcriptomic analysis also did not disclose any resistance development since cells in contact with modified surfaces exhibited some genes involved in microbial resistance equally or less expressed, as compared to the ones recovered from control surfaces. Regarding phagocytosis, it was found that macrophages adhesion to unmodified PDMS tend to cluster which may compromise their mobility and subsequently their phagocytic activity. After PDMS functionalization, a higher number of adhered macrophages were found and more evenly distributed along the functionalized surfaces, helping thus better clearance of bacteria. In conclusion, bacteria found on these surfaces did not develop resistance towards the compounds used to functionalize PDMS, being even more susceptible to vancomycin and macrophages action, which strengthens the great potential of our coating strategy to fight BAI.

References

[1] Alves, D, Magalhães, A, Grzywacz, D, Neubauer, D, Kamysz, W and Pereira, MO, Co-immobilization of Palm and DNase I for the development of an effective anti-infective coating for catheter surfaces, *Acta Biomaterialia*, 44, 313-322, 2016.