Calf Serum Type Affects Protein Degradation, Bacterial Growth and Polyethylene Wear in Knee Simulator Testing

Introduction

The generation of inflammatory polyethylene (PE) wear-particles in modular total knee replacements (MTKR) can lead to osteolysis and eventual implant failure. Thus, there is a great interest in evaluating MTKR in vitro, gaining some indication on the possible clinical performance. Thus, guidelines for displacement-controlled wear simulator testing have been standardized [1]. However, the according standard recommends the use “calf serum”, without giving ranges on the specific protein constituents, such as albumin and different types of globulin. There has been significant attempt on identifying influential protein related-factors on PE wear of total hip joints [2, 3], but not for MTKR. In addition, antimicrobial agent such as sodium azide (SA) may also be added [1], but has shown to be ineffective in pin-on-disc wear testing [4]. In this study, three types of frequently used calf serum with various protein constituents were used. We attempted to identify protein compounds that perhaps serve as effective boundary lubricants and may affect PE wear rates and evaluated the effectiveness of SA.

Materials and Methods

Bovine calf serum (BCS), newborn calf serum (NCS) and alpha-calf serum (ACS, closest to human albumin-a-globulin levels [5]; all HyClone, Logan, UT) were diluted with distilled water to a protein concentration of 19 ± 2 g/l. SA (0.2%) was added to retard bacterial growth. To bind Fe²⁺ and to inhibit calcium deposits EDTA (20mM) was also added. Wear was evaluated (Tab. 1) for 6 million cycles (Mc) on an AMTI (Waltham, MASS) knee simulator according to ISO-14243-3 [1] while being located in the basement of a hospital.

Table 1: Serum-test protocol.

<table>
<thead>
<tr>
<th>Test</th>
<th>Mc</th>
<th>Utilized serum</th>
<th>Left bank (3 stations)</th>
<th>Right bank (3 stations)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0-3</td>
<td>BCS</td>
<td>BCS</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3-4,5</td>
<td>ACS</td>
<td>NCS</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4,5-6</td>
<td>BCS</td>
<td>BCS</td>
<td></td>
</tr>
</tbody>
</table>

The tested MTKR was of cruciate retaining design (A.M.K.®, DePuy Orthopaedics, Warsaw, IN; 10mm, ram extruded GUR 1050, gas-plasma sterilized). Protein degradation was evaluated after 2.5-3Mc and 5.5-6Mc for BCS-tests and after 4-4.5Mc for both the NBC-tests and ACS-tests, utilizing bicinchoninic acid (BCA kit, Pierce Chemicals). The colony forming units (CFU) were estimated on LB-agar after an incubation of 18h from 10⁰ microbial load of each serum. API-20E was used to identify the bacterium. The pH-value was recorded at that start and end of the tests. Electrophoresis (SDS-PAGE, BioRad) was utilized to separate the proteins according to their molecular weight for the starting materials (0Mc), supernatants (0.5Mc) and pellets (0.5Mc). A general linear model coupled with the Fisher’s LSD test as the post-hoc method was used to determine differences in wear rate due to consecutive test conditions at each bank. The wear rate was determined after least square-fitting a line through the weight loss of individual implant pairs.

Results

The protein degradation correlated with the initial β-γ-globulin concentration (R = 0.917, p < 0.001) (Fig.1).

Discussion

There was no bacterial growth at the start of the testing (0h), but was observed in all three serum types after 0.1Mc (~28h). The bacterium was identified as Enterobacter cloacae (E. cloacae). The CFU had an exponential relationship with the protein degradation (Fig.2). The wear test was accompanied with a pH-change in all three serum types with the least in ACS.

![Figure 1: Protein degradation vs. the initial β-γ-globulin concentration.](image)

![Figure 2: Colony-forming units vs. protein degradation.](image)

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References: