Polyethylene Wear is Affected by the Type of Calf Serum used in Knee Simulator Wear Testing

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Introduction: Guidelines for displacement-controlled wear simulator testing have been standardized by the ISO [1]. However, this standard recommends the use of "calf serum" as a fluid lubricant, without giving ranges of the specific protein constituents, such as albumin and the different types of globulin. There have been significant efforts to isolate influential protein related-factors on PE simulator wear for hip [2, 3] but not for knee implants. In the present study, three types of frequently used calf serum with various protein constituents are used [4-6]. The purpose of the present study is to identify the specific protein compounds that influence knee simulator wear and speculate on their role in boundary lubrication.

Materials and Methods: Bovine calf serum (BCS), newborn calf serum (NCS) and alpha-calf serum (ACS), that was closest to human albumin-α-globulin levels [5], (all from HyClone, Logan, UT) were diluted with distilled water to a protein concentration of 19 ± 2 g/l. Sodium azide (SA) was added at 0.2% to retard bacterial growth along with EDTA (20 mM) to inhibit Ca deposits. Wear was evaluated (Table 1) in the various serum types for 6 million cycles (Mc) on an AMTI (Waltham, MA) knee simulator following the ISO [1].

Table 1: Test protocol and wear rates.

Test	Mc	Left side implants		Right side implants	
		Serum	Wear [mg/Mc]	Serum	Wear [mg/Mc]
1	0-3	BCS	22.43 ± 2.67	BCS	21.71 ± 0.94
2	3-4.5	ACS	13.44 ± 0.79	NCS	17.05 ± 3.25
3	4.5-6	BCS	23.84 ± 1.55	BCS	19.25 ± 4.07

Six cruciate-retaining knee implants were tested (AMK, DePuy, Warsaw, IN; 10mm, ram extruded GUR 1050, gas-plasma sterilized). Protein degradation was evaluated for all three serum types after 0.5Mc utilizing bicinchoninic acid (BCA kit, Pierce Chemicals). The colony forming units (CFU) were estimated using LB-agar. API-20E was used to identify the bacterium. The pH-value was recorded at that start and end of the tests. Electrophoresis was utilized to separate the proteins according to their molecular weight. A general linear model coupled with the Fisher`s LSD test as the post-hoc method was used to determine differences in wear due to the various test conditions on each side after least square fitting wear values.

Results: The wear of the left and right implants (Table 1) changed from test 1 to 2 ($p = 0.007$ and $p = 0.019$, respectively). The protein degradation behaved in a similar manner ($p < 0.001$ and $p = 0.001$, respectively). The protein degradation and PE wear rate for BCS were not significantly different from test 1 to 3 ($p \geq 0.116$). The protein degradation correlated with the initial β-γ-globulin concentration ($R = 0.917$, $p < 0.001$) (Fig 1). Electrophoresis confirmed the precipitation of primarily β-γ-globulins in all three serums.

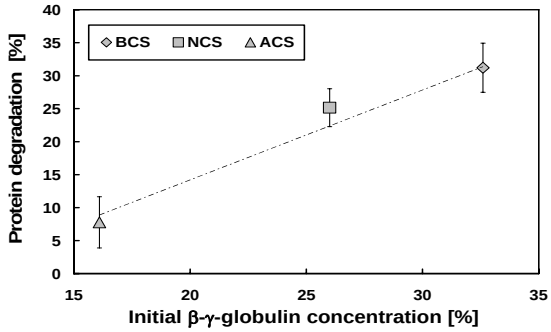

Fig 1: Protein degradation *vs* initial β-γ-concentration.

After 0.1Mc, a bacterium was observed in all three serum types. It was identified as Enterobacter.cloacae, strain JK (*E.cloacae* JK). The CFU had an exponential relationship with the initial β-γ-concentration (Fig 2). The wear test was accompanied with changes in pH for all three serum types, the least being for ACS.

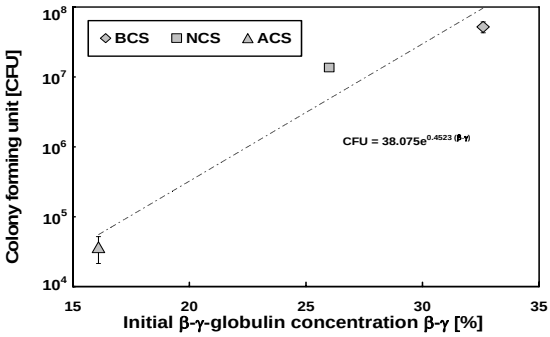

Fig 2: Colony-forming units *vs* initial β-γ-concentration.

The initial albumin-α-globulin concentrations correlated negatively with the PE wear rates ($R = -0.915$, $p < 0.001$).

Discussion: Albumin-α-globulin proteins appeared to be resistant to shear and bacterial attack, suggesting that they serve as the dominant boundary lubricant constituents. It is proposed that shear combined with a biochemical attack of *E.cloacae* JK may have caused the larger change in pH-value for BCS and NCS compared with ACS. In particular, it appeared that primarily β-γ-globulins were sheared and then served as the preferred nutrient for the *E.cloacae* JK. SA was ineffective after 0.1Mc for all serum types, suggesting the need for an alternative. Since ACS has the closest albumin-α-globulin levels to human and the least bacterial protein degradation, it is recommended for knee simulator wear testing to promote fidelity with the sterile *in vivo* conditions.

References: [1] ISO 14243-3(2004). [2]Liao P. JBMR. 1999;48:465. [3]Clarke IC. Wear. 2001;250:188. [4]Bell JC. IMechE[H]. 2001;215(4):513. [5]Yao JQ. Wear 2003;255:780. [6] Mazzucco DC. CORR. 2004;429:17.