Technical Note

The influence of phospholipid concentration in protein-containing lubricants on the wear of ultra-high molecular weight polyethylene in artificial hip joints

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Abstract: There is considerable interest in the wear of polyethylene and the resulting wear-debris-induced osteolysis in artificial hip joints. Proteins play an important role as boundary lubricants *in vivo* in the pseudosynovial fluid, and these are reproduced in *in vitro* tests through the use of bovine serum. Little is known, however, about the effect of phospholipid concentrations within proteinaceous solutions on the wear of ultra-high molecular weight polyethylene (UHMWPE). The effects of protein-containing lubricants with 0.05, 0.5 and 5 per cent (w/v) phosphatidyl choline concentrations on the wear of ultra-high molecular weight polyethylene (UHMWPE) were compared with 25 per cent (v/v) bovine serum which had 0.01 per cent (w/v) lipid; the effects were compared in a hip joint simulator with smooth ($n = 4$) and scratched ($n = 3$) femoral heads. The control bovine serum lubricant produced UHWMPE wear of 55 and 115 $mm^3/10^6$ cycles on the smooth and rough heads respectively. The increased phospholipid concentration significantly reduced the wear rate. At the higher concentration (5% w/v phosphatidyl choline) the average wear was reduced to less than 2 $mm^3/10^6$ cycles. Even with the relatively low concentrations of 0.05% w/v phosphatidyl choline the wear was reduced by at least threefold compared with the bovine serum tests for both the smooth and rough femoral heads. There may be considerable differences in the phospholipid concentrations in patients' synovial fluid and this is highly likely to produce considerable variation in wear rates. *In vitro*, differences in the phospholipid concentration of lubricants may also cause variation in wear rates between different simulator tests.

Keywords: wear, ultra-high molecular weight polyethylene (UHMWPE), lubricant, phospholipid, protein, hip prostheses

NOTATION

CL	confidence limit
R_a	arithmetic mean surface roughness
R_p	peak height of the scratch lip
UHMWPE	ultra-high molecular weight polyethylene

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1 INTRODUCTION

There is currently much interest in the role of wear and wear-debris-induced osteolysis in the long-term failure of artificial hip joints [1]. Historically the majority of bearings used in artificial hip joints have used ultra-high molecular weight polyethylene (UHMWPE) acetabular cups articulating against metallic femoral heads. Micrometre- and submicrometre-sized UHMWPE wear particles have been shown to cause adverse cellular reactions in periprosthetic tissues that lead to osteolysis and aseptic loosening [1]. Traditionally the UHMWPE has been sterilized with γ-irradiation in the presence of air.

This has been shown to cause oxidation and ageing which not only increases the wear rate, but produces a greater percentage volume of smaller particles which are more biologically active [**2**]. This has generated considerable interest in alternative materials such as oxidative-resistant UHMWPEs and cross-linked polyethylenes [**3**], metal-on-metal bearings [**4**] and ceramic-on-ceramic bearings [**5**].

There has, however, been little attention paid to the role of boundary lubricants in controlling the wear of UHMWPE. It has been recognized for a number of years that water does not produce adequate boundary lubrication in hip joint simulations and replication of *in vivo* wear. Bovine serum has therefore most commonly been used as a lubricant in *in vitro* simulations. Substantial differences in the wear debris generated in water and in bovine serum, which reflect the different wear mechanisms that are occurring, have recently been demonstrated [**6**]. The boundary lubricants that occur in bovine serum and in periprosthetic fluid include proteins of various molecular weights and phospholipids. The levels of protein in human synovial fluid have been reported to be between 2 and 3.5 g/dl [**7**] and in leg prenodal lymph, 1.4 g/dl [**8**]. The concentration of protein in the bovine serum solution has been shown to have a significant effect on wear of polyethylene in *in vitro* tests, with higher concentrations reducing the wear rate [**9**]. It has also recently been shown that a single molecular weight protein [gelatin, MW 30 000] did not provide an effective physiologically relevant boundary lubrication regime [**10**]. *In vivo*, and *in vitro* with bovine serum as a model lubricant, the proteins of different molecular weight and phospholipids provide complex and interactive boundary lubricating mechanisms. Hills [**11**] has recently reviewed the lubrication and friction of phospholipids in the body, and Higaki [**12**] has demonstrated the lubricating capacity and reduction in friction for combinations of phospholipids and γ-globulin.

Although the role of phospholipids acting as a boundary lubricant was identified over 20 years ago [**13, 14**], little is known about its effect on wear in artificial joints in physiologically relevant protein-containing solutions. This is particularly important since patients may well have different concentrations of phospholipids in the pseudosynovial fluid surrounding artificial joints. Furthermore, in *in vitro* tests the concentration of phospholipid is not well controlled and there is evidence that the degradation of lubricants *in vitro* and microbiological contamination of bovine serum in *in vitro* tests can significantly increase the level of lipid from 0.012 ± 0.07 to 0.025 ± 0.05 g/dl [**15**].

The aim of this study was to investigate the effect of phospholipid concentration within a protein-containing lubricant on the wear of UHMWPE in a physiological hip joint simulator.

2 MATERIALS AND METHODS

Seven size 28 mm GUR 1120 UHMWPE acetabular cups were used; the cups were γ-irradiated in air and of identical design, material and age. The study was undertaken and completed within 2 years of sterilization to minimize effects of oxidative ageing. Prior to this study the cups had been run for 6×10^6 cycles in the same hip joint simulator in bovine serum for validation of the wear simulation methodologies [**16**]. Prior to testing the cups were soaked in 0.1% w/v sodium azide solution for 14 days to allow moisture uptake and stabilization prior to being fixed into metallic cup holders using acrylic cement. The cups were positioned such that the centre of the cup was on the centre of rotation of the simulator.

Seven size 28 mm cobalt–chrome femoral heads of identical design and batch numbers were used. Four of the heads were used undamaged and three were scratched as described previously using a diamond stylus [**16**]. Three equally spaced scratches extending 180° from the equator over the pole and back to the equator were generated. This model of scratching has been shown to increase the wear rate of UHMWPE more than twofold compared with undamaged heads [**16**]. This was similar to the increase in wear rate found with damaged heads in a series of explanted prostheses [**17**].

The results taken for the final 2×10^6 cycles of the previous study when articulating against smooth and scratched femoral heads in 25% v/v bovine serum (in 0.1% w/v sodium azide) were taken as control data for this study. The same components were then tested in the same simulator for a further 3×10^6 cycles using protein-based lubricants with three different concentrations of phosphatidyl choline.

The Prosim hip joint simulator with two independently controlled motions and a single axis of loading was used. The acetabular cups were mounted in the anatomical position with the cup above the head at an angle of 35° to the horizontal plane. The details of the internal/external rotation, flexion/extension and loading waveforms have been described in detail previously [**16, 18**]. The motion inputs produced an open eccentric elliptical wear path which for UHMWPE acetabular cups produced similar wear rates to those found in a three-axis simulator [**18**]. These simulators have also been shown to generate predominantly submicrometre and micrometre UHMWPE wear particles which were comparable with the wear particles found in tissues around retrieved prostheses [**19**].

In this study the heads and cups were run for a further 3×10^6 cycles, changing the concentration of the lipid in the lubricant and measuring the wear volume every 10^6 cycles. The concentration of the phospholipid in the lubricant was the only variable in this study, all other variables were kept constant. Bovine serum has a mixture of proteins of different molecular weights and a low level of phospholipids. Newborn calf serum (Seralabs) diluted to 25% v/v with 0.1% w/v sodium azide was used as the standard lubricant for simulator testing in the authors' laboratory [**16, 18**]. It has a lower level of calcium than other calf sera and was diluted to 25% v/v to give a protein concentration of approximately 1.5 g/dl which was at the low end of the physiological range [**7, 8**]. The bovine serum also contained 0.01 g/dl of lipid, which has been shown to increase more than twofold during a test period of 4 days due to degradation and microbiological contamination [**10, 20**]. In order to investigate the effect of phospholipid concentration on wear, a stable well-defined protein solution was used. Gelofusin (B. Braun Medical Limited), a single molecular weight solution of gelatin (MW 30 000), was diluted to 25% v/v in 0.1% w/v sodium azide solution. The three concentrations of phosphatidyl choline used were 5, 0.5 and 0.05% w/v, all significantly higher than the 0.01–0.02% w/v lipid found in bovine serum. It has previously been shown that gelofusin alone was not an adequate boundary lubricant [**10**], and hence the need to use 25% w/v bovine serum as a control. Each lubricant was run for 330 000 cycles (4 days) and then changed. The volume of lubricant used in each simulator chamber was 250 ml. The order in which the different lubricants were tested is given in Table 1.

The mean wear rate was determined in each test interval (10^6 cycles) using a coordinate measuring machine to map the internal geometry of the cup [**16, 18**]. The wear rate for each lubricant was averaged for each set of components. The mean wear rate for each lubricant was compared using a one-way analysis of variance, considering the smooth femoral heads ($n = 4$) and rough femoral heads ($n = 3$) separately. Statistical significance was determined using the T-method to determine minimum significant difference (MSD) between the means. Significance was taken at the level $p < 0.05$.

3 RESULTS

The mean wear rate, ± 95 per cent confidence limits, of the UHMWPE cups with the smooth femoral heads with each of the four lubricants is shown in Fig. 1. Lipid concentration had a highly significant effect on wear rates ($p < 0.001$). The wear rates of all three phospholipid-containing Gelofusin lubricants were significantly less than for the bovine serum control. As the phospholipid concentration increased the wear rate reduced. The wear rate of the 5% w/v phospholipid was significantly less than that of the 0.05% w/v phospholipid.

Table 1 Lubricants studied and order of tests

Number of cycles ($\times 10^6$)	Lubricant
0–2	25% v/v bovine serum
2–3	25% v/v Gelofusin with 5% w/v phosphatidyl choline
3–4	25% v/v Gelofusin with 0.5% w/v phosphatidyl choline
4–5	25% v/v Gelofusin with 0.05% w/v phosphatidyl choline

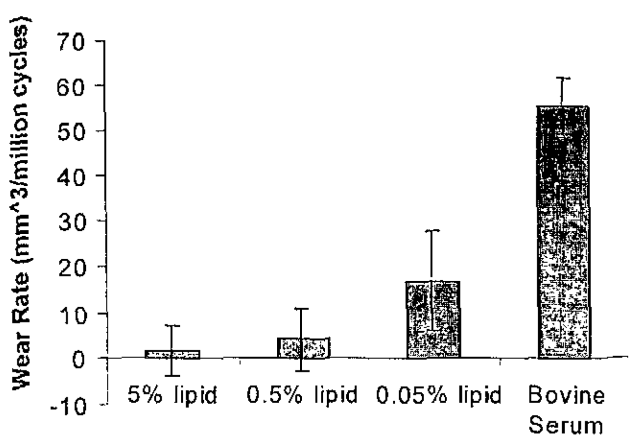

Fig. 1 The mean wear rate of UHMWPE acetabular cups articulating against smooth femoral heads (± 95 per cent CL). Minimum significant difference ($p < 0.05$) = 6.56

The mean wear rate, ± 95 per cent confidence limits, of the four UHMWPE cups with the scratched femoral heads for each of the four lubricants is shown in Fig. 2. The control data wear rates for the bovine serum were more than twice those of the smooth heads. As with the smooth heads, the phospholipid concentration had a significant effect on wear rate ($p < 0.001$). The wear rates for all three phospholipid-containing Gelofusin lubricants were significantly less than for bovine serum. As the phospholipid concentration increased the wear rate reduced; the wear rate with 0.5% w/v phosphatidyl choline concentration was significantly less than the wear rate for the 0.05% w/v concentration. The experiment was designed to keep all the variables constant apart from the lubricant. The peak height of the scratch lips,

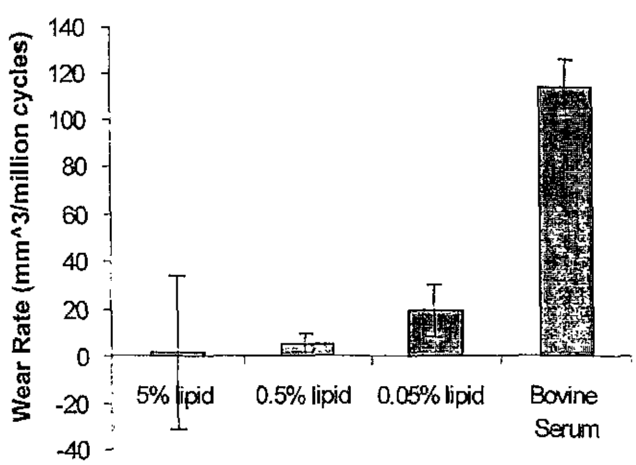

Fig. 2 The mean wear rate of UHMWPE acetabular cups articulating against scratched femoral heads (± 95 per cent CL). Minimum significant difference ($p < 0.05$) = 7.00

R_p, was 2.5 μm at the start of the study, and only reduced slightly to 2.2 μm by the start of the 5% w/v phospholipid test. However, by the end of the 5×10^6 cycles the height of the scratched lips had reduced significantly, presumably due to them being worn away or broken off by the articulation. As a result the final wear with the 0.05 per cent phospholipid may well have been reduced due to loss of the scratch lips. Nevertheless it was still significantly greater than the wear rate for the 0.5% w/v phosphatidyl choline. On the smooth heads there was a threefold reduction in wear in the 0.05% w/v phosphatidyl choline compared with the bovine serum solution, while with the scratched heads there was a fivefold reduction in wear in the 0.05% w/v phosphatidyl choline lubricant compared with the bovine serum solution. The reduction in the height of the scratched lips would have contributed to this additional reduction in wear. There was no significant change in the surface roughness of the smooth femoral heads at the end of the test.

4 DISCUSSION

There has been a considerable body of data in the literature on the wear of UHMWPE sterilized with γ-irradiation (2.5 Mrad) using bovine serum as a lubricant. The control data using bovine serum and smooth femoral heads in this study have previously been shown to compare well with other simulator data [**16**] and the scratched femoral head model has been shown to increase wear by at least a factor of two which is consistent with explant data [**17**]. The analysis of wear particles from this and other similar simulators has shown them to be predominantly sub-micrometre in size [**19**, **20**]. More recently there has been interest in the effect of protein concentration on wear in hip simulators [**9**] with increased levels reducing the wear rates. The concentration of protein used in this study was at the lower end of the range found clinically. There has been much debate about appropriate protein concentration, and this must be set in the context of the total volume of fluid used in a simulator, typically several hundred millilitres compared to a few millilitres *in vivo*. Artificially elevated levels of protein concentration may lead to reduced wear rates [**9**], while inadequate protein as a boundary lubricant may increase the adhesive wear mechanisms and produce unrepresentative wear debris [**9**, **10**].

There is however much less understanding of the effect of lipid concentration on wear of artificial joints in protein-containing solutions. Phospholipid has been cited extensively as a naturally occurring boundary lubricant [**11**, **14**], and has been shown to be an effective lubricant in aqueous solutions [**21**]. Higaki *et al.* [**12**] showed a reduction in friction with phospholipid- and protein-containing lubricants. Little is known about the concentration of phospholipid in pseudosynovial fluid, but it may vary considerably from patient to patient. The concentration of lipid in bovine serum has been measured as 0.01 g/dl, and it has been found to increase with biological contaminants to 0.025 g/dl *in vitro* [**15**, **20**].

The results of this study show a remarkable reduction in wear rate compared with bovine serum with increasing concentration of phospholipid in Gelofusin over the range 0.05 to 5% w/v. There was a significant threefold reduction in wear rate with Gelofusin with 0.05% w/v phospholipid compared with bovine serum; it is not certain that this was solely attributed to the phospholipid concentration, it may also be due to the different molecular weight proteins found in Gelofusin and bovine serum. However, previous studies indicate that this effect on wear rate of the different molecular weight proteins was relatively small [**10**]. These results indicate that the effect of phospholipid was so marked that further studies should be carried out at lower levels of concentration below 0.05 per cent, w/v in both Gelofusin and bovine serum solutions. It is clear that due to the degradation of bovine serum in *in vitro* tests, the phospholipid concentration needs to be measured before and after the test [**15**, **20**].

It is interesting to speculate as to the mechanism of phospholipid lubrication, and whether it acts on the surface itself as a bilayer [**11**, **14**] or indeed whether it acts in conjunction with the proteins to form a complex boundary lubricant [**14**]. Most importantly the articulating interface should not be thought of as a simple two-body problem. The UHMWPE wear particles themselves act as a third body in the contact, with over 10 000 wear particles being shed every step [**17**, **20**]. The interaction of the phospholipid boundary lubrication with these particles, to form an effective self-lubricating third-body screen must not be discounted.

The effect of phospholipid concentration in the lubricant is important *in vivo* and *in vitro*. *In vivo* it may well account for a significant variation in wear rates between patients. *In vitro*, differences in phospholipid concentration for different batches and dilutions of bovine serum and biological degradation of serum will alter phospholipid concentrations and wear rates.

5 CONCLUSIONS

This study has shown a dramatic reduction in the wear of UHMWPE with increasing phospholipid concentrations in a protein-containing lubricant. At the lowest concentration tested 0.05% w/v the wear was at least three times less than with bovine serum lubricant. The studies indicate that at very low concentrations, phospholipid will have a marked effect on UHMWPE wear both *in vitro* and *in vivo*.

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