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Reduced bacterial adhesion on ceramics used for arthroplasty applications

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ABSTRACT

Orthopaedic-implant-related infections are challenging for clinicians: despite progresses in surgical procedures, the mortality rate of patients experiencing periprosthetic joint infections still ranges from 10 to 18%. Generally, infection starts when planktonic bacteria arising from surgery escape immunological surveillance adhering onto implant surface. Bacterial adhesion depends mainly on material's intrinsic surface features depending on its chemical and physical properties. This study compares materials used for bearings of total hip arthroplasty, advanced ceramics (alumina and zirconia-platelet toughened alumina composites), metals (cobalt–chromium–molybdenum alloy) and polymers (highly cross-linked polyethylene), in terms of wettability and protein adsorption. Materials were infected with *Staphylococcus aureus* and *Staphylococcus epidermidis* biofilm for 24 or 48 h. Bacterial adhesion properties were evaluated by means of biofilm viability, morphology, and thickness, in a worst-case surface roughness condition. Thanks to selective protein adsorption, bioceramics reduced bacterial adhesion and subsequent biofilm formation more effectively in comparison with metal and polymer surfaces.

1. Introduction

Implant-related infections are one of the most common reasons for surgical failure (14–29% of total failures) [1], in most cases causing severe disability and leading to a significant reduction in the patient's quality of life. According to the most recent surveys, the mortality rate of patients undergoing primary implant infections ranges from 10 to 18% [2–4]; moreover, if an infection occurs also in the revised implants, this percentage can double or triple [5,6]. In the US alone, more than a million hip and knee arthroplasties are performed yearly [7]. Similarly, the number of patients undergoing orthopaedic surgery in Europe is now almost 200 per 100,000 inhabitants [8,9], and has been steadily increasing over the last 10 years. Due to increasing life expectancy, the World Health Organization (WHO) foresees that osteoarthritis will be the fourth leading cause of disability by 2020.

Total hip or knee arthroplasty still remains the only applicable solution to improve the quality of life of joint-affected patients [10]. However, despite marked progresses in joint replacement surgery, the infection rate during the first 2 years is about 1% for primary implant failures, and 2% after knee replacement [3,4]; moreover, due to the development of multi- or pan- drug-resistant bacterial strains, these rates are expected to rise in the near future. Hospital-acquired infections are now generally considered to be the third-largest cause affecting public health; they are chiefly caused by a group of multi-drug resistant (MDR) pathogenic biofilm producer strains, known as "ESKAPE" (in the acronym of the Infectious Diseases Society of America (IDSA) that identifies the emerging MDR strains *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiellapneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa,* and *Enterobacter* spp.) [11,12]. Unfortunately, orthopaedic medical devices are not an exception, as they are highly subject to biofilm infections, which generally lead to the need for their removal and replacement. The presence of an implant reduces the bacterial concentration needed to induce infection by 100,000 times [1], since bacteria can survive in the periprosthetic environment by adhering to the implant. The necessity to find a post-antibiotic solution to periprosthetic joint infections may thus be relatively urgent.

Biofilm formation begins when planktonic bacteria, originating from the surgical incision site or from independent infection sources, escape immunological surveillance and adhere onto the implant surface [2]. Once adherent, bacteria proliferate and secrete different kinds of macromolecules, principally polysaccharides and glycolipids, known as extracellular polymeric substances (EPS), which embed and protect the

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neo-forming bacteria community [13]. Mature biofilm not only shields bacteria from the host immune system, but also undermines the effectiveness of antibiotics by up to one thousand times compared to planktonic cells. As a consequence, antibiotic treatment of implant-related infections frequently fails, with consequent implant loss and soft tissue invasion by bacterial communities [14,15]. Although biofilms generally contain several different bacterial strains, the most common ones belong to the Staphylococcus genus: *S. epidermidis* and *S. aureus* (together with *Pseudomonas aeruginosa*), are responsible for three out of four cases of medical-device-related infection [2]. Accordingly, the most reliable way to reduce the poor outcome of medical device infections is to prevent bacterial adhesion to implant surfaces, thus reducing the development of mature biofilm.

Although the bacterial strains involved in implant contamination are often host commensals, the adhesion of bacteria to the implant surface depends on many factors related to the biomaterial's intrinsic properties, particularly its chemistry and physical properties (e.g. roughness, surface charge, wettability). It is thus crucial to study in depth the surface features of medical devices, with the aim of improving them in order to avoid bacterial proliferation on their surface.

Metals (Ti-6Al-4V, CoCrMo and stainless steel), polymers (poly (methyl methacrylate, PMMA), ultrahigh-molecular-weight polyethylene (UHMWPE), and ceramics (alumina, zirconia, alumina matrix composites and hydroxyapatite) are the three classes of materials commonly used for orthopaedic implants [16]. Whereas metals are the most widely used material in implantology, some retrospective studies have shown that they are more prone to bacterial adhesion than are ceramics [17–19]. In addition, both metals and polymers are usually affected by significant time-dependent surface degradation, leading to the significant increase of bacterial adhesion (enabled by surface roughening) and various other adverse events caused by the release of ions and particles [17–19]. In contrast, bioceramics, mainly used as bearing couples in artificial joints, have little tendency to degradation, and present peculiar physical-chemical surface properties that are potentially responsible for their antifouling features [20]. Although of crucial importance, there is no literature so far on comparing intrinsic antibacterial properties of systems actually used in orthopaedics.

In this study, monolithic alumina and zirconia-platelet toughened alumina (ZPTA) were compared to metallic cobalt–chromium–molybdenum (CoCrMo) and polymeric cross-linked polyethylene (XLPE) materials, assessing wettability, protein adsorption, and bacterial adhesion. These materials were selected for testing as being among the most widely used for artificial joint applications in hip, knee, and shoulder arthroplasty. Experiments were performed without accomplishing any specimens polishing practice in order to simulate the worst-case in-vivo scenario present in current orthopaedic implants to indisputably focus onto bare selected materials antibacterial properties.*S. aureus* and *S. epidermidis* biofilms were then cultivated for 24 or 48 h on the test materials' surface, and evaluated in terms of viability, morphology, and thickness.

2. Materials and methods

2.1. Samples

The samples comprised: ceramics, both monolithic alumina (Al_2O_3; ISO 6474-1) and zirconia-platelet toughened alumina (ZPTA; ISO 6474-2), marketed under the brand name BIOLOX®forte and BIOLOX®*delta*, respectively; metal cobalt–chromium–molybdenum (CoCrMo, ISO 5832-12) and cross-linked polyethylene (XLPE, ISO 5834-2); all were provided by CeramTec (CeramTec GmbH, Plochingen, Germany) as disks 2 cm in diameter, 6 mm thick. Bare specimens were voluntarily not further polished in order to mimic worst-case surface roughness condition (i.e. ground surface). The bare materials surface profilometric features assessed using a laser optical profilometer (UBM-Microfocus Compact, NanoFocus AG, Germany), accordingly to ISO Standard 25178 are reported in Table 1 and Fig. 1. Specimens were sterilized by gamma rays (25 kGy, as per ISO 11737-1) and stored at room temperature in sterile packages until use.

Table 1
Specimens'surface roughness.

Specimens (bare materials)	Ra (mean ± sd) (μm)
XLPE	0.48 ± 0.08
CoCrMo	0.27 ± 0.01
ZPTA	0.19 ± 0.01
Alumina	0.21 ± 0.02

2.2. Surface analysis

2.2.1. Surface wettability

Small disks of 20 mm diameter and 6 mm thickness were ground and polished with diamond paste to reach a surface roughness (Ra) of less than 5 nm, which have been measured by means of AFM Nanosurf Mobile S (Liestal, Switzerland), installed on an Isolation Platform Halcyonics Micro 40 (Göttingen, Germany). The contact angle measurements were performed in a goniometer (DSA25, Krüss) equipped with an automatic drop dispenser using de-ionized water; images were analysed using Krüss DSA4 proprietary software. All measurements were made using 1 μl as drop volume, within the first ten seconds after deposing the drop; analyses were done in a closed room where temperature and relative humidity was kept reasonably constant at 23 °C (± 3) and 39% (± 7) of relative humidity.

Due mainly to the presence of surface contamination, contact angle hysteresis measurement in dynamic conditions may be markedly influenced by surface irregularities [21]. To eliminate this drawback, the cleaning method providing the lowest hysteresis was applied to equally mirror-finish polished surfaces, so as to remove contamination as far as possible [22], following the CeramTec cleaning procedure for orthopaedic components.

The contact angles on each side of the drops were measured separately, without assuming symmetry; one sample per material was used and each surface was tested at least at five different locations, making sure not to test the same spot twice.

2.2.2. Protein adsorption

To determine the different absorption capacity of the specimens, disks were placed in the wells of a 6 multiwell plate (NuncDelta, ThermoScientific) and incubated in 6 ml/well of foetal bovine serum (FBS, Sigma) for 1 h at 37 °C. The total amount of adsorbed proteins was then quantified by the colorimetric bicinconinic acid assay (BCA, Thermo Scientific) [23]. Briefly, after incubation in FBS, the proteins adsorbed on the specimen surface were lysed in 1 ml of Ripa Buffer (50 mMHepes, 150 mM NaCl, 0.1% SDS, 1% Triton-X100, 1% sodium deoxycholate, 10% glycerol, 1.5 mM MgCl2, 1 mM EGTA, 1 mMNaF, 1% PMSF, 0.5% Na_3VO_4, 1% protease inhibitor mix) and gently collected using a cell scraper. To determine the amount (expressed as μg/cm^2) of protein in each specimen, a standard curve was generated using bovine serum albumin (Albumin Standard, Thermo Scientific, 0–2 mg/mL) and mixed with BCA kit reagents (Thermo Scientific). The absorbance of all specimens and that of the standard curve, were measured at 570 nm by spectrometer (SpectraCount, Packard Bell, USA) and the test specimen protein amount was calculated as a function of the standard curve.

To investigate any selective adsorption of pro- or anti- cell adhesion proteins, 10 μg of each protein extract were dissolved in Laemmli buffer 5× (62.5 mM Tris-HCl, pH 6.8, 25% glycerol, 2% SDS, 0.01% Bromophenol Blue), heated at 95 °C for 5 min, resolved on 8% SDS-PAGE, and transferred to a PVDF membrane. Lastly, the membrane was stained with Comassie blue and analysed with Image j software (NIH)