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On the mechanics of bacterial biofilms on non-dissolvable surgical sutures: A laser scanning confocal microscopy-based finite element study

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ABSTRACT

Biofilms are bacterial communities encapsulated within a self-secreted extracellular polymeric substance and are responsible for a wide range of chronic medical device related infections. Understanding and addressing the conditions that lead to the attachment and detachment of biofilms from biomedical surfaces (orthopaedic implants, sutures, intravenous catheters, cardio-vascular stents) has the potential to identify areas of the device that might be more prone to infection and predict how and when biofilms might dislodge. In this study, an integrated software methodology was devised to create image-based microscopic finite element models of real biofilm colonies of *Staphylococcus aureus* attached to a fragment of surgical suture. The goal was to predict how deformation of the suture may lead to the potential detachment of biofilm colonies by solving the equations of continuum mechanics using the finite element method for various loading cases. Tension, torsion and bending of the biomaterial structure were simulated, demonstrating that small strains in the suture can produce surface shear stresses sufficient to trigger the sliding of biofilms over the suture surface. Applications of this technique to other medical devices are discussed.

1. Introduction

1.1. Device-related biofilm infections

Bacterial infection is a common complication associated with all types of implanted medical devices, regardless of function, material composition (i.e., metals, plastics and elastomers) or anatomical location [1]. Device-related bacterial infections are often difficult to diagnose and treat because the bacteria are present as biofilms that are directly attached to the device or the periprosthetic tissue [2]. Biofilms are communities of bacteria held to a surface and encapsulated within a self-secreted extracellular polymeric slime matrix composed of polysaccharides, proteins and nucleic acids [3]. When in biofilms, even antibiotic sensitive strains of bacteria become highly tolerant to antibiotics. Bacteria can detach from biofilms as single cells or as aggregates in which they retain their antibiotic tolerance. While the controlled detachment of biofilms from medical devices may be seen as desirable for resolving an infection, uncontrolled detachment might result in the release of clusters of biofilm bacteria spreading the infection. Inglis et al. [4] captured particles of detaching biofilm in the gas flow from endotracheal tubes recovered from Intensive Care Unit patients and concluded that the introduction of these biofilm particles to the airways could explain the high rates of ventilator-associated pneumonia in intubated patients. In a more recent study, Wang et al. [5] used bioluminescent strains of wild type *Staphylococcus epidermidis* and a detachment-deficient mutant in a mouse model to demonstrate the importance of detachment in systemic infection from biofilm on subcutaneously implanted catheter pieces. Mechanically induced detachment of biofilm is typically considered in the context of shear stress caused by flow of an overlying fluid phase [4,6]. There has been little consideration of the effect of mechanical flexing of the underlying surface on biofilm detachment. Many medical devices that are prone to biofilm infections are composed of materials that readily undergo struc-

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tural deformation, which carries the possibility of causing biofilm detachment. These include intravenous venous catheters and shunts, which are composed of elastomers that can flex and be stretched to high strains, endotracheal tubes composed of more rigid materials, but designed to flex readily for placement, and non-dissolvable surgical sutures and meshes, which have very high tensile stress, but are pliable for surgical manipulation or the weave allows them to flex with the body motion. Recently, Kathju et al. [7–9] reported three case studies in which they identified biofilms on surgical sutures following open hernia repair as a cause of chronic infection. In two cases, the infection had formed chronic draining sinus tracts from the sutures, suggesting that pathogens were being disseminated from the biofilm.

Surgical site infection is a major complication following open hernia repair occurring in 13.4% of patients (data from 434 patients grouped from 10 independent studies) with 3.5% (of 257 patients) requiring mesh removal [10]. To investigate how flexing, bending and stretching of sutures *in silico* might cause biofilms to detach, biofilms of a clinical strain of *Staphylococcus aureus* were grown on a surgical suture in the laboratory. Then, laser scanning confocal microscopy (CLSM) was used to obtain three-dimensional (3D) data sets, which were subsequently used to create a 3D computational mesh of the biofilm and suture for finite element modelling using mechanical properties from literature values.

1.2. Mathematical and finite element modelling of biofilms

The modelling of biofilms is a relatively recent research area, which aims to offer microbiologists and engineers quantitative assays to study, understand, predict and help to design techniques to control biofilms in medicine and industry. Biofilms are complex adaptive living systems with cellular, physical and chemical processes that interact non-linearly and, by doing so, create feedback loops, which can quickly become too intricate to understand intuitively [11]. Modelling these types of system is a way to deconstruct their complexity so that basic processes or mechanisms can be extracted to further understanding [12]. As pointed out by Duddu et al. [13], there are three main approaches to modelling the behaviour of biofilms: cellular automata [14–28], agent-based models [24,29–35] and continuum models [13,16,28,32,36–44]. Departing from discrete-based models, continuum-based models assume the continuity of fundamental physical quantities associated with micro-organism populations (e.g., density, strain, stress, electric potential) over a specific length scale: the scale of the continuum. These quantities are governed by partial and/or ordinary differential equations. The only practical way to solve large systems of partial differential equations for materials, conditions and geometries of arbitrary complexity is to use computer-based numerical techniques such as the ubiquitous finite element method [45]. Only a limited number of studies have used finite element techniques to study biofilms, and most of them have been restricted to two-dimensional (2D) problems and/or have focused on transport phenomena, growth of biofilms and/or their mechanical response to fluid flow [13,37–40,43]. In their pioneering study, Böl et al. [44] developed an image-based 3D multi-physics finite element model of a biofilm to study the detachment conditions of the biofilm from its substrate under the influence of a laminar fluid flow. In the numerical study, it was shown that the structural and material properties of a biofilm subjected to flow-induced shear stress are the key parameters controlling its detachment.

The structural changes in biofilms induced by growth and other mechano-biological processes significantly alter the stress distribution in response to shear flow, which in turn leads to drastic changes in the propensity of the biofilm to detach.

1.3. Objectives of the study

Mathematical and computational models of biofilm have so far overlooked the effects that the mechanical strains of the surface on which biofilms attach might have on the detachment of biofilm, particularly with regard to biomaterials and engineered surgical devices. In this paper, a simple methodology combining CLSM, image processing and finite element techniques is proposed to capture and analyse the mechanical behaviour of microbial biofilms attached to the surface of a non-resorbable surgical suture material. The major novelty of the present work is the ability to capture the complex micro-scale geometry of biofilms and surfaces with potentially complex geometries for finite element analyses without disrupting them, or the substrate to which they attach.

2. Materials and methods

2.1. Bacterial strains and growth in culture

Biofilms were grown from *Staphylococcus aureus* (CGS.Sa03, BS187), a clinical methicillin resistant (MRSA) strain recovered by standard microbiological culture of explanted pieces of polypropylene mesh from a patient being operated for cellulitis and abscess after mesh placement. The genome of CGS.Sa03 has been sequenced and reported [46]. The doubling time of *S. aureus* CGS.Sa03 was 1.4 ± 0.4 h (n = 6).

2.2. Slime-forming ability

Slime production by the CGSSa03 was assessed using the Congo red agar (CRA) assay [15]. Briefly, strains were streaked out onto brain heart infusion (BHI) agar (Oxoid, Basingstoke, Hampshire, UK) containing Congo red 0.8 g l^{-1} (Sigma–Aldrich, St. Louis, MO, USA), which was autoclaved separately then added to the cooled BHI. Slime-producing colonies are black and rough in appearance, while non-slime-forming colonies are red and smooth [47]. The CRA assay for slime production on the MRSA isolate identified both red and black colonies on the same plate in approximately equal proportions (data not shown).

A red colony and a black colony were picked and re-streaked out and, subsequently, a single black colony phenotype was obtained, suggesting that the strain was capable of phase variation with regard to slime formation.

2.3. Preparation of suture and growth of biofilm

Pieces of size 4 Ti-Cron™ braided polyester suture were aseptically cut into ~2-cm lengths and placed in 35-mm polystyrene Petri plates containing 5 ml of sterile BHI broth growth medium (Oxoid, Basingstoke, Hampshire, UK). Sterile tweezers were used to sink the suture, so that it was fully submerged. The plate was inoculated by adding 100 μl of an overnight BHI broth culture of *S. aureus* CGS.Sa03. The plate was incubated at 37 °C, 5% CO_2 in a humidified incubator on an orbital shaker. After 24 h, the spent medium was removed by pipette aspiration and immediately replaced with fresh sterile BHI. The plate was then again incubated for a further 24 h before repeating the process. After the third cycle of incubation (corresponding to 3 days of growth), the suture was removed from the plate, gently blotted dry on one side and mounted on the bottom of a clean Petri plate by carefully placing on a smear of vacuum grease. The suture was rinsed twice to remove planktonic cells with two 5-ml exchanges of Hanks Balanced Salt Solution (HBSS) with $CaCl_2$ and $MgCl_2$ and without phenol red (Cat. # 14025, Invitrogen, Carlsbad, CA, USA). The sutures were then stained by pipetting two to three 20-μl drops of LIVE/DEAD®