Tribology International 65 (2013) 97–104

Contents lists available at ScienceDirect

Tribology International

journal homepage: www.elsevier.com/locate/triboint

Antimicrobial agents and low-molecular weight polypeptides affect polyethylene wear in knee simulator testing

J.-M. Brandt [a,*], K.K. Mahmoud [b,1], S.F. Koval [b], S.J. MacDonald [c], J.B. Medley [d]

[a] Concordia Joint Replacement Group, 1155 Concordia Avenue–Suite 310, Winnipeg, Man., Canada R2K 2M9
[b] Department of Microbiology and Immunology, University of Western Ontario, London, Ont., Canada
[c] Division of Orthopaedic Surgery, London Health Sciences Centre, University of Western Ontario, London, Ont., Canada
[d] Department of Mechanical and Mechatronics Engineering, University of Waterloo, Ont., Canada

ARTICLE INFO

Article history:
Received 17 August 2012
Received in revised form
5 February 2013
Accepted 15 February 2013
Available online 21 March 2013

Keywords:
Polyethylene wear
Lubrication
Knee
Microbiology

ABSTRACT

A series of eight wear tests with six different calf serum lubricants were performed on a displacement-controlled knee simulator. The calf serum lubricants varied in constituent fractions, dilutive media, and antimicrobial agent. The difference in low-molecular weight polypeptide concentration was determined in all calf serum lubricants and this allowed exploration of its role in the PE wear process. Sodium azide was not an effective antimicrobial agent. The subsequently used antibiotic–antimycotic mixture was initially effective in eliminating bacterial growth but its efficacy became exhausted as the testing period progressed. In the present pilot study, a predator bacterium *Bdellovibrio bacteriovorus* was shown to prey on the antibiotic resistant bacterium, suggesting its potential use in knee simulator wear testing.

1. Introduction

Total knee replacements (TKRs) are frequently implanted in patients suffering from end-stage osteoarthritis. The number of patients requiring TKRs is rapidly increasing [1], and thus, any features of the implants that contribute to the need for revision surgery, even minor ones, can cause problems for tens of thousands of patients. Wear of the ultra high molecular weight polyethylene (PE) tibial insert has been shown to lead to wear particle-induced bone resorption (osteolysis) around the TKR, which can cause implant loosening. Over time, a revision surgery may be necessary, which can be both expensive and potentially debilitating [2]. To address this issue, knee wear simulators have been developed to evaluate new TKR designs and bearing materials.

The standard protocol for displacement controlled knee simulator wear testing (ISO 14243-3 [3]) describes the type of loading, displacements, and protein-containing calf serum lubricant to be used, all of which have been shown to affect PE wear [4–9]; however, there are outstanding issues. Diluting the calf serum with phosphate buffered-saline solution (PBS) instead of deionized water (as recommended by ISO 14243-3) increases the lubricants osmolality to clinically relevant osmolality levels [10]. Also, adding hyaluronic acid (HA), a natural constituent of both healthy and diseased human synovial fluid [10,11], increases the thermal stability of calf serum lubricant [10], and can lead to increased PE wear in knee simulator wear testing [6,7]. Recent studies [8,9] have shown that proteins (also referred to as high-molecular weight (HMW) polypeptides, mainly $>2{,}000$ Da in molecular weight [8]) degrade during knee simulator wear testing, and are associated with increased PE wear [9]. Some of the degraded HMW polypeptides can build aggregates and precipitate out, causing the calf serum lubricant to change from a translucent, yellowish liquid to opaque in appearance. After the wear tests, the degraded HMW polypeptides can easily be removed from the used calf serum lubricant for analysis by centrifugation. Apparently, not all degraded HMW polypeptides appear to build aggregates that precipitate out [8]. Some degraded polypeptides, which are short-amino acid chunks (Fig. 1), can still remain in solution, even after centrifugation, due to their relatively small mass. These short-amino acid chunks are referred to as low-molecular weight (LMW) polypeptides [9]. Although the amount of HMW polypeptide degradation has shown to increase with PE wear [9], the role of LMW polypeptides in implant tribology remains uncertain.

Historically, sodium azide (SA), a very toxic compound [12], has been used as the antimicrobial agent in the wear testing of PE [13]. Recent findings [8] have shown that SA does not inhibit microbial growth due to the presence of a Gram-negative bacterium, *Enterobacter cloacae* JK-1 (*E. cloacae* JK-1). Replacing SA with a more effective antibiotic–antimycotic (AA) mixture results in

* Corresponding author. Tel.: +1 204 926 1243.
E-mail address: brandtjm@gmx.net (J.-M. Brandt).
[1] Present address: Department of Biology, Medgar Evers College of The City University of New York, 1638 Bedford Avenue, Brooklyn, NY 11225, United States.

0301-679X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.triboint.2013.02.019

an increased LMW polypeptide concentration, while the total HMW polypeptide degradation remained largely unchanged [9]. Bacteria are known to secrete proteases that degrade (hydrolyze) polypeptides and then the bacteria take up/transport the amino acids or, in the present case, LMW polypeptides into the cell thus, reducing the LMW concentration when SA was used as the inhibitor [9]. Although this is a circumstantial finding, the effects of LMW polypeptides on the colloid-mediated boundary lubrication process [9,14–18] remain uncertain. Generally, many substances used to inhibit microbial growth are synthetically or semi-synthetically produced, and are referred to as antibiotics [19,20]. No one type of antibiotic inhibits all microorganisms, and some organisms are naturally resistant to certain types of antibiotics. The environment surrounding the TKR can generally be considered sterile and thus, microbial contamination in knee simulator wear testing does not replicate the clinical situation [8], except when infection may be present [21,22]. The detection of a Gram-negative bacterium in knee simulator wear testing is a problem because this species is able to develop antibiotic resistance over time [23–25], and additional antibiotic treatment strategies are rather limited [26]. The spread of antibiotic resistance among Gram-negative bacteria found in laboratory settings could become a serious health risk to the operating personnel [27,28]. Clearly, these risks could be associated with the use of antimicrobial agents in wear testing laboratories, and thus, some sort of alternative *biocontrol* agent should be developed. One obvious path to inhibit microbial growth in knee simulator wear testing is to inhibit the growth of Gram-negative bacterium such as *E. cloacae*. For this purpose, a *biocontrol* agent used in the food processing industry, known as the predator bacterium *Bdellovibrio bacteriovorus* (often referred to as just *Bdellovibrio*; approximately 1 μm in length and 0.2 μm in width) [29–32], could potentially be applied. This predator bacterium has the fascinating ability to prey on other Gram-negative bacteria, including pathogenic and antibiotic resistant bacteria [30,32], but is harmless to humans. The life cycle of *Bdellovibrio* can be separated into two major phases: (a) free-swimming phase to search and attack the prey and (b) the growth phase spent inside the periplasm, the space between the plasma membrane and the outer membrane of the prey (Fig. 2) [29–32]. During the free-swimming phase, *Bdellovibrio* collides with the prey cell and attaches itself irreversibly to the surface. *Bdellovibrio* penetrates the outer membrane of the prey and kills the prey by halting its respiration and growth. While residing in the periplasm the *Bdellovibrio* grows and divides by exploiting the cells' macromolecules for nutrients and essential building blocks. *Bdellovibrio* grows inside the dead cell in a structure termed a bdelloplast. After all resources of the prey have been exhausted, the *Bdellovibrio* lyses the remains of the prey cell and swims away to target new prey [29–32].

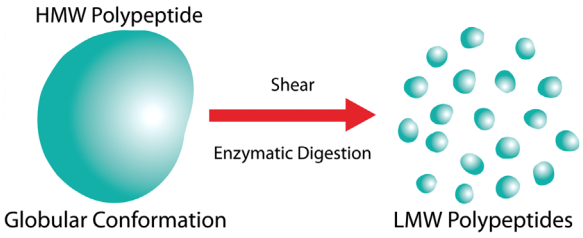

Fig. 1. The effects of shear and enzymatic digestion 1 on the polypeptide structure. Parts of the native, high-molecular weight protein (HMW polypeptide) change from globular conformation into short, polypeptide chunks, referred to as low-molecular weight (LMW) polypeptides.

The primary purpose of the present study was to describe the effects of antimicrobial agents (SA vs. AA) on PE wear with calf serum lubricants that were different in composition. This involved presenting the results of a series of previous wear tests [8,9] and new data on the antibiotic resistance and strategy for *biocontol* in implant wear testing. The related LMW polypeptide concentration was also presented to identify their role in implant tribology. Furthermore, recent knee simulator wear tests have shown the growth of Gram-negative bacterium, despite the use of SA [8]; as a result, microbial growth was monitored during wear testing with AA to detect the potential development of an antibiotic resistant bacterium. Some antimicrobial efficacy tests were performed to gain insight in the mechanisms that could lead to such antibiotic resistance. This was complemented by a pilot study to investigate the ability of *Bdellovibrio* to lyse pre-grown prey cells in buffered

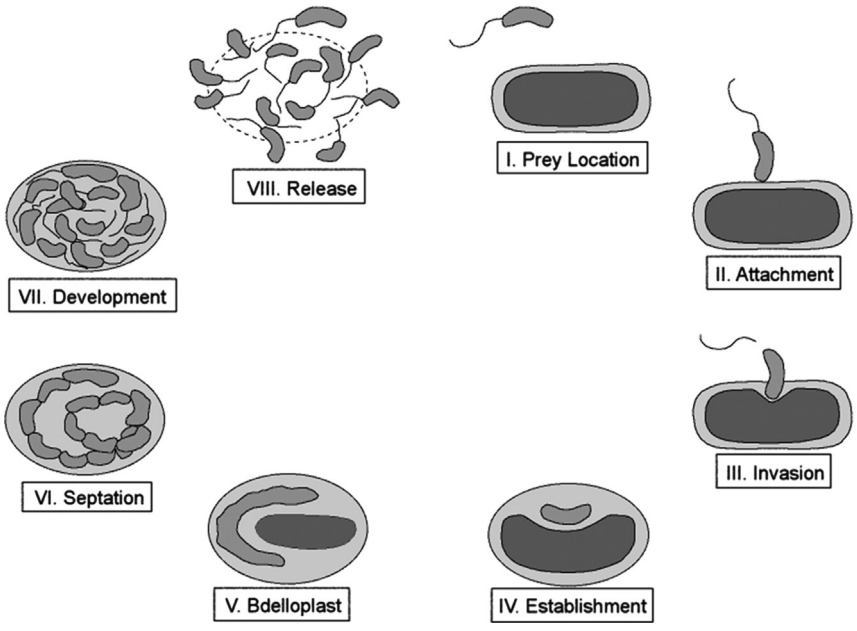

Fig. 2. Schematic of the two-phase life cycle of *Bdellovibrio*, which consists of a free-swimming phase in aqueous medium and a growth phase inside its Gram-negative prey bacterium (Source: Max Planck Institute for Developmental Biology, Tübingen, Germany; with permission from "Science" [32]).