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Potential Synthetic Biolubricant as an Alternative to Bovine Serum

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Abstract: It is crucial that orthopaedic implant materials are tested in conditions that replicate the natural body's environment as closely as possible. Bovine serum is currently recommended for use by the International Organisation for Standardisation (ISO) for the wear testing of these implant materials, however, the rheological properties of bovine serum do not match fully those of the body's natural lubricant, synovial fluid. This study looks at a potential alternative to bovine serum for the testing of orthopaedic implant materials; 0.5% gellan gum fluid gel. Wear tests using multidirectional motion were conducted on ultra-high molecular weight polyethylene (UHMWPE) pins rubbing against stainless steel plates. Roughness measurements were performed during testing along with particle analysis of the testing lubricant. At two million cycles (equivalent to 121.3 km of sliding), the mean wear factor for the four UHMWPE pins was 0.25 (standard deviation (SD) 0.03) $\times 10^{-6}$ mm^3/Nm and there was no evidence of any transfer film on the plate surfaces. The wear factor produced by 0.5% gellan gum fluid gel was lower than that measured in previous studies using bovine serum as the lubricant but greater than the wear factor shown in published work using a similar alternative lubricant (sodium alginate mixed with gellan gum). Work on the development of a suitable alternative lubricant to bovine serum will continue.

Keywords: synthetic biolubricant; ultra-high molecular weight polyethylene; wear testing; pin-on-plate; orthopedic; gellan gum

1. Introduction

The number of artificial joint procedures necessary to alleviate the pain, discomfort, and disability suffered by many people is increasing year on year. Those surgeries reported in the National Joint Registry of England, Wales, Northern Ireland, and the Isle of Man (NJR) are testament to this. In 2010, 98% of the procedures that were undertaken in these countries were entered into the NJR; this is known as the compliance. There were 65,229 primary hip replacements and 72,980 primary knee replacements that were performed in England and Wales in 393 hospitals in 2010 [1]. Five years later, in the 2015 NJR (96% compliance) [2], the numbers were reported to be 88,763 primary hip procedures and 96,986 primary knee surgeries performed in 414 hospitals in England and Wales; and a further 9 hospitals in Northern Ireland [2]. This is an increase in reported surgeries of 27% for primary hip replacements and 25% for primary knee replacements in the NJR over the past five years.

In addition to the primary procedures discussed above, revision operations are needed due to failure of the artificial joints. In 2010 the revision burden (number of revision operations in relation

to the total number of primary and revision operation procedures performed) was 10% for hips and 5.9% for knees [1]. In the 2015 NJR the revision burden was reported as 9.7% for hips and 6% for knees [2]. Therefore there is an on-going need for revision surgery due to artificial joint failure. It is important to understand why these joints are failing and tribological testing of the artificial joints and their materials can offer some explanations.

The most common material combination used for both hip and knee replacements is metal-on-ultra-high molecular weight polyethylene (UHMWPE). In the majority of cases, the small number of prostheses that fail have failed due to wear particle induced osteolysis [3,4]. This is the body's immunological response to the wear particles produced during surface-to-surface rubbing and it is UHMWPE wear that is the main player in this [5]. In order to determine the likely performance of alternative material combinations or prosthesis designs there is an on-going need for testing of artificial joint components and materials.

Tribological testing is widely used to assess the wear performance of different material combinations. To assess the expected in vivo joint performance, orthopedic implant materials need to be tested under conditions that replicate the natural body environment as closely as possible. Natural synovial joints, such as hip and knee joints, are lubricated with synovial fluid. In the laboratory it is not possible to test the wear performance of artificial joints with synovial fluid as the lubricant because there is only a minimal amount of synovial fluid present in the joint (a few millilitres) and this is not sufficient for the volume of lubricant needed in the wear test. Also, ethical approval is needed to obtain this fluid from patients. Bovine serum is currently recommended for use by the International Organisation for Standardisation (ISO) for the wear testing of these materials [6,7]. Currently, bovine serum is the best lubricant to provide clinically relevant wear rates with wear debris of a similar size and shape to that found in vivo [8,9]. However, there are issues with choosing bovine serum as a lubricant. These include batch-to-batch variation (meaning that tests using bovine serum from different batches will not be performed under the exact same conditions), cost, safety, and animal welfare. Most importantly, degradation of the proteins within the serum occurs [10] and necessitates regular replacement of the lubricating fluid; adding more time and expense.

There are many published papers that discuss possible alternatives to bovine serum [11–18] but the search for an alternative lubricant still continues. It is well understood that any proposed alternative lubricant will need to be safe; produce wear rates and particles that match those produced in the body; and be cost effective with no ethical limitations. The work published in this article hopes to add to the collection of data available in the search for an alternative lubricant.

In this study a new alternative lubricant was tested (0.5% gellan gum fluid gel). This lubricant was based on the lubricant tested by Thompson et al. (2015) [16]. The lubricant tested in the study by Thompson et al. (2015) [16] (0.75% gellan gum/2% alginate mixture) has been shown to have similar non-Newtonian properties to synovial fluid, but it gave lower wear rates than that expected for bovine serum [16,19]. Gellan gum in particular, has attracted recent attention in the biomedical sector and has been investigated as a biomaterial [20]. Gellan has the ability to form strong ionotropic gels with divalent cations and monovalent cations, and also has weak gel properties if no cations are added [19]. If a shear force is applied to gel forming biopolymers such as gellan gum during the sol-gel transition, the material formed is a suspension of microgel particles which behave in bulk as a pourable viscoelastic fluid. Furthermore the size of the microgel particles can be easily controlled by simply changing the concentration of the polymer or by the rate of cooling and/or shear rate during fluid gel formation [21]. Consequently, such materials have been explored for application within the food and pharmaceutical industries due to their interesting rheological and tribological properties [22–24]. In the present study, wear tests were performed on a four station pin-on-plate machine. In addition to the wear tests, particle analysis was undertaken to investigate how closely the wear particles using 0.5% gellan gum fluid gel as the lubricant resemble those produced using bovine serum and those produced in the body. The wear test and particle analysis allow for an interesting investigation into the use of this new lubricant as an alternative to bovine serum.

2. Results

2.1. Viscosity Results

The viscosity of 0.5% gellan gum fluid gel was measured at different shear rates to evaluate its shear dependency and allow a comparison with the non-Newtonian (shear-thinning) properties of synovial fluid. These results are shown in Figure 1. This figure shows the viscosity of 0.5% gellan gum fluid gel over a range of shear rates (0.1 s^{-1} to 100 s^{-1}) and shows that this lubricant has a shear-thinning behavior.

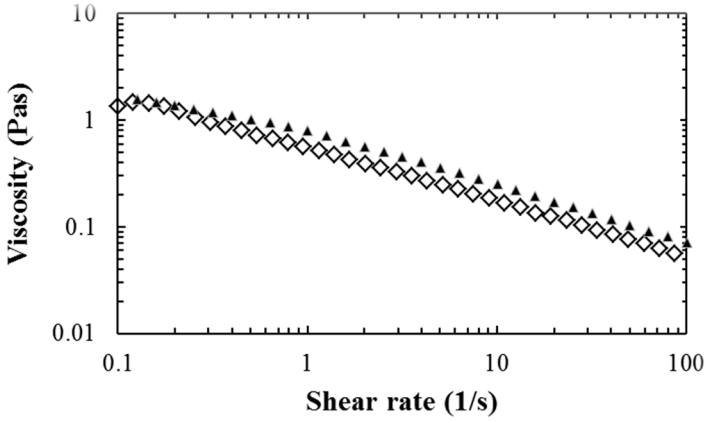

Figure 1. Viscosity vs. shear rate at 37 °C for 0.5% gellan gum fluid gel (open diamonds) and for aspirated synovial fluid (SF) (filled triangles). Synovial fluid data adapted from Smith et al., 2014 [19].

2.2. Wear Results

The wear of the UHMWPE test pins is shown in Figure 2. The control pins showed a relatively small increase in weight over the duration of the wear test (close to one order of magnitude lower than the change in weight of the test pins) and this weight increase was due to lubricant absorption. This has been accounted for whilst plotting the volume loss of the UHMWPE pins (Figure 2). At the end of the two million cycle (121.3 km) wear test, the mean wear factor for the four UHMWPE test pins was 0.25 (standard deviation (SD) 0.03) $\times$ 10^{-6} mm^3/Nm.

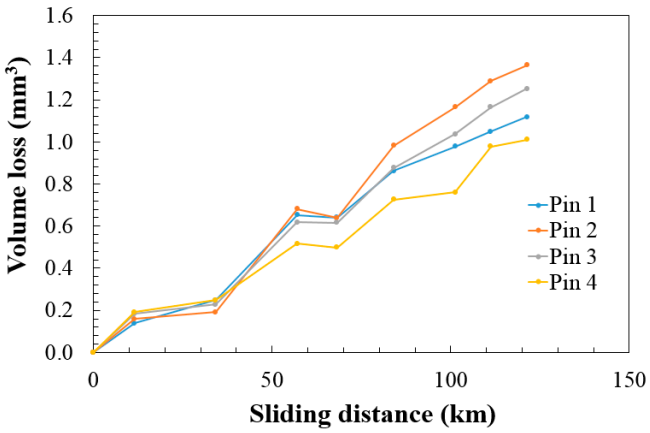

Figure 2. Volume loss versus sliding distance for the ultra-high molecular weight polyethylene (UHMWPE) pins.

The stainless steel plates showed minimal wear (mean total volume loss of 0.09 (SD 0.09) mm^3) over the duration of the wear test. Although the control plate did not show much change in weight

($\pm$0.01 mm^3 in volume) this gain in weight was taken into account when calculating the wear factors. Table 1 shows the wear factors for the four pins and four plates tested in this study.

Table 1. Wear factors for the UHMWPE pins and stainless steel plates.

Station	Wear Factor ($\times 10^{-6}$ mm^3/Nm)	
	Pins	Plates
1	0.24	0.008
2	0.28	0.008
3	0.26	0.048
4	0.21	0.015
Mean	0.25	0.019
Standard deviation	0.03	0.019

2.3. Surface Roughness Analysis

2.3.1. UHMWPE Pins

The surfaces of the UHMWPE pins were analysed using a Zygo 5000 non-contacting profilometer (Zygo Corporation, Middlefield, CT, USA) and the results are shown in Table 2. For the control pins, there was no significant difference in either S_a; S_{sk} or S_{ku} measurements taken prior to testing when compared to the same measurements taken at two million cycles ($p > 0.05$). Prior to testing, the mean S_a of the test surfaces was similar to the control pins (1293 nm cf. 1244 nm). This dropped significantly to a value of 462 nm at two million cycles ($p < 0.05$). It is worth noting that none of these S_a measurements exceeded the recommended maximum of 2000 nm for polymeric bearing surfaces, as dictated by ISO 7206-2 [25]. An overall increase in skewness was observed for the test surfaces, with an initial S_{sk} value of -0.10 prior to testing; significantly increasing to +1.44 at two million cycles ($p < 0.05$). There was an overall increase in kurtosis over the duration of the experiment, with: an initial S_{ku} value of 2.74; increasing to 12.18 at one million cycles; then dropping to 7.67 at two million cycles. Although the intermediate S_{ku} value was much higher than that at two million cycles, the S_{ku} values acquired post testing exhibited a significantly higher mean than that of those acquired prior to testing ($p < 0.05$). Prior to testing, the surfaces of the pins showed a lay pattern characterized by concentric trenches (Figure 3); this pattern had completely worn away at the intermediate measurement interval (one million cycles) (Figure 3), accounting for the overall reduction in average roughness, and for the increased skewness. Multi-directional scratching can be seen on the worn surfaces (Figure 3).

Table 2. Surface roughness values for the UHMWPE pins.

	Cycles	UHMWPE Pins (Test)			UHMWPE Pins (Control)		
		Mean (95% CI)	*t*	*p*	Mean (95% CI)	*t*	*p*
S_a (nm)	**0**	1293 (1079 to 1508)	6.11	<0.05	1244 (894 to 1594)	−0.05	0.96
	1×10^6	582 (353 to 811)			1156 (783 to 1529)		
	2×10^6	462 (303 to 620)			1256 (908 to 1604)		
S_{sk}	**0**	−0.10 (−0.30 to 0.11)	−5.93	<0.05	0.142 (0.01 to 0.27)	0.11	0.92
	1×10^6	1.04 (0.48 to 1.59)			0.282 (0.12 to 0.45)		
	2×10^6	1.44 (0.97 to 1.91)			0.129 (−0.09 to 0.34)		
S_{ku}	**0**	2.74 (2.48 to 3.00)	−4.34	<0.05	2.370 (2.05 to 2.69)	−0.19	0.85
	1×10^6	12.18 (2.85 to 21.51)			2.470 (2.23 to 2.71)		
	2×10^6	7.67 (5.46 to 9.88)			2.420 (2.06 to 2.78)		

N.B. Student's *t*-test was conducted to compare the surface roughness measurements taken before testing (0 cycles) to after testing (2×10^6 cycles). *t* refers to the *t*-test statistic; *p* refers to the *p*-value.

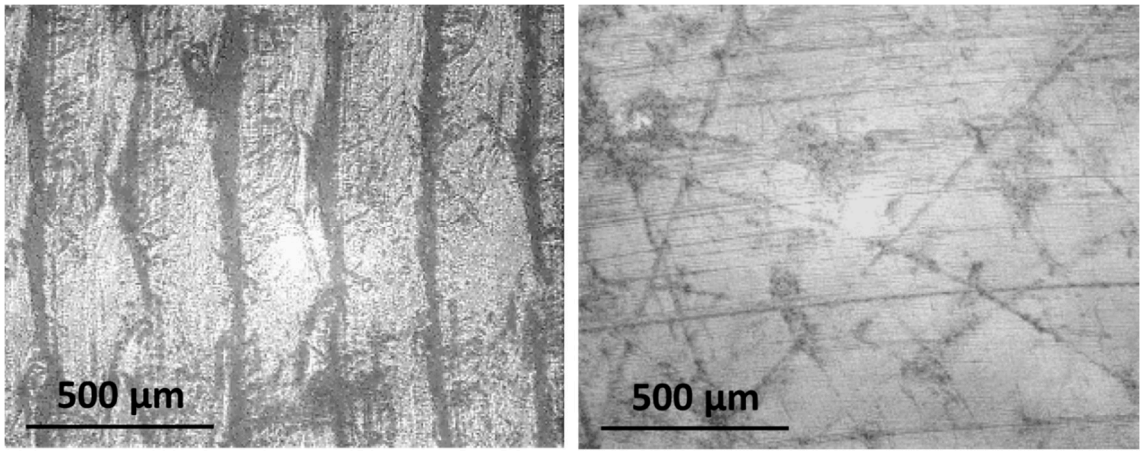

Figure 3. Micrographs showing an unworn UHMWPE pin exhibiting a characteristic concentric lay pattern (**A**) and a worn UHMWPE pin at two million test cycles (**B**).

2.3.2. Stainless Steel Plates

The surface roughness results for the stainless steel plates are shown in Table 3. These were also measured using the Zygo 5000 non-contacting profilometer (Zygo Corporation, Middlefield, CT, USA). These plates were polished prior to testing to an average of 0.01 µm S_a. As with the UHMWPE pins, there was no significant difference in either S_a, S_{sk}, or S_{ku} for the measurements of the stainless steel control plates taken prior to testing when compared to the same measurements taken at two million cycles ($p > 0.05$). There was also no significant difference between the measured S_a values from the test plates taken prior to testing when compared to the same values acquired at two million cycles ($p > 0.05$). It is worth noting that none of these S_a measurements exceeded the recommended maximum of 50 nm for metal bearing surfaces, as dictated by ISO 7206-2 [25]. A small yet significant decrease in skewness (S_{sk}) was observed for the test plates over the two million cycles ($p < 0.05$). The greatest observed change was to the S_{ku} values. The test plates increased significantly, by an entire order of magnitude, over the two million test cycles ($p < 0.05$) from 17.38 to 113. Prior to testing, the surfaces of the stainless steel plates were unmarred (Figure 4); at two million cycles, multidirectional scratches were present (Figure 4). These scratches account for the reported decrease in skewness, and for the increase in kurtosis over the duration of the experiment.

Table 3. Surface roughness values for the stainless steel plates.

	Cycles	Stainless Steel Plates (Test)			Stainless Steel Plates (Control)		
		Mean (95% CI)	***t***	***p***	**Mean (95% CI)**	***t***	***p***
S_a (nm)	**0**	14.47 (8.44 to 20.49)			7.44 (3.44 to 11.44)		
	1×10^6	16.27 (13.54 to 19)	1.44	0.16	11.87 (6.15 to 17.59)	−1.88	0.08
	2×10^6	9.88 (7.51 to 12.25)			12.32 (9.18 to 15.45)		
S_{sk}	**0**	−1.62 (−2.76 to −0.48)			−0.43 (−0.89 to 0.03)		
	1×10^6	−3.32 (−3.77 to −2.87)	2.07	<0.05	−1.62 (−2.33 to −0.91)	0.90	0.38
	2×10^6	−4.15 (−6.26 to −2.05)			−0.85 (−1.63 to −0.06)		
S_{ku}	**0**	17.38 (5.22 to 29.54)			12.91 (4.41 to 30.23)		
	1×10^6	105 (4.20 to 206)	−3.56	<0.05	12.37 (4.73 to 20.10)	−0.66	0.52
	2×10^6	113 (61.70 to 164.30)			20.25 (6.96 to 33.54)		

N.B. Student's *t*-test was conducted to compare the surface roughness measurements taken before testing (0 cycles) to after testing (2×10^6 cycles). *t* refers to the *t*-test statistic; *p* refers to the *p*-value.

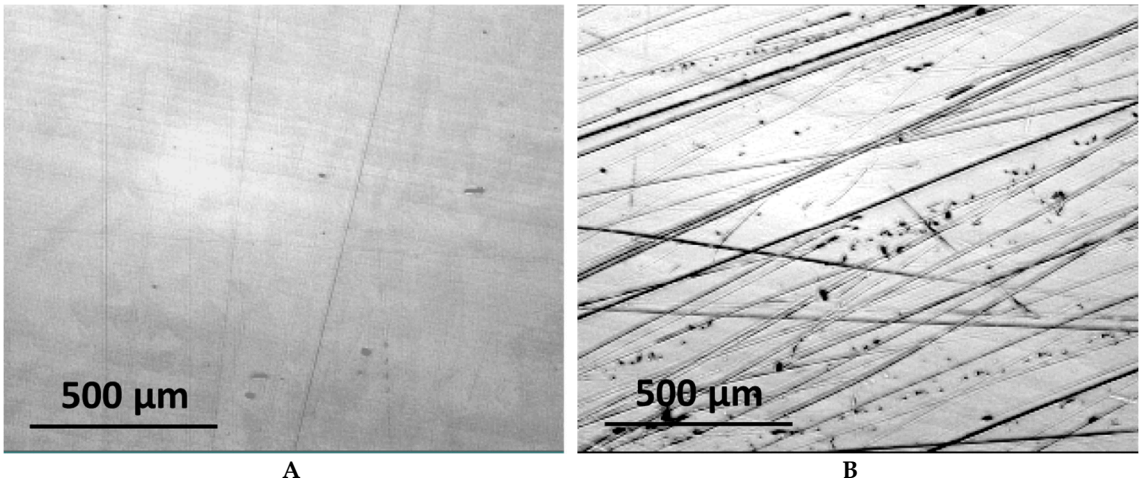

Figure 4. Micrographs showing an unworn stainless steel plate (**A**) and a worn stainless steel plate at two million test cycles (**B**).

2.4. Wear Particle Analysis

The lubricant analysis post-wear revealed particles in the range of 100–300 nm in diameter (Figure 5A) and larger particles in the micron range (Figure 5B).

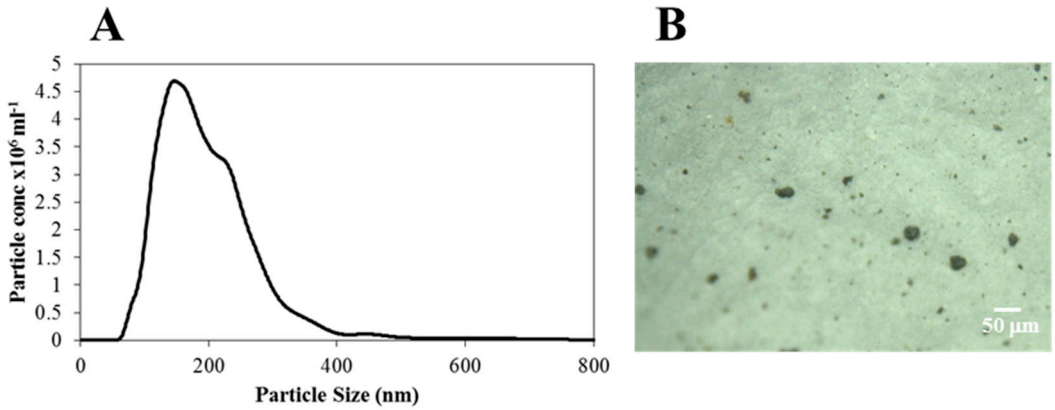

Figure 5. (**A**) Size distribution of nanoparticulate wear debris in the gellan gum fluid gel lubricant post-wear measured using nanoparticle tracking analysis; (**B**) Micro particulate wear debris in the gellan gum fluid gel post-wear using light microscopy.

3. Discussion

3.1. Viscosity Results

The fluid gel lubricant, 0.5% gellan gum, was shown to have similar non-Newtonian characteristics distinctive to aspirated synovial fluid across the shear rate range tested (0.1 s^{-1} to 100 s^{-1}) (Figure 1). It must be noted, however, that the shear rates experienced in the pin-on-plate machine are likely to be much greater than 100 s^{-1}. The pin-on-plate machine provides reciprocal motion therefore the shear rate will vary throughout each testing cycle. When calculating the shear rate throughout the cycle, this shear rate depends heavily on the lubricant film thickness. Using the film thickness of 0.09 µm calculated for metal-on-UHMWPE hip joints at the greatest velocity during the stance phase of the walking cycle [26] (which is likely to be greater than the film thickness produced in the pin-on-plate machine), along with the average speed of reciprocation of the pin-on-plate machine (56 mm/s) will lead to a shear rate over three orders of magnitude greater than the maximum shear rate used to

measure viscosity in this study (100 s^{-1}). Using the rheometer available, it was not possible to test the lubricants at these high shear rates so it is unknown how closely the 0.5% gellan gum fluid gel matches the viscoelastic properties of synovial fluid at these high shear rates.

3.2. Wear Results

During the two million cycle wear test (121.3 km of sliding), the mean wear factor of the four UHMWPE pins was 0.25 $\times$ 10^{-6} mm^3/Nm. This can be compared to the wear factors of 1.1 $\times$ 10^{-6} mm^3/Nm and 1.6 $\times$ 10^{-6} mm^3/Nm when using dilute bovine serum as the lubricant in the same rig [27,28]. For failed and explanted Charnley hips, the wear factor has been found to be 2.1 $\times$ 10^{-6} mm^3/Nm [29]. Interestingly, for UHMWPE sockets functioning well and retrieved at autopsy, it has been found that the wear rate is 45%–69% less than those that have failed [30] leading to a wear factor of between 0.95 $\times$ 10^{-6} mm^3/Nm and 1.45 $\times$ 10^{-6} mm^3/Nm for well-functioning hip implants [27].

Previous work reporting on the same material combination (UHMWPE against stainless steel) lubricated with a 0.75% gellan gum/2% alginate mixture gave wear factor values for UHMWPE of 0.099 $\times$ 10^{-6} mm^3/Nm [16]. The UHMWPE wear factor measured for 0.5% gellan gum fluid gel is approximately 2.5 times greater than 0.75% gellan gum and is closer to the values reported for bovine serum.

It is clear from Table 2 that the plates showed slight wear, although this was very low at an average of 0.019 $\times$ 10^{-6} mm^3/Nm. A more important finding was that, as shown by surface roughness analysis, the plate surfaces had no evidence of a transfer film. In the previous pin-on-plate studies using 0.75% gellan gum/alginate as the lubricant, the wear of the plates was not reported [16] but there was evidence of a transfer film. Other work investigating the wear properties of UHMWPE pins against stainless steel plates using bovine serum as the lubricant also have not reported the wear of the plates but they do report that no transfer film was present [27,28]. The plate weight change was actually measured in these previous studies, but as it was not reported it is likely that the plates showed zero wear or wear within the precision of the balance used. Other work investigating the wear of UHMWPE pins against CoCr discs using a circularly translating pin-on-disc machine (Super CTPOD) reported that at the end of the test the discs had no change in weight [31].

Although the novel 0.5% gellan gum fluid gel lubricant shows promising wear results with no evidence of a transfer film, it shows lower UHMWPE wear than bovine serum.

3.3. Surface Roughness Analysis

It is clear from the roughness measurements provided in Table 2 that for the UHMWPE test pin surfaces, the 3D average roughness (S_a) has decreased post-wear with an increase in 3D skewness (S_{sk}), along with an increase in 3D kurtosis (S_{ku}). The decrease in S_a has been shown in previous pin-on-plate studies using bovine serum as the lubricant [31,32] and is often seen in the UHMWPE components of hip, knee, and finger prostheses with CoCr counterfaces [33–36]. Also, the increase in S_{sk} has been shown by others [33,35]. The S_{ku} measurement is not often quoted but has been found to range from 3.29 to 18.2 for revised UHMWPE hip liners [37] and 27.6 to 32.5 for retrieved UHMWPE liners in another study [33]. During the wear test in this study, we observed an increase in S_{ku} from less than 3 prior to testing, and reaching a peak of 12 at one million cycles, giving values similar to those measured in the worn areas of hip prostheses by Edidin et al. [37]. The increase in kurtosis of the UHMWPE pins in this study suggests that, post-wear, the surface has a peaked distribution (the peaks or valleys on the surface profile are mainly angular and sharp), similar to the surfaces of retrieved UHMWPE components of artificial hip joints measured by Edidin et al. [37]. It must, however, be noted that kurtosis is sensitive to isolated peaks and isolated valleys (outliers).

For the stainless steel plates, from Table 3 it can be seen that the S_a is very similar for the un-worn and worn surfaces; the S_{sk} decreases post-wear and the S_{ku} increases. Previous pin-on-plate studies using bovine serum as the lubricant have also shown this pattern of little change in the S_a values and

it was suggested that these surface roughness results indicated that there was no transfer film on the plate surface [27,28,31]. As the S_a remains similar for the duration of the wear test, this means that there was little change to the average roughness of the plates. However, this does not mean that no changes in surface topography have taken place. The changes in S_{sk} and S_{ku} may offer further insight as to how the surface has changed during the wear process. A decrease in S_{sk} and an increase in S_{ku} along with the surface profilometry images show that multi-directional scratching of the plates has occurred. To the authors' best knowledge, for published UHMWPE on stainless steel pin-on-plate studies, there is no information available on the effect of wear on skewness or kurtosis of the plate surface. Works investigating the surface roughness of metal counterfaces in metal-on-UHMWPE hip and knee joints have, however, measured skewness and kurtosis. These studies support the work reported here in that they have shown a move to a more negative skewness [33,38] and an increase in kurtosis [33].

In summary, the changes in surface roughness encountered by both the pins and the plates using 0.5% gellan gum fluid gel as the lubricant is similar to what has been seen in other relevant biomaterial wear screening tests and also some explanted hip and knee prostheses.

3.4. Wear Paricle Analysis

The wear particles produced when using 0.5% gellan gum fluid gel as the lubricant were of two distinct particle sizes, with the first population ranging between 100–300 nm and a second population in the region of 10–30 μm. This was comparable with the wear particles produced using bovine serum as the lubricant in another pin-on-plate study testing the same materials [39]. The greatest percentage volume of particles (approximately 70%) from the most relevant test, using non-irradiated UHMWPE pins against smooth plates, was in the 0.1–1.0 μm size range with some particles (approximately 10%) in the 1.0–10 μm size range [39]. In addition to this, a recent review paper discussing the wear particle analysis of hip and knee joints, both from in vitro wear simulator testing and in vivo periprosthetic analyses, showed that the particles generated were not uniform in size [40]. The debris size ranged from nanometers to micrometers and many match the sizes reported in this study [40].

Analysis of the wear debris when using 0.75% gellan gum/2% alginate mixture as the lubricant in the Thompson et al. (2015) study [16] showed particle sizes ranging from 50 to 400 nm. No larger particles were found however, it must be noted that the particle analysis performed was done solely by using the nanoparticle tracking analysis tool leading to a maximum particle size of 2000 nm in diameter, therefore any larger particles will not have been measured in the Thompson et al. paper.

With respect to the work performed in this paper, it must be noted that particles in the size range from 2–11 μm were difficult to measure with either of the techniques used for the wear particle analysis.

3.5. Limitation

This test could be seen as having a small sample size. This sample size (four test pins and four test plates) has, however, been used in previous published works [28,41–46]. These tests were sufficient to indicate that the new lubricant (0.5% gellan gum fluid gel) produced wear factors closer to those measured with bovine serum than 0.75% gellan gum/alginate tested in a previous study [16].

4. Materials and Methods

4.1. Materials

Seven UHMWPE pins (four test pins and three control pins) were machined from a cylindrical bar to 18 mm in length and 6 mm in diameter. The UHMWPE was GUR 1020. It was not irradiated. The four test pins were articulated against four 316 stainless-steel test plates with an additional plate used as a soak control. The plates were 50 mm × 25 mm × 3 mm in size.

4.2. Test Lubricant

The lubricant tested was thixotropic and was 99.5% water and 0.5% low acyl gellan. This 0.5% gellan gum was formulated as a suspension of microgel particles. These are prepared by introducing a shear force while a gelling biopolymer passes through its sol-gel transition. The result is a material that behaves in bulk as a viscoelastic fluid but has a cross-linked gel microstructure. These are often referred to as fluid gels or sheared gels [21]. Moreover, the gel particles were prepared at a particle size similar to the original surface roughness of the pins (1–2 μm) as previously reported by Thompson et al. (2015) which may provide improved boundary layer lubrication [16].

The test lubricant fluid gel was prepared by adding low acyl gellan gum (CP Kelco, Atlanta, GA, USA) at 0.5% w/w to deionised water at 85 °C while stirring. Once fully dissolved, 0.25% w/w sodium chloride (Sigma-Aldrich, Irvine, UK) was added. The solution was then allowed to cool to 20 °C, whilst being sheared at a shear rate of 1000 s^{-1}. Once cooled, the fluid gel lubricant was recovered and stored at room temperature prior to use.

Viscosity measurements were taken on a Bohlin Gemini Nano HR rheometer using the 55 mm parallel plate geometry across shear rates ranging from 0.1 s^{-1} to 100 s^{-1}. These measurements were taken at 37 °C.

To ensure that the lubricant fluid gel particles were in the size range in the region of 1–2 μm, the samples were inspected using light microscopy using an optical microscope (Keyence VHX digital microscope RZ 250× –2500× real zoom lens in high dynamic range) according to the method by Mahdi et al. (2014) [21]. Briefly, the lubricant was dispersed in 10 mL of 50 mM $CaCl_2$ (Sigma-Aldrich, Irvine, UK) and then centrifuged at 13,000 rpm for 5 min. The pellet was then examined under the microscope.

A light microscopy image showing the microgel particles (<2 μm) in the 0.5% gellan gum fluid gel lubricant prior to testing is presented in Figure 6.

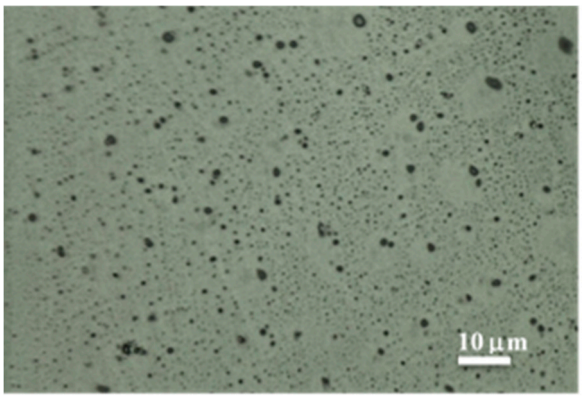

Figure 6. Light microscopy image of the un-used lubricant fluid gel microgel particles showing an approximate size of 1–2 μm.

4.3. Apparatus

4.3.1. Pin-on-Plate Wear Test Machine

The 0.5% gellan gum fluid gel lubricant was tested in a four-station, multidirectional, pin-on-plate wear test rig (see Figure 6) [16,28] to determine the effect on the wear of UHMWPE pins against stainless steel plates. To provide the multidirectional motion, rotational motion was applied to the test pins and reciprocating sliding was applied to the plates. The pin rotation was set at 1 Hz, through the use of a 12 V motor via a pair of spur gears. The test plates were subjected to a reciprocating motion of frequency 1 Hz with a stroke length of 28 mm and a resulting mean sliding speed of 56 mm/s. Test pins were each held in a pin holder and were loaded against the stainless steel test plates. The load applied

was 40 N, which was achieved by the placement of a weight at the end of a lever arm, as shown in Figure 7 [16]. A 40 N load produces a contact stress of 1.4 MPa, and research has shown an artificial hip joint is exposed to a contact stress of 1–2 MPa [47]. The control pins and plate were soaked in the lubricant but no motion or load was applied to these pins or plate. The wear test was conducted for two million cycles (equivalent to 121.3 km sliding distance).

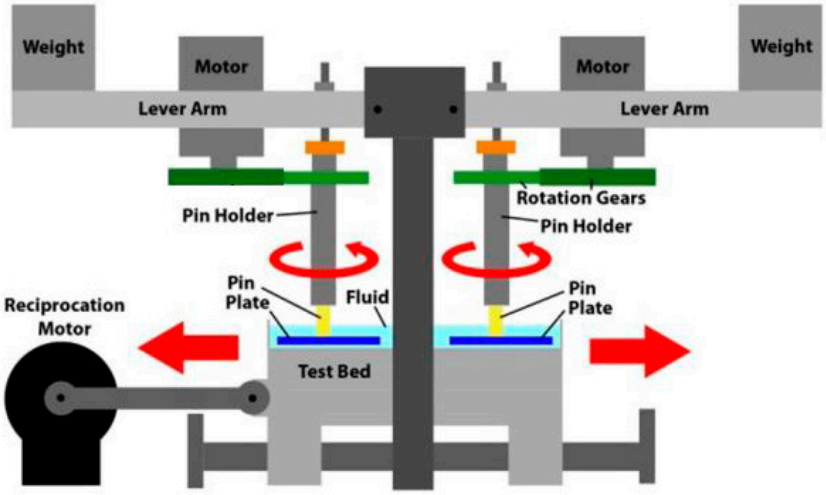

Figure 7. Schematic of the four station, multidirectional wear testing machine [16].

In order to effectively evaluate the novel lubricant; a consistent cleaning, drying, and weighing protocol was followed (please see Appendix A).

The wear was measured gravimetrically using a Denver TB-215D balance. Any weight gain due to lubricant absorption, which would result in an incorrect wear measurement, was taken into account with the use of control pins and a control plate. These three control pins and one control plate were positioned in the lubricant, separate to each other with no loading. The weight gain in these controls was subtracted from the weight measurements of the test pins and plates allowing for a truer wear measurement. Pins and plates were weighed approximately every 60 hours on a balance with a sensitivity of 10 µg. Three readings were taken and an average weight was calculated. The density of the UHMWPE pins tested in this study was 949 kg/m^3 and the density of the stainless steel plates was 8000 kg/m^3. The compensated weight loss was plotted against sliding distance. Using linear regression, the wear rate (mm^3/km) was then calculated for each of the four pins and each of the four plates and the wear factors were also derived using the equation below.

$$k = \frac{V}{LD} \tag{1}$$

where k is the wear factor (mm^3/Nm), V is the volume lost (mm^3), D is the sliding distance (m) and L is the load (N).

The samples were then reassembled in the wear testing rig, fresh lubricant was added and the testing continued.

4.3.2. Surface Roughness Measurements

A Zygo NewView 5000 white light interferometer (Zygo Corporation, Middlefield, CT, USA) was used to conduct the topographical analysis prior to the commencement of the test, at one million cycles and at two million cycles. Five topographical plots were taken along the wear track of each of the four stainless steel test plates. Plots were also obtained from the corresponding UHMWPE pin surfaces: one taken from the centre; and four peripheral measurements taken, equispaced at 90°. Five plots

were also obtained from one control plate; and three control pins. The measurement window for each topographical plot obtained was 1.58 mm × 1.19 mm; covering a spatial area of 1.88 mm^2.

Three key roughness parameters: 3D average roughness (S_a), 3D skewness (S_{sk}), and 3D kurtosis (S_{ku}) were measured, with ISO standard methods of calculation followed [48]. The standard governing bearing surfaces for prostheses (ISO 7206-2) [25] specifies R_a as the key surface roughness measurement, stating a maximum value of 50 nm for the metallic femoral component and 2000 nm for the UHMWPE component. R_a is the average surface roughness measured on a line profile and this equates to S_a when the measurement is taken over an area (such as on the Zygo NewView 5000). The second measurement parameter that was taken, the S_{sk}, can provide further detail of the measured surface [49]. A positive S_{sk} is indicative of more peaks present above the mean profile line; negative S_{sk} is indicative of more valleys present below the mean profile line; and zero skewness indicates symmetrical height distribution about the mean profile line. S_{sk} can be used to distinguish between surfaces that have the same average roughness, S_a, value but exhibit different roughness profiles. The final measurement, S_{ku}, describes the sharpness of the distributed heights [50,51]. If $S_{ku} < 3$ then the surface is considered to have relatively few high peaks and low valleys. Conversely, if $S_{ku} > 3$ then the surface is considered to have relatively many angular, sharp high peaks and low valleys [51]. A further explanation of these surface roughness measurements, along with the equations used to calculate them, is given in a recent publication [49].

4.4. Statistical Methods

The Student's *t*-test was used to compare the sample populations of S_a, S_{sk}, and S_{ku} taken prior to testing (zero cycles) to the respective sample populations acquired post testing (two million cycles). Both the *t*-statistic and *p*-value was recorded in each instance; with the latter used to determine the level of statistical significant difference between the before- and after-wear sample populations.

4.5. Wear Particle Analysis

Wear debris accumulated in the test lubricants were sized using nanoparticle tracking analysis (Nanosight LM10, Malvern Instruments, Malvern, UK). This nanoparticle tracking analyser allows the measurement of particles in the size range from 10 nm to 2000 nm in diameter. In order to analyse the wear debris, the polysaccharides were removed from the test lubricant by ethanol extraction using the method adapted from Thompson et al. (2015) [16]. Briefly, lubricant samples were collected during the wear test at each lubricant changeover. The samples were pooled before extraction giving the results as cumulative over the full two million cycle wear test. This lubricant was diluted 1:100 with ultrapure water (18.2 MΩ·cm). A fivefold volume of cold ethanol (95% v/v) was then added and the precipitated gellan gum was removed. The remaining solution was filtered using a Buchner funnel with a pore size of 11 µm. The filtrate was collected and the ethanol removed using a rotary evaporator. The remaining wear debris was then re-suspended in ultrapure water prior to analysis on the nanoparticle tracking analyser.

In addition to this, any larger wear debris retained on the filter paper was imaged using an optical microscope (Keyence VHX digital microscope).

5. Conclusions

This work provides a useful result in the search for an alternative lubricant. Both the wear results and surface roughness measurements on the plates indicated that no transfer film was present on the plate surfaces, as is seen with bovine serum. However, the novel 0.5% gellan gum fluid gel lubricant showed lower wear than has been previously shown when using bovine serum as the lubricant. Therefore, the search for an alternative lubricant will continue.

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Abbreviations

The following abbreviations are used in this manuscript:

ISO	International Organisation for Standardisation
UHMWPE	ultra-high molecular weight polyethylene
NJR	National Joint Registry
ASTM	American Society for Testing and Materials

Appendix Appendix A

Cleaning, Drying and Weighing Procedure

- At each weighing interval the test bath was removed.
- A 20 mL syringe was used to extract the test fluid from the test and control baths, this fluid was placed into labelled specimen jars.
- The plates were then removed from the test bath and the pins from the pin holders.
- The pins and plates were cleaned in acetone and weighed on a balance with a sensitivity of 10 µg.
- The test bath was also cleaned between tests.
- Each test piece was weighed three times; a mean of these three values was calculated for each specimen.
- After the cleaning, drying, and weighing procedure was complete, the plates were re-located in the correct position in the bath.
- The bath was then fixed into place on the test rig and the pins fixed into the pin holders.
- 20 mL of new lubricant was added to each bath and the test rig was re-started.

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