An alginate lyase functional coating catalysis-independent to prevent *P. aeruginosa* adhesion

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Bacterial colonisation of indwelling devices remains a serious threat in clinical field as it is commonly associated to persistent infections, called biomaterial-associated infections (BAI). *Pseudomonas aeruginosa* is the most common gram-negative bacillus associated with BAI and its emergence as a nosocomial pathogen is a growing concern. This opportunistic pathogen can produce a capsule-like polysaccharide called alginate that contributes to mucoid biofilm structure and persistent nature of infections. Given alginate's contribution to bacterial virulence, it has long been considered as a promising target for interventional therapies. Alginate lyase, an enzyme able to degrade alginate, has been shown to detach mucoid biofilms from abiotic surfaces and increase their antibiotic susceptibility.

In this work, a new approach for alginate lyase was explored. Instead of using this enzyme for the treatment of pre-established mucoid biofilms, the ability of alginate lyase to prevent *P. aeruginosa* adhesion to a surface was investigated. For that purpose, a polydopamine (pDA) dip-coating strategy was applied for functionalization of biomaterials with alginate lyase. Polycarbonate (PC) substrates were immersed in an alkaline solution of dopamine to form a thin layer of pDA and then transferred into a solution of alginate lyase. Surface characterization was performed with XPS, contact angle measurement and SEM. Two reference strains of *P. aeruginosa*, a mucoid strain (ATCC 39324) and a non-mucoid (27853) as well as four clinical isolates, were used to assess the anti-adhesive properties of the functional coatings.

Surface characterization confirmed the successful and efficient grafting of alginate lyase onto pDA-coated PC substrates. Untreated PC substrates allowed the adhesion of both reference strains and most of bacteria were found alive on these surfaces. Polydopamine-coated substrates had no significant effect on bacterial adhesion compared to the unmodified substrates. Substrates functionalized with alginate lyase exhibited anti-adhesive properties, causing a significant inhibition of the mucoid strain adhesion. Interestingly, substrates immobilized with this enzyme also proved to inhibit the adhesion of the non-mucoid strain and pDA-coated PC substrates immobilized with heat-inactivated enzyme also prevented the attachment of both bacterial strains. These results suggested that alginate lyase immobilized on pDA-coated substrates was able to impair *P. aeruginosa* adhesion regardless its mucoid phenotype and therefore it could be applied in a different context than cystic fibrosis. For instance, this enzyme could be used to develop functional coatings able to prevent *P. aeruginosa* infections associated to biomaterials. To confirm this hypothesis, the attachment of 4 clinical strains of *P. aeruginosa,* isolated from peritoneal dialysis catheters, on alginate lyase functional coatings were also evaluated. Alginate lyase immobilized on the substrates impaired the attachment of the clinical strains with the exception of one as its adhesion to the unmodified PC was already low.

In this work, the versatile chemistry of polydopamine was successfully exploited to functionalize biomaterial surfaces with alginate lyase to impart them with anti-adhesive properties. The antibacterial performance of these alginate functional coatings was catalysis-independent which highlights the importance of further studies to better understand its mechanism of action against *P. aeruginosa* strains.

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