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Macrophage phagocytic activity toward adhering staphylococci on cationic and patterned hydrogel coatings *versus* common biomaterials

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ABSTRACT

Biomaterial-associated-infection causes failure of biomaterial implants. Many new biomaterials have been evaluated for their ability to inhibit bacterial colonization and stimulate tissue-cell-integration, but neglect the role of immune cells. This paper compares macrophage phagocytosis of adhering *Staphylococcus aureus* on cationic-coatings and patterned poly(ethylene)glycol-hydrogels *versus* common biomaterials and stainless steel in order to identify surface conditions that promote clearance of adhering bacteria. Staphylococci were allowed to adhere and grow on the materials in a parallel-plate-flow-chamber, after which murine macrophages were introduced. From the decrease in the number of adhering staphylococci, phagocytosis-rates were calculated, and total macrophage displacements during an experiment determined. Hydrophilic surfaces had the lowest phagocytosis-rates, while common biomaterials had intermediate phagocytosis-rates. Patterning of poly(ethylene)glycol-hydrogel coatings increased phagocytosis-rates to the level of common biomaterials, while on cationic-coatings phagocytosis-rates remained relatively low. Likely, phagocytosis-rates on cationic coatings are hampered relative to common biomaterials through strong electrostatic binding of negatively-charged macrophages and staphylococci. On polymeric biomaterials and glass, phagocytosis-rates increased with macrophage displacement, while both parameters increased with biomaterial surface hydrophobicity. Thus hydrophobicity is a necessary surface condition for effective phagocytosis. Concluding, next-generation biomaterials should account for surface effects on phagocytosis in order to enhance the ability of these materials to resist biomaterial-associated-infection.

1. Introduction

Biomaterials play an important role in human life to support and restore function after wear, trauma or surgical intervention, the most common examples being total hip- or knee prostheses made of a combination of polymeric and metallic biomaterials. Biomaterial implants and devices provide foreign surfaces, alien to the human body, to which bacteria can adhere and start forming biofilms. Accordingly, biomaterial-associated infection (BAI) is the number one cause of failure of biomaterial implants and devices presenting high costs to the healthcare system. Bacterial contamination of a biomaterial surface during surgical implantation has been recognized as an important route of contamination, but whether or not such contamination eventually results in BAI depends on the outcome of the "race for the surface" between tissue integration and biofilm formation [1]. If tissue cells win this race, the implant surface will be covered by a cellular layer and is then less vulnerable to biofilm formation and associated infection. Alternatively, in the inverse case, bacteria will colonize the implant surface and tissue cell functions are hampered by bacterial virulence factors and excreted toxins [1–3]. BAI is often difficult to treat, as the biofilm mode of growth protects pathogenic microorganisms against both the host defense system and antibiotics [4]. In most cases, the final outcome of BAI is the removal of the implant in order to eradicate infection and subsequent replacement. Consequently, an important next challenge in biomaterials development is to preserve or enhance the ability of an implant or device to facilitate tissue integration while simultaneously inhibiting colonization by bacteria [1,5]. In an era of an increasing prevalence of antibiotic-resistant strains [6] and considering the protection offered to colonizing bacteria by their biofilm mode of

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growth, these innovative next-generation biomaterial surfaces should have an efficacy that eliminates the need to use post-operative antibiotics.

Many next-generation biomaterials or coatings have been proposed over the past decades (see Campoccia et al. [7] and Hasan et al. [8] for excellent reviews). Several non-adhesive modifications of biomaterial surfaces have been developed to mitigate bacterial colonization, such as poly(ethylene)glycol (PEG) coatings [9–11]. However, while they inhibit bacterial colonization, they simultaneously prevent tissue integration unless appropriately patterned. Recently, it has been observed that bacterial colonization can be confined to small adhesive patches in a PEG-hydrogel coating that at the same time provide sufficient anchoring points for tissue cells to adhere, spread and grow [12–14]. Another category of innovative surfaces is constituted by cationic coatings, either of biological [15] or synthetic [16] origin and possessing the unique quality of killing adhering bacteria upon contact [17]. There is a minimal cationic charge density required for bacterial contact-killing, but also a maximal one to ensure survival of tissue cells on such surfaces [18]. This leaves a narrow-window of positive charge density available for clinical application.

The pathogenesis of BAI is complex, however, and the outcome of the race for the surface depends not only on how tissue cells and contaminating bacteria interact on a biomaterial surface, but also on how a biomaterial influences the host immune system [19]. Following biomaterial implantation, tissue trauma and injury trigger a cascade of events that activate the immune system [20]. Macrophages are one of the most predominant immune cells that arrive within minutes to hours after surgery at an implant site and can remain at a biomaterial surface for several weeks to orchestrate the inflammatory process and foreign body reactions [20–23]. During infection, macrophages detect bacteria via cell surface receptors that bind to bacterial ligands and opsonins [21–23]. Subsequently, macrophages ingest pathogens and activate cellular functions such as proliferation, secretion of proteins and cytokines, and respiratory burst to destroy phagocytized organisms and recruit other cells from the adaptive immune system [21,23]. Therefore, bacteria-biomaterial-immune cell interactions are important factors in the pathogenesis of BAI. Immune cell interactions with bacteria on a biomaterial surface are extremely hard to study as they require complicated culture conditions in which neither immune cells nor bacteria are put at too big an (dis)advantage with respect to each other. As a consequence, such studies are rarely done [24,25]. Yet, co-culture studies are urgently needed to advance next-generation biomaterials or coatings to clinical use and possess the potential of reducing the number of animal studies required, since many new biomaterials or coatings can be discarded beforehand on the basis of improved in vitro models, such as a co-culture one [5]. Co-culture studies involving bacteria and tissue cells have been performed under static conditions [26], under flow in macroscopic flow perfusion systems [27] or in microfluidic devices [28] and importantly have shown results that are consistent with clinical studies [25,29]. Moreover, biomaterial surface conditions have been revealed on which the presence of low levels of adhering *Staphylococcus epidermidis* enhances tissue integration [30,31] or completely negates positive effects of cell-adhesive sites on tissue integration indicated in mono-culture studies [32]. Co-culture studies involving bacteria and macrophages [33] have revealed differences in clearance of adhering staphylococci from a surface between murine macrophages and human phagocytes, which require differentiation from their monocyte or promyelocytic state during an experiment. In addition, surface thermodynamic analysis indicated that phagocytosis of adhering pathogens is determined by the interplay of physical attraction between pathogens and phagocytes and the influence of bacterial chemo-attractants [34–36].

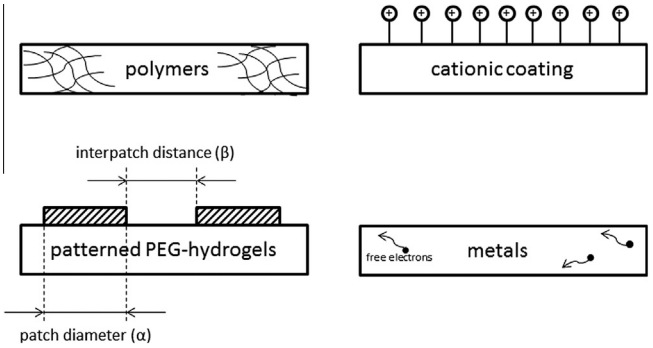

Fig. 1. Schematic drawings of common polymers (silicone rubber, polymethylmethacrylate, tissue culture polystyrene), a cationically coated surface, differently patterned polyethylene glycol coatings and a metal surface, emphasizing the unique features of these different classes of materials involved in this study with respect to their interaction with bacteria and macrophages.

Next-generation biomaterial coatings like patterned PEG-hydrogel coatings and cationic coatings, have never been subjected to co-culture studies with macrophages and bacteria. Therefore the aim of this paper is to compare macrophage phagocytosis activity toward adhering staphylococci on cationic coatings and patterned PEG-hydrogels *versus* common biomaterials (polymers with different hydrophobicity and stainless steel) in order to identify surface conditions that promote clearance of adhering bacteria (see Fig. 1 for a schematic of the different surfaces involved). *Staphylococcus aureus* was chosen as a pathogen as it is frequently found in infections associated with biomaterial implants and devices. The murine macrophage cell line J774 was chosen because this cell line readily phagocytoses *S. aureus* [37–39], while being activated by lipoteichoic acid and other cell wall antigens of Gram-positive bacteria [40–44]. In this study we focused on the macrophage activity toward adhering staphylococci on different materials in bi-cultures. We did not include tissue cells, like osteoblasts as in other studies [24], as such tri-cultures would have complicated the analyses and generally yields high standard deviations that would not allow a quantitative approach to our current research question.

2. Materials and methods

2.1. Polymeric and metallic biomaterials

Bacterial-macrophage interaction was evaluated on different common polymeric and metallic biomaterial surfaces: silicone rubber (SR, water contact angle 103°), polymethyl methacrylate (PMMA, water contact angle 73°), tissue culture polystyrene (TCPS, 48°), stainless steel (SS, 71°) and glass (37°), though not a common biomaterial. All surfaces were cleaned in 2% RBS 35 detergent solution (Omnilabo International BV, Breda, The Netherlands) under sonication and rinsed abundantly with ultrapure water, submerged in 70% ethanol and washed again with sterile ultrapure water. Finally, surfaces were placed inside a parallel plate flow chamber ($175 \times 17 \times 0.75\ mm^3$) and rinsed with phosphate-buffered saline (PBS, 10 mM potassium phosphate, 150 mM NaCl, pH 6.8).

2.2. Cationically coated glass surfaces

Cationic hyperbranched polyethyleneimine (PEI) coatings were prepared as described by Asri et al. [45]. Briefly, glass slides were activated with Piranha treatment (3:1 of 98% sulfuric acid