# The Effect of Lubricant Composition on the Wear Behaviour of Polyethylene for Orthopaedic Applications

By

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# Abstract

The composition of orthopaedic wear testing lubricants used to mimic synovial fluid (SF) is known to significantly affect *in vitro* polyethylene (PE) wear; however, some wear testing standards may be promoting the use of lubricants that are not clinically relevant. The present thesis evaluated the biochemical composition of human osteoarthritic and periprosthetic SF in order to propose changes to lubricant specifications in current wear testing standards. Using this data, pin-on-disc wear tests were conducted to explore the effects of more clinically relevant lubricants on PE wear. Results showed that wear decreased using a more clinically relevant lubricant. Samples of these lubricants were biochemically evaluated and compared to the SF results previously obtained, which showed that current standards for wear testing lubricants are biochemically different from SF. The findings from the present thesis encourage the modification of standardized lubricant specifications to improve wear testing protocols and guarantee clinically relevant wear testing.


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*I have been truly blessed by this experience.*

# Dedication

*To my Grandparents:*

*Graham (公公) and Linda (婆婆) Ma*

*Paul (爺爺) and Chui-Yuet (奶奶) Wong*

*And In Loving Memory of Ann Lugsdin*
*(1938-2005)*

*Philippians 4:13*

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No copyrighted material was used in the present thesis.

# Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

# List of Symbols

| | |
|---|---|
| $\mu_k$ | Kinetic coefficient of friction |
| $\eta$ | Viscosity |
| $\upsilon$ | Sliding velocity |
| P | Normal load per unit width |
| Å | Angstrom |

# List of Abbreviations

| | |
|---|---|
| **AA** | Antibiotic/Anti-mycotic |
| **ACS** | Alpha calf serum |
| **ASTM** | American Society of Testing and Materials |
| **BCA** | Bicinchoninic Acid Assay |
| **BCS** | Bovine calf serum |
| **CoCr** | Cobalt chromium |
| **CoCr-PE** | Cobalt chromium - Polyethylene |
| **Cp** | Heat capacity |
| **CPE** | Conventional (non-crosslinked) polyethylene |
| **DSC** | Differential Scanning Calorimetry |
| **DW** | Deionized water |
| **EDTA** | Ethylenediaminetetraacetic acid |
| **g** | Grams |
| **GLM** | General linear model |
| **HA** | Hyaluronic Acid |
| **HXPE** | Highly crosslinked polyethylene |
| **ISO** | International Organization for Standardization |
| **kg** | Kilogram |
| **kGy** | Kilogray |
| **L** | Liter |
| **Mc** | Million cycles |

| | |
|---|---|
| **mg** | Milligram |
| **$mm^2$** | Square millimeters |
| **mmol** | Millimoles |
| **Mrad** | Mega rad |
| **N** | Newtons |
| **OA** | Osteoarthritis |
| **OA-SF** | Osteoarthritic synovial fluid |
| **OxZr** | Oxidized Zirconium |
| **PBS** | Phosphate buffered saline solution |
| **PE** | Polyethylene |
| **POD** | Pin-on-disc |
| **PP-SF** | Periprosthetic synovial fluid |
| **Ra** | Average Roughness |
| **Rpmax** | Maximum peak height |
| **Rz** | Ten-point roughness height |
| **SA** | Sodium azide |
| **SEM** | Scanning electron microscope |
| **SF** | Synovial fluid |
| **THR** | Total hip replacement |
| **TJA** | Total joint arthroplasty |
| **TJR** | Total joint replacement |
| **TKA** | Total knee replacement |
| **Tm** | Temperature midpoint |
| **XPE** | Moderately crosslinked polyethylene |

# List of Appendices

# Chapter 1

# Introduction

## 1.1 Clinical Implant Wear and *In Vitro* Wear Testing

Over the last several decades, changes in patient demographics have been reported showing that patients undergoing total joint arthroplasties (TJAs) are 20% heavier, more physically active, and have a 25% greater life expectancy [1]. These current trends indicate a need for more resilient total joint replacements (TJRs) to accommodate everyday activities.

The typical lifetime of a TJR can reach 15 years or more, barring any clinical complications such as infection, instability, and wear-induced osteolysis [2-4]. The incidence of clinical complications is outweighed by the number of successful implantations; however, the incidence of revision surgeries continues to be an economic problem worldwide [2-4].

Revision surgeries are time-consuming and expensive [5-6]; Kurtz et al. [6] predicted that, in addition to rising costs, the number of total hip and total knee revision surgeries in

the United States would significantly increase by 137% and 601%, respectively, between 2005 and 2030. Revision surgeries are also associated with a higher patient morbidity [5]; these surgeries can cause unnecessary pain, and for some patients, a loss of income. With these economic and health-related issues, in addition to changing patient demographics, it is evident that improvements need to be made to current TJR materials and designs in order to enhance their long-term function and durability.

The wear performance of new TJR materials are often evaluated in *in vitro* wear tests that are capable of simulating some *in vivo* conditions. Pin-on-disc (POD) testing is a cost-effective way to screen new materials prior to manufacturing; successful candidate materials are then manufactured into TJR components that undergo further wear testing in joint simulators, where the kinetics and kinematics of the joint can be reproduced. These simulator wear tests are more difficult to conduct due to building and maintenance expenses, and sample preparation [4]. Nonetheless, *in vitro* wear testing can provide invaluable information regarding the overall performance of TJRs prior to mass commercialization and implantation.

The present thesis focuses on the POD testing of orthopaedic bearing materials, specifically on cobalt chromium alloy-polyethylene (CoCr-PE) bearing couples. Research focusing on joint simulator wear testing appears to be making strides toward reproducing clinical wear [5]; however, it has been found to be increasingly difficult to assess the clinical relevance of POD testing due to its simpler mechanics and implementation. Currently, ASTM F732 [6] is the only standard available for the POD testing of polymeric materials; however, the specifications provided are quite general in nature, specifically in the section discussing lubricant composition.

## 1.2 Synovial Fluid and Wear Testing Lubricants

Synovial fluid (SF) is the body's natural joint lubricant, which is produced by a membrane (the synovium) that surrounds synovial joints such as the hip, knee, shoulder and elbow. In healthy synovial joints, SF reduces the friction caused by joint articulations [7, 8]; however, the composition of SF changes with the onset of disease [9], such as osteoarthritis (OA). This change in composition can significantly affect the lubricating properties of SF, which may influence PE wear [9-11]. Mazzucco et al. [9] analyzed the protein concentration of osteoarthritic synovial fluid (OA-SF) and periprosthetic synovial fluid (PP-SF) from knee joints and found that there was no significant difference between the two types of SF. SF also contains a specific polysaccharide called hyaluronic acid (HA), which is the main constituent that gives SF its characteristic viscosity. Upon analyzing the concentration of HA in OA-SF and PP-SF from knee joints, results showed that the HA composition in SF was lower in PP-SF than in OA –SF [9]. Other studies had similar results with OA-SF and PP-SF obtained from hip joints [12, 13]. Evidently, there is some compositional change following TJA, and it is important to consider the composition of PP-SF in *in vitro* orthopaedic wear testing due to the fact that under physiological conditions, the TJR interacts with PP-SF following arthroplasty.

In *in vitro* wear testing, the use of SF as the wear testing lubricant would be ideal; however, among ethical concerns, large volumes of SF obtained from a single source cannot be obtained due to physiological constraints [14]. Patient-to-patient variability may also introduce confounding variables into the test [9, 15, 16]. Because of these

issues, bovine sera are most often used to reproduce the composition of SF in *in vitro* wear tests.

To reach clinical protein levels, bovine sera are diluted to target protein concentration levels with distilled water. While this bovine sera-based lubricant is capable to producing comparable wear characteristics seen *in vivo*, the composition of this lubricant may not be clinically relevant [14, 15, 17-21]. This issue is further agitated by the fact that some wear testing standards [2, 6] do not specify protein concentration levels that are reported by clinical studies. A biochemical study by Brandt et al. [20] found that different types of some commercially available calf sera contain different protein concentrations and different protein constituents than OA-SF obtained from knee joints; additionally, the thermal properties of some calf sera were quite different from the thermal properties of OA-SF. Knee simulator wear tests were subsequently performed that compared wear rates between two types of calf sera [19]; results showed that an alpha calf serum-based lubricant, which exhibited similar thermal properties [18] and protein composition to human SF, produced lower wear rates than bovine calf serum-based lubricant, which was found to be quite different from human SF. The addition of HA was also found to have a significant impact on knee simulator wear rates [22, 23]. Despite this research, recommendations for wear testing continue to be quite broad because there is still some uncertainty regarding the effects of protein constituents, HA [24, 25], and thermal properties on *in vitro* PE wear.

## 1.3 Thesis Objectives and Outline

The objectives of the present thesis were to examine the biochemical composition and thermal properties of human OA-SF and PP-SF, and to use this information to investigate the effect of a more clinically relevant lubricant composition on *in vitro* PE wear using a POD apparatus. By simplifying the mechanics of wear testing, it may be possible to clarify some of the uncertainties that are delaying changes to current lubricant recommendations made in wear testing standards [2, 6, 26].

In the present thesis, it was necessary to provide some background information on the fundamentals of tribology, in addition to a review of both clinical and *in vitro* wear testing studies, as shown in Chapter 2. Chapter 3 outlines the materials and methods used to implement the synovial fluid analysis and wear tests conducted in this thesis. The results from the synovial fluid analysis and wear tests described in Chapter 3 are presented in Chapter 4, followed by a detailed discussion of these results in Chapter 5. A summary of the findings in the present thesis are given in Chapter 6.

# Chapter 2

# Literature Review

## 2.1 Introductory Remarks

The scope of the literature review in the present thesis requires a diverse investigation into tribology and biochemistry, and how they relate to and affect orthopaedic wear testing. In order to understand the fundamentals of wear testing, the definition and scope of tribology must be appreciated. Many systems are developed on the fundamentals of tribology, including the movement of natural and artificial joints, where lubrication affects the severity of wear and friction. The lubrication regimes pertaining specifically to the human hip and knee joints are reviewed and discussed, which then leads to an investigation into the role of synovial fluid (SF) and its biochemical composition. The literature was then extended to how SF is replicated for *in vitro* wear testing using bovine sera and various dilutive media and additives. Specific attention was placed on the composition and thermal properties of these synthetic lubricants, and how these properties affect the wear behaviour of different types of PE. Finally, the review finishes

with how wear tests are implemented, and a discussion on current issues with pin-on-disc (POD) testing.

## 2.2 Tribology of Total Joint Replacements

Tribology is an interdisciplinary study of how wear, friction, and lubrication affect interacting surfaces that move relative to each other [27]. These interactions can cause material removal, and in many cases, a loss of mechanical performance; these losses are generally reported as wear. The severity of wear is influenced by the energy lost during surface interaction caused by friction and microstructural interactions, which can be controlled by using a lubricant that acts as a layer of solid, liquid, or gas that limits contact between two articulating surfaces. In the context of total joint replacements, the overall design of artificial joints is significantly affected by the tribological properties present of the human joint.

### 2.2.1 Lubrication in Total Joint Replacements

There are three types of lubricant regimes: fluid film lubrication, mixed lubrication, and boundary lubrication (Figure 2.1). These lubricant regimes are often characterized by a system's ability to maintain a continuous fluid film that prevents contact between two articulating surfaces. The Stribeck curve, named after Richard Stribeck, is a fundamental tool that provides a visualization of these regimes. The curve illustrates friction as a function of the unitless Sommerfeld number [28] (Figure 2.1):

$$\mu_k = f\ \eta, v, P\ ; Sommerfeld\ Number = \frac{\eta * v}{P} \qquad (E.2.1)$$

where $\mu_k$ is the kinetic coefficient of friction, $\eta$ is the lubricant viscosity, $\upsilon$ is the sliding velocity, and P is the normal load per unit width.

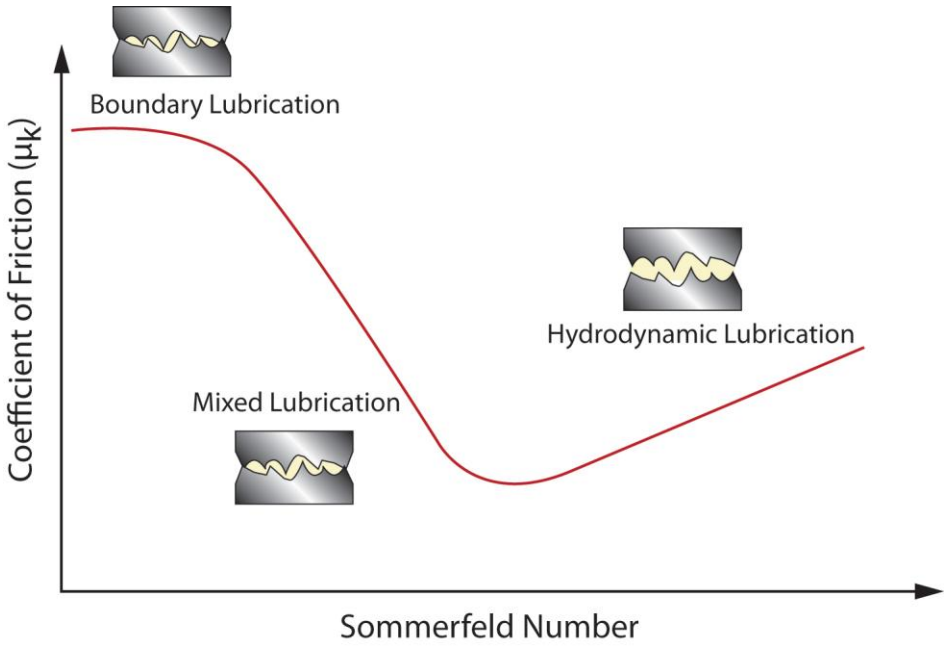


**Figure 2.1: The Stribeck curve showing the lubrication regimes under different coefficients of friction (μk) and Sommerfeld Numbers.**

Fluid film and mixed lubrication modes are typical of healthy human hip and knee joints [29-32]. The cartilage and the joint's natural lubricant, synovial fluid (SF) work collectively to maintain these modes of lubrication, which produce low friction and a high resistance to wear [32]; however, osteoarthritic joints suffer from excessive cartilage loss and changes to the viscosity of SF [33]. Over time, boundary lubrication becomes the main mode of lubrication; this mode of lubrication is preserved, even after total joint arthroplasty (TJA) [31].

Boundary lubrication occurs when two articulating surfaces are separated by a thin film of lubricant, permitting contact between two articulating surfaces (Figure 2.1). Under this regime, the viscosity of SF plays a less important role, and as a result, the

composition of SF, and the material and mechanical properties of total joint replacements (TJRs) need to be able to retain the function of the natural joint [20].

### 2.2.2 Archard's Wear Law

In general, material wear is often reported as a wear factor, k. In 1956, Archard [34] suggested that material wear was linearly proportional to contact pressure and sliding distance for flat-on-flat articulations. The wear factor, k, was developed to communicate the severity of wear:

$$V = kA_r l = kl\frac{W}{H} \qquad (E.2.2)$$

where V is volumetric wear [$m^3$], k is the wear factor, $A_r$ is the apparent contact area [$m^2$], W is the load [N], H is the hardness of the softer surface [Pa], and l is the sliding distance [m]. Typically, k should be equal to or less than 0.001, but should not exceed unity [27]. Low k-values suggest that the wear of the softer surface is quite low, whereas k is much higher for excessive wear.

Archard's wear law was adapted for PE wear [35] using the relationship:

$$V = k \times d \times W \qquad (E.2.3)$$

where V is the volumetric wear [$mm^3$], d is the sliding distance [m], and W is the constant load [N]. In this relationship, the factors relating to material properties are incorporated into k [36].

*In silico* wear simulations found that the relationship between wear, sliding distance and load correlated well with the wear observed on a retrieved tibial insert [37] and *in*

*vitro* wear tests [38]. Clinically, k is approximately $2.0 \times 10^{-6}$ $mm^3/Nm$ for the PE wear of acetabular cups in total hip replacements [39, 40].

### 2.2.3 Reasons for Failure

The wear resistance of TJRs has been known to influence the prevalence of implant failures, and is currently one of the subjects being addressed [4, 9, 36, 41-58]. Wear-induced osteolysis [59] is often linked to aseptic loosening, which is a common cause of failure in hip and knee replacements [60-65]. Large quantities of micrometer-sized wear particles can be generated from the articulating bearing surfaces; these wear particles can trigger an auto-immune response that causes the surrounding bone to resorb and deteriorate around the implant, leading to implant loosening [59, 66-68]. Implant loosening has also been found to initiate bone resorption due to changes in fluid pressure [69]. To address the concerns associated with wear-related implant failure, TJRs should be subjected to vigorous *in vitro* wear testing.

Sterilization of the PE component of TJRs has also been found to have a significant impact on wear and surface damage [70-76] (Figure 2.2). In the 1960s, non-crosslinked PE TJR components were sterilized using a gamma-in-air sterilization technique with a typical dose of 25-40 kGy (2.5-4.0 Mrad). It was found that, initially, sterilization with radiation improved material properties and wear resistance through the crosslinking of PE chains; however, in the mid-1990s, it was found that, over time, such sterilization techniques degraded the physical, chemical, and material properties of the PE components due to oxidation [77]. Free radicals from broken PE chains were formed due

to the gamma radiation. These free radicals reacted with the oxygen present in body fluids or during shelf storage, causing the PE components to become brittle [72]. The brittle nature of the PE components essentially increased wear, delamination, and the formation of PE wear particles, which can cause osteolysis [77]; because of this, other sterilization techniques, such as ethylene oxide and gas plasma, were developed in order to maintain the beneficial material properties of crosslinked PE. Heat treatments following the crosslinking of PE have also been used to remove residual free radicals to stabilize the PE [78].

**Figure 2.2: Image of a retrieved total knee and total hip replacement. The retrieved total knee replacement consists of a CoCr femoral component (left, top) and an ethylene oxide crosslinked PE tibial insert (left, bottom). The retrieved total hip replacement is shown with a CoCr femoral head and stem (right, top) with a crosslinked gamma sterilized PE acetabular insert (right, bottom). Note the characteristic yellowing and delamination of the gamma sterilized acetabular insert (right, bottom) in comparison to the ethylene oxide sterilized tibial insert (left, bottom).**

### 2.2.4 Wear Mechanisms

Wear mechanisms are the fundamental processes by which a material can experience wear. These mechanisms describe how and why articulating surfaces behave under certain tribological conditions. Surface analysis techniques, such as surface profilometry and scanning electron microscopy (SEM), can verify the presence of these wear mechanisms. There are four main types of wear mechanisms: adhesive, abrasive, tribochemical, and fatigue.

Adhesive wear is characterized by the shearing of asperities (tiny sharp projections that arise from the material surface) through the direct contact of two moving surfaces under load (Figure 2.3). The adhesion between the two surfaces can cause material transfer from one surface to the other through adsorption, where ionic, covalent, hydrogen, or Van der Waals forces act between the two surfaces. Van der Waals forces typically act on metal-polyethylene bearing couples [27]. On retrieved TJRs, the presence of burnishing (highly polished areas) on the PE component typically indicates adhesive wear [79, 80].

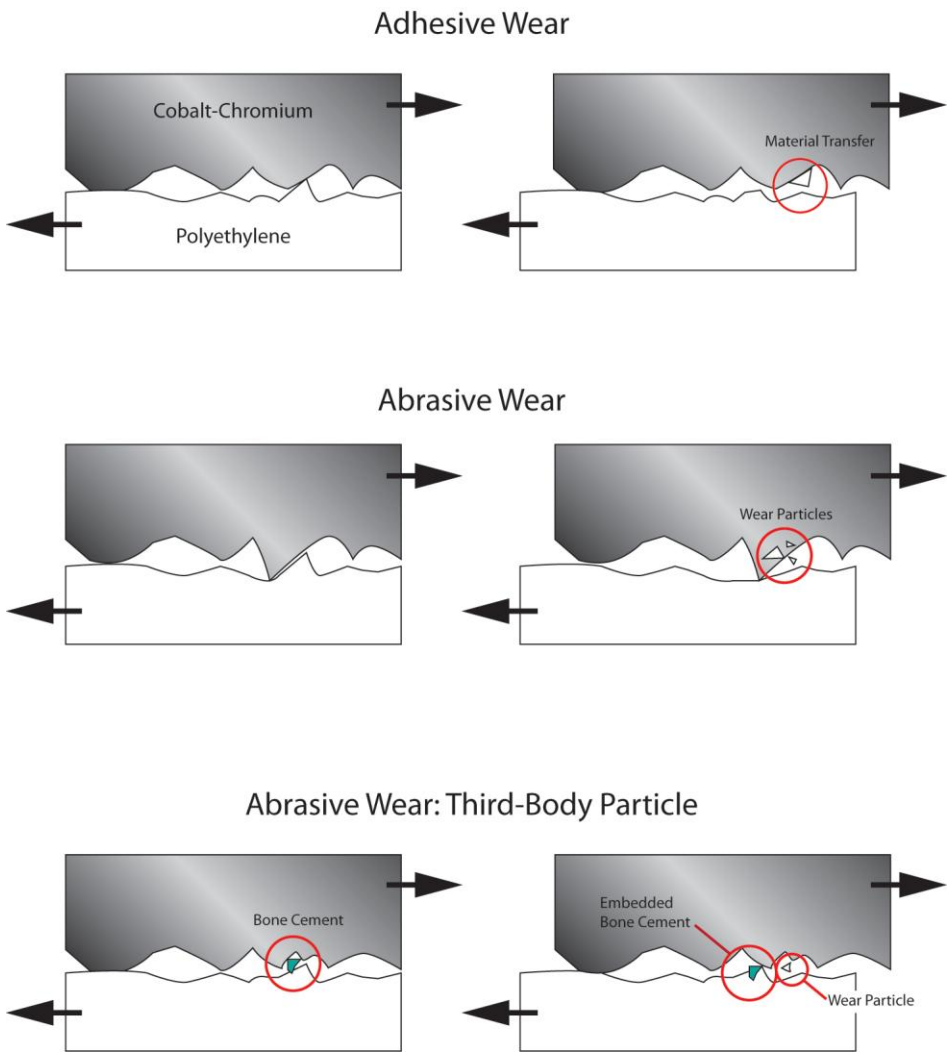


**Figure 2.3: Illustration showing adhesive and abrasive wear mechanisms for cobalt-chromium alloy articulating against polyethylene.**

Surface asperities or loose particles have the potential to cut into an opposing softer surface, which results in material removal during sliding (Figure 2.3). This is known as abrasive wear. In TJRs, this wear mechanism can be identified by the presence of indentations, grooves or scratches on the PE surface, and /or scratches on the metal

counterface; in some cases, third-body wear particles (such as bone cement or metallic particles) can be found embedded in some of the indentations [23, 81] (Figure 2.3).

The biological environment surrounding the human joint is naturally corrosive to TJRs with metal components [82]. Tribochemical wear is caused by a chemical reaction between the articulating surfaces; this chemical reaction is often triggered by heat generated from the articulating surfaces, and the presence of a corrosive lubricant [82, 83]. Under low sliding speeds, the corrosion by-product can form a protective oxide layer that can decrease wear; however, at higher speeds, this oxide layer can break down, acting as third-body particles and increasing wear [84].

Fatigue wear is often used to describe excessive material removal due to repeated cyclic loading during sliding or rolling [27, 85]. The normal stresses resulting from this cyclic loading can initiate cracks on or beneath the material surface; over time, these cracks can propagate and cause material delamination. In TJRs, sterilization techniques involving gamma irradiation accelerated the oxidation of the PE components, which ultimately reduced its mechanical properties *in vivo*, causing delamination [71-73, 75, 86]. Sterilization techniques have since been improved with the development of gas-plasma and ethylene-oxide sterilization, which have been shown to have no detrimental effect [76] or some beneficial effects on the mechanical properties of PE, such as higher toughness [73].

### 2.2.5 Wear Testing

*In vitro* wear testing allows researchers to more closely examine specific tribological conditions that influence failures in TJRs, such as PE wear; investigating these aspects can provide valuable information that can lead to the optimization of current and new implant designs. Currently, total joint wear simulators provide an approximate kinetic and kinematic representation of human joints. One of the earliest total hip joint simulators was built by the University of Leeds in 1968, which was followed by the development of an electro-hydraulic knee joint simulator in the mid-1970s [87]. Since then, the operation of a total joint simulator has become more complex in order to more accurately reproduce *in vivo* kinematic and kinetic conditions. With these complexities, however, comes greater expenses in building and maintaining these machines; sample preparation is also time consuming [4]. For these reasons, wear testing in total joint simulators is reserved for the final wear testing of total joint replacements (TJRs).

POD testing provides a more cost-effective material screening method, and is often used to quantitatively screen a material's wear performance prior to joint simulator testing. A POD apparatus can be a single station [50] or multi-station machine [88] that typically consists of a PE pin articulating against a flat metal disc or plate. POD testing allows researchers to simplify, isolate, and control for certain parameters and conditions (Figure 2.4), which can then be better understood and accounted for during joint simulator testing where a greater number of variables, such as implant geometry and other implant-related design features, are introduced into the testing environment; however, because many of these design-related variables are absent from POD testing,

the ability to exactly replicate *in vivo* conditions is somewhat limited. While these limitations may restrict comparisons to *in vivo* measurements, wear rates and wear factors generated from POD tests can be extrapolated to joint simulators to some extent, and are valuable for comparisons between different materials for screening purposes undergoing the same test.

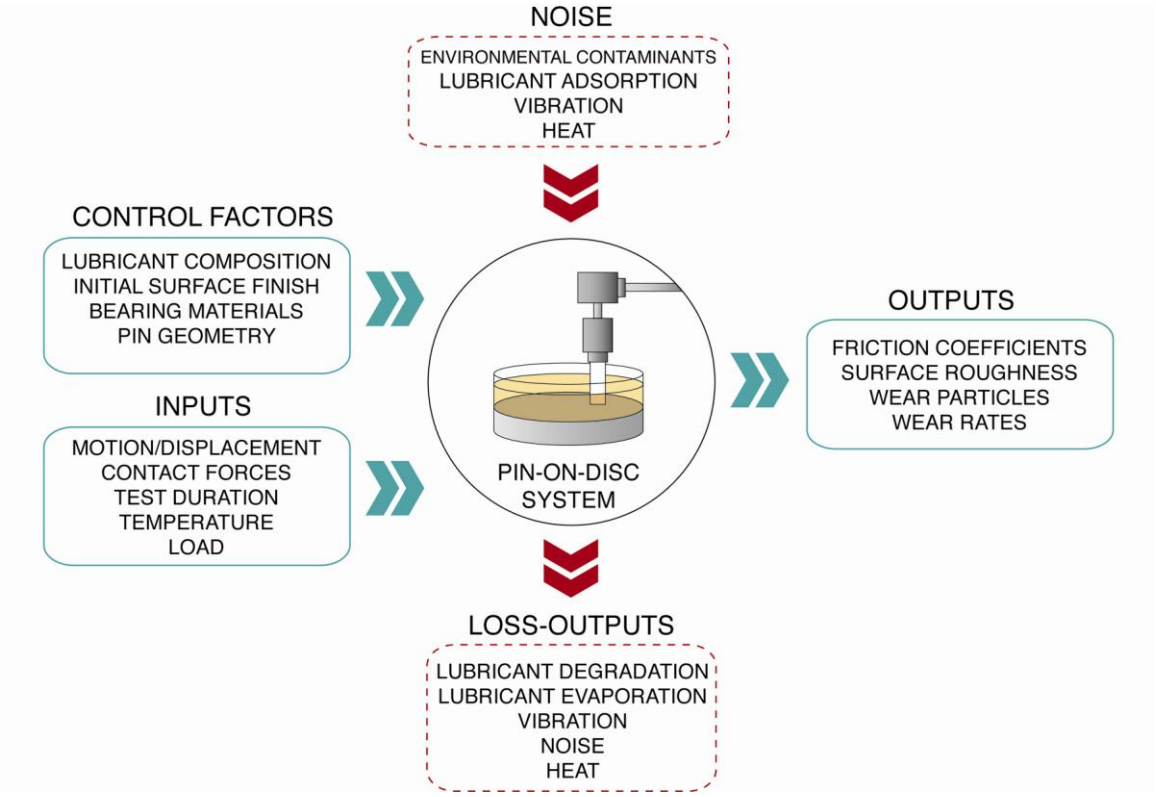


**Figure 2.4: Schematic of the parameters and conditions involved in pin-on-disc testing.**

## 2.3 The Role of Synovial Fluid in Wear

Synovial joints, such as the hip, knee, shoulder, and elbow, are the most common type of joint in the human body. Unlike cartilaginous joints, synovial joints consist of a capsule that surrounds the entire joint. Inside this capsule is the synovial membrane (Figure 2.5), or synovium, which produces SF, a natural lubricant that helps reduce the friction between the articular cartilages of synovial joints. Approximately 0.2-10 mL of SF are generated per day in the hip or knee joint [14].

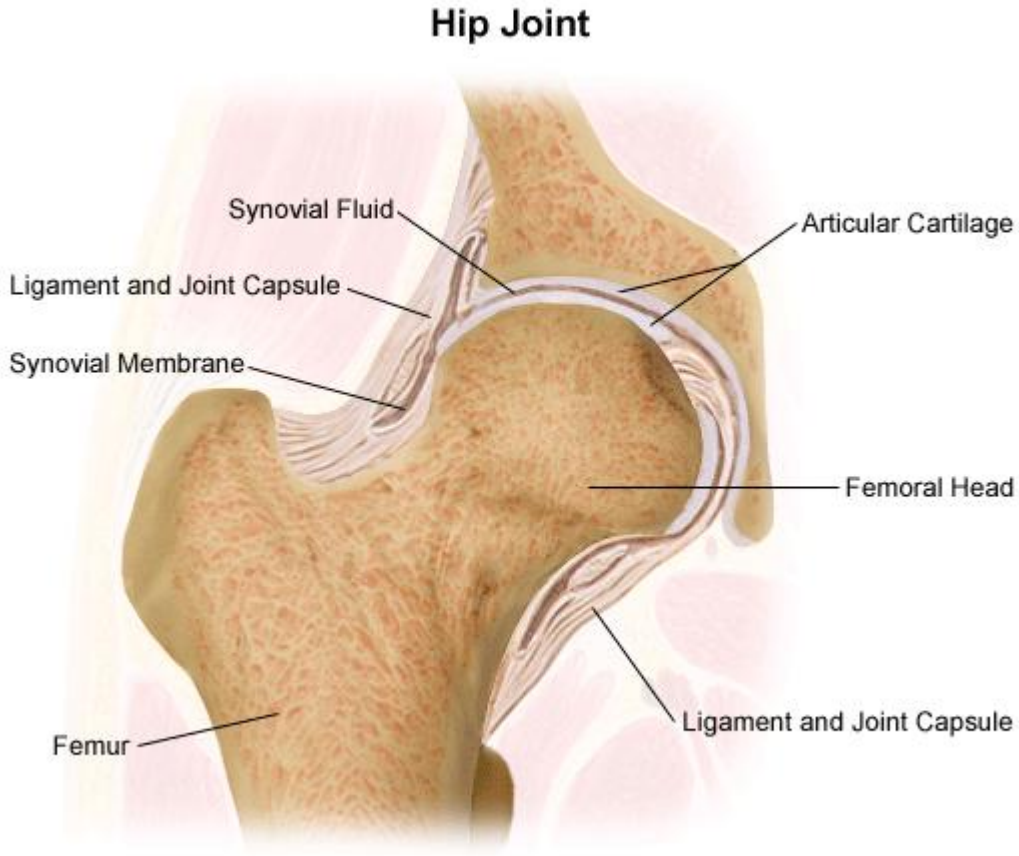


**Figure 2.5: Image from Martini et al. [1] showing simple anatomy of the hip joint. Note that the synovial membrane (the synovium) surrounds the femoral head.**

In healthy joints, the body is able to maintain the lubricating properties of SF, such as its low co-efficient of friction [7, 8]; however, in diseased or osteoarthritic joints, the composition of SF changes [9], therefore affecting its ability to lubricate the joint effectively.

SF is a complex fluid composed of a number of solutes such as proteins, phospholipids, and hyaluronic acid (HA) [10, 89]. It was suggested that these elements affect the lubricating properties of SF [89].

Proteins are large molecules that are composed of one or more chains of amino acids. The main protein constituents have been classified as human serum albumin, α1-globulin, α2-globulin, β-globulin, and γ-globulin, and have been documented in patients with rheumatoid [90] and osteoarthritis [20]. In osteoarthritic SF (OA-SF), the protein concentration is much higher than in healthy SF [9, 91].

Although the composition of SF is influenced by disease, a previous study [92] found that there was no significant difference between the pH of normal, traumatic, and osteoarthritic synovial fluid, and that the pH levels varied between 7.50-7.69.

### 2.3.1 Biochemical Composition of Synovial Fluid

The biochemical composition of SF plays an important role the tribology of healthy and diseased human joints. There is some evidence that other individual or combinations of SF constituents are able to change the rheology of SF and, therefore, the mode of lubrication [9-11], which may have a significant impact on PE wear. Since the synovium is generally retained during TJA, it is able to produce hyaluronic acid (HA), or

hyaluronan, that is similar to those produced by the synovium of healthy joints [93]. HA is the main constituent that determines the rheology of synovial fluid [9, 91, 94], and plays a specific role in increasing *in vitro* wear [22]; the mechanism of HA in SF is detailed elsewhere [10]. The molecular weight of HA was found to range between 1.7 to 2.0 MDa in OA-SF and periprosthetic SF (PP-SF) [9], and 1.12 MDa and 2.63 MDa in OA-SF and PP-SF in hip joints, respectively [95].

The amount of HA is known to be affected by disease, such as osteoarthritis [91, 96-98]; Mazzucco et al. [9] found that, while protein and phospholipid concentrations were similar in patients undergoing primary and revision total knee arthroplasty (TKA), the HA composition in SF was lower in patients undergoing revision TKA (0.9 ± 0.4g/L) than in patients undergoing primary TKA (1.3 ± 0.5g/L). Similar results have been shown in patients undergoing primary and revision total hip arthroplasty (THA) [12, 13]. Even though boundary lubrication is maintained after TJA [31], it remains to be seen if the composition of SF differs between patients undergoing primary or revision THA and TKA.

Naturally, SF would be an ideal lubricant for *in vitro* wear testing; however, there are some issues regarding its use in such applications. Yao et al. [16] performed a number of wear and rheological tests comparing human periprosthetic SF to other lubricants, and found it difficult to collect and preserve a large volume of SF for wear testing, which requires approximately 40-600 mL per wear station for simulator wear testing [14]. Other researchers have found that the use of SF for *in vitro* wear testing is simply not feasible; some of the concerns regarding ethical use, patient-to-patient variability, and SF availability are discussed in more detail elsewhere [9, 15, 16]. These concerns illustrate a

need for an alternative, yet more representative lubricant for *in vitro* wear testing that mimics SF.

Bovine sera-based lubricants have been, and are currently the most widely used lubricants in orthopaedic material testing. Unlike distilled water and saline solutions [99-102], bovine serum has been found to produce wear behaviours and wear mechanisms that were comparable to those observed on retrieved prosthetic joints [44, 100, 103]; however, over the last decade, the clinical relevance of bovine serum has come under debate [14, 15, 17-20]. While bovine serum is able to mimic clinical wear to a degree, its composition [11, 16, 19, 46, 104-107], in addition to its biochemical [20], and thermal properties [18, 108-110], have been found to be quite different from human SF. The use of more cost-effective lubricants, such as egg whites and soy protein, had also been investigated, but none were found to be suitable alternatives to bovine serum [111]. It is pertinent that further research be more focused on defining a lubricant that is biochemically and thermally similar to SF, in addition to producing similar wear characteristics that are seen *in vivo*. These underlying properties can have a considerable effect on wear, and by extension, the incidence of wear-related implant failures.

### 2.3.2 Effect of Lubricant Composition on Wear

The type and composition of bovine serum used in *in vitro* wear testing significantly influences the wear performance of hip and knee implants [9, 16, 17, 19, 20, 46, 105, 107]. Brandt et al. [20] recently showed that protein concentrations differ between commercially available calf sera: bovine calf serum, newborn calf serum, alpha calf

serum (ACS) , and iron-supplemented alpha calf serum (ACS-I) [20]. When undiluted, these calf serum protein concentrations range from 41-69g/L, whereas SF protein concentrations range between 20-35g/L for OA and PP hip and knee joints [9, 12, 20, 46].

The ASTM standard acknowledges that serum composition can fluctuate between brands and suppliers, and recommends a dilution of up to 75% (v/v) for wear testing [6]; however, the general nature of this ASTM guideline has caused some uncertainty as to what protein concentrations are appropriate for wear testing, and how sera should be diluted, resulting in an assortment of dilution ratios and protein concentrations between studies (Appendix A.1). In addition, following the ASTM recommendation may result in producing lubricants that are diluted beyond clinically relevant protein concentrations. To address these issues, Saikko [46] proposed that dilutions should be based on protein concentration rather than dilution ratios or percentages, and that protein concentrations should not drop below 20g/L.

The protein constituent fractions that make up these sera are also quite different from SF (Figure 2.6). Results from the biochemical study by Brandt et al. [20] showed that, of the commercially available calf sera, the protein constituent fractions of ACS and ACS-I were the most comparable to protein constituent fractions in SF, and that these fractions significantly influenced wear rates in knee simulator wear testing [19]. The influence of these fractions on wear rates in POD and hip simulator wear tests have yet to be investigated. The most recent ASTM standard does not provide recommendations regarding the documentation or use of protein constituent fractions in test lubricants; only

a handful of POD studies reviewed in this thesis document the protein constituent fractions present in the lubricants used in their studies [46, 56, 57].

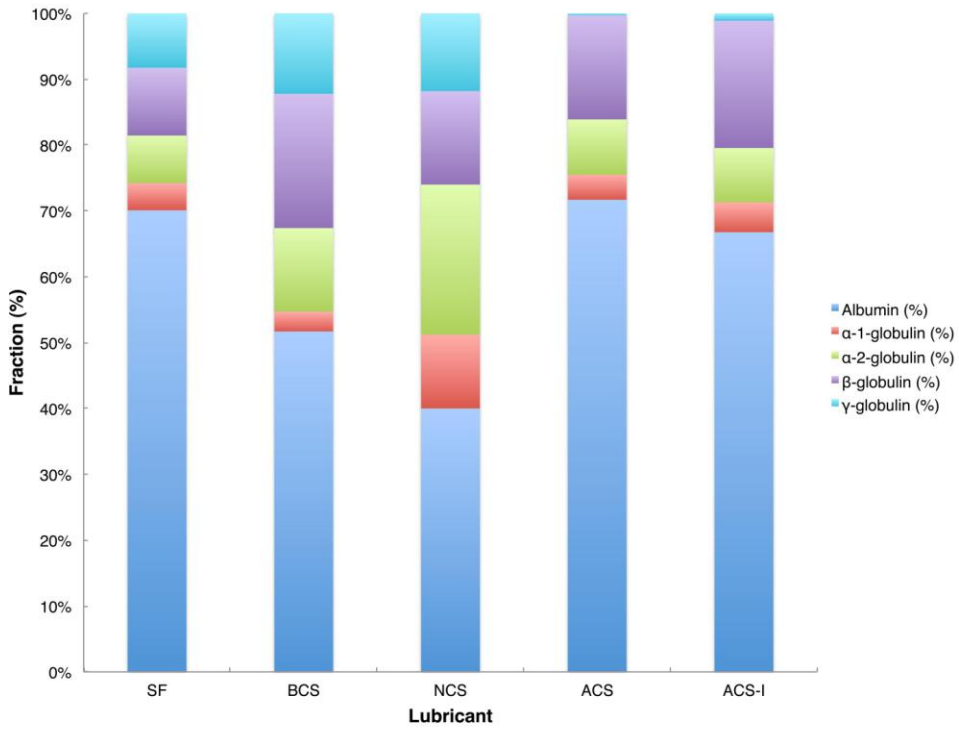


**Figure 2.6: Comparison between constituent fractions of human osteoarthritic synovial fluid and different types of some commercially available calf serum (BCS = bovine calf serum; NCS = newborn calf serum; ACS = alpha calf serum; ACS-I = iron supplemented alpha calf serum; ACS-I = iron supplemented alpha calf serum); figure replicated from data produced by Brandt et al. [18].**

## 2.3.3 The Effect of Hyaluronic Acid on Wear

The bovine sera currently used in *in vitro* wear testing contain no traces of HA [57] due to the fact that it is derived from bovine blood and not synovial fluid. Because of this difference, the rheological properties of bovine serum are quite different from human SF.

There are a limited number of *in vitro* wear tests that investigate the effects of HA on PE wear [22, 24, 25]; many of these tests, however, implemented different test

conditions and parameters. A hip joint simulator test by Wang et al. [24] and a POD test by Joyce [25] found that, when HA was added to 90% ACS and 33% bovine serum respectively, there was no significant effect on wear rates; on the other hand, a knee simulator test by DesJardins [22] found that the addition of HA to 50% bovine serum had a seven-fold increase on the wear of PE. A POD study by Sawae et al. [11] found that a lubricant containing HA and saline solution was able to reduce the friction and wear of PE. HA is known to bind to a number of proteins, and its rheological contribution appears to be dependent on protein concentration [10]; this interaction may explain the negative correlation found between concentrations of HA, and protein and phospholipid concentrations in both primary and revision TKA patients [9]. This correlation may be the cause of discrepancies between wear tests and test protocols; however, there is a need for more research to clarify the role of HA in serum-based lubricants, and its effect on PE wear while maintaining protein concentration levels that are seen clinically.

### 2.3.4 The Effect of Lubricant Thermal Stability on Wear

Bovine-serum-based lubricants are susceptible to protein degradation due to increased temperatures produced at the CoCr-PE interface *in vitro* [112]; temperatures have also been known to intensify at the ball-cup interface of total hip prostheses *in vivo* [113]. *In vivo*, protein degradation may occur; however, Levick et al. [114] had suggested that the SF is replenished at a rate of 2 mL/hour, which may eliminate most of the degraded proteins present in the fluid. Protein degradation has a tendency to accelerate wear [106], and therefore, it can be implied that increased temperatures generated from the CoCr-PE

articulations contribute to accelerating wear [83]. The unfolding of proteins can be directly related to its thermal stability, which can be altered by its pH [110] and ionic strength [108, 109, 115, 116]. A recent study by Brière et al. [18] used differential scanning calorimetry detect and quantify the point at which proteins unfold in human SF and calf serum, and found the unfolding curves to be relatively similar.

A number of factors can alter the thermal stability of a protein. A study by Pico [110] showed that pH can alter the thermal transition temperature of human serum albumin, a main constituent of SF. Altering the composition of a lubricant can also influence the stability of a protein: the addition of salts and other solutes affect ionic strength and, therefore, osmolality (osmoles of solute per kilogram of solvent). A study by Giancola et al. [109] showed that increasing the ionic strength increased the thermal transition temperature and enthalpy change, which implies a more thermally stable protein solution.

Evidently, the clinical relevance of a lubricant is dependent on a number of characteristics that are more complex than just the composition and protein concentration; the thermal stability of a lubricant may help clarify the issue of protein degradation in *in vitro* wear testing, and may provide a better understanding of the role that individual constituents play in PE wear. It appears that some of the lubricant recommendations provided by the ASTM standard [6] do not represent the clinical situation. The mechanism of lubrication and its influence on wear are not yet fully understood, and therefore, more extensive research in this area is encouraged.

## 2.4 Pin-on-Disc Wear Testing

POD testing poses many challenges to researchers despite being simpler to setup and operate than total joint wear simulators. Presently, ASTM F732 [6] is the only standard that provides guidelines for POD wear testing of polymeric materials used in total joint prostheses. These recommendations outlined in this standard are rather general and provide limited guidance; these generalizations have made it quite difficult to compare and interpret results between studies due to the large variation in experimental parameters and conditions [17, 117] (Appendix A.1-3). Nonetheless, certain trends emerged in which specific combinations of parameters and variables, such as lubricant composition [9, 16, 17, 19, 20, 46, 105, 107], wear paths [4, 9, 36, 41-57], contact pressure [11, 36, 41-43, 45, 46, 48-51, 54-58], test temperature [106], etc., significantly influenced the tribology and the subsequent wear of the bearing materials.

### 2.4.1 Wear Paths and Cross-Shear Motion

One of the main kinematic issues in POD testing relates to implementing a wear path that is able to simplify motions observed *in vivo* while producing similar wear rates and wear factors as those seen clinically. Earlier pin-on-plate tests have shown little success with unidirectional and reciprocating wear paths [118-120]. These types of wear paths produced wear rates as low as 0.1mg/million cycles (Mc) [103, 121] (or 0.11 $mm^3$/Mc if assuming a PE density of $\rho = 0.935 mg/mm^3$ [74, 122]) due to the preferential alignment of polymer chains in PE, as explained by Wang et al. [81, 123]. While these types of wear paths may be relevant in other applications, research has shown that for orthopaedic

bearing materials, the wear rates occurring in TJRs range between two to three magnitudes higher than the wear demonstrated using unidirectional and reciprocating wear paths [103, 121, 123, 124]. These higher wear rates were attributed to cross-shear motion, where the PE surface experiences shear and tensile stresses in many directions, causing shear rupture and the formation of PE wear particles [123]. Because of the significant effect of cross-shear on wear, a variety of multi-directional wear paths are now implemented in order to replicate the cross-shear phenomenon typically seen in TJRs [4, 9, 36, 41-57]. Results from these wear tests showed that PE wear accelerated substantially in comparison to wear tests using unidirectional and reciprocating wear paths (Appendix A.2). To date, any multi-directional motion that is able to reproduce clinical wear is acceptable, according to ASTM F732-00 [6].

#### 2.4.1.1 Rectangular Wear Paths

A study by Bragdon et al. [44] was the first to implement a rectangular wear path in order to emphasize the importance of bi-directional motion on the wear rate of PE in total hip replacements (THRs). Although the wear path did not simulate the quasi-rectangular or elliptical wear paths that are reported to occur in the hip, they were able to generate a wear rate that was two orders of magnitude higher than wear rates reported for unidirectional motion (approximately 10mg/Mc [44] vs. 0.1mg/Mc [103, 121]); on the other hand, the wear rates reported from their own multi-directional hip simulators were approximately 3.5-times higher than the wear rates generated using the rectangular wear path [124]. Wear particles generated from the rectangular wear test were of similar size and morphology as those found *in vivo* [125].

A number of researchers have since integrated the rectangular wear path into their POD tests [36, 45, 54-56]. Yao et al. [56] examined the effect of fluid absorption on the wear resistance of conventional, non-crosslinked PE and highly cross-linked PE (HXPE) using a 15mm x 15mm square wear path to account for the multidirectional sliding motion in THRs. They had found that hydrated pins experienced more wear, and that wear was greater for conventional, non-crosslinked PE in comparison to HXPE.

Mazzucco and Spector [36] explored the relationship between contact area and stress on the volumetric wear of PE using a 10mm x 10mm square wear path. The wear path was selected to match the path traced by a femoral head on an acetabular cup [126]. Wear rates between this study and previous POD tests were difficult to compare due to differing test parameters, such as wear paths, normal loads, and lubricant replenishing protocols.

Other studies by Turell et al. [45] and Korduba et al. [54] investigated the effect of various rectangular aspect ratios on the wear rate of conventional PE and HXPE, respectively. Turell et al. [45] hypothesized that volumetric wear was linearly related to wear path geometry based on an earlier theory proposed by Wang [127] that suggested maximum PE wear should occur under square wear paths. They had found that wear rates were indeed dependent upon the selected wear path; however, a linear relationship between the wear path shape and PE wear could not be established. A more recent study by Korduba et al. [54] tested conventional PE and HXPE along rectangular wear paths of similar aspect ratios used by Turell et al. [45]. Results for conventional, non-crosslinked PE correlated well with the study by Turell et al.; however, cross-shear was found to have a minimal to no effect on the wear of HXPE.

In order to improve the predictive power of *in vitro* wear tests, which are mainly based on Archard's wear law [34], Dressler et al. [55] developed a protocol to investigate whether a variable wear rate exists. The pins would repeatedly slide along a linear path to obtain a certain sliding distance; once the sliding distance was achieved, the pin would stop, rotate 90 degrees, and slide back and forth again in the new direction until the designated sliding distance was reached. In essence, a square wear path was produced. Dressler et al. [55] determined that, for moderately cross-linked PE, wear increases after a change in direction; however, with increased sliding distance, the wear suddenly drops to almost zero in less than 5mm of sliding [55]. These results challenge the way wear is currently interpreted, which assumes a linear relationship between wear and sliding distance.

#### 2.4.1.2 Elliptical Wear Paths

Much work has been based on circular translating wear paths, which, unlike other studies, does not rotate the plate relative to the pin [43, 46, 49, 107, 128, 129]. This wear path prevents the preferential alignment of polymer chains, and produces wear factors closer to those found clinically in retrieved Charnley prostheses [39]. Saikko et al. [47] also studied the effect of wear path geometry on wear using ellipses of varying aspect ratios (the ratio between a shape's width and height), and compared the them to the aspect ratio of flexion-extension to abduction-adduction amplitudes in walking [130]. They had found that aspect ratios ranging between 1.0 and 3.8 produced wear factors that were similar to those found clinically [39, 131], and that aspect ratios greater than 3.8 had the potential to produce wear factors that were not clinically relevant [47].

Kilgour and Elfick [52] explored the changing microstructure and wear rate of PE and mildly crosslinked PE (XPE) using an elliptical wear path to better approximate the quasi-elliptical paths of hips *in vivo* [126, 132]. A wear factor of 1.6 x $10^{-6}$$mm^3$/Nm was generated for conventional PE, which was found to be in good agreement with clinical wear factors of 2.1 x $10^{-6}$$mm^3$/Nm [133]. Scanning electron microscope images of the PE surfaces subjected to the elliptical wear path showed random polymer chain orientation, i.e. no preferential alignment was observed; on the other hand, microstructural differences were not as distinct on XPE surfaces subjected to linear and elliptical motion. It is suggested that crosslinking limits the ability for polymer chains to re-orient, which may explain the lower wear rates observed [134].

#### 2.4.1.3 Hybrid Wear Paths

Marrs et al. [41] combined a linear wear path with pin rotation in their wear tests to investigate the counterface roughness and multi-directional motion on conventional and crosslinked PE. This hybrid wear path produced a frictional force that constantly changed direction with respect to the PE surface, similar to what Wang et al. [123] determined based on a computer model of the natural hip joint, and yielded wear factors close to those found clinically under rougher surface conditions.

Kang et al. [127] developed a computer model to quantify cross-shear based on Wang's theory of PE wear and multi-directional sliding, which determined the wear path specifications for their multi-directional wear tests [51]. The wear path was similar to the path used by Marrs et al., incorporating a pin rotation of up to ±55° for the purpose of integrating the cross-shear effect observed in TJRs. Although the wear factors generated

were similar to those generated by Turell et al.'s wear tests [45], the wear factor for conventional PE were one order of magnitude less than the maximum wear factor obtained from *in vivo* [135] and *in vitro* studies [49, 124]. Kang et al. indicated that the differences may be due to the dissimilarities in test parameters and conditions.

#### 2.4.1.4 Randomly Generated Wear Paths

A 16-station POD machine, designated as the RandomPOD, was developed by Saikko et al. [53] in order to investigate the effects of random motion and random load on wear. The RandomPOD is capable of producing any wear path and loading profile. As such, sliding velocities and accelerations can vary depending on the input parameters. Wear factors were compared between four different test conditions: circular translation under static loading, circular translation under random dynamic loading, random motion under static loading, and random motion under random dynamic loading. It was found that random motion under random dynamic loading resulted in a wear factor that was 1.6 times greater than circular translation under static loading; however, wear factors produced from random loading were not statistically different from the wear factors produced from static loading [53]. The wear mechanisms and wear factors produced were found to be similar to clinical observations for all four test conditions [40, 136-138]; however, simply reproducing clinical wear mechanisms and wear factors does not dictate clinical relevance. Computational [126, 132] and knee simulator wear tests [139] have found that the movement of the hip and knee joint follows a certain path; these studies show that joint motion can be complex, but not random.

## 2.4.2 Other Test Parameters

### 2.4.2.1 Loading and Contact Stress

Dynamic and constant loads have been used interchangeably in POD testing— constant loads for its simplicity [11, 36, 41-43, 45, 46, 48-51, 54-58], and dynamic loads for its ability to reproduce *in vivo* conditions [4, 44, 49, 52, 53]. Saikko et al. [53, 140] maintains that the type of load used during POD testing is of secondary importance to the type of wear path implemented in the experimental design; wear factors generated from the same wear path were not found to be significantly different, despite differences in loading (static or random loading). While this may hold some validity, it is important to also consider the importance of replicating contact stresses and cross-shear exerted by TJRs *in vivo*.

Clinical retrieval studies have shown that contact stresses and contact area may have a significant influence on wear in TJRs [141, 142]; however, the individual effects of contact area [127], contact stress [143, 144], and sliding distance [55] on wear rates are still under debate. Conventionally, it was thought that increasing normal load and area lead to higher wear rates [143]; however, with the current advances in POD testing, studies have shown that wear is dependent on a number of parameters. Some studies have shown that higher contact stresses can lead to a reduction in wear since the area exposed to abrasive surface asperities is reduced [144]. It has also been found that a greater contact area [129, 145] can lead to a reduction in wear. Another study exhibited an increase in volumetric wear with increasing contact area within a relevant range of

contact stresses [36]. More recently, Dressler et al. [55] found that wear rates decreased suddenly with increased linear sliding distance, and increased drastically following a change in direction under constant loading.

Based on these findings, it is evident that a number of parameters can influence wear, and that the actual mechanism of wear is much more complex than what has been proposed by currently accepted theories. In order to clarify the relationships between test parameters and wear rates, test conditions must closely mimic *in vivo* conditions by implementing appropriate contact stresses, contact areas, and dynamic loading. Incorporating dynamic loading, however, introduces an additional complexity into POD testing by changing the modes of lubrication [52], which may or may not be a natural occurrence in human joints.

#### 2.4.2.2 Surface Roughness

The effects of surface roughness on PE wear have been investigated by a number of studies [41, 43, 146-148]. Generally, it has been shown that rougher CoCr counterfaces increase the wear of PE; however, it appears that there are other variables that enhance the effect of surface roughness on wear.

In a study by Cooper et al. [146], the use of water as a lubricant formed a PE transfer film that was not seen on retrieved TJRs; this transfer film essentially increased the average surface roughness (Ra) of the CoCr surface, producing a 7-fold increase in wear. Subsequently, a protein-containing lubricant was used, which eliminated the presence of the transfer film. As a result, the Ra remained constant, as did the average wear rate throughout their tests.

Turell et al. [148] compared the differences in wear factors between square and rectangular wear paths under smooth and rough counterface conditions. They found that, under rougher conditions, rectangular wear paths produced wear factors up to 3-times greater than wear factors produced under smoother surfaces; this difference was less pronounced for more square-like wear paths, where wear factors for rough counterfaces were only 1.25-times greater than wear factors for smooth counterfaces. Evidently, counterface roughness has a greater impact on the differences between wear rates produced by rectangular wear paths in comparison to square wear paths.

Marrs et al. [41] investigated the effect of smooth and roughened CoCr counterfaces on the wear rates of conventional PE and mildly cross-linked PE (XPE) using various loads (80 or 160 N), and frequencies (1 or 2 Hz) between tests. Under unidirectional motion, no significant difference was found between the wear rates of PE and XPE on smooth counterfaces. The opposite was true using rougher counterfaces, where a significant difference was detected; this appeared to be similar to the results found by Turell et al. [148]. Under multidirectional motion, wear rates were significantly different on smooth counterfaces, but surprisingly not significantly different on rough counterfaces even though this condition produced the highest wear rates. These results may be explained by the study performed by Saikko et al.[43], which investigates the effect of counterface roughness on the wear of conventional PE and XPE under multidirectional motion.

A retrieval study by Hall et al. [39] found a weak correlation between femoral head roughness and the wear factor of conventional PE ($R^2$ = 0.14). Saikko et al. [43] suggested that this correlation may be weakened by the fact that *in vitro* wear tests do not

incorporate the variables that influence wear *in vivo*, such as patient activity level, and where the roughened areas are located. In addition to this, CoCr counterfaces are often artificially roughened prior to performing wear tests. In the study by Saikko et al.[43], this initial surface roughening remained fairly constant during testing. This may not occur *in vivo*, where sudden changes in surface roughness may cause rapid wear.

Knee simulator wear tests [149, 150] have found that type of surface roughness has a significant effect on PE wear. The roughness of a surface can be characterized by the height/depth of peaks and valleys. In these knee simulator wear tests [149, 150], wear rates increased with the increased presence and height of peaks or asperities. A recent implant retrieval study[151] comparing the performance of CoCr and oxidized zirconium (OxZr) femoral components found that, although the OxZr components appeared rougher, the prevalence of valleys had the potential of improving the wettability of the surface, potentially reducing the generation of PE wear particles.

Based on the literature presented, it appears that the effect of surface roughness on PE wear is highly dependent on the wear path shape, lubricant composition, material properties, and type of surface roughness implemented into the wear test. Further understanding of this effect can be attained by changing very few variables in order to maintain consistency throughout testing.

#### 2.4.2.3 Pin Geometry

A majority of the POD tests reviewed in this thesis perform wear tests using cylindrical, flat-tipped pins; however, to the authors' knowledge, discussion related to the effect of different pin geometries on wear is limited.

Some of the earlier POD tests done by Saikko [128] and Saikko and Ahlroos [107] performed POD tests using PE pins with a tapered edge to produce a similar contact geometry to machined PE acetabular cups. In addition, the tapered edge gradually increased contact area with increased wear, which resulted in a steady decrease in contact pressure— an effect that was likely to occur in TJRs over time [128]. These wear tests were found to produce similar damage features to those found on retrieved PE acetabular cups; however, other comparisons, such as wear particle characterization, were not performed. Later studies by Saikko [46, 49] and Saikko et al. [43, 47, 53, 129, 140] performed wear tests using flat-tipped pins. Marrs et al. [41] also performed wear tests using pins with a tapered edge; however, reasons for selecting this geometry were not provided.

Cornwall et al. [58] contrasted a number of different geometries in order to replicate the motions of a TKR: sliding, gliding, and rolling. A tapered pin was used for one sliding test in order to minimize the effects edge loading. A spherical-tipped pin made from CoCr was used for a gliding test, which provided a fatigue-type contact loading that has been known to occur in TKRs [66, 152]. Rolling was replicated using a ball-on-flat contact. Rolling provided the largest wear rate in comparison to the other kinematic contact conditions; gliding had the second highest wear rate (Appendix A.3) [58]. In the event that a ball-on-flat contact cannot be achieved, it appears that a pin with a spherical tip would be desired for a better representation of the knee or hip joint, as it simplifies the calculation of contact stress and contact area [57]; however, in terms of pin material, it would be beneficial to use PE pins rather than metal pins, since a metal pin would continually deform a PE disc and possibly skew the wear results. This continual

deformation would present a confounding parameter in the form of a viscoelastic effect[57].

#### 2.4.2.4 Polymer Fluid Absorption

PE wear is often assessed gravimetrically using a high-precision analytical balance; however, PE has a natural tendency to readily absorb fluid, despite its hydrophobic nature, and may influence wear measurements. Because of this behaviour, PE components will often undergo a pre-soaking period to account for any mass gain due to fluid absorption [36, 41, 45, 52, 54, 55, 153-155].

Absorption is time [56, 156], fluid [17, 154, 157], temperature [154], and material dependent [158]. Fluid-sorption rates were found to be higher in distilled water than in bovine serum [157], and are lower for HXPE in comparison to conventional, non-crosslinked PE [158]. After 1 Mc of pin-on-flat tests, wear rates were shown to increase up to 2.5 times when hydrated pins were used in comparison to non-hydrated pins, although these effects were less apparent with increasing test duration [56]. This may not occur in joint simulator testing, where fluid absorption may continue to occur throughout testing. Yao et al. [56] suggested that the hydration of the PE pins prior to wear testing may affect the chemical or physical properties of the PE pin surface, which can change how the surface responds to the composition of the lubricant, or how susceptible the surface is to wear, respectively. From a clinical perspective, patients who have undergone TJA may spend weeks under restricted activity [159], and months of rehabilitation before being able to walk with full weight bearing [160]. During the recovery period, it is possible that the PE insert absorbs fluid during periods of little to no weight bearing; the

fluid uptake during this recovery period may be best reproduced in *in vitro* wear testing by pre-soaking PE components prior to wear testing.

Many of the POD tests reviewed here have implemented a load-soak design into their experiments; however, the main difference lies in the duration in which a PE component is exposed to hydration. Some PE components are soaked for a few hours, while others are immersed for months. Currently, there are no standardized specifications that provide guidelines as to how long a PE component should be hydrated; however, suggestions have been made by Brandt et al. [154].

### 2.4.3 The Clinical Relevance of Pin–on–Disc Tests

PE wear is most frequently reported in combination with Archard's wear law [34], as shown in Section 2.2.2, which suggests that PE wear rates are linearly proportional to contact pressure and sliding distance; however, studies by Turell et al. [45] and Korduba et al. [54] attempted to confirm a linear relationship between cross-shear and wear based on a theory by Wang [127], and found that the model did not accurately predict wear for PE and HXPE, respectively. Furthermore, a study by Dressler et al. [55] provided evidence that wear is higher when there is a change in sliding direction, and becomes almost negligible as linear sliding distance increases up to 5mm, contrasting the linear relationship assumed in Archard's wear law. These studies show that the widely accepted methods used to interpret and predict wear rates and wear factors may not be adequate, even though these conventional methods have been standardized [2, 6, 26].

For the purposes of the present thesis, it was deemed necessary to relate POD wear results, which are based on the Archard's law, to clinical data [70, 161-163]. To make such comparisons, the linear penetration rates of total hip and knee replacements were assessed to approximate their *in vivo* wear behaviour [70, 161-163]. The amount of clinical wear (wear and creep) is often presented as a linear penetration rate (wear depth per year, [mm/year or mm/Mc]), whereas POD wear rates are presented as wear or volumetric wear per sliding distance (mg/Mc or $10^{-6}$ $mm^3$/m), or as a wear factor, described as volumetric wear per unit load and sliding distance ($10^{-6}$ $mm^3$/Nm).

Linear penetration rates were calculated from the wear rates or wear factors from 17 of the 21 POD tests reviewed (3 studies did not specify a wear rate or wear factor). For wear rates given in mg/Mc, a PE density of 0.935mg/$mm^3$ was adapted [19]. The linear penetration of PE pins from the POD tests was calculated by dividing the reported PE wear rate [$mm^3$/Mc] by the contact area ($mm^2$), or by multiplying the reported wear factor ($10^{-6}$ $mm^3$/Nm) by the load (N) and sliding distance (mm), divided by the contact area. Clinical penetration rates were converted from mm/year to mm/million cycles (Mc) by approximating patient activity at 2 Mc/year [164].

Plots generated from this analysis revealed that some POD tests generated linear penetration rates that were within the range of clinical penetration rates (Appendix A.4). These POD tests and their corresponding parameters were identified, and an attempt was made to compare these parameters in the hope of determining similarities; however, it was found that very few tests had similar test parameters and conditions, and a comparison was not feasible. For example, lubricant protein concentrations from POD tests reviewed in the present thesis range from 4 - 69 g/L (Appendix A.1), while contact

pressures range from 1.1 - 32 MPa (Appendix A.3). Evidently, wear rates, wear factors, and linear penetration rates alone cannot determine the clinical relevance of a POD test; therefore, a more in-depth analysis of the test parameters and conditions was required in order to explore how current POD practices and procedures influenced wear behaviour.

Based on the information collected (Appendix A.1-3), and from the linear penetration data (Appendix A.4), it can be seen that interpreting and comparing wear results continues to be difficult due to the wide variety and endless combinations of parameters and conditions used by each research group, such as lubricant composition, wear path shape, sliding distance, contact pressure, and sliding velocities. While it can be argued that comparisons can only be made between POD tests with similar parameters and conditions, it appears that very few POD studies are similar enough to make such comparisons. In more general terms, interpreting wear results according to well-documented clinical data and clinical conditions appears to be the most common way to interpret wear results in individual studies. Care should be taken when using this method as some clinical conditions may not or could not be reproduced in the POD test design, such as appropriate lubrication, implant conformity, kinetic, or kinematic conditions. It is recommended that POD wear results include discussions regarding their similarities (or dissimilarities) to *in vivo* conditions, which will help clarify and rank their clinical relevance.

## 2.5 Concluding Remarks

Many of the issues discussed in the present thesis may be addressed by enhancing current standards with more specific values and guidelines. The current ASTM standards [6] were recently revised to reflect developments in research; however, the standard remains quite general in nature. Providing specific values or more defined limits, where appropriate, may lend some structure that is much needed in POD testing, and will aid in "closing the gap" between individual studies; this may facilitate performing comparisons between studies to further establish the fundamentals of POD testing.

# Chapter 3

# Materials and Methods

## 3.1 Introductory Remarks

This chapter is divided into three main sections: SF collection, POD testing, and biochemical analyses. The methods used to obtain OA-SF and PP-SF from patients undergoing primary and revision hip or knee arthroplasty are described in Section 3.2. Section 3.3 describes the POD wear testing procedures, lubricant mixtures, and gravimetric analysis. Additional analyses were also performed to further characterize the effect of the different lubricants on wear. These analyses include surface roughness measurements, microbial susceptibility, and damage assessment via scanning electron microscopy. In Section 3.4, the SF samples obtained in Section 3.2 are analyzed for protein concentration, protein constituent fractions, osmolality, and thermal stability. The same biochemical tests described in Section 3.4 were also performed on samples of the different lubricants used in Section 3.3 in order to compare them with the composition of

the SF samples obtained in Section 3.2. The statistical analysis methods used for the wear tests and biochemical analyses are detailed in Section 3.5.

## 3.2 Synovial Fluid Collection

### 3.2.1 Patient Characteristics

Ethics approval was obtained from the Biomedical Research Ethics Board at the University of Manitoba (approval #B2011:058) prior to conducting this study. Forty SF samples were aspirated from male (n=20) and female (n=20) patients undergoing primary hip (n=10), primary knee (n=10), revision hip (n=10), and revision knee (n=10) arthroplasties (Table 3.1) by four independent surgeons (Drs. Eric Bohm, Colin Burnell, David Hedden, and Thomas Turgeon). The mean patient age was 66.7 years (range: 38-85 years), and the mean BMI was 34.4 $kg/m^2$ (range: 24.8-67.9 $kg/m^2$). All patients underwent primary hip or knee arthroplasties due to osteoarthritis. The main reasons for revision hip and knee arthroplasties include aseptic loosening (n=6), osteolysis (n=5), pain (n=2), instability (n=2), PE wear, implant fracture, acetabular erosion, patellar malposition, and one unknown reason (no chart available). Patients who had previously received injections for pain relief less than one year prior to surgery, or patients with suspected infection or other inflammatory arthritis were excluded from the study.

**Table 3.1: Patient characteristics of synovial fluid samples.**

| Sample ID | Gender | Age | BMI | Side | Joint | Fluid Type |
|---|---|---|---|---|---|---|
| 1 | M | 38 | 31.2 | L | H | OA-SF |
| 2 | M | 74 | 28.4 | L | H | OA-SF |
| 3 | M | 51 | 31.5 | R | H | OA-SF |
| 4 | F | 57 | 35.6 | L | H | OA-SF |
| 5 | F | 72 | 30.9 | L | H | OA-SF |
| 6 | F | 82 | 26.9 | L | H | OA-SF |
| 7 | M | 63 | 25.6 | R | H | OA-SF |
| 8 | M | 84 | n/a | L | H | OA-SF |
| 9 | F | 85 | 42.7 | R | H | OA-SF |
| 10 | F | 56 | 32.0 | L | H | OA-SF |
| 11 | M | 60 | 39.2 | L | H | PP-SF |
| 12 | M | 72 | 31.2 | L | H | PP-SF |
| 13 | M | 81 | 33.2 | L | H | PP-SF |
| 14 | M | 61 | 26.6 | L | H | PP-SF |
| 15 | F | 63 | 27.9 | R | H | PP-SF |
| 16 | F | 69 | 36.5 | L | H | PP-SF |
| 17 | F | 71 | 26.9 | L | H | PP-SF |
| 18 | F | 76 | 28.2 | L | H | PP-SF |
| 19 | F | 78 | 35.2 | L | H | PP-SF |
| 20 | F | 76 | 33.5 | R | H | PP-SF |
| 21 | M | 79 | 32.6 | R | K | OA-SF |
| 22 | M | 72 | 32.6 | L | K | OA-SF |
| 23 | M | 45 | 40.2 | L | K | OA-SF |
| 24 | F | 59 | 67.9 | R | K | OA-SF |
| 25 | M | 62 | 41.8 | L | K | OA-SF |
| 26 | F | 65 | 27.6 | R | K | OA-SF |
| 27 | F | 59 | 58.6 | L | K | OA-SF |
| 28 | M | 76 | n/a | L | K | OA-SF |
| 29 | F | 78 | 36.3 | R | K | OA-SF |
| 30 | M | 61 | 26.0 | L | K | OA-SF |
| 31 | F | 77 | 24.8 | R | K | PP-SF |
| 32 | M | 50 | n/a | L | K | PP-SF |
| 33 | M | 56 | 37.4 | L | K | PP-SF |
| 34 | M | 69 | 55.4 | L | K | PP-SF |
| 35 | M | 82 | 27.3 | R | K | PP-SF |
| 36 | F | 58 | 30.4 | R | K | PP-SF |
| 37 | F | 59 | 28.0 | L | K | PP-SF |
| 38 | M | 56 | 31.8 | L | K | PP-SF |
| 39 | F | 57 | 35.1 | R | K | PP-SF |
| 40 | F | 78 | 32.6 | L | K | PP-SF |

BMI = Body-mass index (kg/m2); M = male patient; F = female patient; L = left; R = right; H = hip; K = knee; OA-SF = osteoarthritic synovial fluid; PP-SF = periprosthetic synovial fluid

## 3.3 Pin-on-Disc Testing

A total of five POD tests were performed using various lubricants in order to evaluate the effect of each lubricant on wear. All tests were performed with ultra-high molecular weight PE pins articulating against cobalt chromium alloy (CoCr) discs. Three tests were designated as validation tests, where the performance and wear rates of the testing equipment were assessed and compared to the same equipment, parameters, and conditions used by DePuy Synthes (Warsaw, IN). Two additional tests were subsequently performed, and utilized four different lubricants to successively illustrate the effect of lubricant composition on wear. All test parameters and conditions implemented remained the same for each test except for the PE material used, and the composition of the lubricants.

### 3.3.1 Test Apparatus

A six station POD apparatus (OrthoPOD™, PD-05-06, Serial #041053, Advanced Mechanical Technology Inc., Watertown, MA) was used for each POD test performed due to its ability to implement multi-directional cross-shear motion and dynamic loading. The OrthoPOD (Figure 3.1) was installed in the Implant Simulator Laboratory located at the Orthopaedic Innovation Centre (OIC).

Each wear station is controlled by a planetary gear that is driven by a single sun gear; as a result, all wear stations undergo the same rotary motion. Vertical loading is applied to each wear station by air pressure on a precision piston.


**Figure 3.1: The AMTI OrthoPOD located in the Implant Simulator Laboratory at the Orthopaedic Innovation Centre.**

The wear stations can be contained within individual acrylic basins, or collectively in one large acrylic basin. The large basin was used for the POD tests in the present thesis to allow any degraded proteins to settle in the basin instead of being confined to the surface of the discs. In order to prevent lubricant evaporation, a second acrylic cover was provided by the manufacturer. This cover also acts as an external protective shield that fits over the wear testing chamber (Figure 3.2). The OrthoPOD was calibrated before each test was performed by following the instructions given in the operating manual.


**Figure 3.2: Overview of typical pin-on-disc test setup (top); wear station components (bottom).**

### 3.3.2 Bearing Materials

Three types of PE materials were used: non-crosslinked conventional PE (CPE; DePuy Synthes, Warsaw, IN), and 2 types of mildly crosslinked UHMWPE. CPE is made from non-crosslinked GUR 1050, and was used to characterize and quantify the substantial effect that material properties have on wear. XLK (DePuy Synthes, Warsaw, IN) is a mildly crosslinked (5Mrad) GUR 1020, and is the material used in the tibial insert of the P.F.C. Sigma® Knee System. Marathon (DePuy Synthes, Warsaw, IN) is made from mildly crosslinked (5Mrad) GUR 1050, and is the PE used in the acetabular insert of the Pinnacle® Acetabular Cup System (DePuy Synthes, Warsaw, IN). Tests 4 and 5 utilized XLK and Marathon, respectively. These materials were machined into flat-tipped PE pins with a diameter of 3/8” (9.525 mm) and a length of 1/2” inch (12.7 mm). Generally, PE inserts for the aforementioned total knee and hip replacement systems are gas-plasma sterilized; however, the PE pins that were provided did not undergo any sterilization processes.

All pins were pre-soaked in distilled water (DW; Cat #CA11006-294, VWR, Mississauga, ON) for at least two weeks at either room temperature or at 37°C prior to testing in order to reach a steady-state absorption [17].

Six discs were manufactured from implant grade CoCr (ASTM F1537 or F75), and were 1-1/2” (38.1 mm) in diameter and 1/2" (12.7 mm) in height. The discs were polished to an average roughness (Ra) $\leq$ 0.01 μm, which was verified using a contact profilometer (Surfcom 2900SD2, Carl Zeiss Industrial Metrology LLC, Oberkochen, Germany) just prior to testing.

### 3.3.3 Test Parameters and Conditions

A waveform developed and used by DePuy Synthes was programmed to apply dynamic loading at a maximum load of 330 N (maximum contact pressure of 4.63 MPa), and a 10 mm x 10 mm square wear path conducted at a frequency of 1.6 Hz (Figure 3.3. This waveform was uploaded into the AMTI POD software program (Version 3034, Advanced Medical Technology Inc., Watertown, MA).

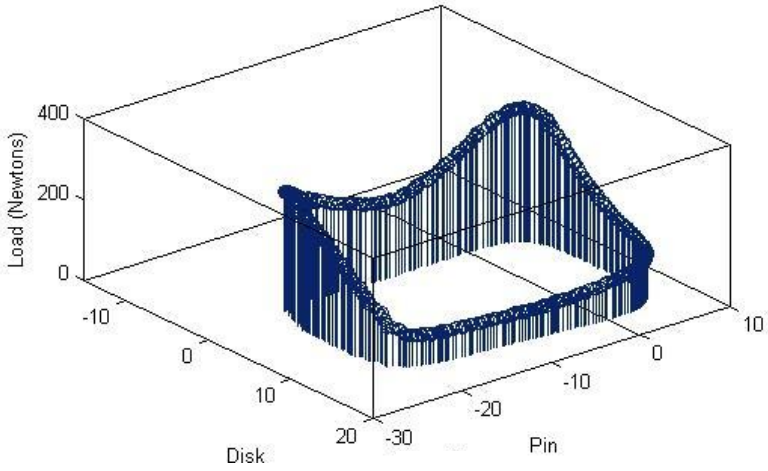


**Figure 3.3: 3D plot showing load relative to pin and disc position (image courtesy of DePuy Orthopaedics).**

Each wear test was made up of intervals consisting of 330 000 cycles (0.33 Mc). The validation tests (Tests 1-3) were divided into 6 x 0.33 Mc intervals for a total test duration of 1.98 Mc. Tests 4 and 5 were divided into 4 lubricant subtests, where the same lubricant composition was used for a set number of intervals. The first lubricant subtest consisted of 6 x 0.33 Mc intervals (1.98 Mc) in order to achieve a steady wear rate; the remaining three lubricant subtests consisted of 3 x 0.33 Mc intervals (0.99 Mc) to

successively show the effect that each lubricant had on wear. In total, tests 4 and 5 were run for approximately 5 Mc each (Table 3.2).

The software was programmed to collect data at a rate of 200 data sets/second for a duration of 2 seconds. Data collection occurred 10 times throughout the interval for each wear station after every 33 000 cycles.

All tests were performed at 37 ± 0.3°C using a temperature control unit (ThermoCube 200, Solid State Cooling Systems, Wappingers Falls, NY).

**Table 3.2: Summary of pin-on-disc tests performed.**

| Test No. | Pin | Disc | Lubricant Composition | Protein [g/L] | Duration [Mc] |
|---|---|---|---|---|---|
| 1 | CPE | CoCr | BCS+DW+EDTA+SA | 54 | 2 |
| 2 | XLK | CoCr | BCS+DW+EDTA+SA | 54 | 2 |
| 3 | Marathon | CoCr | BCS+DW+EDTA+SA | 54 | 2 |
| 4 | XLK | CoCr | ACS+PBS+AA | 34 | 2 |
| | | | ACS+PBS+HA+AA | | 1 |
| | | | ACS+PBS+2HA+AA | | 1 |
| | | | ACS+DW+AA | | 1 |
| 5 | Marathon | CoCr | ACS+PBS+AA | 34 | 2 |
| | | | ACS+PBS+HA+AA | | 1 |
| | | | ACS+PBS+2HA+AA | | 1 |
| | | | ACS+DW+AA | | 1 |

CPE = conventional non-crosslinked polyethylene; XLK = mildly crosslinked GUR 1020; Marathon = mildly crosslinked GUR 1050; CoCr = cobalt chromium alloy; BCS = bovine calf serum; ACS = non-iron supplemented alpha calf serum; DW = distilled water; PBS = phosphate buffered saline solution; EDTA = Ethylenediaminetetraacetic acid; HA = hyaluronic acid at 1.5g/L; 2HA = hyaluronic acid at 3g/L; SA = sodium azide; AA = antibiotic/antimycotic; Mc = million cycles

### 3.3.4 Test Setup and Installation

All PE pins were pre-soaked in DW for a minimum of 2 weeks before testing to allow the pins to become fully saturated. In the validation tests (Tests 1-3), the PE pins were

soaked for a minimum of 30 days at room temperature, and left undisturbed. PE pins used in the lubricant investigation tests (Tests 4 and 5), the PE pins were soaked in DW for a minimum of 2 weeks at 37°C; a stirring rod was used to create some agitation in order to increase the amount of absorption in a short period of time [154]. For each test, a total of 9 pins were randomly selected, weighed, and assigned an identification number. Six pins were assigned to each wear station in the OrthoPOD, while three pins were designated as "soak pins" to account for fluid absorption during testing. Just prior to testing, the "soak" pins were placed into a 250 mL glass beaker containing approximately 150 mL of lubricant. The pins were weighed down using a jar lid to ensure full submersion. To prevent evaporation during testing, the beaker was covered with a sheet of Parafilm® (Cat # 470152-246, VWR, Mississauga, ON). The soak station was then placed on the chassis of the OrthoPOD, which supplied constant heat at 37 ± 0.3°C via a temperature control unit (ThermoCube 200, Solid State Cooling Systems, Wappingers Falls, NY).

The pins and discs were mounted into the wear stations at the same orientation throughout each test. To facilitate this, the bearing materials were each marked with an etched reference line. The pins and discs remained with the same wear station throughout each test.

The lubricant used for each test was replaced with new, unused lubricant after every 0.33 Mc interval in order to limit the effect of protein degradation on wear. Lubricant levels were monitored daily for evaporation, but no significant change was observed during each test. As a result, the lubricant did not require any kind of replenishment.

Upon the completion of a wear test, the discs were sent to DePuy Synthes to be re-polished to a Ra ≤ 0.01 μm. New, unworn pins and discs were then installed for the next test.

### 3.3.5 Lubricant Mixtures

All lubricants used in the present thesis were prepared according to ASTM F732 [6] just prior to the wear tests, and consisted of either triple 0.1 μm sterile filtered bovine calf serum (BCS; Lot #AWA92916; Cat #SH30073, HyClone®, Logan, UT) or non-iron supplemented alpha calf serum (ACS; Lot #AVC63364, Cat #SH30212, HyClone®, Logan, UT).

With recommendations made by DePuy Synthes, a solution consisting of distilled water (DW; Cat #CA11006-294, VWR, Mississauga, ON), ethylenediaminetetraacetic acid (EDTA; Cat #CA97061-022, VWR, Mississauga, ON), and 0.2% wt. sodium azide (SA; Cat #CASX0299-1, VWR, Mississauga, ON) was mixed, and was used to partially dilute BCS (Hyclone, Logan, UT) to a protein concentration of 65 g/L (90% BCS). DW was then used to dilute the BCS further to a protein concentration of 54 g/L (75% BCS). Initial protein concentrations for all calf sera were provided by the supplier in a certificate of analysis (Table 3.3).

**Table 3.3: Composition of the undiluted calf sera used in each wear test.**

| | BCS | ACS |
|---|---|---|
| Lot # | AWA92916 | AVC63364 |
| Total Protein (g/L) | 72 | 38 |
| Albumin (%) | 44 | 67.20 |
| α1-globulin (%) | 5.57 | 7.24 |
| α2-globulin (%) | 9.59 | 4.25 |
| β-globulin (%) | 16.41 | 18.85 |
| γ-globulin (%) | 24.43 | 2.45 |
| Osmolality (mmol/kg) | 298 | 286 |
| pH | 7.45 | 7.65 |

Following the validation tests, different lubricants were used to evaluate their effect on wear (Table 3.2). Non-iron supplemented alpha calf serum (ACS; Hyclone, Logan, UT) was diluted to a protein concentration of 34 g/L (89% ACS), as per Brandt et al. [20], using either DW or phosphate buffered saline solution (PBS; Cat #72060-034, VWR, Mississauga, ON). An antibiotic/antimycotic (AA) solution (Gibco® Antibiotic/Antimicotic 100X, Cat # 15240-062, Life Technologies, Burlington, ON) containing penicillin, streptomycin, and Fungizone® (amphotericin B) was added in place of SA to prevent microbial growth. 5 mL of AA was added to every 500 mL of ACS lubricant just prior to starting the POD test to maintain efficacy. Some of the lubricants contained HA (Lot #024055, Part #80081, Lifecore Biomedical Inc., MW = 1.56 x $10^6$ Daltons), which was added at a concentration of 1.5 g/L (HA Lubricant) or 3 g/L (2HA Lubricant) (Table 3.4). The HA was dissolved in the lubricant by stirring for 8 hours at 37°C, similar to the methods used by Desjardins et al. [22].

**Table 3.4: Summary and nomenclature of lubricants used in POD tests.**

| Test No. | Lubricant | Serum | Dilutive Media | Protein [g/L] | HA [g/L] | Microbial Inhibitor |
|---|---|---|---|---|---|---|
| 1, 2, 3 | BCS Lubricant *(BCS+DW+EDTA+SA)* | BCS | DW | 54 | - | SA |
| 4,5 | PBS Lubricant *(ACS+PBS+AA)* | ACS | PBS | 34 | - | AA |
| 4, 5 | HA Lubricant *(ACS+PBS+HA+AA)* | ACS | PBS | 34 | 1.5 | AA |
| 4, 5 | 2HA Lubricant *(ACS+PBS+2HA+AA)* | ACS | PBS | 34 | 3 | AA |
| 4, 5 | DW Lubricant *(ACS+DW+AA)* | ACS | DW | 34 | - | AA |

### 3.3.6 Gravimetric Analysis

After each interval, and at the end of each test, standard protocols were used to clean and weigh each pin (Appendix B). The cleaning protocol involved ultrasonic cleaning (Ultrasonic Cleaner, Serial #1013A036X, VWR, Mississauga, ON), cleaning with critical-cleaning liquid detergent (Liquinox™, Alconox, Jersey City, NJ), cleaning with alcohol (70% v/v reagent alcohol, Cat #2546.70-1, Ricca Chemical Company, Arlington, TX), and desiccation (Desi-Vac Container, Model #3164, VWR, Mississauga, ON) for 30 minutes.

A microbalance (XP205, Mettler Toledo, Mississauga, ON) with a resolution of 0.01 mg was used to assess the weight loss of each pin. The precision and accuracy of the microbalance was established by measuring a 10g and 100g reference weight (ASTM class 1, Mettler Toledo, Mississauga, ON) five times every 15 minutes over a period of three consecutive days. Before each measurement, the microbalance was internally and

externally calibrated to the specified weight according to the manufacturer's instructions. The weight, temperature, relative humidity, mean, and standard deviation were recorded (Table 3.5).

**Table 3.5: Repeated measurements of the 10 g and 100 g reference weights to establish the precision of the microbalance.**

| Day | Measurement | 10g | 100g | °C | %r.H. |
|---|---|---|---|---|---|
| 1 | 1 | 9.99999 | 99.99995 | 25.5 | 21.0 |
| | 2 | 10.00000 | 99.99994 | 25.5 | 21.0 |
| | 3 | 9.99999 | 99.99995 | 25.5 | 21.0 |
| | 4 | 10.00001 | 99.99994 | 25.5 | 21.0 |
| | 5 | 9.99999 | 99.99994 | 25.5 | 21.0 |
| 2 | 1 | 10.00000 | 100.00001 | 26.5 | 28.5 |
| | 2 | 9.99999 | 100.00001 | 26.5 | 28.5 |
| | 3 | 9.99999 | 100.00000 | 26.0 | 28.0 |
| | 4 | 9.99999 | 100.00000 | 26.0 | 29.0 |
| | 5 | 9.99999 | 100.00000 | 26.0 | 29.0 |
| 3 | 1 | 9.99999 | 99.99991 | 25.5 | 18.5 |
| | 2 | 9.99999 | 99.99991 | 25.5 | 18.5 |
| | 3 | 9.99998 | 99.99991 | 25.5 | 18.5 |
| | 4 | 9.99998 | 99.99991 | 25.5 | 19.5 |
| | 5 | 9.99998 | 99.99992 | 25.5 | 18.5 |
| | **AVERAGE** | **9.99999** | **99.99995** | **25.7** | **22.8** |
| | **SD** | **0.00001** | **0.00004** | **0.4** | **4.4** |
| | **MIN** | **9.99998** | **99.99991** | **25.5** | **18.5** |
| | **MAX** | **10.00001** | **100.00001** | **26.5** | **29.0** |
| | **RANGE** | **0.00003** | **0.00010** | **1.0** | **10.5** |
| | **Range (mg)** | **0.03** | **0.10** | **-** | **-** |

Before each weight assessment session, the microbalance was internally calibrated to ensure accuracy and precision. 10 g, 100 g and 200 g reference weights (ASTM class 1, Mettler Toledo, Mississauga, ON) were used to verify the calibration.

Three measurements were recorded for each pin. The average of these measurements was used to calculate total wear, which takes material wear and fluid absorption into consideration [165].

### 3.3.7 Surface Roughness Measurements

Surface roughness measurements were performed on each CoCr disc according to ISO 4287 [166] using a contact profilometer (Surfcom 2900SD2, Carl Zeiss Metrology LLC, Oberkochen, Germany). Just prior to performing the measurements, the profilometer was calibrated using a depth specimen plate (Model #E-MC-S57A, Carl Zeiss Metrology LLC, Oberkochen, Germany).

A diamond stylus with a tip radius of 2 µm was used to trace the surface of the CoCr discs at a resolution of 0.1 nm and a maximum force of 0.75 mN. The stylus traced a straight path of 20 mm across each disc at a speed of 60 µm/s. The data was processed with a least square straight tilt correction and a cutoff wavelength of 0.08 mm. Six measurements were acquired for each disc (Figure 3.4). Measurement parameters (Ra, Rz, and $Rp_{max}$; Table 3.6) were obtained before and after each test, or before and after each lubricant interval.

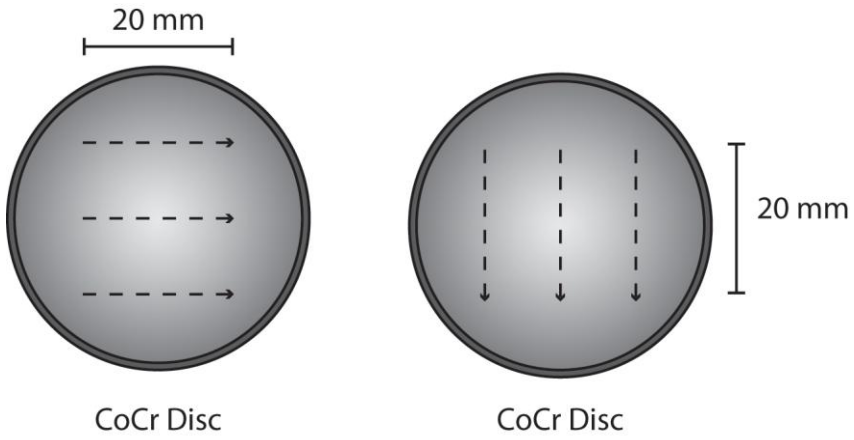


**Figure 3.4: Schematic of roughness measurement locations on surface of CoCr discs (top view).**

**Table 3.6: Description of roughness parameters.**

| Abbreviation | Parameter | Description |
|---|---|---|
| Ra [µm] | Average roughness | Average roughness of the sampling length. |
| Rz [µm] | Ten-point height | The difference in height between the average of the five highest peaks and the five lowest valleys in the sampling length. |
| $Rp_{max}$ [µm] | Maximum height of peaks | Maximum peak height in the sampling length. |

### 3.3.8 Scanning Electron Microscopy

A scanning electron microscope (JSM-6610LV, JEOL Inc., Peabody, MA) was used to capture the surface characteristics of the unworn and worn PE pins. The PE pins were either carbon-coated or gold sputter-coated with a 52nm (520 Å) thick layer. A secondary electron imaging mode was used at an accelerating voltage of 5 kV. Working distances ranged from 9-10mm for x40 and x2000 magnifications.

### 3.3.9 Melt-Annealing

It is often difficult to assess the wear of PE with ultra-low wear due to plastic deformation, especially on burnished surfaces. Re-melting experiments conducted by Muratoglu et al. [167] have shown that the original machining marks on unworn PE surfaces were restored on highly cross-linked explants. In order to confirm that surface damage was indeed due to wear and not creep [168], worn XLK and Marathon pins used in Tests 4 and 5 were re-melted in a convection oven. The oven was then preheated to 80°C; the temperature was maintained for 10 minutes. For every 2 minute increment, the temperature was increased by 5°C until the oven temperature reached 145°C. At this temperature, the PE pins began to turn translucent. When the entire pin was translucent, the temperature was maintained for an additional 5 minutes, after which the oven was turned off and the PE pins were left to cool slowly in the oven overnight.

### 3.3.10 Microbial Contamination

Unworn and worn lubricant samples were collected at the beginning and end of each validation test, and at the beginning and end of each lubricant subtest in tests 4 and 5. The samples were then labeled with the lubricant composition, the test number, the interval, and the date it had been collected. The samples were frozen at −21°C.

Frozen lubricant samples were then sent to the Clinical Microbiology Laboratory at the Health Sciences Centre in Winnipeg. The samples were frozen at −80°C until the day of processing. Just prior to processing, the samples were thawed at room temperature. The samples were then refrozen after processing.

A volume of 300 µL of each unworn and worn lubricant sample was thoroughly mixed and spread over a Tryptone Soya Agar plates containing 5% sheep blood (Cat #MP2012, Oxoid, Nepean, ON). The plates were incubated at 35°C in ambient air overnight, and then assessed for growth. If no growth was detected, the plates were re-incubated for an additional 24 hours. Following this period, the plates were assessed again for growth and were reported as "no growth detected". If ample growth was detected on the plate, the sample was re-plated using volumes of 100 µL and 10 µL.

Each organism grown was identified using the Microbiology Identification Methods used at the Health Sciences Centre. Other types of growth media may have been used as necessary. These media were manufactured in the Clinical Microbiology laboratory at the Health Sciences Centre. Antimicrobial susceptibility testing (Vitek® 2, bioMérieux Canada Inc., St. Laurent, QC) was performed and reported where appropriate.

## 3.4 Biochemical Analyses

In order to ensure analysis of precipitate-free SF samples, aliquots of each SF sample were pipetted into 1.5 mL microtubes and spun down in a centrifuge (Centrifuge 5430 R, Eppendorf, Hamburg, Germany) at 20 000 rcf (13 722 rpm) for 20 minutes at 4°C. Any precipitates within the samples formed a pellet following centrifuging; the liquid lying above the pellet (the supernatant) was pipetted into microtubes for further analysis or storage. This processing method was also used to separate any degraded proteins or precipitates from the lubricant samples obtained before and after each POD test.

### 3.4.1 Protein Concentration and Degradation

A Pierce bicinchoninic acid (BCA) protein assay (BCA™ protein assay kit, Cat #PI23225, Pierce Chemicals, Rockford, IL) was used to determine the protein concentration of each SF sample, and the protein concentration and degradation of the lubricant samples collected before and after each POD test, similar to the method used by Brandt et al. [20]. BCA protein assays depend on the biuret reaction, where $Cu^{2+}$ is reduced to $Cu^{1+}$ when it binds to proteins present in an alkaline solution (the biuret reaction). BCA then reacts with $Cu^{1+}$ to form an intense reaction that turns the solution purple. Protein concentration is determined by measuring the absorbance of the purple solution (Figure 3.5); absorbance increases with increasing protein concentration.

Each sample was diluted 100-times with phosphate buffer saline (PBS) solution (10 μL sample + 990 μL PBS). Twelve-25 μL aliquots of each diluted sample were pipetted into the wells of a microplate (Cat #66025-626, VWR, Mississauga, ON). 200 μL aliquots of the BCA working reagent were subsequently added to each well in order to initiate the biuret reaction. Following incubation at 37 ± 2°C for 30 minutes, the absorbance of each sample was measured using a microplate photometer (SpectraMax Plus 384, Molecular Devices; Sunnyvale, CA) set to a wavelength of 562 nm; twelve measurements were obtained for each sample, which were converted to protein concentrations (in g/L) based on a standard curve produced from bovine serum albumin diluted with PBS at concentrations of 1, 0.75, 0.50, 0.375, 0.25, 0.125, and 0.0625 g/L.


**Figure 3.5 BCA assay microplate showing various shades of purple for different protein concentrations. Note the dark-to-light shades of purple on the top row signifying the decreasing protein concentrations of the bovine serum albumin standards. The middle five rows show samples at similar protein concentrations, followed by the last row showing a sample with higher concentrations.**

Protein degradation in the wear testing lubricants was calculated using the equation 3.1:

$$\%\, Degradation = 1 - \frac{P_{worn}}{P_{unworn}} \times 100 \quad (E.3.1)$$

where $P_{worn}$ is the protein concentration of the worn lubricant, and $P_{unworn}$ is the protein concentration of the unworn lubricant.

## 3.4.2 Electrophoresis

An electrophoretic analysis was necessary in order to determine the different types of proteins present in each SF and lubricant sample, and the distribution of each type of protein (constituent fractions).

An agarose gel electrophoresis procedure (pH = 8.5; Hydragel 30 β1-β2, Sebia Norcross, GA), similar to the procedure used by Brandt et al.[20], was used to determine the protein constituent fractions of each SF sample, and each lubricant sample collected before and after each POD test. Each SF sample was treated with a 71 g/L hyaluronidase-PBS solution (Hyalurono-glucosaminidase, H 3506, Sigma-Aldrich, St.Louis, MO) due to the viscous nature of SF; 5μL of the hyaluronidase solution were then added to 100 μL of each SF sample. A hyaluronidase sample was run separately as a control specimen. An agarose gel electrophoresis system (Sebia Hydrasys®, Sebia, Norcross, GA) was used to analyze the SF samples on an alkaline buffered agar-based gel, which was stained with Coomassie blue in order to distinguish the various bands that indicate the protein content. A software program (Sebia, Phoresis Release 4.9.0, Norcross, GA) was used to measure the relative intensities of each protein band.

### 3.4.3 Osmolality

Osmolality measurements were performed in triplicate on 10 μL of each SF sample, and each lubricant sample collected prior to POD testing. The osmometer used in the present study (Vapro® 5520, Wescor, Logan, UT) determines the difference between the freezing point of a pure solvent and the freezing point of a mixed solution containing that solvent. This difference is known to be directly proportional to the molar concentration of the solution, and is also a direct measure of ionic strength which, consequently, influences the thermal stability of SF [108, 109, 115, 116]. A 290 mmol/kg (OPTI-Mole™, Wescor,

Logan, UT) reference sample was used to calibrate the instrument prior to testing, and throughout the procedure to ensure accurate measurements.

### 3.4.4 pH Measurements

The pH level of all SF samples obtained in Section 3.2 were not measured due to low SF volumes; however, the pH level of all wear testing lubricants prepared in Section 3.3.5 was monitored with a pH meter (SevenEasy S20, Mettler Toledo, Columbus, OH), which was calibrated using three reference solutions with a pH of 4.01, 7.00, and 10.01. Following each pH measurement, the electrode tip was rinsed with DW. The electrode remained in DW until the next measurement. The pH of all lubricants was adjusted to 7.63 ± 0.1 in order to maintain clinically relevant levels [92] and to ensure pH compatibility between the dilutive media and the calf sera. Sodium hydroxide pellets (Cat #CASX0590-3, VWR, Mississauga, ON) were added gradually to raise the pH level to 7.63 ± 0.1 when necessary.

### 3.4.5 Thermal Stability

Differential scanning calorimetry (DSC) (MicroCal, GE Healthcare, Northampton, MA, USA) was used to measure the thermal stability of OA and PP SF and all wear testing lubricants using a similar procedure described by Brière et al.[18] and Brandt et al.[20]. Aliquots of the SF and lubricant samples were sent to the Biomolecular Interactions and Conformations Facility in London, Ontario, where they were thawed, vortexed and then diluted with PBS to a total protein concentration of 6 g/L. The samples were degassed by

gentle stirring under vacuum prior to analysis. DSC curves were generated for samples over a range from 10-90°C at a scan rate of 60°C/h. Buffer-buffer reference scans were generated using the same conditions, and were then subtracted from sample endotherms. Non-linear least squares regression analysis was used to process the raw data in Origin 5.0 (MicroCal GE Healthcare, Northampton, MA, USA) by first subtracting a buffer-buffer reference scan; the data was then fitted to a non-two-state transition model. Select SF samples were measured in triplicate, and showed reproducibility; therefore, only single replicates of the other SF samples were completed due to limited sample volume. Thermograms were generated for each SF sample depicting 2 to 3 peaks of transition midpoint temperatures (Figure 3.6). These peaks indicate that the unfolding of the sample is a multi-stage process, and can illustrate how resistant the proteins are to degradation at certain temperatures.

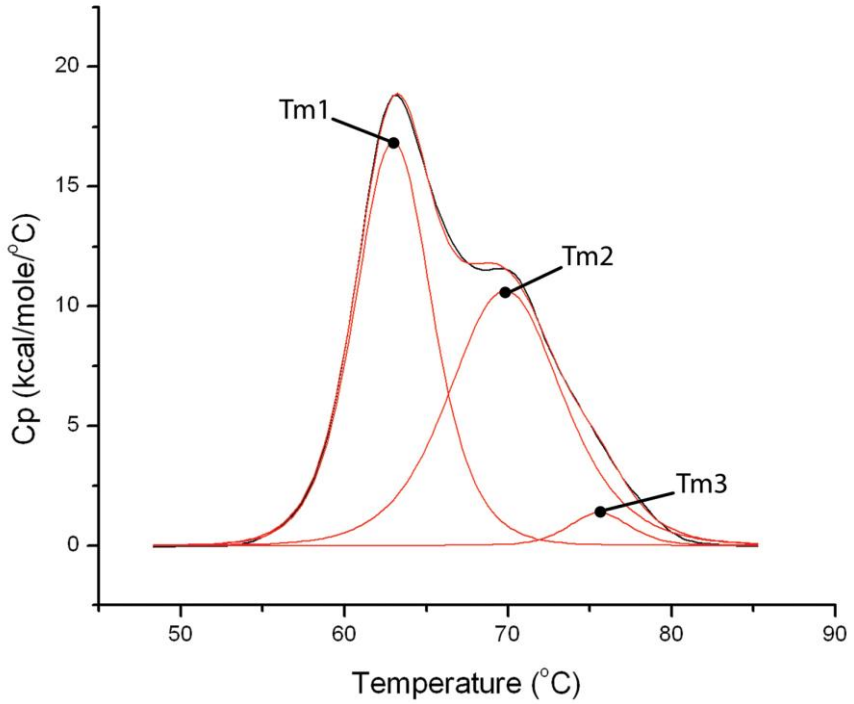


**Figure 3.6: Thermogram of an osteoarthritic synovial fluid sample obtained from the knee joint. Similarly shaped curves were seen for all synovial fluid samples collected. Note that Tm1 occurs at a maximum heat capacity (Cp).**

## 3.5 Statistical Analyses

A statistics software program (SPSS Version 20, SPSS®, Chicago, IL) was used to analyze the data obtained from the wear tests, surface roughness, and biochemical analyses conducted. Prior to conducting any statistical analyses, the Kolmogorov-Smirnoff test was used to assess the distribution of the data sets. A Student's t-test, paired samples t-test was used to analyze data obtained from two measurements of the same sample; however, for repeated measurements obtained from the same sample, a repeated measures GLM (GLM) procedure was used. Two types of post-hoc methods were used to find significant differences between groups: Tukey's test was applied when sample sizes and within-group variances were equal, and Tamhane's T2 test was applied when sample sizes and within-group variances were unequal. For small data sets that were not normally distributed, Mann-Whitney U and Wilcoxon-Rank tests were used in order to compare the medians between groups. The mean and 95% confidence intervals were calculated and reported for all measurements, with the level of significance set at $\alpha = 0.05$.

# Chapter 4

# Results

## 4.1 Introductory Remarks

Chapter 4 consists of three separate sections that report on the clinical study, and the validation and lubrication wear tests. In Section 4.2, the results from the biochemical analyses of the collected 40 synovial fluid samples were presented; comparisons were made between the protein composition, osmolality, and thermal stability of OA-SF and PP-SF.

Section 4.3 presents results from the validation POD tests. Wear rates, surface characterization, and microbial growth were analyzed. In addition, biochemical tests were performed for the BCS lubricant in order to determine its protein composition, degradation, osmolality, and thermal stability.

In Section 4.4, wear rates, surface characterization, and microbial growth results were obtained from the lubrication investigations, which utilized lubricants composed of ACS and various dilutive media. Biochemical analyses were also performed on these

lubricants in order to compare the protein composition, degradation, osmolality, and thermal stability to the synovial fluid collected in Section 4.2, and the BCS lubricant analyzed in Section 4.3.

# 4.2 Synovial Fluid Investigation

## 4.2.1 Introductory Remarks

SF samples were aspirated from patients who were undergoing primary or revision TJA in order to characterize and quantify the biochemical composition and thermal properties of OA-SF and PP-SF.

From a clinical perspective, a previous study [9] had suggested that the synovial capsule, which produces SF, is able to recover and retain its function following primary arthroplasty; by comparing the composition and thermal properties of OA-SF and PP-SF, additional evidence can be provided to support or negate this finding.

From a wear testing perspective, the data collected from this study can provide a foundation for the development of a more clinically relevant wear testing lubricant.

## 4.2.2 Biochemical Analyses

SF volumes obtained from patients varied between 1.5-8 mL. SF samples obtained from hip joints were often lower in volume than SF samples obtained from knee joints. Most samples appeared light yellow in colour, although a few SF samples were light red in

colour due to the presence of blood contamination that could not be removed, even after centrifuging; however, these samples were still included in the analysis.

### 4.2.2.1 Protein Concentration

The protein concentration of each SF sample collected in Section 3.2 was measured using the Pierce BCA assay. The mean total protein concentrations for OA-SF obtained from the hip and knee joints was 32.35 ± 4.25 and 26.61 ± 2.75 g/L, respectively; for PP-SF obtained from the hip and knee joints, the mean total protein concentrations were 33.00 ± 6.09 and 29.56 ± 7.17 g/L, respectively (Figure 4.1; Table 4.1). No significant difference was detected between the total protein concentrations of OA-SF and PP-SF samples for both joints ($p \geq 0.450$); as such, the mean total protein concentration for all SF samples was 30.38 ± 2.44 g/L (Figure 4.1).

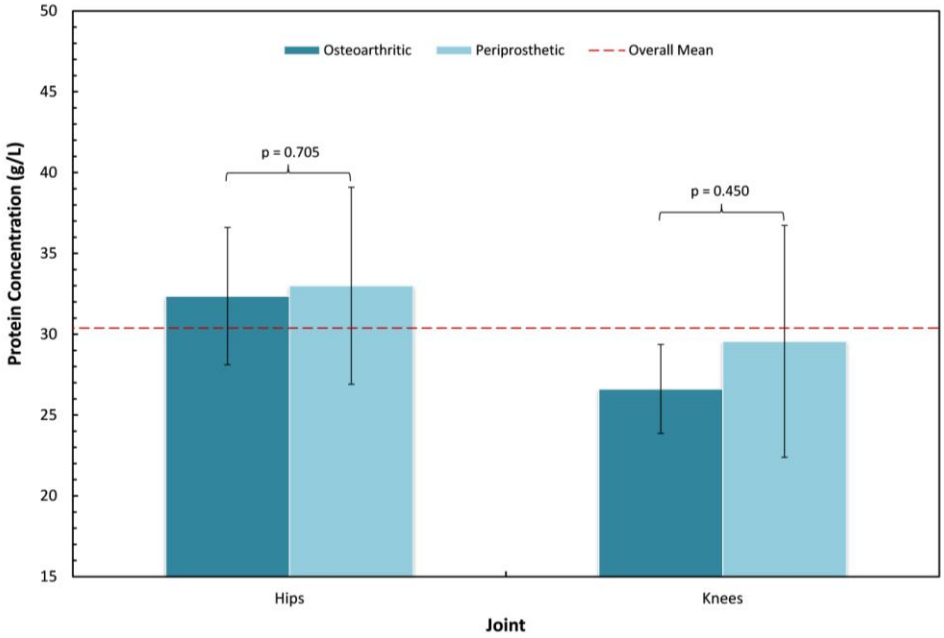


**Figure 4.1: Average total protein concentrations of the osteoarthritic and periprosthetic synovial fluid samples collected from the hip or knee joints of patients 1-40. The dashed line shows the mean protein concentration for all samples with error bars showing 95% confidence intervals. The p-values obtained compare the medians of osteoarthritic and periprosthetic synovial fluid for each joint (Mann-Whitney U).**

### 4.2.2.2 Electrophoresis

Performing electrophoretic analysis on the SF samples facilitated the separation and measurement of albumin, α-1-globulin, α-2-globulin, β-globulin, and γ-globulin fractions for each SF group (Table 4.1; Figure 4.2). Electrophoretic results for all SF samples showed bands of albumin, α-1-globulin, α-2-globulin, β-globulin, and γ-globulin with average fractions of 71.15 ± 1.58%, 2.13 ± 0.11%, 6.04 ± 0.42%, 10.74 ± 1.23%, and 9.95 ± 0.54%, respectively. No significant difference was found between the constituent fractions of OA-SF and PP-SF obtained from the hip joint ($p \geq 0.058$, Mann-Whitney U). For OA-SF and PP-SF obtained from knee joints, no significant difference was detected between α-1-globulin, α-2-globulin, and γ-globulin ($p \geq 0.096$, Mann-Whitney U); however, significant differences were found between albumin ($p = 0.023$, Mann-Whitney U) and β –globulin ($p = 0.003$, Mann-Whitney U).

**Table 4.1: Summary of the protein composition in osteoarthritic and periprosthetic synovial fluid obtained from hip and knee joints.**

| | Osteoarthritic | | Periprosthetic | |
|---|---|---|---|---|
| | **Hips** | **Knees** | **Hips** | **Knees** |
| **Protein** | 32.35 ± 4.25 | 26.61 ± 2.75 | 33.00 ± 6.09 | 29.56 ± 7.17 |
| **Albumin** | 69.67 ± 2.53 | 74.40 ± 1.79 | 69.93 ± 5.36 | 70.59 ± 2.75 |
| **α1-globulin** | 2.00 ± 0.29 | 2.21 ± 0.25 | 2.04 ± 0.14 | 2.28 ± 0.27 |
| **α2-globulin** | 6.97 ± 0.76 | 5.14 ± 0.57 | 6.01 ± 0.94 | 6.02 ± 1.00 |
| **ß-globulin** | 11.26 ± 2.31 | 8.32 ± 0.68 | 12.70 ± 4.49 | 10.68 ± 1.16 |
| **γ-globulin** | 10.10 ± 1.13 | 9.93 ± 1.34 | 9.32 ± 1.07 | 10.43 ± 1.26 |

Average protein concentrations from the present thesis are given in g/L with 95% confidence intervals, unless otherwise stated. Constituent fractions for albumin, α1-globulin, α2-globulin, ß-globulin, and γ-globulin are given as percentages with 95% confidence intervals. References for historical values are denoted by superscripts.

* Standard deviations given in lieu of 95% confidence intervals.

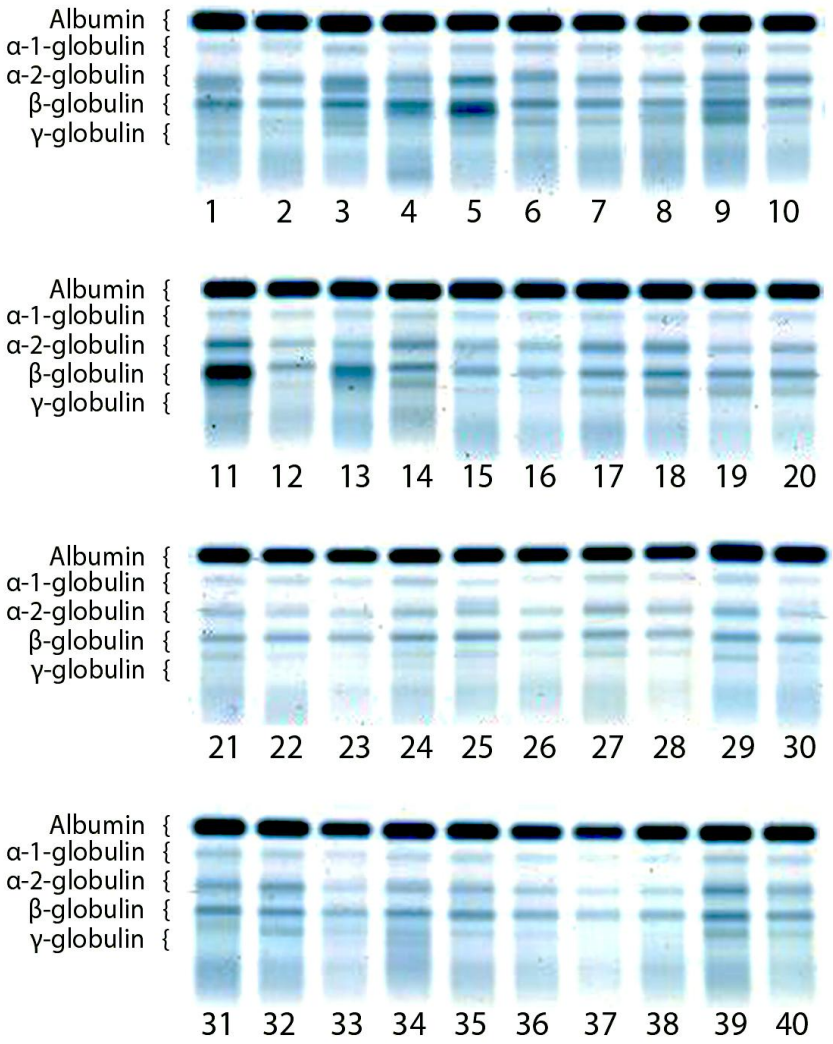


**Figure 4.2: Electrophoretic results for all forty samples showing the characteristic migration of the protein constituents; sample numbers correspond to patient characteristics described in Table 3.1.**

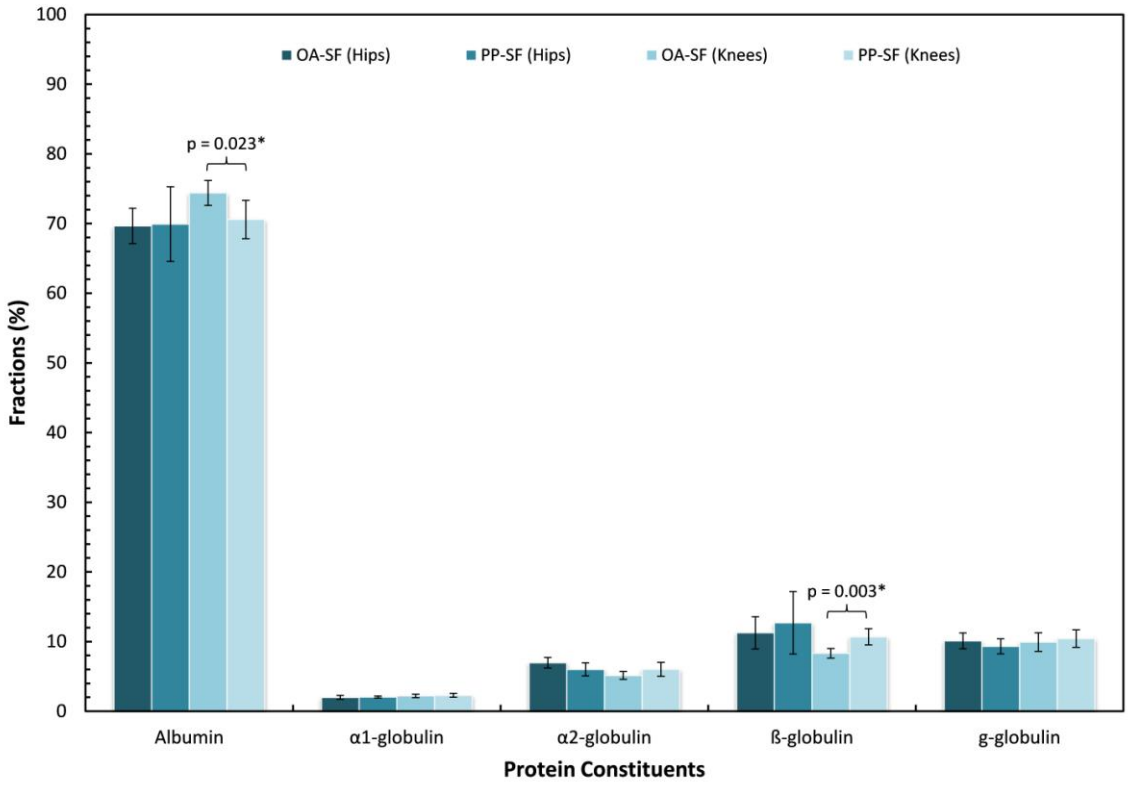


**Figure 4.3: Average protein constituent fractions of osteoarthritic and periprosthetic synovial fluid samples obtained from the hip or knee joints of patients 1-40. Error bars show 95% confidence intervals.**

### 4.2.2.3 Osmolality

Osmolality measurements were used to determine the ionic strength for the SF samples in each SF group (Table 4.2). The mean osmolality for all SF samples was 280.84 ± 4.43 mmol/kg (Figure 4.4); no significant difference was found between the osmolality of OA-SF and PP-SF ($p \geq 0.241$; Mann-Whitney U).

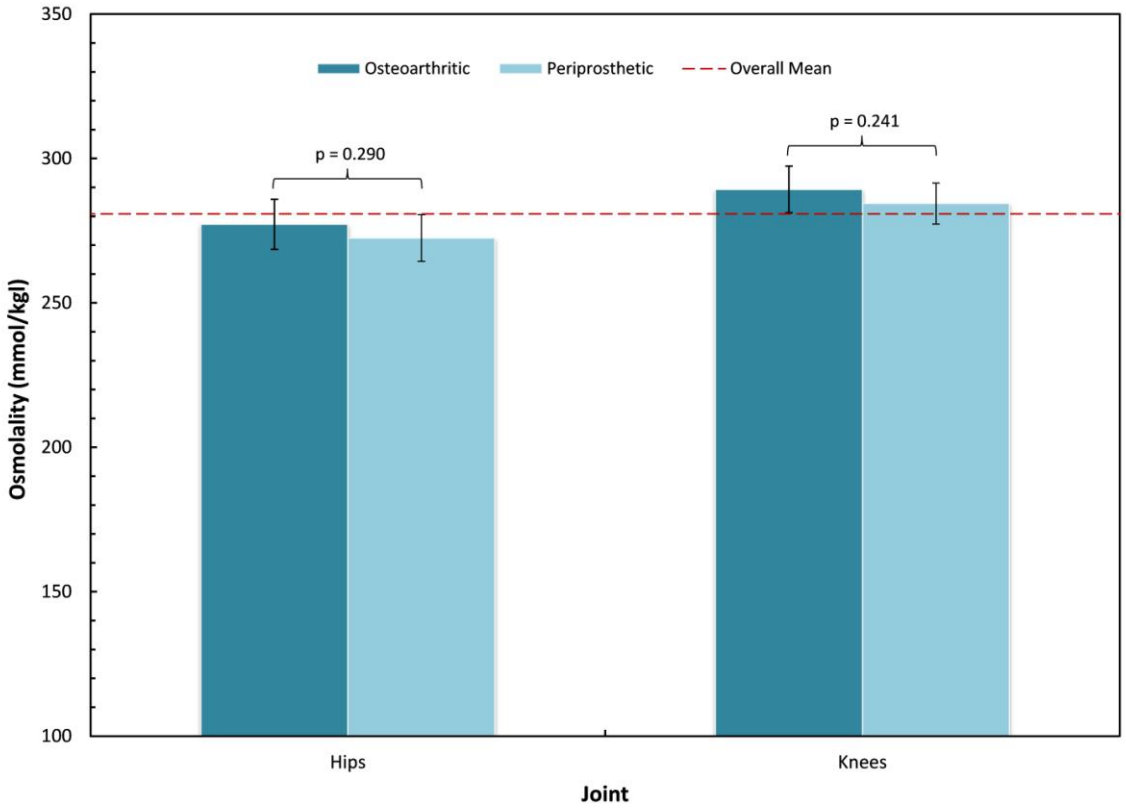


**Figure 4.4: Osmolality of osteoarthritic and periprosthetic synovial fluid samples obtained from the hip or knee joints of patients 1-40. The dashed line shows the mean osmolality for all samples with error bars showing 95% confidence intervals. The p-values obtained compare the medians of osteoarthritic and periprosthetic synovial fluid for each joint (Mann-Whitney U test).**

### 4.2.2.4 Thermal Stability

DSC measurements produced results quantifying the transition midpoint temperatures, enthalpy, and entropy of the SF samples in each SF group (Table 4.2). The mean transition midpoint temperature at the maximum heat capacity (Tm1) was 68.3 ± 1.76°C for all SF samples, and no significant difference was found between OA-SF and PP-SF for both hip and knee joints ($p \geq 0.078$).

**Table 4.2: Summary of thermal properties in osteoarthritic and periprosthetic synovial fluid obtained from hip and knee joints.**

| | Osteoarthritic | | Periprosthetic | |
|---|---|---|---|---|
| | **Hips** | **Knees** | **Hips** | **Knees** |
| **Osmolality** | 277.2 ± 8.68 | 289.3 ± 11.79 | 272.47 ± 8.06 | 284.4 ± 7.07 |
| **Tm1** | 64.32 ± 1.58 | 63.32 ± 0.25 | 63.84 ± 0.91 | 63.81 ± 0.59 |
| **Tm2** | 67.75 ± 1.71 | 67.94 ± 1.35 | 65.70 ± 3.42 | 67.55 ± 2.63 |
| **Tm3** | 74.91 ± 2.47 | 74.37 ± 1.20 | 70.92 ± 2.94 | 73.75 ± 2.09 |
| **Enthalpy, ΔH** | 1662 ± 316 | 1371 ± 187 | 1286 ± 296 | 1497 ± 470 |
| **Entropy, ΔS** | 4.92 ± 0.93 | 4.08 ± 0.56 | 3.81 ± 0.87 | 4.44 ± 1.39 |

Average osmolality, temperature midpoints (Tm1, Tm2, and Tm3), enthalpies (ΔH), and entropies (ΔS) from the present thesis are given in mmol/kg, °C, kJ/mol, and kJ/mol·K, respectively, with 95% confidence intervals, unless otherwise stated. References for historical values are denoted by superscripts.

* Standard deviation given in lieu of 95% confidence intervals.

Enthalpy (ΔH) is typically a measurement of the total energy absorbed by a system based on the area under the DSC curves, and can indicate the thermal stability of the proteins present in the sample. The mean ΔH for all SF samples was 1422.5 ± 152.6 kJ/mol (Table 4.2); no significant difference was detected between the ΔH of OA-SF and PP-SF for both hip and knee joints ($p \geq 0.131$; Figure 4.5).

The mean entropy (ΔS) was found to be 4.22 ± 0.45 kJ/mol·K (Table 3); no statistical difference was found between the ΔS of both OA-SF and PP-SF samples ($p \geq 0.131$; Figure 4.6).

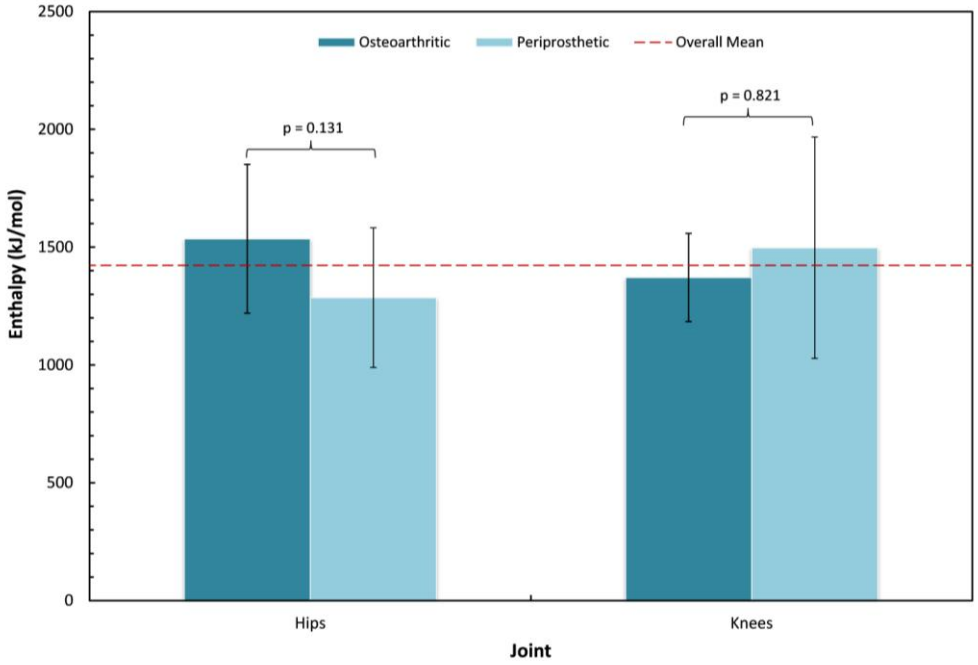


**Figure 4.5: Enthalpy measured for osteoarthritic and periprosthetic synovial fluid samples obtained from the hip or knee joints of patients 1-40. The dashed line shows the mean enthalpy for all samples with error bars showing 95% confidence intervals. The p-values obtained compare the medians of osteoarthritic and periprosthetic synovial fluid for each joint (Mann-Whitney U test).**

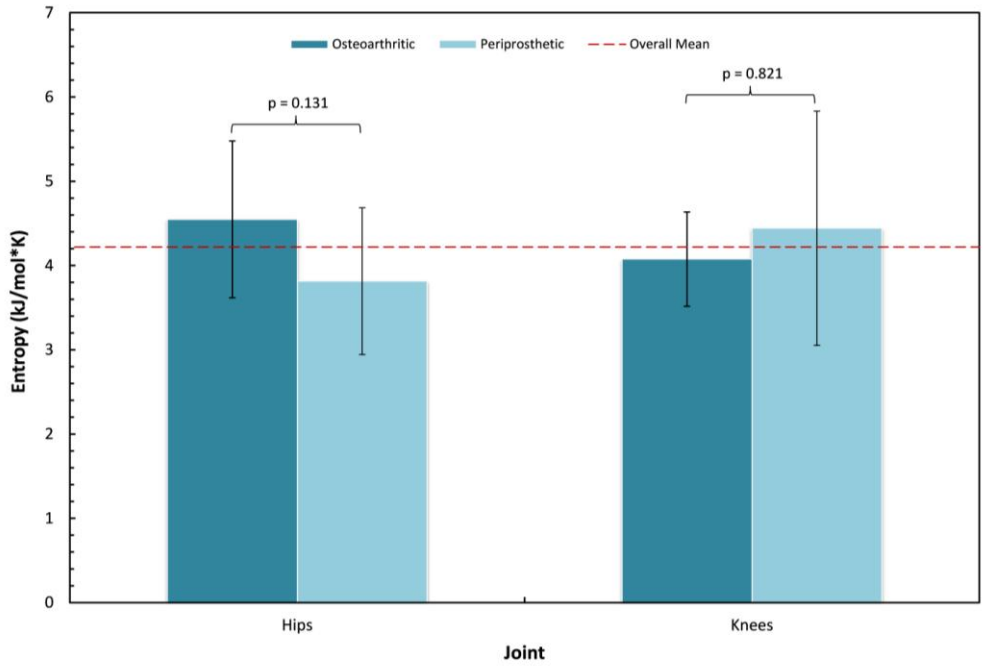


**Figure 4.6: Entropy measured for osteoarthritic and periprosthetic synovial fluid samples obtained from the hip or knee joints of patients 1-40. The dashed line shows the mean enthalpy for all samples with error bars showing 95% confidence intervals. The p-values obtained compare the medians of osteoarthritic and periprosthetic synovial fluid for each joint (Mann-Whitney U test).**

### 4.2.3 Concluding Remarks

Results from the biochemical analyses of OA-SF and PP-SF have shown that the values obtained for protein composition [9, 20, 46, 169], osmolality [20, 170], and thermal stability [20] were comparable to previous studies; in addition, to the author's knowledge, the protein constituent fractions, osmolality, and thermal stability for PP-SF in both hip and knee joints were quantified for the first time [9, 20, 95, 170].

No significant difference was found between OA-SF and PP-SF, suggesting that the synovial capsule is able to maintain its previous function following arthroplasty.

## 4.3 Validation Wear Tests

### 4.3.1 Introductory Remarks

As part of the OrthoPOD commissioning process, validation wear tests were performed in order to verify that the wear rates produced at the OIC were comparable to historical wear rates generated by DePuy Snythes under the same test parameters and conditions. The wear performance of three different PE pins was evaluated. These tests utilized a bovine calf serum-based lubricant that was similar in composition to the lubricants currently used by DePuy Synthes. Soak tests were conducted for Tests 2 and 3, and were necessary in order to account for fluid absorption that occurred during testing. Calculating total wear was performed by adding the average fluid uptake (or loss) to the mass lost by each wear station pin [165]. Surface profilometry and SEM imaging were used to characterize the surface of unworn and worn CoCr discs. Macroscopic and SEM

imaging were performed on new, unworn PE pins and PE pins worn after 1.98 Mc to further characterize the surface before and after wear testing. Lubricant samples were collected just prior to wear testing, and after 0.33 Mc. These samples were sent to the Clinical Microbiology Laboratory in Winnipeg in order to identify the types of organisms growing in the lubricant. Biochemical tests were then conducted on unworn and worn lubricant samples collected at 0 Mc and after 0.33 Mc. Protein degradation and constituent fraction degradation were quantified for unworn and worn lubricant samples. Osmolality and thermal stability measurements were obtained for unworn lubricant samples only.

### 4.3.2 Fluid Absorption

Prior to each validation test, a pre-test soak control was performed where all PE pins were weighed and then soaked, undisturbed, in DW for a minimum of 30 days at room temperature. The pins were weighed again immediately before wear testing. Fluid absorption appeared to be highest in CPE when compared to XLK and Marathon pins; additionally, there were no significant differences in fluid uptake between XLK and Marathon pins ($p = 0.211$, Mann-Whitney U; Figure 4.7).

Soak controls were also implemented during each POD test in order to account for fluid absorption. For each validation test, three pins were designated as “soak pins”, and were submerged in BCS lubricant; after each 0.33 Mc interval, the soak pins were cleaned, desiccated, and weighed. During the first validation test using CPE (Table 3.2),

there was some confusion regarding the procedure for gravimetric assessment of the soak pins; therefore, fluid absorption was only reported for XLK and Marathon.

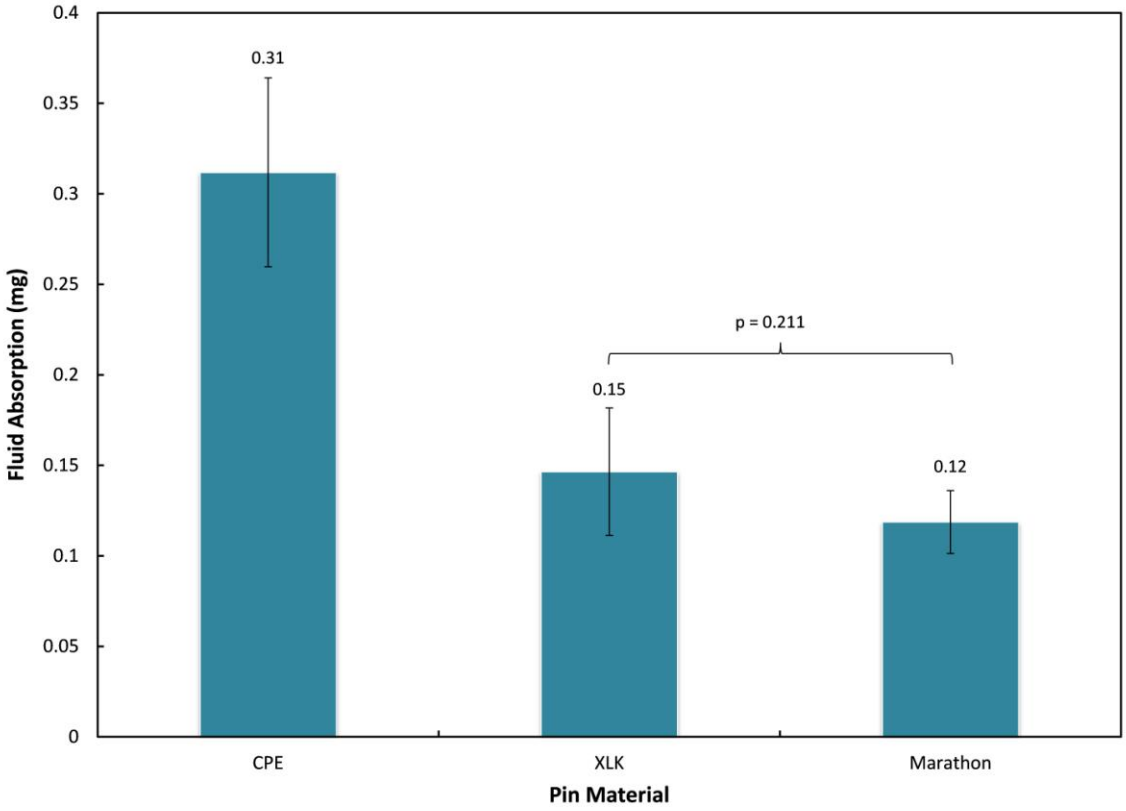


**Figure 4.7: Fluid absorption during pre-test soak control for CPE, XLK, and Marathon pins after a minimum of 30 days of soaking, just prior to testing.**

The mass of each XLK and Marathon soak pin appeared to be unaffected by fluid absorption (Figure 4.8), with mass gains up to 0.09 mg for XLK, and 0.08 mg for Marathon. These minute changes in mass had little to no effect on the net wear of the PE pins, and because of this, it was deemed unnecessary to further report these values for the remaining POD tests.

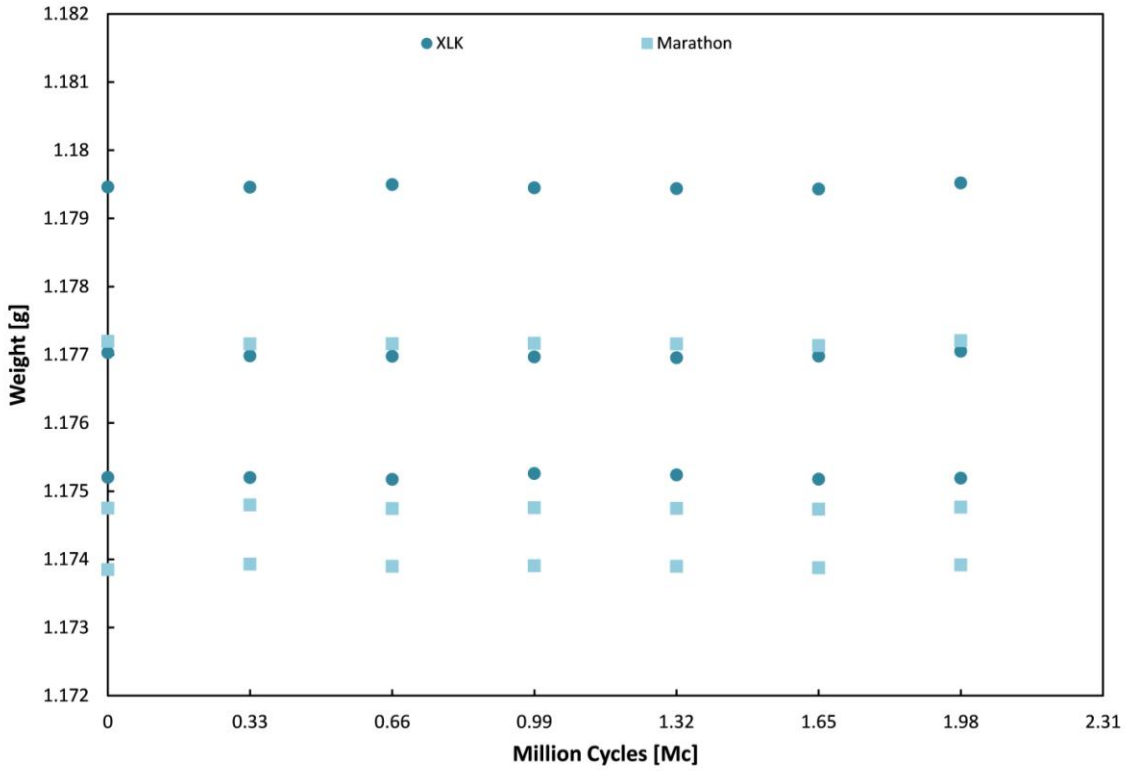


**Figure 4.8: The weight of each XLK and Marathon soak pin measured after every 0.33 Mc interval throughout the validation tests using the BCS lubricant. Note that the weight of each pin remained relatively unchanged throughout testing.**

### 4.3.3 Wear Rates

For each validation test, a set of six PE pins and six CoCr discs were selected and assigned to each wear station on the OrthoPOD; each pin and disc remained with the same wear station for the entire 1.98 Mc test duration. All pins were weighed after every 0.33 Mc interval (Figures 4.9-11). Wear rates for each station were obtained from the slope of the least squares linear-regression lines fitted through the data (Appendix C.1); there was no significant difference in the wear rates produced between each station ($p \geq 0.885$; GLM-Tukey).

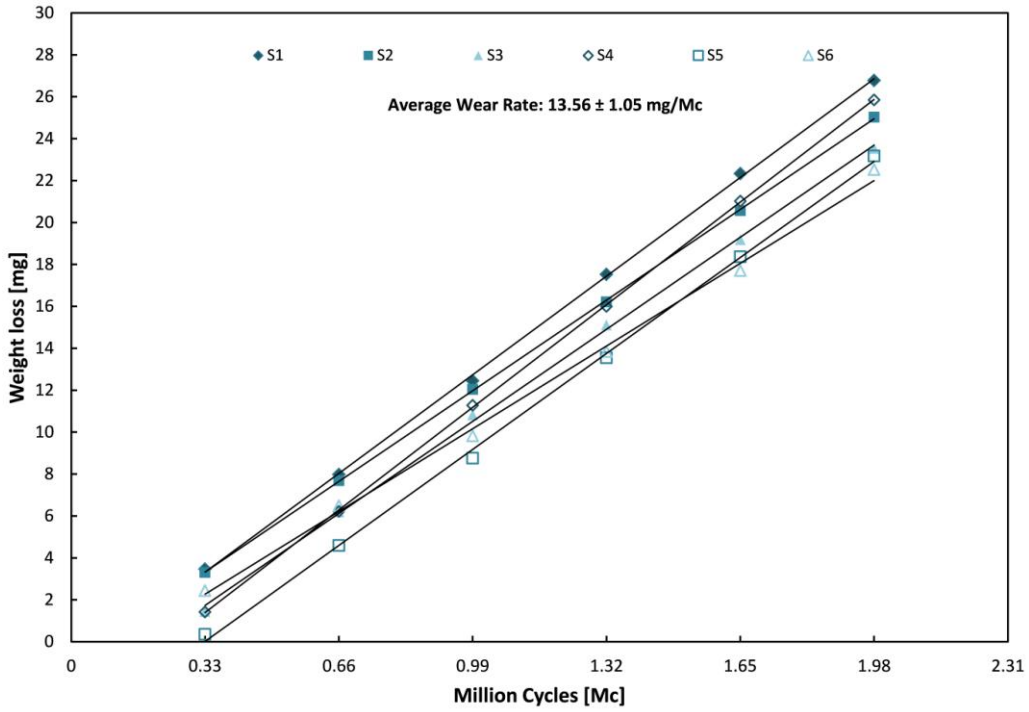


**Figure 4.9: The wear of all six CPE pins over 1.98 Mc with linear regression lines fitted through the data from 0.33 to 1.98 Mc. Fluid absorption was not accounted for in the wear data.**

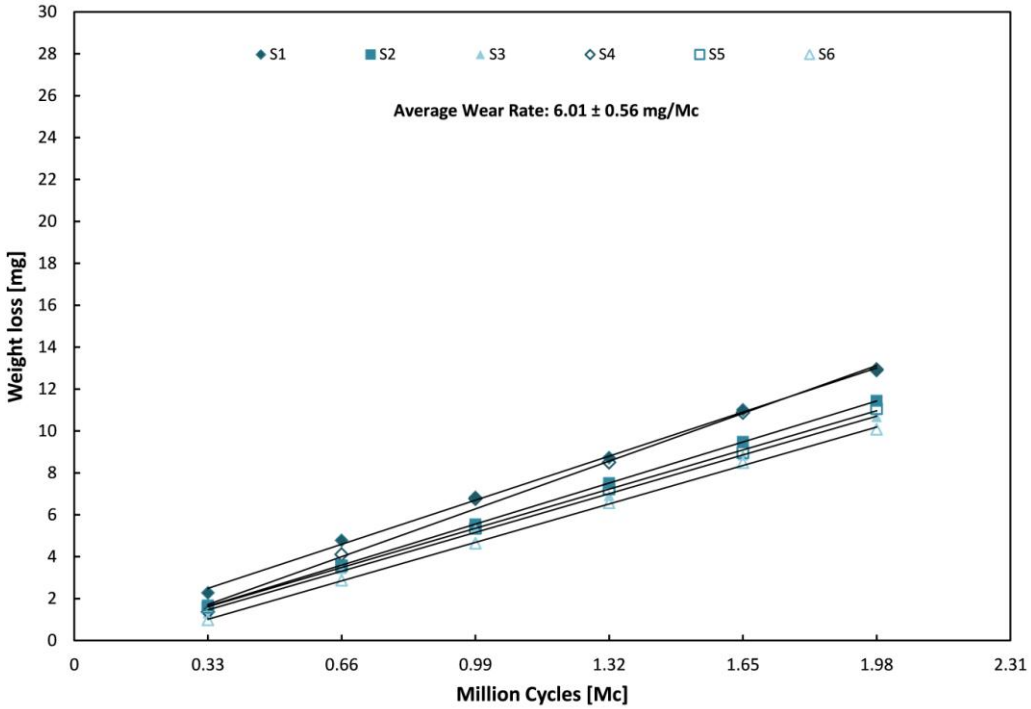


**Figure 4.10: The wear of all six XLK pins over 2Mc with linear regression lines fitted through the data from 0.33 Mc to 1.98 Mc. Fluid absorption was accounted for in the wear data.**

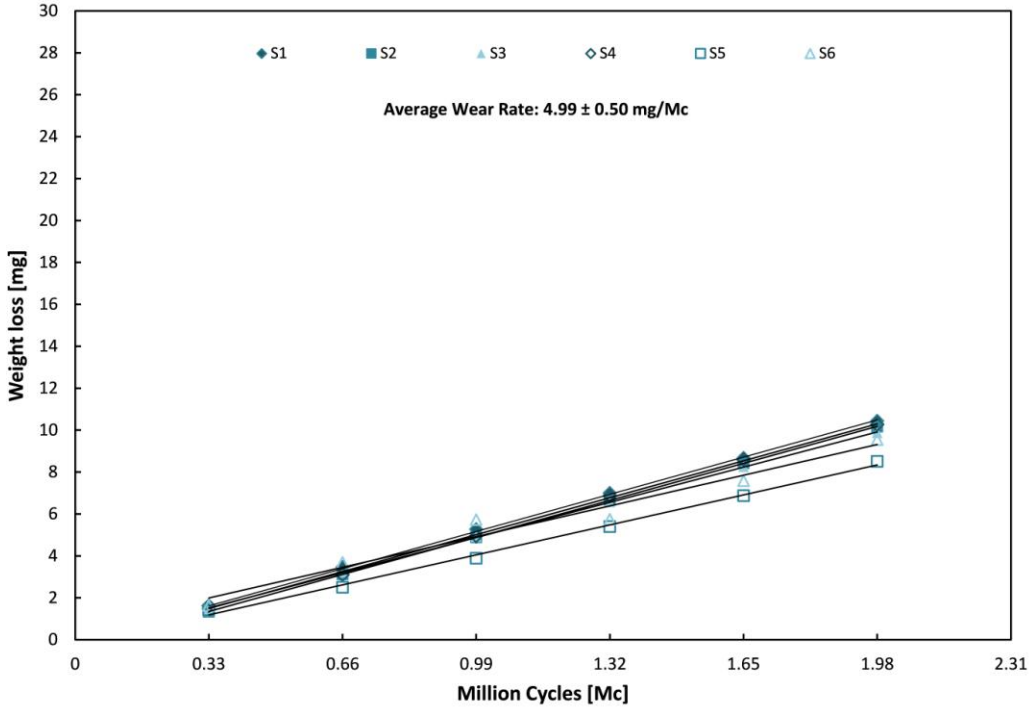


**Figure 4.11: The wear of all six Marathon pins over 2Mc with linear regression lines fitted through the data from 0.33 Mc to 1.98 Mc. Fluid absorption was accounted for in the wear data.**

The total mean wear rate for CPE was 13.56 ± 1.05 mg/Mc, and was over 2-times higher than the total mean wear rates produced by the crosslinked PE pins. Total mean wear rates for XLK and Marathon were 6.01 ± 0.56 mg/Mc and 4.99 ± 0.50 mg/Mc respectively, and were found to be significantly different ($p = 0.004$, Mann-Whitney U; Figure 4.12).

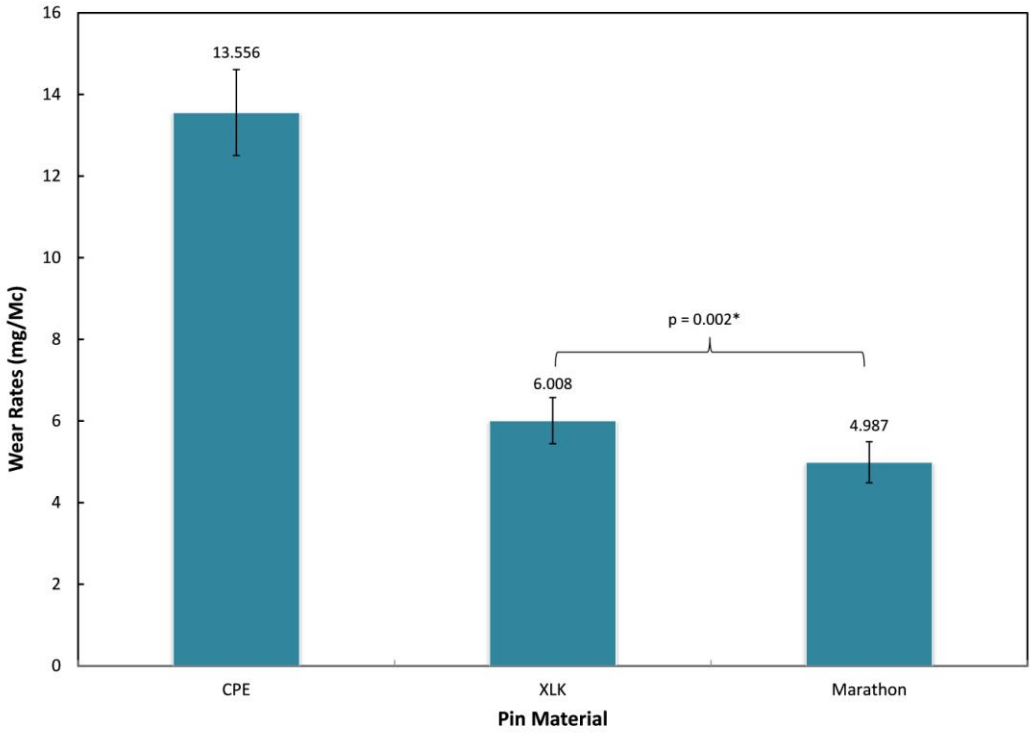


**Figure 4.12: Total mean wear rates for CPE, XLK, and Marathon from 0 to 1.98 Mc. CPE is shown to have the highest wear rate. Note that the total mean wear rates for XLK and Marathon were significantly different (p = 0.002, Mann-Whitney U).**

### 4.3.4 Surface Characterization

Surface profilometry and scanning electron microscopy were used to characterize the unworn and worn surfaces of the CoCr discs. Roughness measurements (Ra, Rz, and $Rp_{max}$) were collected just prior to each validation test and after each validation test at 1.98 Mc. Ra, Rz, and $Rp_{max}$ were selected as the roughness parameters that best described the surface of each CoCr disc, where Ra is the average roughness, Rz is the difference between the five tallest peaks and the five deepest valleys, and $Rp_{max}$ is the maximum peak height.

SEM was performed on both unworn and worn CoCr discs (Figure 4.13) and PE pins (Figure 4.19). The unworn CoCr disc showed light scratching on the surface at x40 magnification, which may have been caused by the polishing process (Figure 4.13a). At x2000 magnification, there was a distinct presence of carbides (Figure 4.13c). A deep scratch was present on the worn surface of the CoCr disc (Figure 4.13b), which was barely visible macroscopically; the image was enhanced to x2000 magnification (Figure 4.13d), which revealed the presence of carbide particles and deep pits and scratches. These damage features were present at the corner of the square wear path, where the PE pin experiences cross shear motion.

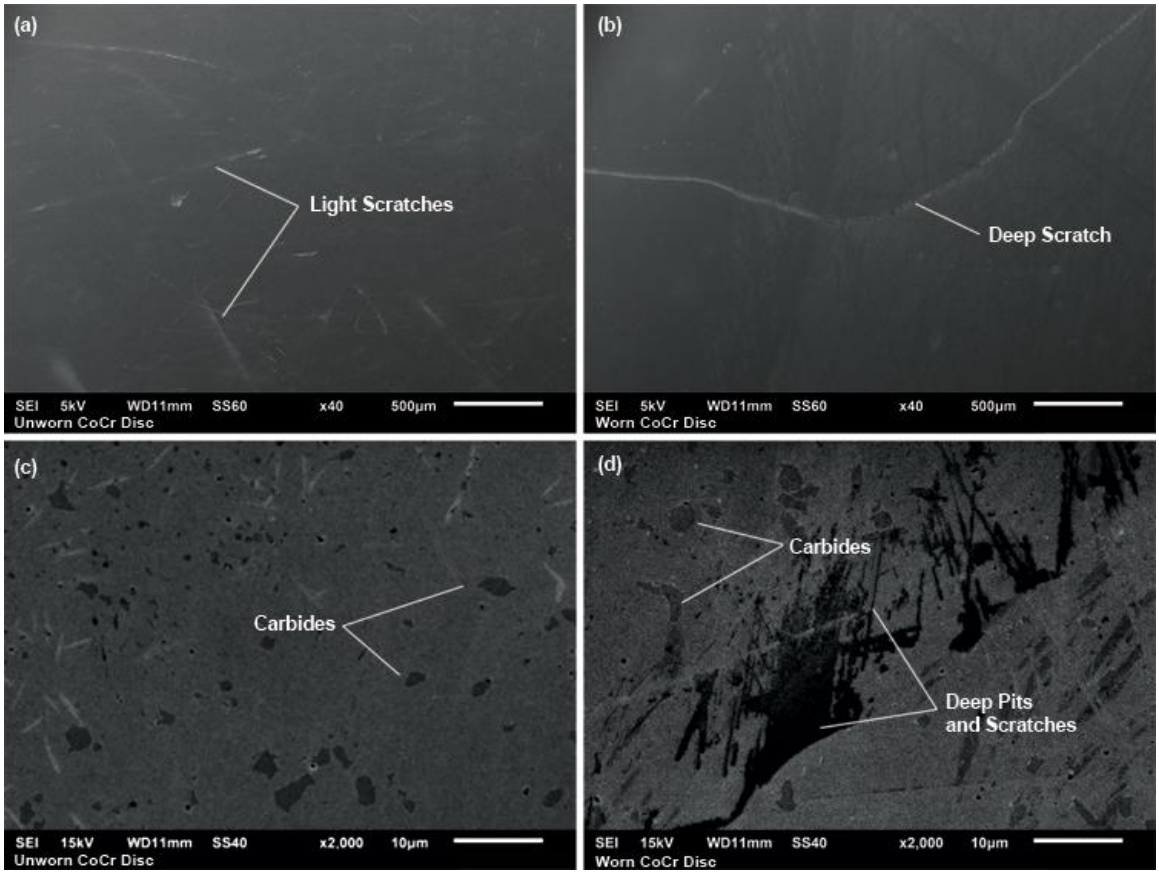


**Figure 4.13: SEM images of unworn and worn CoCr discs at x40 and x2000 magnification. (a) An unworn disc is shown to have light scratches, possibly due to polishing; (b) A deep scratch is present on the surface of a worn disc following 1.98 Mc of wear testing; (c) Carbides appear to be embedded into the surface of an unworn disc; (d) The deep scratch present in (b) is magnified to show deep pits and scratches at the point of cross-shear motion.**

Roughness measurements collected from Test 1 (CoCr-CPE) showed no significant difference between the initial and final Ra ($p = 0.212$, paired t-test); however, there was a significant difference between the initial and final Rz and Rpmax values ($p < 0.001$, paired t-test; Figure 4.14). In the case of Test 2 (CoCr-XLK), all roughness parameters were significantly different from the initial and final values ($p \leq 0.047$, paired t-test; Figure 4.15). Interestingly, Test 3 (CoCr-Marathon) there was a significant difference between the initial and final $Rp_{max}$ measurements ($p < 0.001$; paired t-test), but no difference between the initial and final Ra and Rz values ($p \geq 0.148$, paired t-test; Figure 4.16).

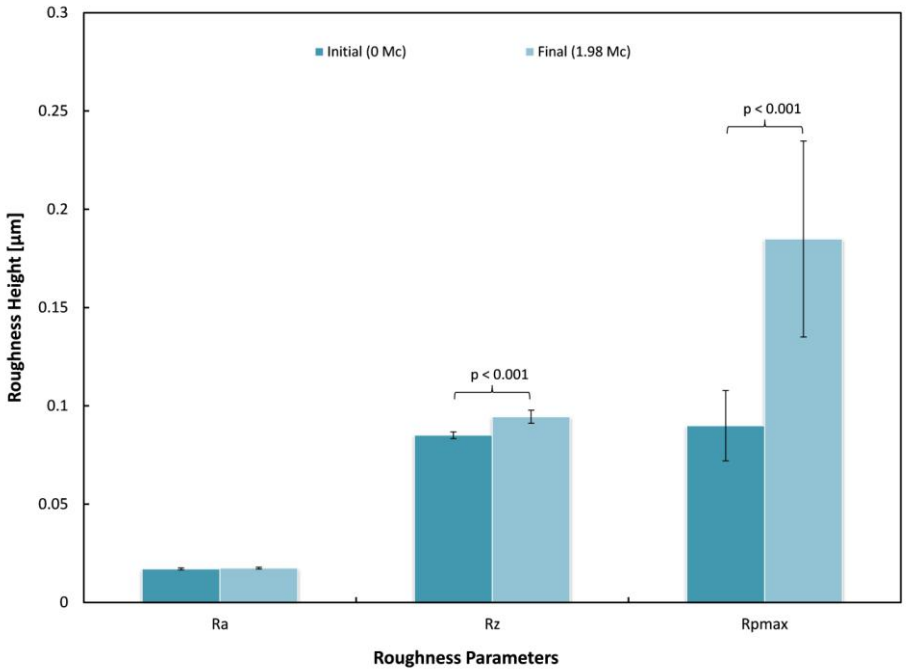


**Figure 4.14: Roughness parameters Ra, Rz, and $Rp_{max}$ collected from the CoCr discs used in validation Test 1 (CPE). Note the significant differences between the Rz and Rpmax parameters ($p < 0.001$).**

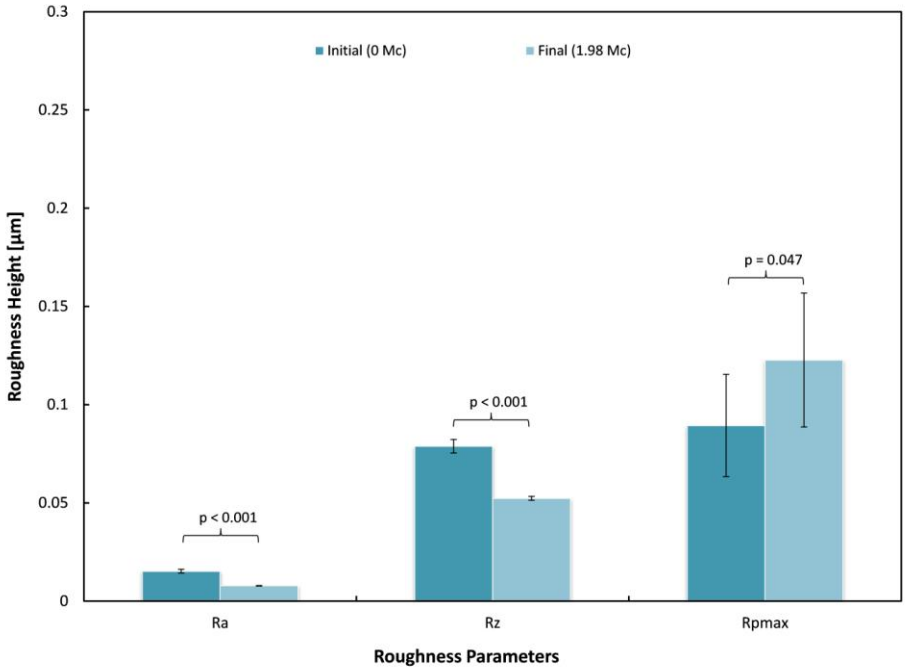


**Figure 4.15: Roughness parameters Ra, Rz, and $Rp_{max}$ collected from the CoCr discs used in validation Test 2 (XLK). Note that the initial Rpmax was significantly different from the final $Rp_{max}$ ($p < 0.001$), while the other parameters were not significantly different ($p \geq 0.148$).**

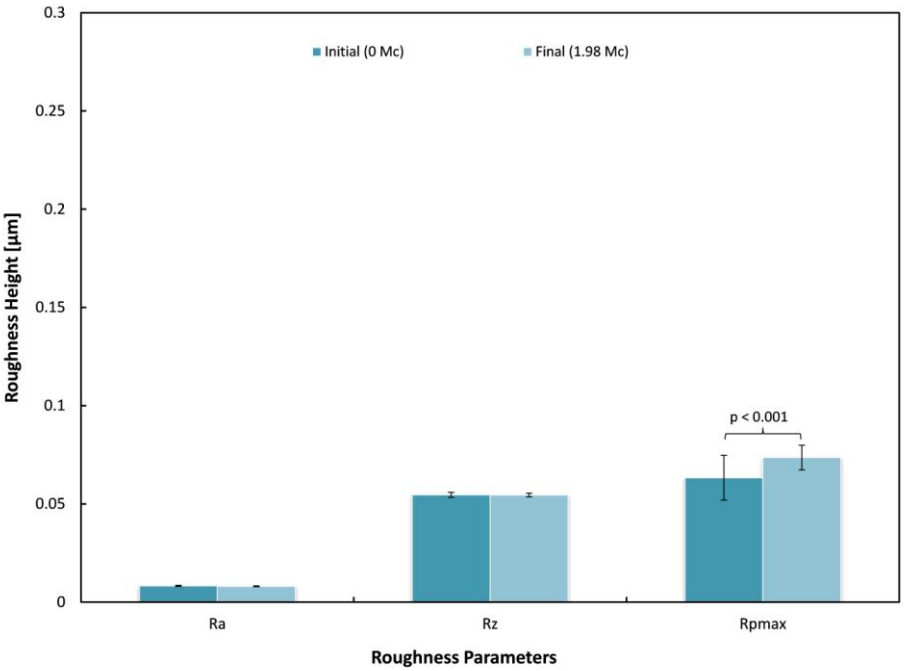


**Figure 4.16: Roughness parameters Ra, Rz, and $Rp_{max}$ collected from the CoCr discs used in validation Test 3 (Marathon). All initial roughness parameters were found to be significantly different from the final roughness parameters ($p \leq 0.047$).**

On the PE counterfaces, well-defined machining marks were visible macroscopically (Figure 4.17) and at x40 magnification (Figure 4.18). These machine marks appeared to be completely absent from the surface of the worn pins due to burnishing. Large-scale protrusions were present on the surface of worn CPE pins, and appeared in patches throughout the surface. These large-scale protrusions appeared on the surface of worn XLK pins as well, but were less scattered and more centralized than on the worn CPE pins. Mild protrusions were present on the surface of worn Marathon pins, and appeared to be scattered across the burnished surface.

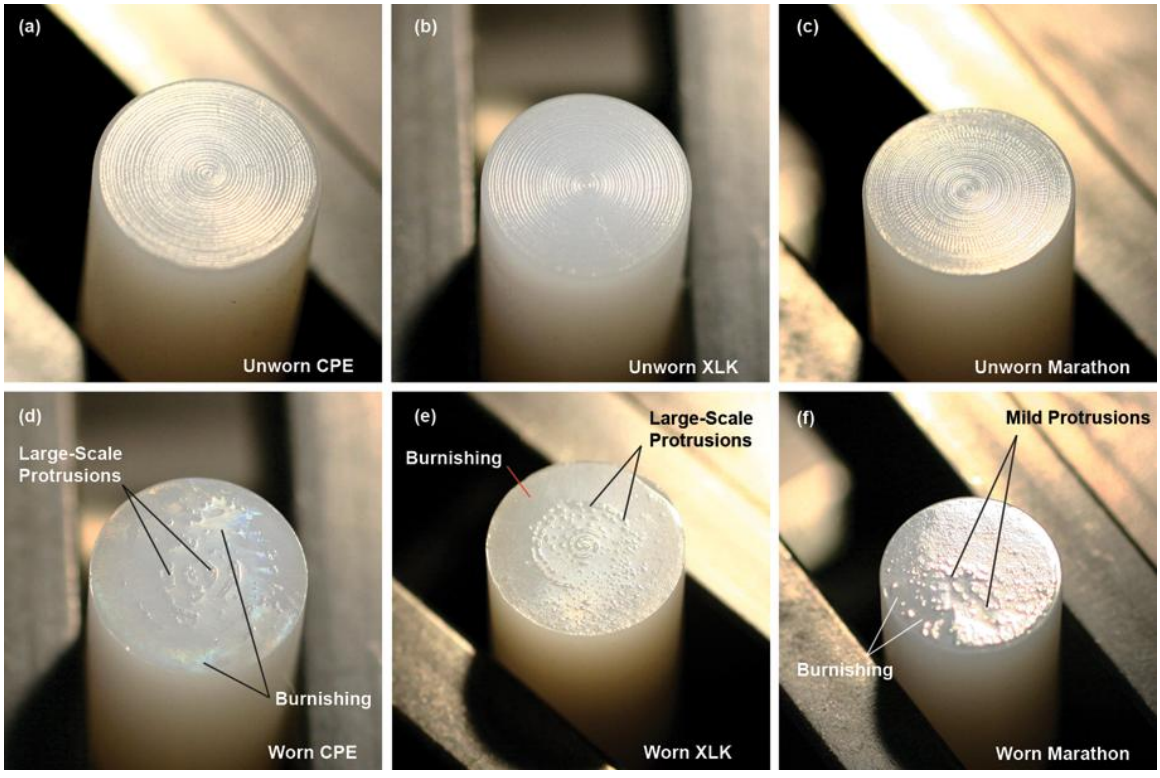


**Figure 4.17: Macroscopic images of unworn and worn PE pins. Well-defined machining marks are apparent on the surfaces of new (a) CPE, (b) XLK, and (c) Marathon pins. All worn PE pins appeared to be burnished with (d) large-scale protrusions on CPE and (e) XLK, and milder protrusions on (f) Marathon.**

SEM images of the unworn PE pins showed machining marks at x40 magnification; at the same magnification, the damage features previously identified (Figure 4.17) were shown in greater detail (Figure 4.18d-f. On the worn CPE pin, the large-scale protrusions were indeed elevated from the burnished surface (Figure 4.18d); however, at x2000 magnification, ripples were observed on the surface of these protrusions (Figure 4.18g).

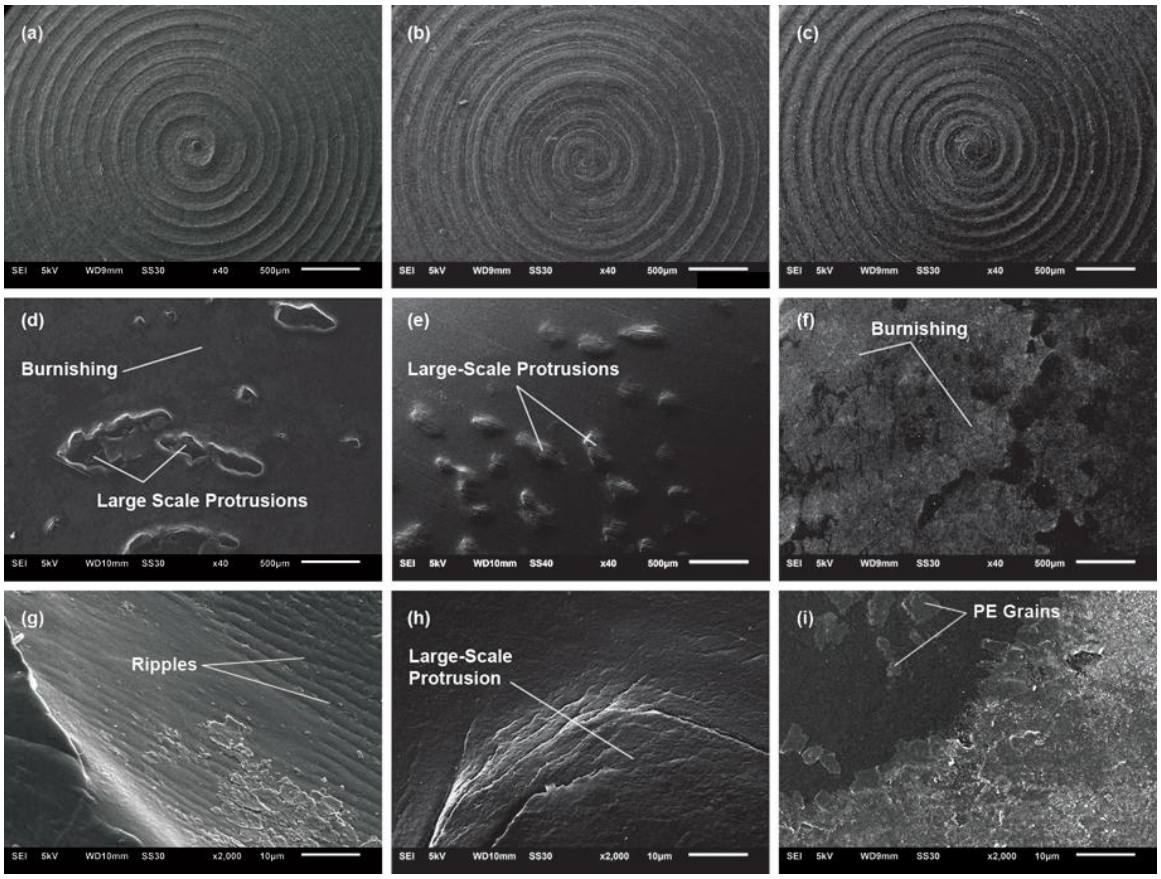


**Figure 4.18: SEM images of unworn and worn PE pins at x40 and x2000 magnification. (a-c) Unworn surfaces of CPE, XLK, and Marathon PE pins, respectively. (d) Surface of a CPE pin showing irregularly shaped large-scale protrusions present after 1.98 Mc. (e) Worn XLK surface illustrating large-scale protrusions that appeared more round. (f) Details of the worn surface on a Marathon pin. (g) Ripples are evident on the surface of the large-scale CPE protrusions. (h) Magnification of large-scale protrusion on worn XLK without the presence of ripples. (i) Marathon PE grains being pulled away from the burnished surface.**

Large-scale protrusions were also evident on the surface of the worn XLK pin at x40 magnification (Figure 4.18e), but no ripples were detected on the surface of the protrusions at x2000 magnification (Figure 4.18h). The mild protrusions found on the worn Marathon pins did not appear to be elevated from the surface (Figure 4.18f), although small pits seemed for form within these protrusions. At x2000 magnification, the PE grains on the protrusions appeared to be pulling away from the burnished surface (Figure 4.18i), which may have given the protrusions their raised appearance.

### 4.3.5 Microbial Growth

To assess the efficacy of the presence of SA in the BCS lubricant, unworn and worn lubricant samples were obtained at 0 Mc and after 0.33 Mc, and were sent to the Clinical Microbiology Laboratory at the Health Sciences Centre in Winnipeg for microbial identification. *Staphylococcus aureus*, coagulase-negative *staphylococci*, diphtheroids, and a bacillus species were grown and isolated in the unworn and worn lubricant samples collected in Test 1 (Table 4.3); interestingly, no growth was detected in both the unworn and worn lubricant samples collected in Tests 2 and 3 (Table 4.3).

**Table 4.3: Summary of organisms grown in each validation test.**

| Test No. | Pin Material | Lubricant Type | Organism(s) Isolated |
|---|---|---|---|
| 1 | CPE | Unworn | S. aureus, CNS, Diphtheroids |
| | | Worn | CNS, Bacillus species |
| 2 | XLK | Unworn | No growth |
| | | Worn | No growth |
| 3 | Marathon | Unworn | No growth |
| | | Worn | No growth |

S. aureus = Staphylococcus aureus; CNS = Coagulase-negative staphylococci;

## 4.3.6 Biochemical Analyses

### 4.3.6.1 Protein Concentration and Degradation

BCA assays were used to verify the protein concentration of the unworn BCS lubricant and the protein concentration of the worn BCS lubricant after 0.33 Mc. For each validation test, twelve measurements were conducted for each sample of worn and unworn lubricant, and compared to a standard curve; the resulting protein concentrations were then averaged for each sample. Unworn and worn protein concentrations were then compared to determine the overall protein degradation as a percentage.

The initial protein concentrations for the BCS lubricant were found to be within 1.05% of the target protein concentration (54 g/L). The protein concentrations obtained for the unworn and worn lubricant samples were not found to be significantly different between tests ($p \geq 0.242$; Mann-Whitney U). Generally speaking, the validation Test 1 (CPE) had the highest protein degradation at 19.28%, followed by validation Test 2 (XLK) at 18.89% and Test 3 (Marathon) just slightly below at 18.63% (Figure 4.19).

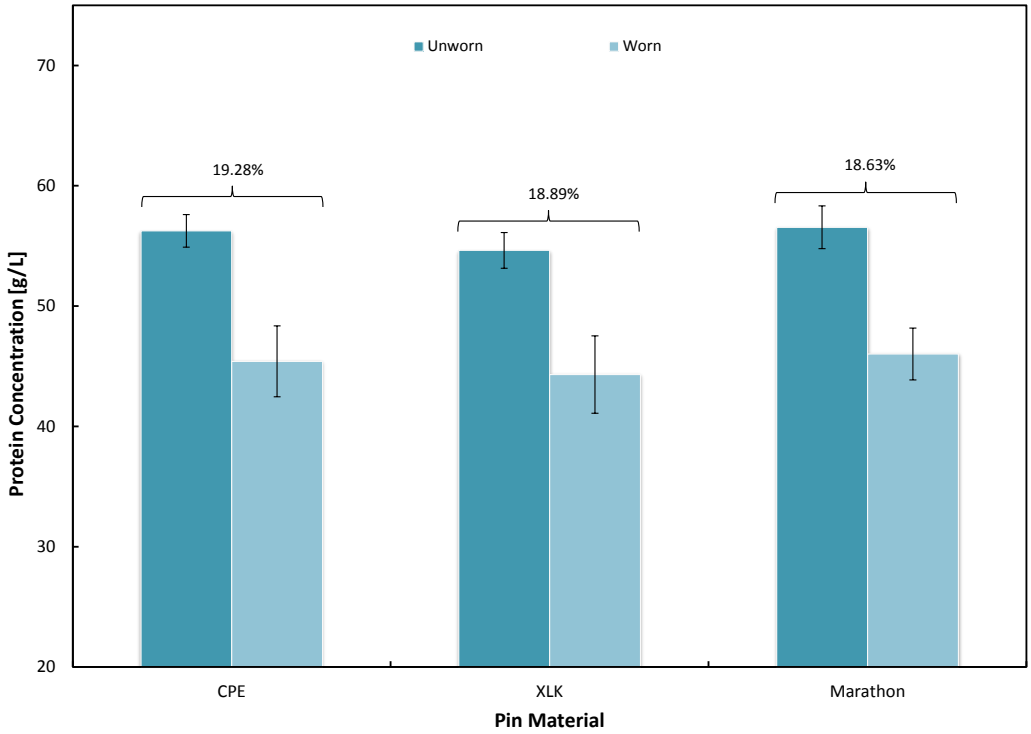


**Figure 4.19: Protein concentration and degradation for each PE material. Protein degradation is depicted as a percentage.**

### 4.3.6.2 Electrophoresis

Single electrophoretic measurements were obtained from unworn and worn BCS lubricant samples in order to quantify the degradation of the protein constituents after 0.33 Mc. For all validation tests, γ-globulin had the greatest degradation percentage at over 30% (Figures 4.20-22). For validation Test 1, 13.36% of α1-globulin had degraded after 0.33 Mc (Figure 4.20), which was a stark contrast to Test 3, where less than 3% of α1-globulin had degraded (Figure 4.22). There was a slight increase in α1-globulin in Test 2 (Figure 4.21), but this may be due to measurement error. Degradation of albumin, α2-globulin, and β-globulin were relatively consistent with degradation between 13-15%, 16-21%, and 19-23%, respectively.

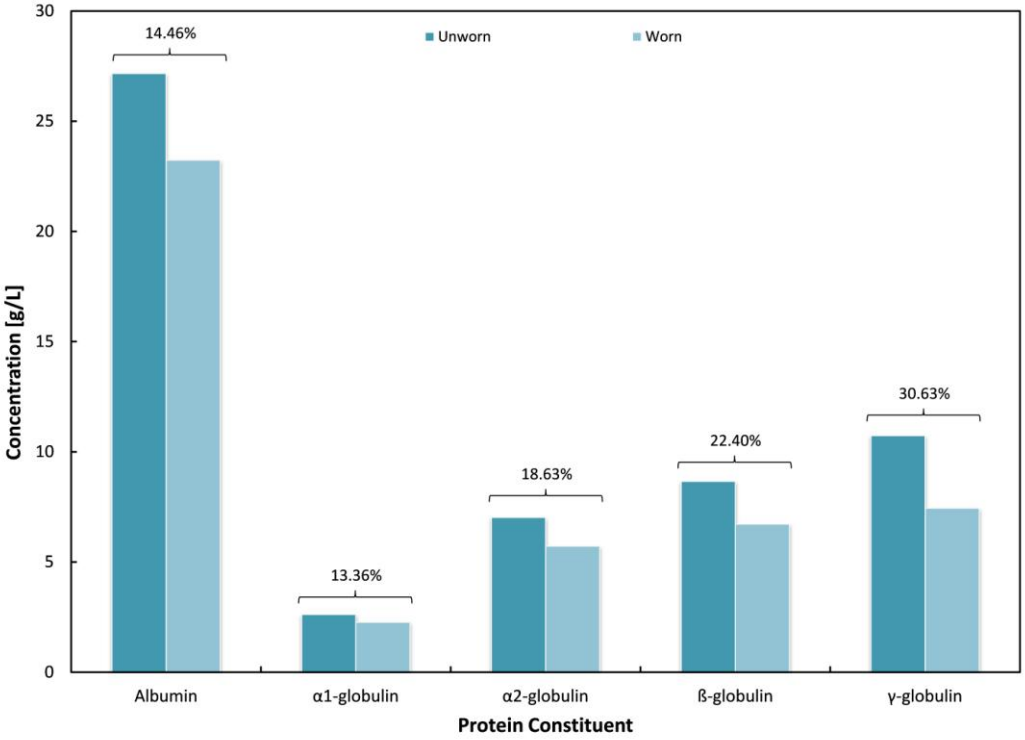


**Figure 4.20: Protein constituent degradation for validation Test 1 (CPE), with concentrations measured at 0 Mc and 0.33 Mc.**

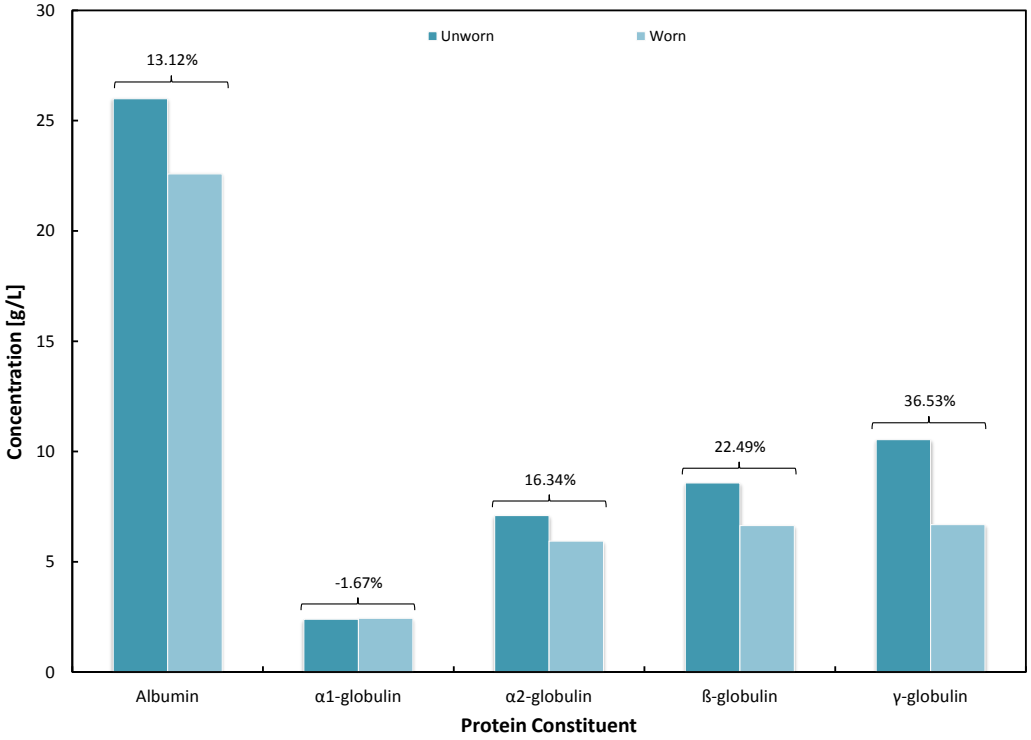


**Figure 4.21: Protein constituent degradation for validation Test 2 (XLK), with concentrations measured at 0 Mc and 0.33 Mc.**

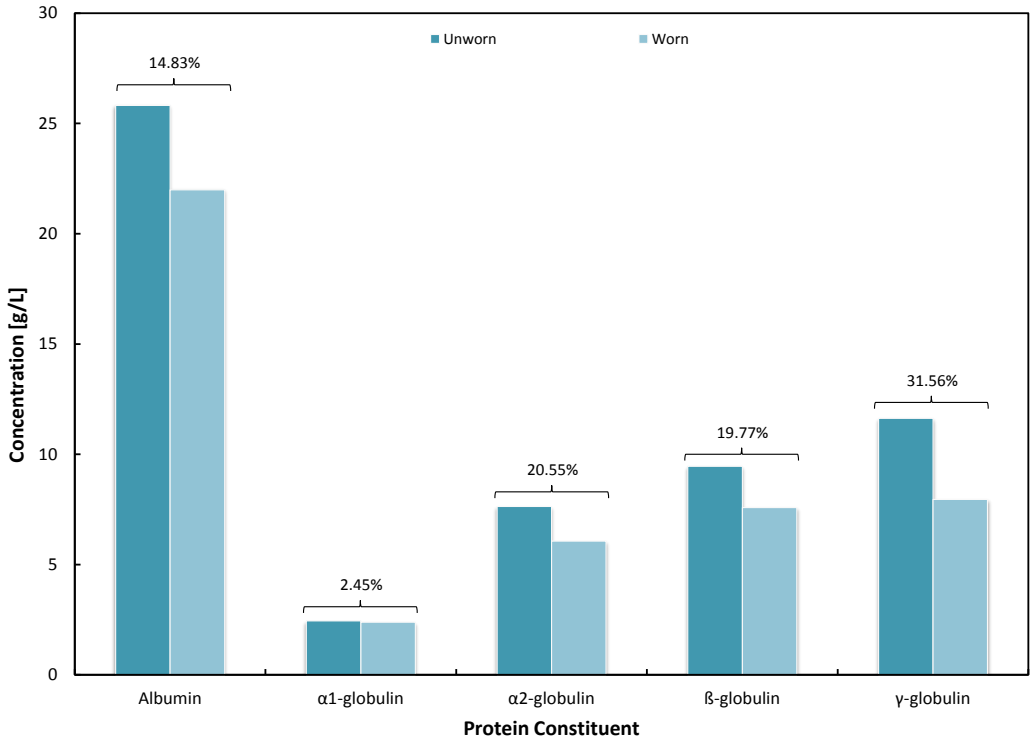


**Figure 4.22: Protein constituent degradation for validation Test 3 (Marathon), with concentrations measured at 0 Mc and 0.33 Mc.**

### 4.3.6.3 Osmolality

In order to determine the ionic strength of the BCS lubricant, osmolality measurements were performed in triplicate on an unworn lubricant sample. The mean osmolality for the BCS lubricant was found to be 267.67 ± 1.44 mmol/kg.

### 4.3.6.4 Thermal Stability

The tendency for a protein to degrade can often be illustrated by conducting DSC measurements, which quantify the Tm's, enthalpy, and entropy required to degrade 50% of the proteins present in a sample. Due to the cooperative protein unfolding of the BCS lubricant, and because Tm1, Tm2, and Tm3 were quite comparable, it was determined

that reporting Tm1 would be sufficient for the purpose of the present thesis. Tm1 was found to be 65.19°C, with a ΔH of 379.80 kJ/mol, and a ΔS of 1.12 kJ/mol·K.

### 4.3.7 Concluding Remarks

Pre-soak tests should be performed in order to reproduce the patient recovery period following arthroplasty, where periods of inactivity may cause the TJR to absorb fluid. These pre-soak tests also allow the PE pins to reach a steady-state absorption prior to wear testing. Soak controls implemented during wear testing appeared to have no significant effect on the resulting wear rates of XLK and Marathon, which may be due to the cross-linking of the PE material. The validation wear tests showed that CPE pins wore over 2-times more than the moderately cross-linked XLK and Marathon when the BCS lubricant was used. A significant difference was found between the wear rates of XLK and Marathon, where wear rates obtained for Marathon were slightly less than XLK.

Assessing surface damage at a macroscopic level may not provide sufficient information regarding the behaviour of certain materials undergoing wear processes. Because of this, it is important to perform surface profilometry and SEM on (1) unworn surfaces to establish a baseline and (2) worn surfaces to make more informed comparisons and evaluations of new and existing TJR bearing materials.

It was deemed necessary to identify the type of growth present during wear testing in order to determine the efficacy of SA, which, although extremely toxic, is a common microbial inhibitor used in BCS lubricants. In a previous study [171], SA was found to be

ineffective during knee simulator wear testing; because of this, the use of an alternative microbial inhibitor may be necessary.

Performing biochemical analyses on wear testing lubricants can provide information regarding accurate lubricant dilutions and protein degradation. BCA assays were found to be relatively reproducible, and electrophoretic analysis was able to effectively separate and quantify the distribution of proteins in the BCS lubricant. Osmolality and DSC measurements were also performed in order to make some comparison to the measurements obtained for SF.

## 4.4 Lubricant Investigations

### 4.4.1 Introductory Remarks

The lubricant investigations consisted of two tests performed for the purpose of exploring the effect of lubricant composition on wear. These tests (Tests 4 and 5; Table 3.2) also implemented the same test parameters and conditions used by DePuy Synthes; however, unlike the validation tests, the lubricants used were alpha calf serum-based as they have been found to be closer in composition to human SF [20]. In addition, the wear performance of two types of cross-linked PE pins articulating against CoCr discs was evaluated. Results from these tests could not be compared to any historical results from DePuy Synthes due to the use of various alpha calf serum-based lubricants, which are not currently used in their wear tests. Wear rates were determined in a similar manner as discussed in Section 4.3 for each lubricant; however, a repeated measures GLM was used

to analyze the data for significant trends due to the fact that the same pins and discs were used throughout each test.

Surface roughness parameters were obtained at the beginning of each test, and after every lubricant interval. Macroscopic and SEM images were only obtained for the PE pins at the end of the DW lubricant wear test.

Lubricant samples were obtained at the beginning and end of each lubricant interval. These samples were sent to the Clinical Microbiology Labortatory in Winnipeg, Manitoba for microbial identification. Samples were also sent to the London Health Sciences Centre and to the Biomolecular Interactions and Conformations Facility in London, Ontario for electrophoretic analysis and DSC measurements, respectively. BCA assays and osmolality measurements were performed by the author at the St. Boniface Research Centre and at the Department of Biological Sciences at the University of Manitoba, respectively.

### 4.4.2 Fluid Absorption

Dry weights for all XLK and Marathon pins were obtained, and then pre-soaked in DW at 37°C for a minimum of 2 weeks prior to wear testing. A stirring rod was used to agitate the DW to create some disturbance. The pins were weighed again immediately before wear testing. The average pre-soak absorption was 0.19 mg and 0.10 mg for XLK and Marathon, respectively, and was found to be significantly different ($p = 0.001$; Mann-Whitney U; Figure 4.23).

Soak controls were implemented during each POD test as described in Section 4.3.2 in order to account for fluid absorption. For each validation test, three pins were designated as “soak pins”, and were submerged in ACS lubricant; after each 0.33 Mc interval, the soak pins were cleaned, desiccated , and weighed (Appendix B) as per Section 3.3.6.

Since minute changes in the mass of XLK and Marathon had been found in Section 4.3.2, it was deemed unnecessary to report fluid absorption values for the lubricant investigation tests.

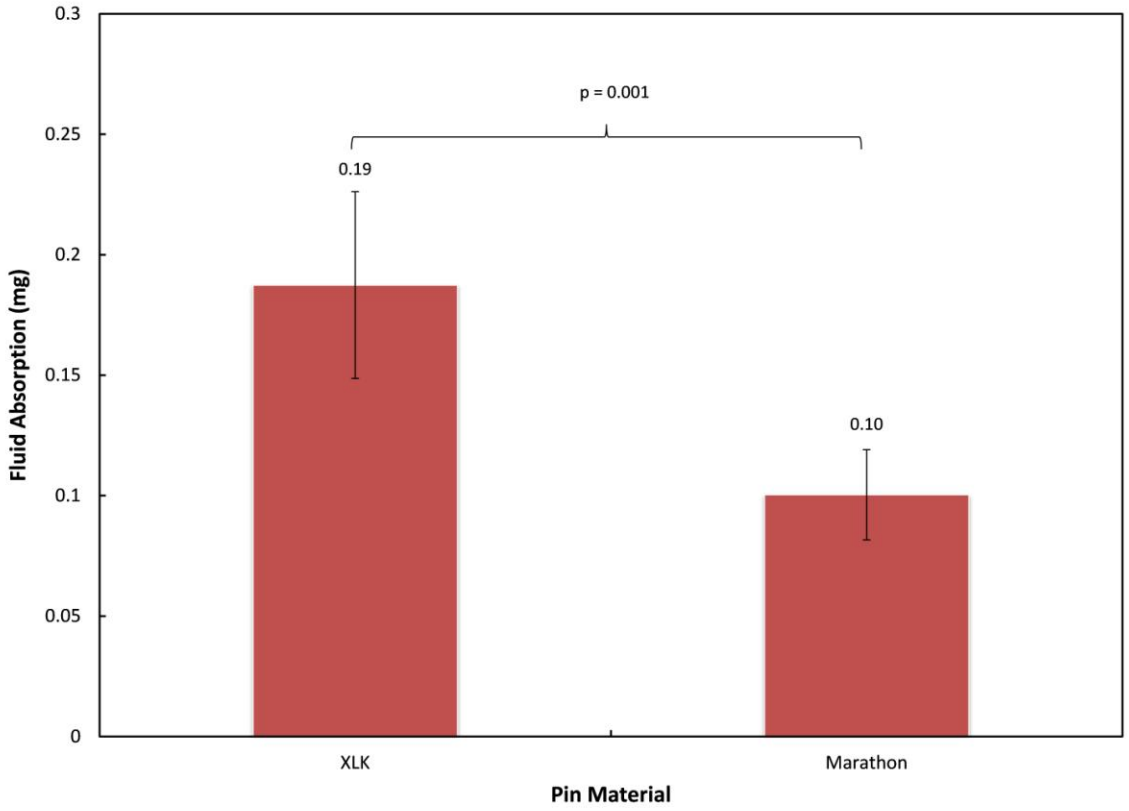


**Figure 4.23: Fluid absorption during pre-test soak control for XLK, and Marathon pins just prior to testing.**

### 4.4.3 Wear Rates

Six PE pins and six CoCr discs were selected and assigned to each wear station on the OrthoPOD, and remained with the same wear station for the entire 5 Mc test duration.

All XLK and Marathon pins were weighed after every 0.33 Mc interval (Figures 4.24-25). Wear rates for each station and for each lubricant interval were obtained from the slope of the least squares linear-regression lines fitted through the data (Appendix C.2); for each test, there was no significant difference in the wear rates produced between each station for each lubricant interval ($p \geq 0.208$; GLM – Tukey).

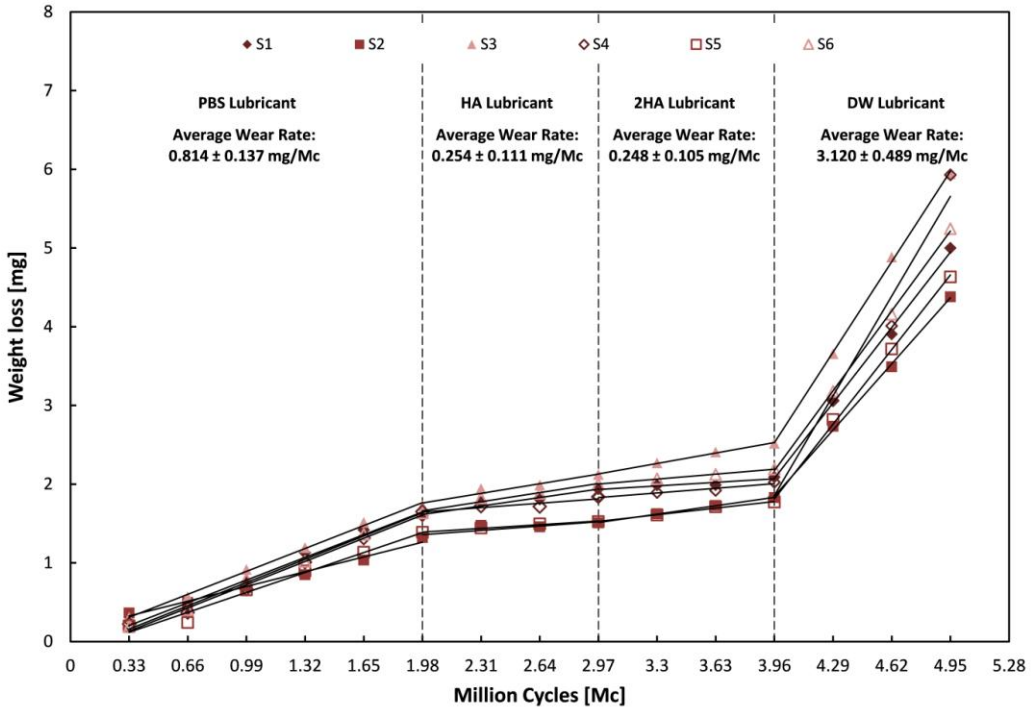


**Figure 4.24: The wear of all six XLK pins over 5 Mc with linear regression lines fitted through each lubricant interval. Fluid absorption was accounted for in the wear data.**

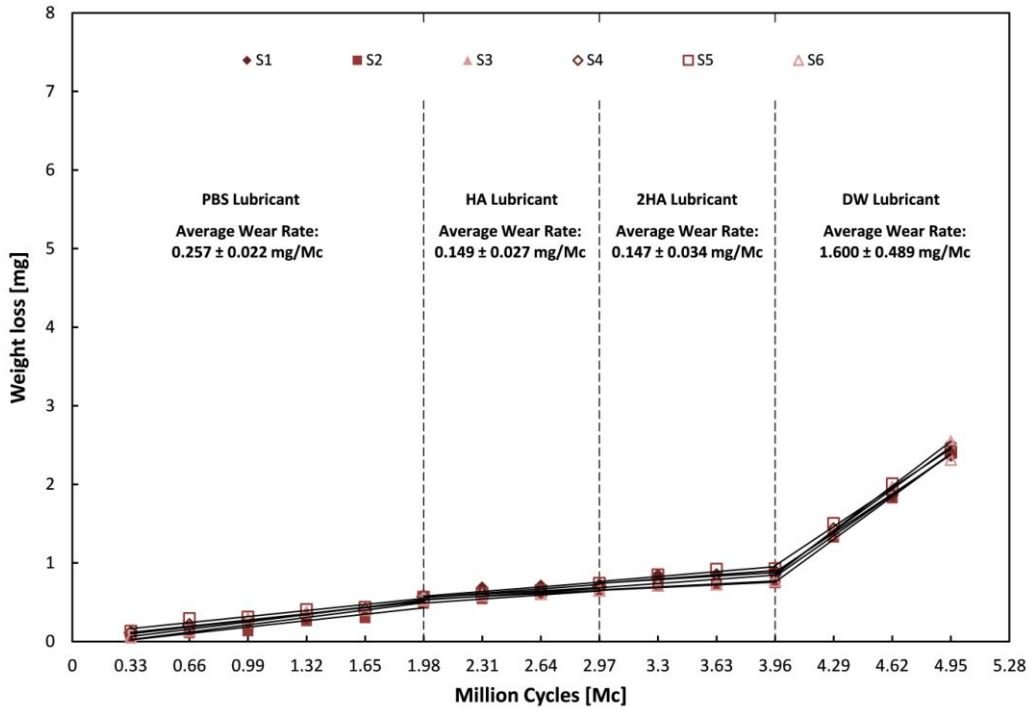


**Figure 4.25: The wear of all Marathon pins over 5 Mc with linear regression lines fitted through each lubricant interval. Fluid absorption was accounted for in the wear data.**

The total average wear rates for all XLK pins were 0.814 ± 0.14 mg/Mc, 0.254 ± 0.11 mg/Mc, 0.248 ± 0.11 mg/Mc, and 3.12 ± 0.49 mg/Mc for the PBS, HA, 2HA, and DW lubricants, respectively (Figure 4.26). Generally, the DW lubricant produced the highest average wear rate, where the XLK pins wore approximately 4-times more than in the PBS lubricant, and over 12-times more than in the HA and 2HA lubricants. Surprisingly, there was no significant difference between the wear rates generated from the HA and 2HA lubricants ($p = 0.999$, GLM-Tamhane's T2). The average PBS lubricant wear rate was significantly different from the HA, 2HA, and DW lubricants ($p \leq 0.018$, GLM – Tamhane's T2; Figure 4.26); the average wear rate produced from the DW lubricant was also significantly different from HA, 2HA, and PBS lubricants ($p < 0.001$, GLM – Tamhane's T2).

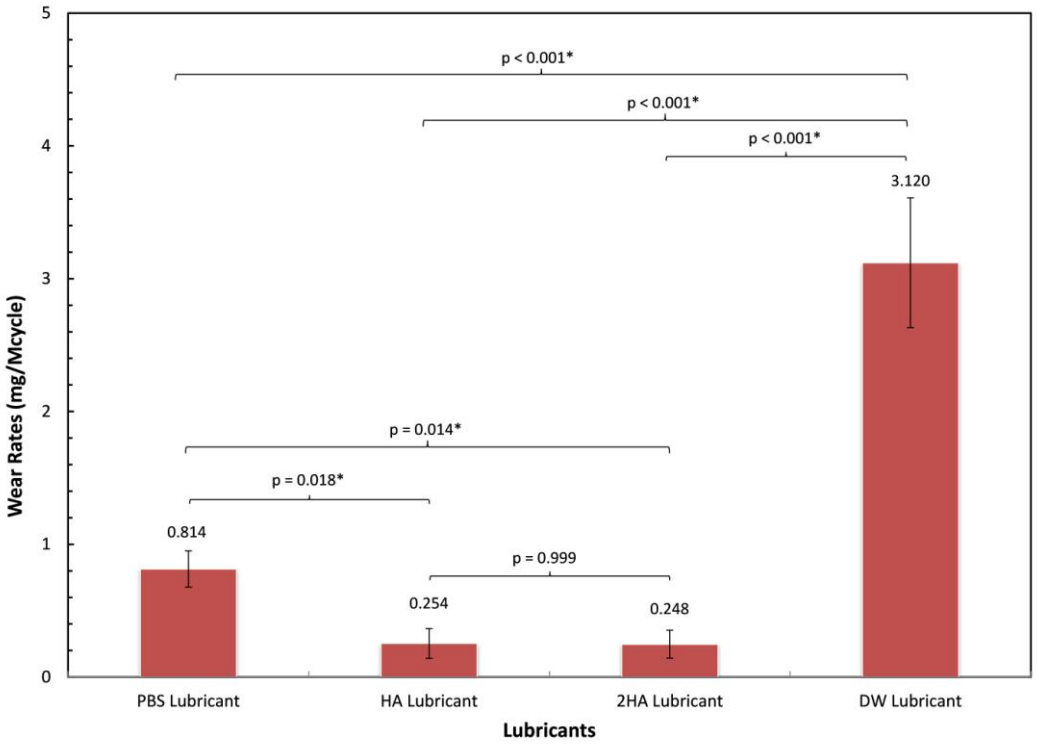


**Figure 4.26: Average XLK wear rates (Test 4) generated from each lubricant. Note that there was no significant difference between the HA and 2HA lubricants ($p = 0.999$). Significant differences were found between the PBS and the HA and 2HA lubricant wear rates ($p \leq 0.018$); the wear rates produced by the DW lubricant were also found to be significantly different from the PBS, HA, 2HA lubricant wear rates ($p < 0.001$).**

The total average wear rates for all Marathon pins were 0.257 ± 0.02 mg/Mc, 0.149 ± 0.03 mg/Mc, 0.147 ± 0.03 mg/Mc, and 1.596 ± 0.09 mg/Mc for the PBS, HA, 2HA, and DW lubricants, respectively. Similar trends were observed in the Marathon test (Test 5) as those in the XLK test (Test 4), where the DW lubricant generated the highest average wear rate at over 6-times higher than the PBS lubricant, and 10-times higher than the HA and 2HA lubricants. Average wear rates produced by the HA and 2HA lubricants were also not significantly different ($p = 1.000$, GLM – Tukey; Figure 4.28)). Unlike Test 4, however, there was no significant difference between the PBS lubricant wear rates and

the HA and 2HA lubricants in Test 5 ($p \geq 0.357$, GLM – Tukey; Figure 4.28). Wear rates produced by the DW lubricant were significantly different from all wear rates generated from the other lubricants ($p \leq 0.001$, GLM – Tukey; Figure 4.27).

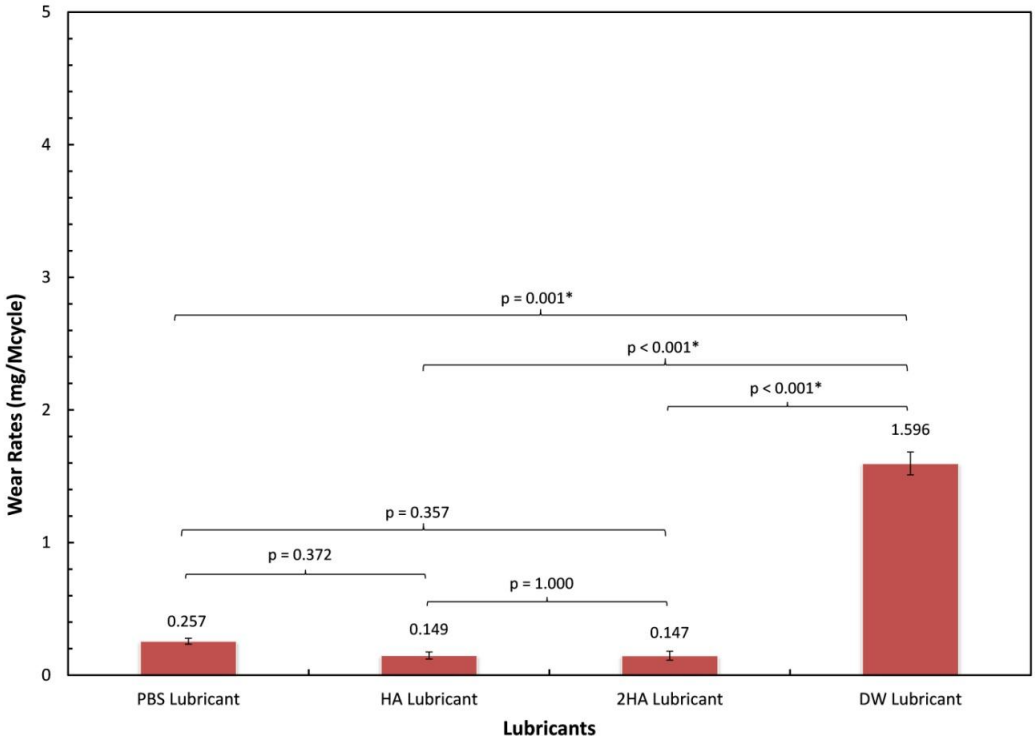


**Figure 4.27: Average Marathon wear rates (Test 5) generated from each lubricant. Note that the average DW lubricant wear rate was significantly different from the average wear rates produced by the PBS, HA, and 2HA lubricants ($p \leq 0.001$).**

For every lubricant, comparisons were made between the wear rates of XLK and Marathon in order to identify significant differences (Figure 4.28). There was a significant difference between the XLK and Marathon wear rates under the PBS ($p = 0.004$) and DW ($p < 0.001$, Mann-Whitney U) lubricant, but no significant differences were observed between the wear rates under the HA ($p = 0.060$, Mann-Whitney U) and 2HA ($p = 0.058$, Mann-Whitney U) lubricants. In addition, there were no significant

differences between the HA and 2HA wear rates for both XLK and Marathon ($p > 0.906$, Mann-Whitney U; Figure 4.28).

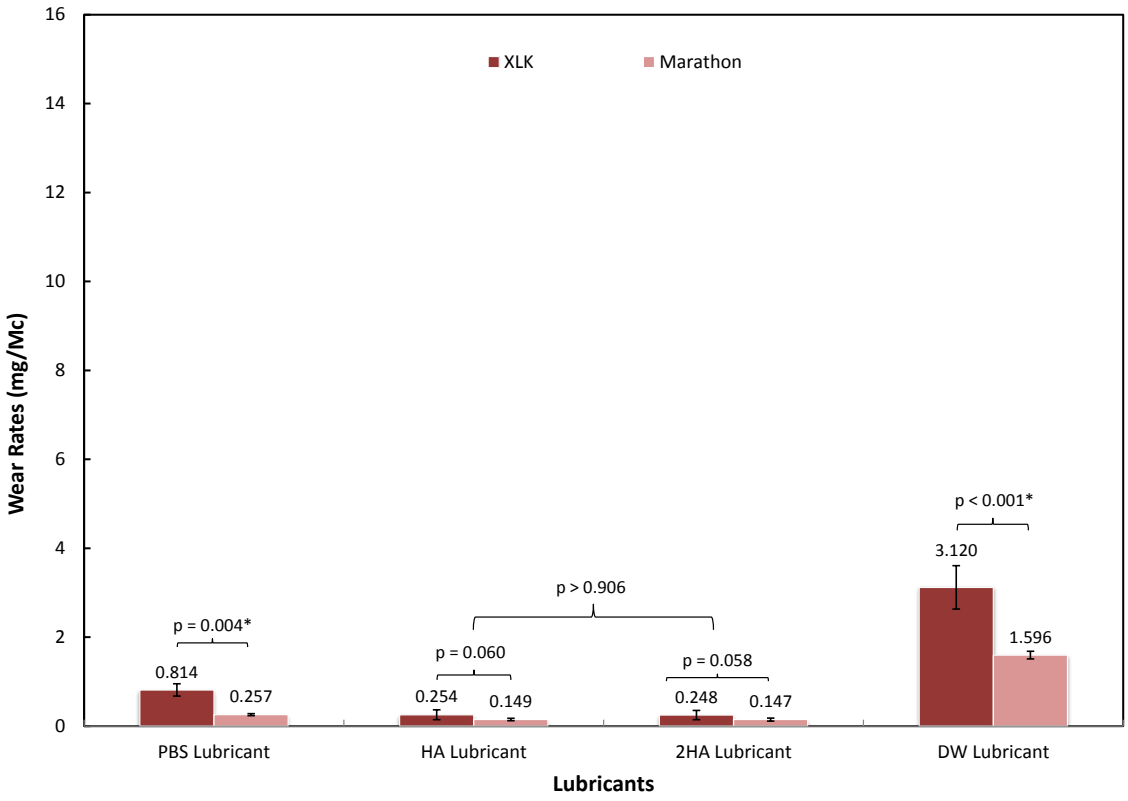


**Figure 4.28: Comparisons between the XLK (Test 4) and Marathon (Test 5) wear rates for each lubricant used in Test 4 and 5. Note that there were significant differences between the XLK and Marathon wear rates for the PBS ($p = 0.004$) and DW ($p < 0.001$) lubricants, but no significant difference between the HA and 2HA lubricants ($p \geq 0.058$).**

## 4.4.4 Surface Characterization

Similar to Section 4.3.4, surface profilometry and scanning electron microscopy were used to characterize the unworn and worn surfaces of the CoCr discs used in Test 4 and 5. Roughness measurements (Ra, Rz, and $Rp_{max}$) were also collected just prior to each test

and after each lubricant interval. The objective was to discern differences in Ra, Rz, and $Rp_{max}$ between each successive lubricant interval.

Macroscopic images were taken of the PE pin surfaces prior to performing SEM (Figure 4.29). On the unworn PE counterfaces, well-defined machining marks were visible macroscopically (Figure 4.29a, b) and at x40 magnification (Figure 4.29a, b). These machine marks appeared to be completely absent from the surface of the worn pins due to burnishing. A combination of mild protrusions and burnishing were present on the surface of worn XLK pins (Figure 4.29c). The Marathon pins showed extensive burnishing on the worn surface (Figure 4.29d); sections located at the center of the pin appeared to be elevated in comparison to the areas along the edge.

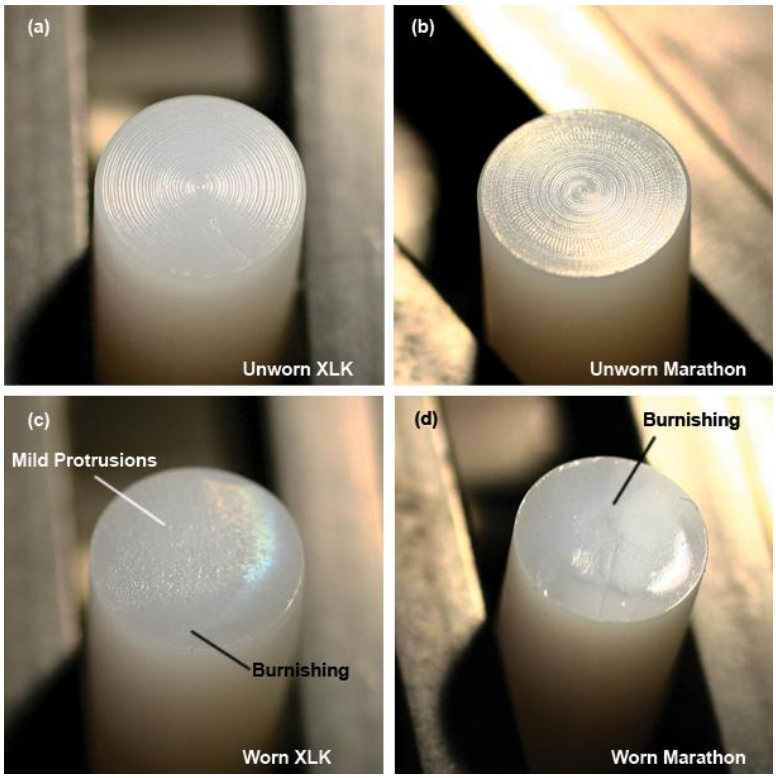


**Figure 4.29: Macroscopic images of unworn and worn XLK and Marathon pins. Well defined machining marks are apparent on the surface of new (a) XLK and (b) Marathon pins. (c) Worn XLK pin showing mild protrusions and burnishing damage features. (d) Worn Marathon pin with a completely burnished surface.**

At x40 magnification, SEM images of the unworn XLK and Marathon pins (Figure 4.30a, b) showed clear machining marks, which appeared to be absent on the worn XLK and Marathon pins after 1.98 Mc (Figure 4.30c, d). On the surface of the worn XLK pin, the stippling that was macroscopically visible was enhanced at x40 (Figure 4.30c), showing raised bumps on the surface. One of these bumps was magnified at x2000 magnification and showed that, while the elevated surface of the bump was smooth, PE grains appeared to be pulled in a number of directions on the lower surface (Figure 4.30e). At 40x magnification, the worn surface of the Marathon pin showed smoother, flatter PE grains. Grain boundaries were also observed at x2000 magnification, surrounded by a smeared layer of PE grains (Figure 4.30f).

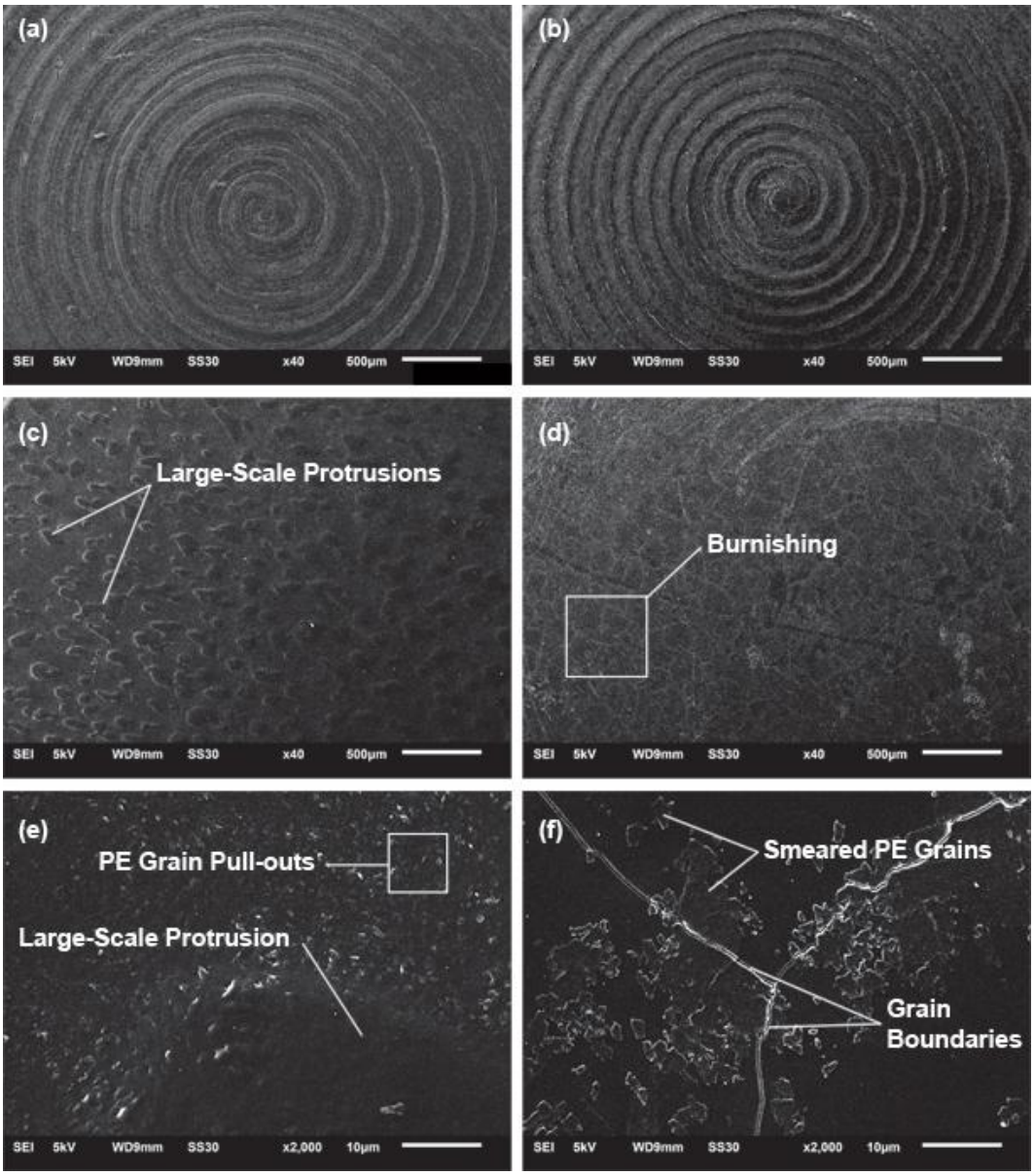


**Figure 4.30: SEM images of unworn and worn XLK and Marathon pins at x40 and x2000 magnification. (a-b) Unworn surface of XLK and Marathon pins, respectively. (c) Worn XLK surface showing large-scale protrusions. (d) Burnished surface of worn Marathon pin. PE grain boundaries can be seen. (e) Magnification of large protrusion on a worn XLK pin. Small grain pull-outs are evident. (f) Grain boundaries on the surface of a worn Marathon pin; grains appear to be smeared across the burnished surface.**

In order to determine that the surface damage was due to wear and not plastic deformation, the worn XLK and Marathon pins were slowly heated and re-melted in a conventional oven. SEM images were then obtained at x40 magnification in order to distinguish any differences that may not have been apparent macroscopically. The worn, re-melted XLK pin showed no distinguishable machining marks on the surface (Figure 4.31a); however, some machining marks were restored on the worn, re-melted Marathon pin (Figure 4.31b).

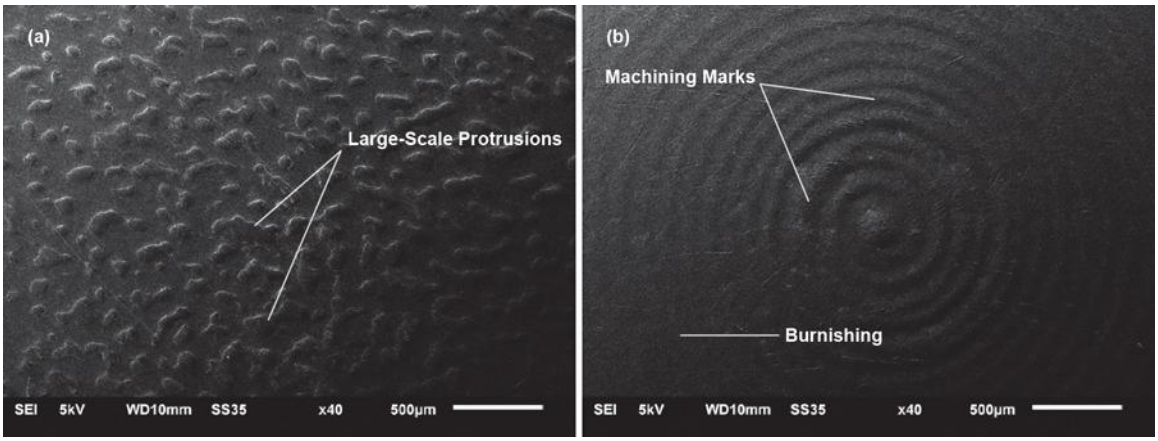


**Figure 4.31: SEM images of worn, re-melted XLK and Marathon pins at x40 magnification. (a) Re-melted XLK pin showing no change in surface characteristics previously observed in Figure 4.33(c). (b) Restoration of machining marks on worn, re-melted Marathon pin with patches of burnishing. PE grain boundaries evident in Figure 4.33(d) are no longer observed.**

Roughness measurements collected from Test 4 (CoCr-XLK) showed an increase from the initial Ra and Rz, but a decrease in $Rp_{max}$ at the end of the PBS lubricant interval. There was a significant difference between the Ra obtained before and after the 2HA lubricant interval, and before and after DW lubricant interval ($p < 0.001$, GLM – Tamhane's T2; Figure 4.32). No other significant differences were found in Ra between successive lubricant intervals ($p \geq 0.126$, GLM – Tamhane's T2). The same differences

were observed for the Rz values obtained before and after the 2HA lubricant interval, and before and after the DW lubricant interval ($p < 0.001$, GLM – Tamhane's T2; Figure 4.32). Surprisingly, there were no significant differences observed between the $Rp_{max}$ values obtained between the initial and successive lubricant intervals ($p \geq 0.127$, GLM – Tamhane's T2).

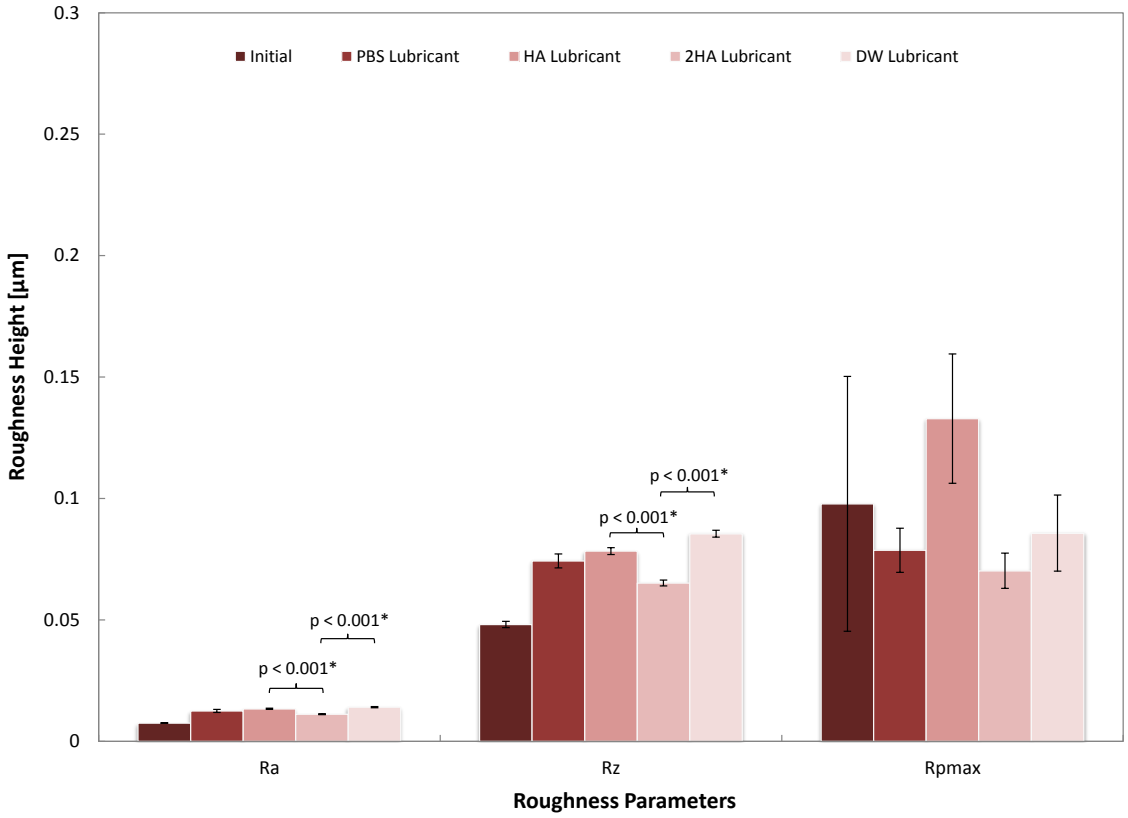


**Figure 4.32: Roughness parameters Ra, Rz, and Rpmax collected from the CoCr discs used in Test 4 (XLK). Note the significant differences between the Ra and Rz values obtained before and after the 2HA and DW lubricant intervals ($p < 0.001$).**

In Test 5 (CoCr-Marathon), a similar trend was observed where Ra and Rz increased at the end of the PBS lubricant interval, but saw a decrease in $Rp_{max}$ values. There was a significant difference between the Ra obtained before and after the HA, 2HA, and DW lubricant intervals ($p \leq 0.014$, GLM – Tukey; Figure 4.33). Interestingly, there were no

significant differences to report for the Rz and $Rp_{max}$ values between each successive lubricant interval ($p \geq 0.377$; GLM - Tukey).

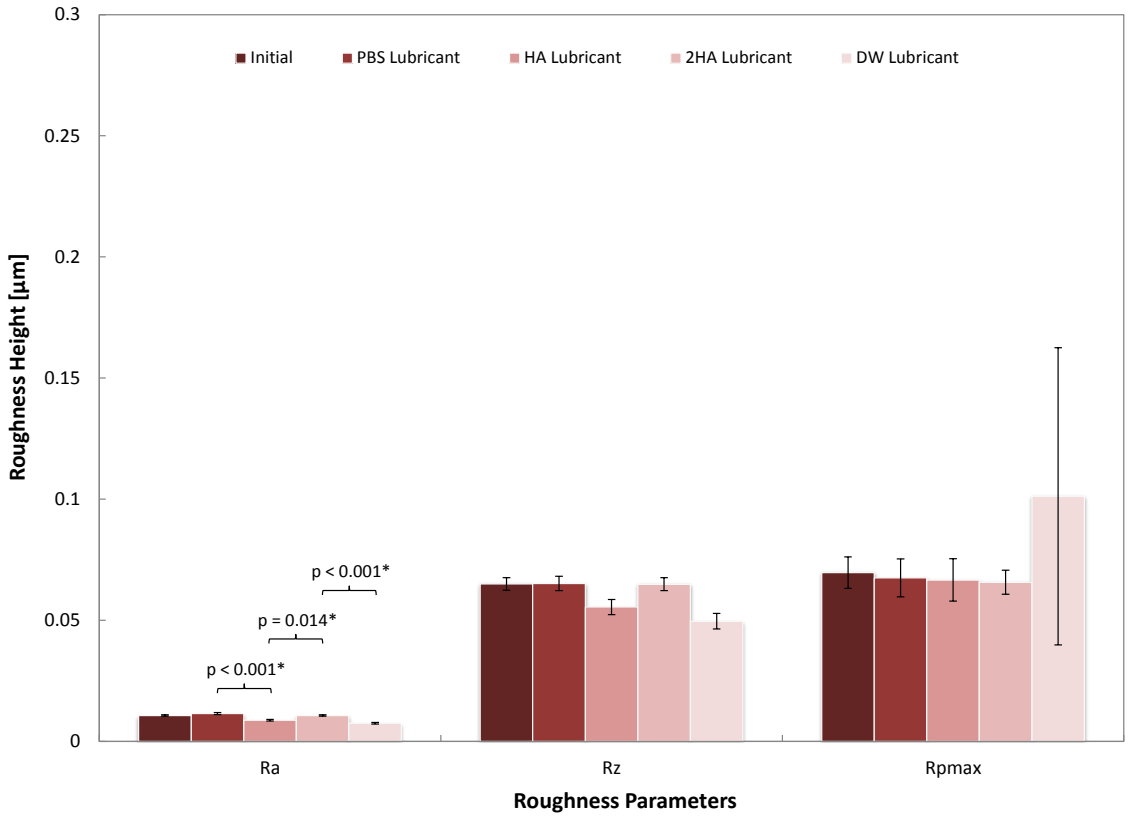


**Figure 4.33: Roughness parameters Ra, Rz, and Rpmax collected from the CoCr discs used in Test 5 (Marathon). Note the significant differences between the Ra obtained before and after the HA, 2HA and DW lubricant intervals ($p \leq 0.014$).**

## 4.4.5 Microbial Contamination

In Tests 4 and 5, an antibiotic/antimycotic solution was used in lieu of SA in order to evaluate whether SA could be replaced with a safer and less toxic alternative. Worn and unworn lubricant samples were collected at 0 Mc and after 0.33 Mc for each ACS lubricant. These samples were sent for microbial identification. While the unworn

lubricant samples initially showed no growth, the worn lubricant samples revealed that a bacillus species grew after 0.33 Mc (Table 4.4) in both tests. No other organisms were grown or identified in these samples.

**Table 4.4: Summary of the organism grown in each lubricant investigation test.**

| Test No. | Pin Material | Lubricant Type | Organism(s) |
|---|---|---|---|
| 4 | XLK | Unworn | No growth |
| | | Worn | Bacillus species |
| 5 | Marathon | Unworn | No growth |
| | | Worn | Bacillus species |

## 4.4.6 Biochemical Analyses

### 4.4.6.1 Protein Concentration and Degradation

Similar to Section 4.3.6.1, BCA assays were used to determine the unworn and worn ACS lubricant protein concentrations at 0 Mc and 0.33 Mc, respectively. The same protocol was followed, where twelve measurements were conducted for each sample, and compared to a standard curve.

The initial protein concentrations for the ACS lubricants were found to be within 1.1% of the target protein concentration (34 g/L). Of the four ACS lubricants used, the PBS and DW lubricants had the highest degradation at 5.57%, and the 2HA lubricant had the lowest degradation at 0.95% (Figure 4.34).

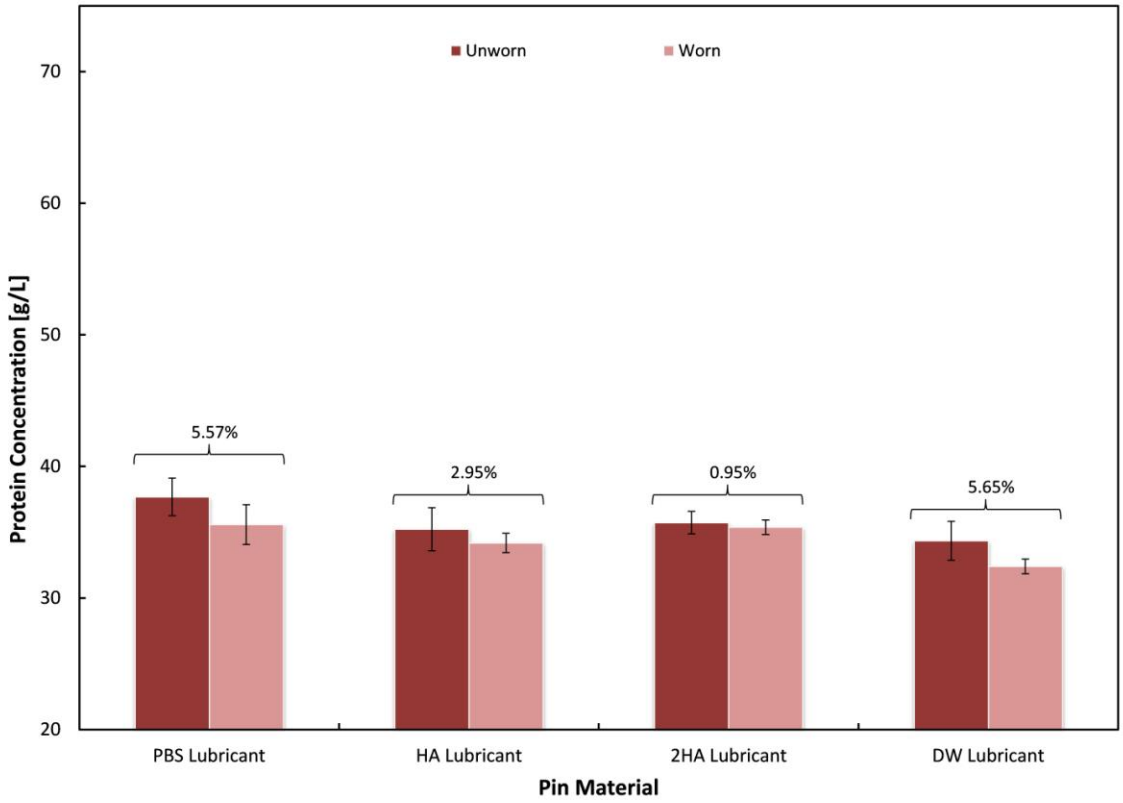


**Figure 4.34: Protein concentration and degradation for each lubricant used in Tests 4 and 5. Protein degradation is depicted as a percentage.**

### 4.4.6.2 Electrophoresis

The degradation of the protein constituents in the ACS lubricants after 0.33 Mc was determined through electrophoretic analysis. Generally, the lowest amount of degradation was observed for the 2HA lubricant; degradation was more pronounced in the DW lubricant. For all ACS lubricants, α2- and γ-globulin had the greatest degradation percentage (Figure 4.35-38). There were slight increases in albumin, and albumin and α1-globulin in the HA (Figure 4.36) and 2HA lubricants (Figure 4.27), respectively. This

small increase may be due to measurement error; as a result, negative degradation percentages were considered to be 0%.

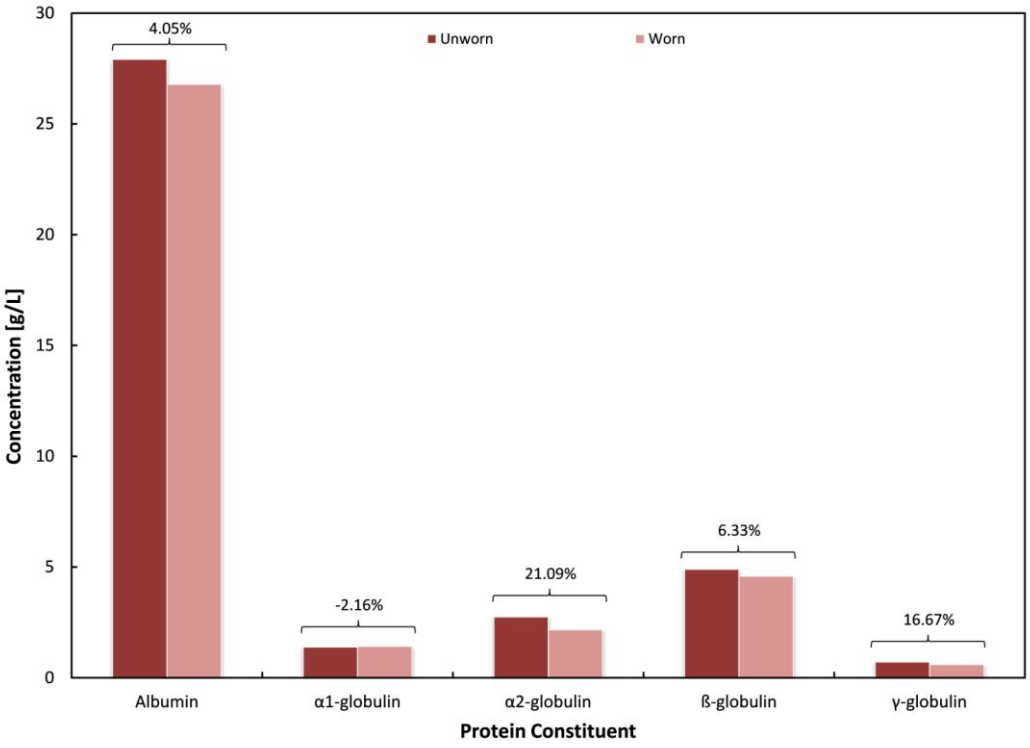


**Figure 4.35: Protein constituent degradation for the PBS lubricant, with concentrations measured at 0 Mc and 0.33 Mc.**

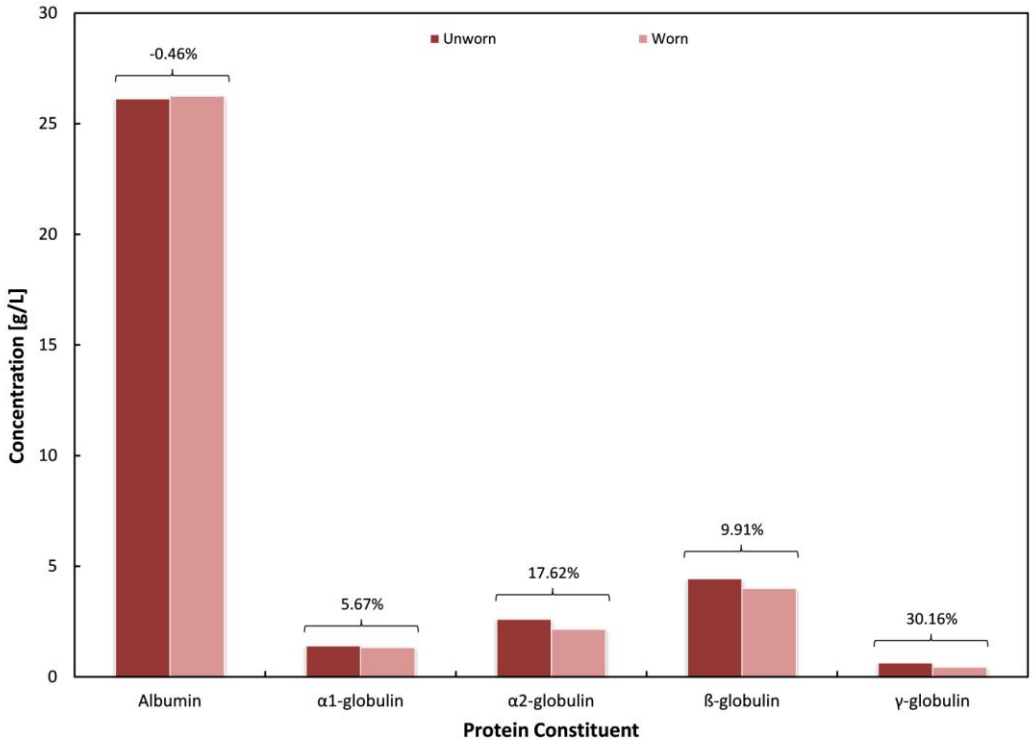


**Figure 4.36: Protein constituent degradation for the HA lubricant, with concentrations measured at 0 Mc and 0.33 Mc. Note that the negative degradation of albumin may be due to measurement error.**

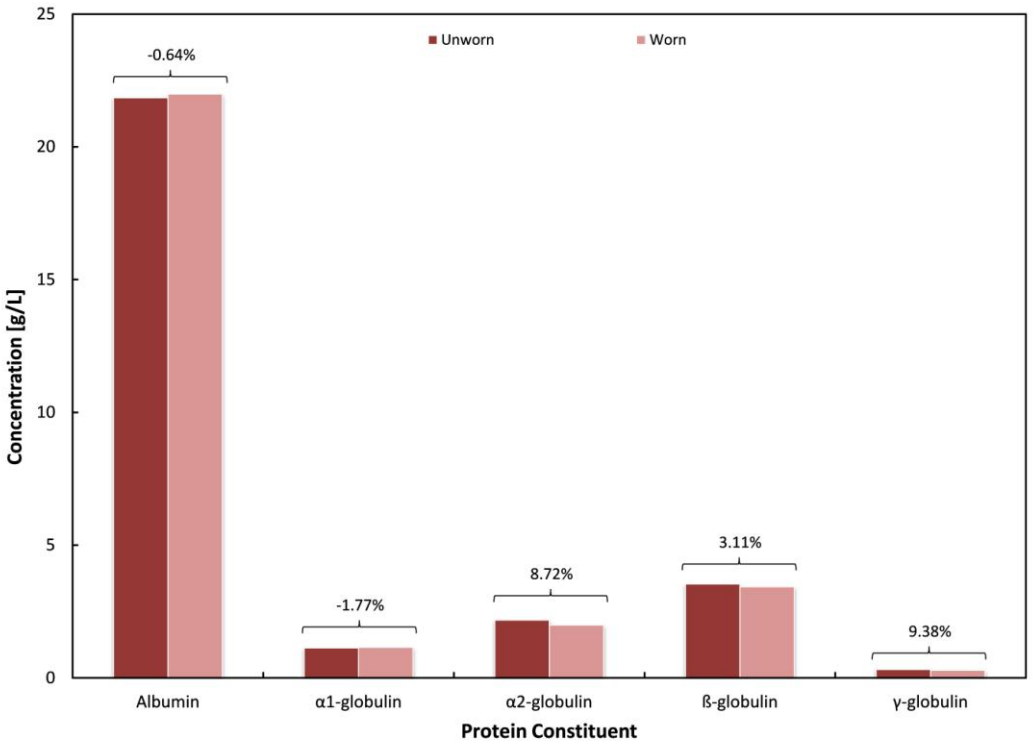


**Figure 4.37: Protein constituent degradation for the 2HA lubricant, with concentrations measured at 0 Mc and 0.33 Mc. Note that the negative degradation of albumin and α1-globulin may be due to measurement error.**

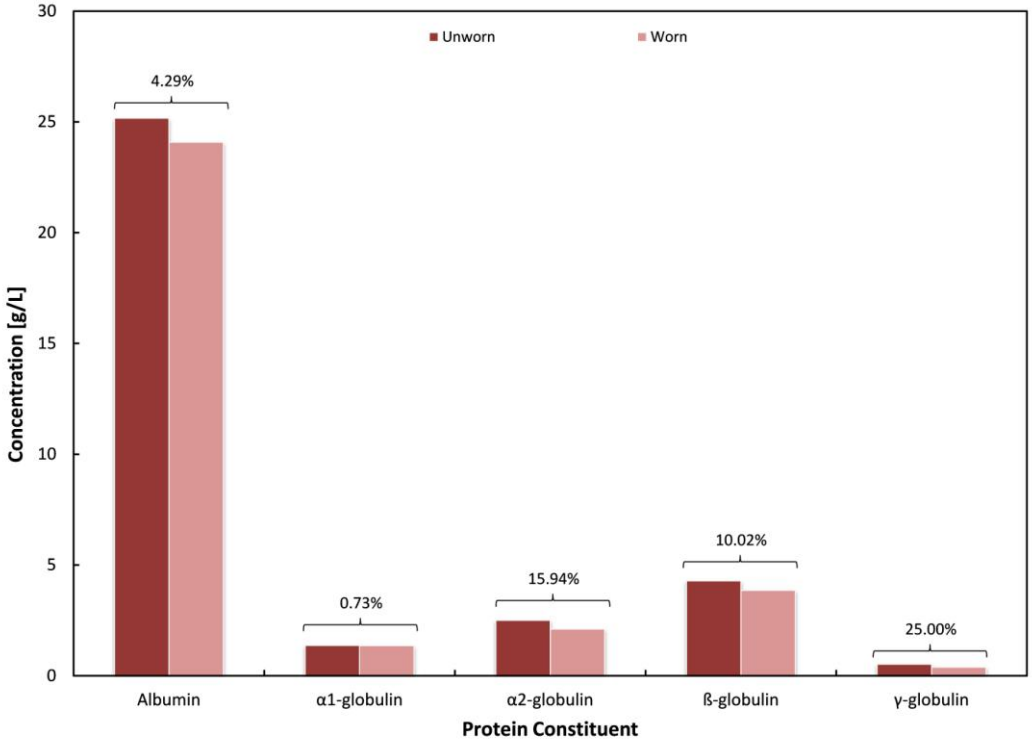


**Figure 4.38: Protein constituent degradation for the DW lubricant, with concentrations measured at 0 Mc and 0.33 Mc.**

### 4.4.6.3 Osmolality

The ionic strength of each unworn ACS lubricant was determined by measuring the osmolality in triplicate. In general, the 2HA lubricant had the highest osmolality, whereas the DW lubricant exhibited the lowest osmolality. The mean osmolality for the PBS, HA, 2HA, and DW lubricants was 293.00 ± 7.45, 295.33 ± 5.17, 302.67 ± 20.23, and 253 ± 8.96 mmol/kg, respectively (Figure 4.39). There was no significant difference in osmolality when comparisons were made between the PBS, HA, and 2HA lubricants ($p \geq 0.142$; ANOVA-Tukey); however, the osmolality of the DW lubricant was significantly different from the other lubricants ($p < 0.001$; ANOVA-Tukey; Figure 4.39)

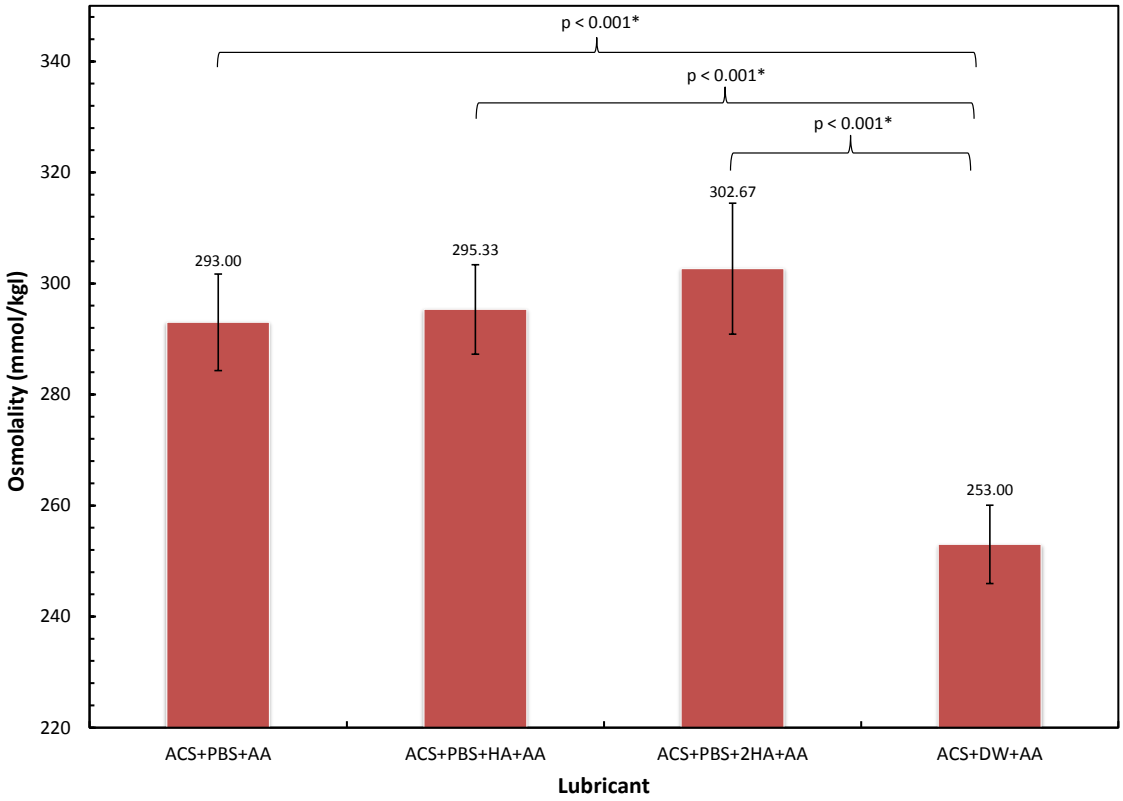


**Figure 4.39: Comparison between the osmolality of each ACS lubricant. Note that the DW lubricant has the lowest osmolality, and is significantly different from the other ACS lubricants.**

### 4.4.6.4 Thermal Stability

DSC measurements on the unworn ACS lubricants were performed by the Biomolecular Interactions and Conformations Facility in London, Ontario. Due to the cooperative protein unfolding of the ACS lubricants, and because Tm1, Tm2, and Tm3 were quite comparable, it was determined that reporting Tm1 would be sufficient for the purpose of the present thesis. The Tm1 was found to be 61.93°C, 60.93°C, 61.11°C, and 60.15°C for the PBS, HA, 2HA, and DW lubricants, respectively (Figure 4.40). The PBS appeared to have the highest Tm1, while the DW lubricant had the lowest Tm1.

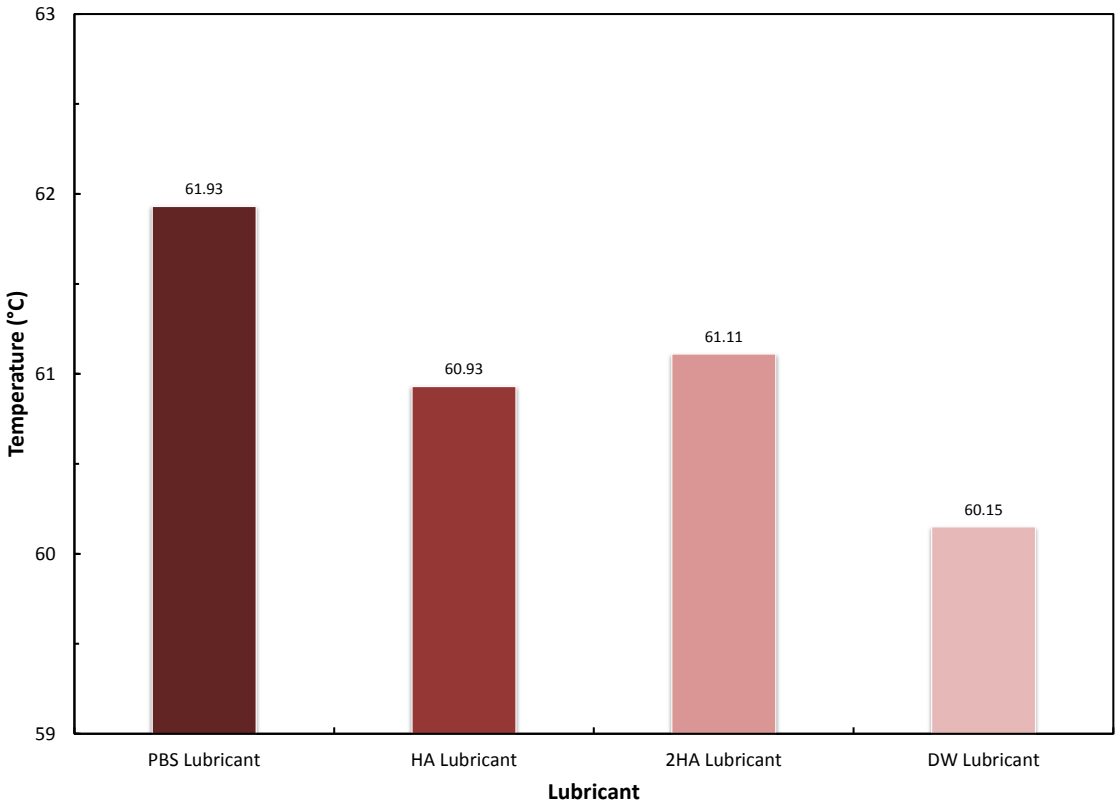


**Figure 4.40: Comparison between the Tm1 temperatures for each ACS lubricant. Note that the DW lubricant has the lowest Tm1 value, and that the PBS lubricant has the highest Tm1 value.**

ΔH was found to be 1060.68 kJ/mol, 992.88 kJ/mol, 1191.98 kJ/mol, and 860.68 kJ/mol for the PBS, HA, 2HA, and DW lubricants (Figure 4.41). ΔS was found to be 3.17 kJ/mol·K, 2.97 kJ/mol·K, 3.57 kJ/mol·K, and 2.58 kJ/mol·K (Figure 4.42)

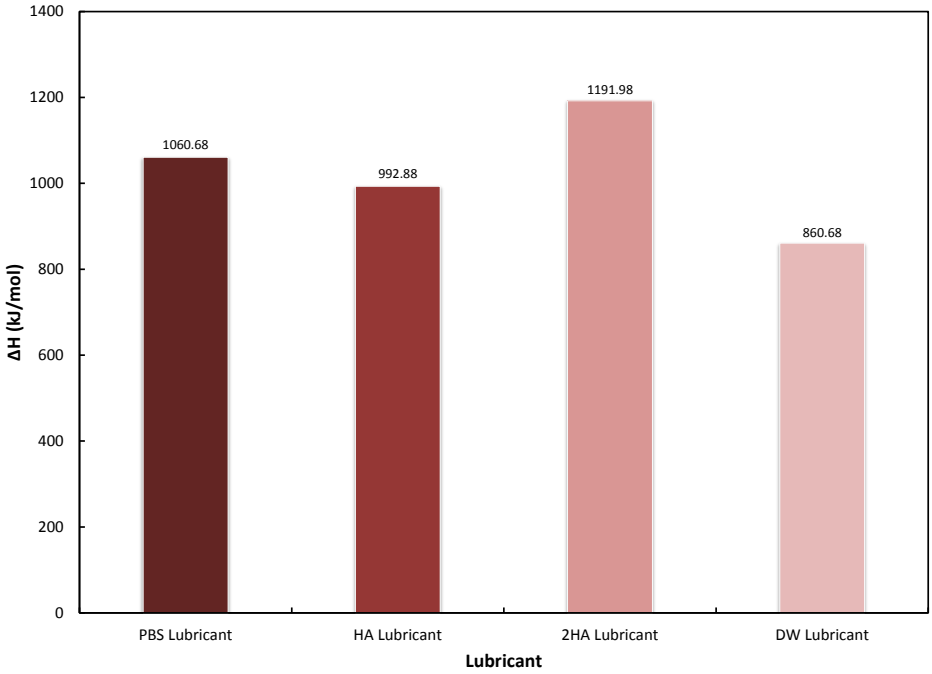


**Figure 4.41: Comparison between the enthalpies for each ACS lubricant. Note that the 2HA lubricant has the highest enthalpy, and that the DW lubricant has the lowest enthalpy.**

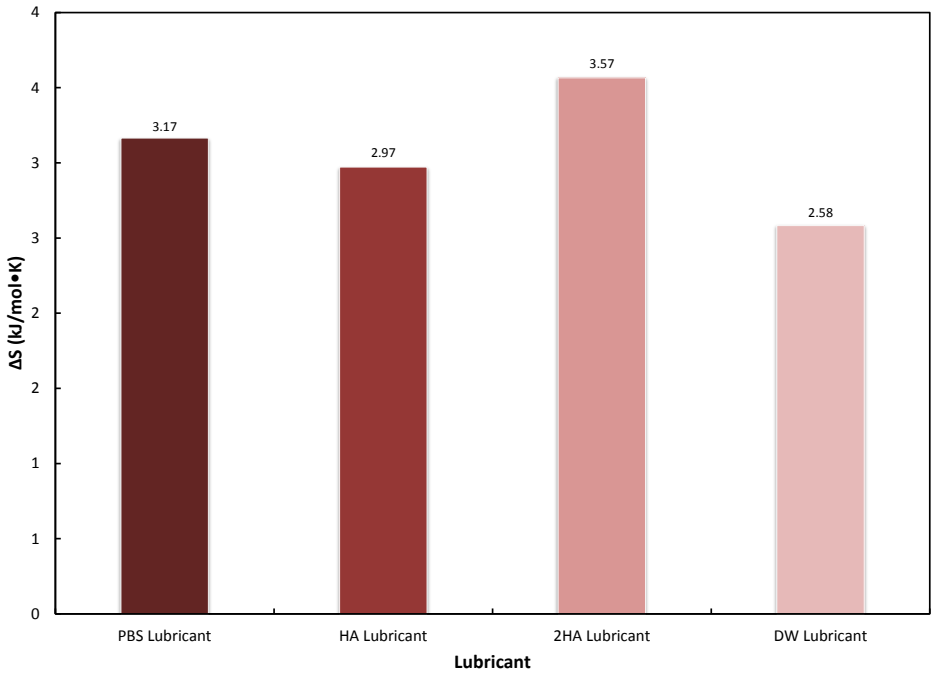


**Figure 4.42: Comparison between the entropies for each ACS lubricant. Note that the 2HA lubricant has the highest entropy, and that the DW lubricant has the lowest entropy.**

## 4.5 Concluding Remarks

The pre-soak tests showed that fluid absorption was highest in XLK, and was found to be significantly different from the fluid absorption experienced in Marathon. This suggests that the soak period may play an important role in establishing the amount of fluid taken up by crosslinked PE pins.

The type of dilutive media appeared to have a significant effect on the wear rates generated from each lubricant interval, where the DW lubricant produced the highest wear rates in comparison to the PBS, HA, and 2HA lubricants. Based on the biochemical analyses, the higher wear observed with the DW lubricant may be due to the lower osmolality; DSC measurements may confirm these findings.

Surface roughness measurements did not reveal consistent relationships between Ra, Rz, and $Rp_{max}$ for each successive lubricant interval. This may suggest that simply reporting one roughness parameter may not give sufficient insight regarding the wear behaviour of TJR bearing materials.

Macroscopic images of the worn PE pins showed that burnishing was present on both XLK and Marathon surfaces; however, stippling appeared to be characteristic of XLK pins, while the surface of Marathon pins appeared to be purely burnished. Re-melting the surfaces of these pins resulted in the restoration of some original machining marks, which shows that the purely burnished surface observed on the Marathon pin was partially due to plastic deformation and some wear; however, no machining marks were observed on the surface of the XLK pin, which suggests that the amount of plastic deformation was rather low.

An antibiotic/anti-mycotic solution was added to each ACS lubricant as a replacement for SA. Microbial analysis showed that no growth was detected in the unworn lubricant; however, a bacillus species grew consistently in the worn lubricant after 0.33 Mc.

Changing the type of calf serum, dilutive media, and protein concentration used in the lubricants appeared to have a significant effect on protein degradation. Osmolality measurements also showed that the type of dilutive media and the presence of HA affected the ionic strength of the lubricant; however, the type of dilutive media and the presence of HA did not appear to affect the enthalpy of the ACS lubricants.

# Chapter 5

# Discussion

## 5.1 Introductory Remarks

The present chapter provides a detailed discussion on the results presented in Chapter 4. The biochemical composition and thermal properties must first be quantified from the synovial fluid investigation, as this component of the present thesis may dictate the clinical relevance of the wear tests performed. Section 5.2 details the discrepancies between clinical data and current *in vitro* wear testing standards; these issues are further highlighted by the biochemical and thermal stability data obtained from the OA-SF and PP-SF analyzed in the present thesis (Section 4.2). Collectively, this data provides a more complete illustration of the differences between SF collected in the present thesis and historical data, and differences between human SF and *in vitro* wear testing lubricants.

In Section 5.3, the results from both the validation and lubricant investigation wear tests were compared and contrasted, with a specific focus on the differences observed due to the lubricant composition and the material properties. Retrieved Marathon acetabular

cups were selected from the Implant Retrieval Database at the Orthopaedic Innovation Centre in Winnipeg in order to compare the clinical surface damage to the *in vitro* surface damage observed on the worn Marathon pins. Retrieved Sigma P.F.C. tibial inserts, which are manufactured from XLK, were not available in Implant Retrieval Database at the time of the present thesis; because of this, a surface damage comparison could not be done with the worn XLK pins.

Section 5.3.6 compares the biochemical and thermal stability results obtained from the BCS and ACS lubricants. To determine the clinical relevance of these findings, the biochemical data was also compared to the biochemical data from the clinical investigation. An explanation was provided regarding the mechanism of HA in simulator and POD wear tests, and how using a more clinically relevant lubricant may, in some cases, give puzzling results, as seen in Section 4.4.3.

## 5.2 Synovial Fluid Investigation

### 5.2.1 Introductory Remarks

*In vitro* wear testing is routinely used to approximate the wear performance of TJRs *in vivo*; however, these predictions are largely influenced by the type and composition of the wear testing lubricant [16, 17, 19, 24, 46, 105-107]. Bovine serum is currently the most common lubricant used in orthopaedic wear testing due to its ability to reproduce wear characteristics found *in vivo* [44, 100, 103]; because of this, the International Organization for Standardization (ISO) and the American Society for Testing and

Materials (ASTM) have standardized the use of bovine serum-based lubricants as a more historical [103] and practical alternative to SF [6, 26, 172]. Though bovine serum and bovine serum-based lubricants are able to mimic clinical wear to some extent, there is much debate regarding its biochemical similarities to human SF [14, 15, 155].

### 5.2.2 Synovial Fluid Protein Composition

A previous study had investigated the protein composition and thermal stability of OA-SF in order to develop a more clinically relevant wear testing lubricant [20]; however, only SF samples from patients undergoing primary total knee arthroplasty were analyzed. Following arthroplasty, the TJR components interact with PP-SF, which is why it is also important to investigate the composition of PP-SF. To the author's knowledge, the protein composition and thermal stability of PP-SF from both hip and knee joints were characterized and quantified for the first time. In addition, the present thesis is the first to look at OA-SF and PP-SF from both hip and knee joints collectively. Comparisons between OA-SF and PP-SF may provide some additional information regarding the extent of the synovium's ability to recover and retain its SF generating ability [9].

The ISO standard for hip simulator wear testing [26] was recently revised to specify a minimum protein concentration of 30g/L; however, the minimum protein concentration remains at 17g/L for knee simulator wear testing [172], which is consistent with the lower range of some clinical levels [9] (Table 5.1). For pin-on-disc wear testing, the ASTM standard [6] recommends a maximum 75% dilution of bovine serum, which can produce protein concentrations well below clinical levels reported in previous studies

[9, 20, 95] (Table 5.1). It would be difficult to accurately estimate the overall *in vitro* performance of new bearing materials and designs using a lubricant that does not reflect clinical levels of protein concentration.

The SF samples obtained from patients undergoing primary and revision TJA showed that no significant difference was found in total protein concentration, which suggests that the synovium is able to recover and maintain its SF generating ability following primary TJA. The protein concentration found in the present study was found to be comparable to those reported for OA-SF [9, 20, 95] and PP-SF [9] in previous studies (Table 5.1), but quite different from the protein concentrations specified in the wear testing standards for knee simulators [172] and, in some cases, for pin-on-disc testing [6]. These results, in combination with those from previous studies [9, 20, 95], show that perhaps a narrower range of values is necessary in order to ensure wear testing lubricant protein concentrations remain within clinical findings.

Protein constituent fractions have generally not been considered in lubricant specifications recommended by wear testing standards [6, 26, 172]. The protein constituent fractions in some commercially available, undiluted serums have been shown to differ significantly from OA-SF [20]. This is concerning, as polyethylene wear rates obtained *in vitro* can be influenced by the protein composition of the test lubricant [11, 16, 19, 20, 46, 104-106]. These lubricants may consist of protein concentrations that are not seen clinically [9, 20, 46, 95]. More recently, certain distributions of protein constituents in some commercially available bovine serums were found to significantly influence wear rates [19].

The main protein constituents present in OA-SF and PP-SF for both hip and knee joints were identified and quantified, and were found to be comparable to levels previously reported for OA-SF from knee joints [20] (Table 5.1). While no significant difference was detected between OA-SF and PP-SF obtained from hip joints in the present study, significant differences were found between the albumin and β-globulin content in OA-SF and PP-SF from knee joints: albumin levels appeared to be lower in PP-SF, accompanied by higher β-globulin levels. These differences may be explained by patient-to-patient variability, although these differences may have also been amplified due to the relatively small sample size in each patient group (n = 10 per group).

The protein constituent fraction data collected in the present study may contribute to the development of a more clinically relevant wear testing lubricant. A similar biochemical study was performed by Brandt et al. [20], where the constituents present in OA-SF were further compared to the constituents present in some commercially available calf serums. Results from that biochemical study showed that the protein constituent fractions in bovine calf serums were quite different from OA-SF, while constituents in alpha calf serums were much closer to clinical findings. A subsequent study showed that the distribution of these protein constituents significantly influenced wear rates in hip [105] and knee simulator wear testing [19]. The influence of these constituents on wear rates in pin-on-disc wear testing have yet to be investigated.

**Table 5.1: Summary of the protein composition in osteoarthritic and periprosthetic synovial fluid obtained from hip and knee joints.**

| | Osteoarthritic | | Periprosthetic | | Historical Data | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Osteoarthritic | | Periprosthetic | |
| | Hips | Knees | Hips | Knees | Hips | Knees | Hips | Knees |
| **Protein** | 32.35±4.25 | 26.61±2.75 | 33.00±6.09 | 29.56±7.17 | 31.8±1.5 [12] | 27±10*[9]<br>34.18±2.23 [20] | 34.4±1.9 [12] | 34±13 [9] |
| **Albumin** | 69.67±2.53 | 74.40±1.79 | 69.93±5.36 | 70.59±2.75 | - | 67.82±1.63 [20] | - | - |
| **α1-globulin** | 2.00±0.29 | 2.21±0.25 | 2.04±0.14 | 2.28±0.27 | - | 4.00±0.22 [20] | - | - |
| **α2-globulin** | 6.97±0.76 | 5.14±0.57 | 6.01±0.94 | 6.02±1.00 | - | 6.80±0.48 [20] | - | - |
| **β-globulin** | 11.26±2.31 | 8.32±0.68 | 12.70±4.49 | 10.68±1.16 | - | 10.81±1.16 [20] | - | - |
| **γ-globulin** | 10.10±1.13 | 9.93±1.34 | 9.32±1.07 | 10.43±1.26 | - | 9.79±0.93 [20] | - | - |

Average protein concentrations from the present thesis and historical data are given in g/L with 95% confidence intervals, unless otherwise stated. Constituent fractions for albumin, α1-globulin, α2-globulin, ß-globulin, and γ-globulin are given as percentages with 95% confidence intervals. References for historical values are denoted by superscripts.

* Standard deviations given in lieu of 95% confidence intervals.

### 5.2.3 Synovial Fluid Thermal Stability

A study by Pico et al. [110] had shown that the ionic strength of a solution contributed to its thermal stability. SF is a naturally protein-rich lubricant that contains solutes that may not be present in bovine serum-based lubricants; these bovine serum-based lubricants are often diluted with distilled water to reach certain protein concentrations, and may not be as thermally stable as SF. A biochemical study by Brandt et al. [20] had shown that bovine serum diluted with distilled water had only half of the osmolality of OA-SF, while 100% bovine serum and bovine serum diluted with phosphate-buffered saline solution (PBS) were comparable to clinical levels. These findings suggested that the nature of the dilutive media had an effect on the thermal stability of a wear testing lubricant, in addition to its clinical relevance.

In the present thesis, osmolality measurements, which were obtained in triplicate, were found to be quite reproducible for each sample. No significant differences were found between all OA-SF and PP-SF samples (Figure 4.4). The osmolality of all SF samples obtained in the present study were found to be nearly 2-times higher than the osmolality of bovine serum diluted with distilled water [20]. The osmolality of all OA-SF and PP-SF samples was found to be 280.84 ± 4.43 mmol/kg, which is of similar magnitude to the 311.25 ± 8.21 mmol/kg [20] and the 297.3 ± 16.93 mmol/kg [170] reported in previous studies for OA-SF obtained from knee joints (Table 5.2). Small variances between the present data and historical data may be attributed, again, to patient-to-patient variability, since osmolality has been shown to fluctuate with physical activity

[173] and disease [170]. Minor differences in measurement protocols may also contribute to the differences between the present data and historical data.

The ability for proteins to resist degradation at high temperatures can play a significant role in how bearing materials perform and behave during wear testing, since precipitated proteins can increase wear by acting as abrasive particles [105, 106]. There are various ways to measure the thermal stability of proteins present in solutions. Liao et al. [105] performed heating experiments on a 90% bovine serum-based lubricant diluted with PBS in lieu of distilled water and found that little or no degradation occurred at temperatures below 60°C. Other studies [18, 20] performed DSC measurements on OA-SF and different wear testing lubricants to determine and compare transition temperatures, enthalpies, and entropies.

In the present study, the thermal stability of OA-SF and PP-SF from both hip and knee joints was determined through DSC measurements in order to better develop the clinical range found in previous studies [18, 20]. Single DSC measurements were performed on each SF sample due to low volumes collected for certain patient groups. For all SF samples, the mean Tm1 was 68.3 ± 1.76°C for all SF samples (Table 5.2), and was not found to be statistically different between OA-SF and PP-SF for both hip and knee joints. These results show that 50% of the proteins present in OA-SF and PP-SF have degraded at this temperature, regardless of the joint. This result appeared to be consistent with the results presented by Liao et al. [105] for 90% bovine serum diluted with PBS. pH levels for all SF samples were not measured due to low volumes obtained, and was considered a limitation of the present thesis.

DSC measurements performed by Brandt et al. [20] showed that, despite having similar ionic strengths to OA-SF, Tm1 obtained from bovine serum diluted with PBS was considerably lower than Tm1 obtained from OA-SF. These results suggest that there may be additional constituents that affect thermal stability, such as the presence of hyaluronic acid (HA). HA is a main constituent of SF that contributes to its viscosity [89, 91, 95], and is known to affect the thermal stability of proteins [174, 175]. In the studies by Brandt et al. [20] and Brière et al. [18], DSC measurements were performed on bovine serum diluted with PBS with the addition of HA. Tm1, $\Delta H$, and $\Delta S$ obtained from this lubricant were found to be similar to those obtained from OA-SF [18, 20]. It was suggested that the thermal stability was more similar to clinical findings due the presence of HA [20]. The molecular weight and concentration of HA present in OA-SF and PP-SF were not determined in the present thesis, and was considered a limitation of the present thesis.

**Table 5.2: Summary of osmolality and thermal properties in osteoarthritic and periprosthetic synovial fluid obtained from hip and knee joints.**

| | Osteoarthritic | | Periprosthetic | | Historical Data | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Osteoarthritic | | Periprosthetic | |
| | Hips | Knees | Hips | Knees | Hips | Knees | Hips | Knees |
| **Osmolality** | 277.2 ± 8.68 | 289.3 ± 11.79 | 272.47 ± 8.06 | 284.4 ± 7.07 | - | 311.25 ± 8.21 [20]<br>297.30 ± 16.93*[170] | - | - |
| **Tm1** | 64.32 ± 1.58 | 63.32 ± 0.25 | 63.84 ± 0.91 | 63.81 ± 0.59 | - | 63.69 ± 0.98 [18, 20] | - | - |
| **Tm2** | 67.75 ± 1.71 | 67.94 ± 1.35 | 65.70 ± 3.42 | 67.55 ± 2.63 | - | 69.21 ± 0.74 [18, 20] | - | - |
| **Tm3** | 74.91 ± 2.47 | 74.37 ± 1.20 | 70.92 ± 2.94 | 73.75 ± 2.09 | - | 74.80 ± 0.98 [18, 20] | - | - |
| **Enthalpy, ΔH** | 1662 ± 316 | 1371 ± 187 | 1286 ± 296 | 1497 ± 470 | - | 958.45 ± 133 [20] | - | - |
| **Entropy, ΔS** | 4.92 ± 0.93 | 4.08 ± 0.56 | 3.81 ± 0.87 | 4.44 ± 1.39 | - | 2.84 ± 0.39 [20] | - | - |

Average osmolality, temperature midpoints (Tm1, Tm2, and Tm3), enthalpies (ΔH), and entropies (ΔS) from the present thesis and historical data are given in mmol/kg, °C, kJ/mol, and kJ/mol·K, respectively, with 95% confidence intervals, unless otherwise stated. References for historical values are denoted by superscripts.

* Standard deviation given in lieu of 95% confidence intervals.

# 5.3 *In Vitro* Wear Testing

## 5.3.1 Introductory Remarks

Two types of *in vitro* wear tests were performed in the present thesis: validation wear testing, and lubricant investigations. The purpose of the validation POD tests was to ensure that the wear rates produced by the newly installed OrthoPOD were comparable to historical wear rates generated by DePuy Synthes internally using the same test parameters and conditions. Three types of PE pins were used in order to establish the effect of wear on non-crosslinked CPE, and the moderately crosslinked XLK and Marathon. A BCS-based lubricant was used, which was diluted to a protein concentration of 54g/L with a DW solution containing EDTA and SA.

In the lubricant investigations, the same test parameters and conditions were used; however, only XLK and Marathon pins were used since CPE is no longer widely used as a bearing material in TJRs. In addition, a unique approach to POD testing was utilized, where the effect of lubricant composition on wear was assessed in a step-wise fashion. Since a previous study [20] had found that ACS was more comparable to OA-SF, the lubricant investigations utilized an ACS-based lubricant diluted with various media to a concentration of 34g/L.

### 5.3.2 Polyethylene Fluid Absorption

Pre-test soak controls were implemented prior to each wear test. In the validation tests, PE pins were soaked, undisturbed, for a minimum of 30 days in DW. Brandt et al. [154] had found that increasing the soak temperature to 37°C and repeatedly disturbing the PE tibial inserts increased the amount of fluid absorbed. Due to the delayed delivery of the PE pins used in the lubricant investigation wear tests, the PE pins were pre-soaked for a minimum of 2 weeks at 37°C with continuous agitation.

Results from these tests showed that CPE pins had the highest fluid uptake in comparison to the moderately crosslinked XLK and Marathon pins within the same time interval. No significant difference was found in the fluid absorption of XLK and Marathon pins ($p = 0.211$, Mann-Whitney U); however, in the lubricant investigations, XLK pins appeared to absorb nearly two-times more fluid than Marathon pins, which was found to be significantly different ($p = 0.001$, Mann-Whitney U). It is possible that this significant difference in fluid absorption was more apparent when continuous agitation and higher soak temperatures were implemented [154]. Nonetheless, the fluid absorbed by XLK and Marathon pins for the lubricant investigations were of the same magnitude as the XLK and Marathon pins used in the validation tests. This suggested that pre-soaking PE pins in DW at a higher temperature with continuous agitation can result in similar fluid uptake as pre-soaking for 30 days at room temperature, undisturbed.

When the fluid uptake from the pre-test soak controls (Figure 4.7) was compared to the soak tests performed during the validation wear tests (Figure 4.8), it was found that fluid absorption decreased during testing. This may have been caused by changing the

soaking fluid from DW in the pre-test soak control to the BCS lubricant during wear testing. This reduction in fluid absorption is consistent with the findings of previous studies [17, 154]. The amount of fluid absorbed during the soak tests had little effect on the resulting wear rates of XLK and Marathon, and was therefore not reported for the lubricant investigation wear tests; however, in following standard protocols [165], the mass gains and losses obtained from the soak test PE pins were accounted for in the wear rate calculations.

### 5.3.3 Wear Rates

For both the validation tests and lubricant investigations, the wear rates produced between each station for each interval were not found to be significantly different ($p \geq 0.885$; GLM-Tukey), which indicates proper function and consistent setup of the POD apparatus. The same PE pins and CoCr discs remained with the same station throughout each test, as it had been found that rotating TKR components between knee simulator wear stations had a significant effect on wear rates obtained for each interval [176].

In the validation tests, CPE pins were found to have the highest wear in comparison to XLK and Marathon pins. CPE wear rates were over 2-times higher than the wear rates produced by XLK and Marathon, which shows that even the moderate crosslinking of PE can have significant improvements in material properties and wear resistance [52]. An attempt was made to compare the wear rates obtained from the validation tests with similar studies; however, it was difficult due to the vast array of test parameters and conditions implemented by other studies (Appendix A). In addition, wear rates from POD

tests were often not reported; wear factors, which are based on Archard's wear law [34], account for loading and sliding distance, and are reported in many studies (Appendix A.3). In the present thesis, wear rates are reported in mg/Mc in order to stay consistent with the standard practices of DePuy Synthes.

A comparison between the validation results for XLK and Marathon and two previous studies could be made. In a POD study by Dressler et al. [55], XLK was found to have a wear rate of approximately 4.6 mg/Mc at a sliding distance of 40mm using 90% bovine serum; however, the present thesis found a much higher wear rate of 6.01 mg/Mc at the same sliding distance using 75% bovine serum (54 g/L). Differences between these wear rates may be due to the type of loading implemented in each test: constant loading was used by Dressler et al. [55], and dynamic loading was implemented in the present thesis. A POD study by Yao et al. [16] explored the effect of crosslinking on GUR 1050; however, the wear rates obtained from the validation tests in the present thesis were nearly five-times higher than those obtained for crosslinked GUR 1050. It is possible that the PE used by Yao et al. [16] was highly crosslinked (10 Mrad) rather than moderately crosslinked (5 Mrad), and the use of constant loading may have also contributed to the lower wear rate found in their study.

New and existing PE materials are often ranked based on their resistance to wear: a higher resistance to wear increases the material's ranking. XLK has historically been found to have slightly higher wear rates than Marathon due to the differences in resins and associated mechanical properties, and therefore, it is ranked lower than Marathon. Fisher et al. [177] compared crosslinked GUR 1020 (2.5-4 Mrad) against crosslinked GUR 1050 (5 Mrad) and found that GUR 1020 produced a wear rate that was two-times

higher than GUR 1050. XLK is manufactured from a GUR 1020 resin, which is crosslinked with an irradiation dose of 50 kGy (5 Mrad). Because of its lower molecular weight (3.5 x $10^6$ g/mol), GUR 1020 has a higher crystallinity [77]; it is also more ductile, and has improved toughness, making it more resistant to fatigue cracking [178, 179]. On the other hand, its lower molecular weight decreases its abrasive wear resistance. Marathon is manufactured from a GUR 1050 resin, and is also crosslinked with a dose of 50 kGy (5 Mrad); however, due to its higher molecular weight (5.5-6 x $10^6$ g/mol), it has an improved resistance to abrasive wear [77]. In the validation tests, XLK produced a wear rate that was 1.2-times higher than Marathon (Table 5.3), which is consistent with the historical data from DePuy Synthes; however, the ranking of XLK and Marathon was more prominent in the lubricant investigation wear tests. The PBS lubricant showed the largest difference in wear rates between XLK and Marathon, where the average wear rate for XLK was more than 3-times higher than the average wear rate for Marathon (Table 5.3). This noticeable difference may be due to a combination of mechanical properties and lubricant composition. To the author's knowledge, this is the first study that has shown the effect of lubricant composition on the ranking of PE materials.

**Table 5.3: Summary of average wear rates for XLK and Marathon, and the difference in magnitude between them.**

| | Lubricant | PE Material | Average Wear Rate (mg/Mc) | Difference in Magnitude |
|---|---|---|---|---|
| Validation | BCS | XLK | 6.008 | 1.2 |
| | | Marathon | 4.987 | |
| Lubricant Investigation | PBS | XLK | 0.814 | 3.2 |
| | | Marathon | 0.257 | |
| | HA | XLK | 0.254 | 1.7 |
| | | Marathon | 0.149 | |
| | 2HA | XLK | 0.248 | 1.7 |
| | | Marathon | 0.147 | |
| | DW | XLK | 3.120 | 2.0 |
| | | Marathon | 1.596 | |

The lubricant investigation wear tests successively compared the effect of different lubricant compositions on PE wear, which is a unique approach that had not been used previously in any POD studies. Throughout each lubricant investigation test, the same PE pins and CoCr discs were used in order to eliminate variables that may be associated with using brand new materials. Of the four types of lubricants used, the DW lubricant produced the highest wear rates, followed by the PBS lubricant for both XLK and Marathon. The higher wear rates produced by the DW and PBS lubricants may be explained by the biochemical nature of the lubricants, which will be discussed subsequently in Section 5.3.6. For XLK, both the DW and PBS lubricants were found to be significantly different from each other, and from the HA and 2HA lubricants ($p \leq 0.018$, GLM – Tamhane's T2; Figure 4.27). In contrast, there was no significant difference between the Marathon wear rates produced from the PBS and the HA

lubricants, and the PBS and 2HA lubricants ($p \geq 0.372$, GLM – Tukey; Figure 4.28). This discrepancy may be explained by the material properties of XLK, where XLK is less resistant to abrasive wear, and would therefore be more influenced by the effects of different lubricant compositions.

Interestingly, the wear rates for the HA and 2HA lubricants were not found to be significantly different for both materials ($p \geq 0.999$, GLM – Tamhane's T2 and Tukey), which suggests that changing the HA concentration present in the lubricant, and therefore changing the viscosity of the lubricant, have little to no effect on PE wear. This effect is consistent with the a hip joint simulator wear test [24] and a POD test [25] that added HA to 90% ACS and 33% bovine serum, respectively. These studies showed that the addition of HA had no significant effect on wear rates; however, the same cannot be said for knee simulator wear testing. Desjardins et al. [22] showed that the addition of 1.5 g/L of HA to 50% bovine serum had a seven-fold increase on PE wear. Brandt [23] had also found an increase in wear by adding HA to the wear testing lubricant, although a two-fold increase was observed. Joyce [25] suggested that the presence of HA may have a more significant impact on wear under fluid film lubrication conditions, and little effect on the boundary lubrication conditions that are present in POD tests. On the other hand, boundary lubrication exists in knee simulator wear testing, where Desjardins et al.[22] and Brandt [23] found a significant increase in wear with the addition of HA. The divergence of the findings in the present thesis and previous studies from the findings by Desjardins et al. [22] and Brandt [23] can be explained by knee joint kinematics, where tractive rolling and its associated forces are absent from POD and hip simulator wear testing. The

exploration of HA in lubricants, and its interaction with tractive rolling will be discussed subsequently in Section 5.3.6.2.

## 5.3.4 Surface Characterization

### 5.3.4.1 Polyethylene Surface Damage

Burnishing appeared to be the dominant surface damage on the worn polyethylene surfaces for both the validation wear tests in Section 4.3 and the lubricant investigation wear tests in Section 4.4. In the validation tests, worn CPE pins appeared to have the most distinctive surface damage characteristics. Irregularly-shaped large-scale protrusions were visible macroscopically, and at higher magnifications, ripples were observed on the surface of these protrusions. The formation of large-scale protrusions were also found on the surface of worn XLK pins, although the rippling effect was absent from the surface of the protrusion. The shape of the protrusions on XLK also appeared more round than the protrusions on worn CPE. Similar protrusions were observed by Scholes et al. [111] when an egg white-based lubricant with a protein concentration of 11 g/L was used; however, the type of PE used in the wear test was not reported. Milder protrusions were evident on the surface of Marathon; however, under the SEM, these protrusions appeared to be smeared on the surface. At x2000 magnification, PE grains appeared to be pulled away from the surface.

In the lubricant investigation wear tests, the same large-scale protrusions were observed on the surface of worn XLK pins. Using the SEM, higher magnifications showed that what was initially perceived as a smooth surface was actually more granular

in appearance. It appeared as though PE grains were being pulled out during the wear process. In contrast, burnishing dominated the surface of the worn Marathon pins. SEM images showed that the PE grains were smeared on the surface; grain boundaries were also visible on the worn surface at x2000 magnification (Figure 4.30f). The differences observed in wear damage suggest that the higher ductility of XLK influenced its ability to resist wear under adhesive-abrasive conditions.

In order to assess the clinical relevance of the surface damage see on worn Marathon pins, retrieved Marathon acetabular cups were selected from the Implant Retrieval Database at the Orthopaedic Innovation Centre in Winnipeg for comparison; retrieved Sigma P.F.C. tibial inserts, which are made from XLK, were not yet available in the Implant Retrieval Database, and so no comparison could be made to the worn XLK pins used in the present thesis. Two acetabular cups were selected based on implantation period. Case 1575 had been implanted for 8 years (Figure 5.1a), and case 1312 had an implantation period of 5 years before being retrieved (Figure 5.1b). Macroscopic images were taken, which revealed the presence of scratching and burnishing.

At x40 magnification, SEM images of both acetabular cups showed PE grains that were smeared in the direction of sliding, similar to that observed on worn Marathon PE pins in the lubricant investigation wear tests (Figure 5.2).

PE grain boundaries were also observed on the acetabular cups and on the Marathon PE pins from Test 5; however, these distinct PE grain boundaries were not observed on the Marathon PE pins from validation Test 3 (Figure 4.19f). This may suggest that the ACS lubricants are better able to produce similar surface damage to that observed from retrieved Marathon acetabular cups.

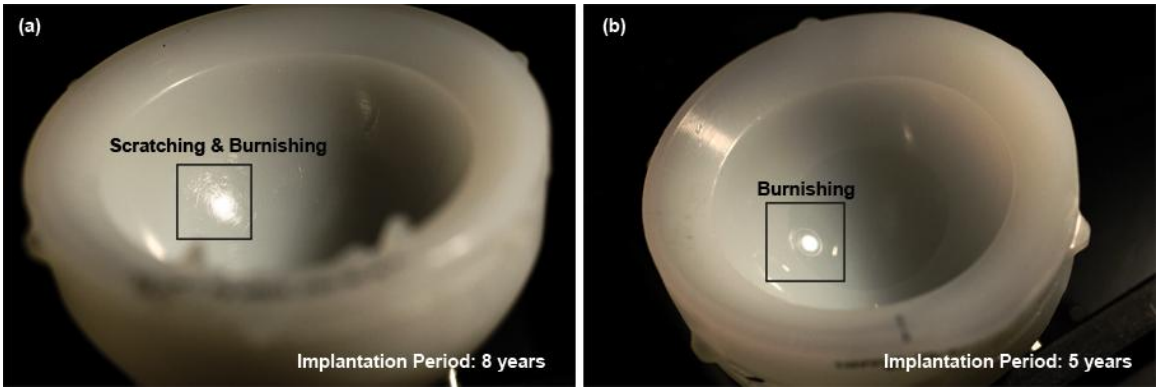


**Figure 5.1: Surface damage of retrieved Marathon acetabular cups. (a) Case 1575 shows scratching and burnishing after 8 years of implantation. (b) Case 1312 shows pure burnishing after 5 years of implantation.**

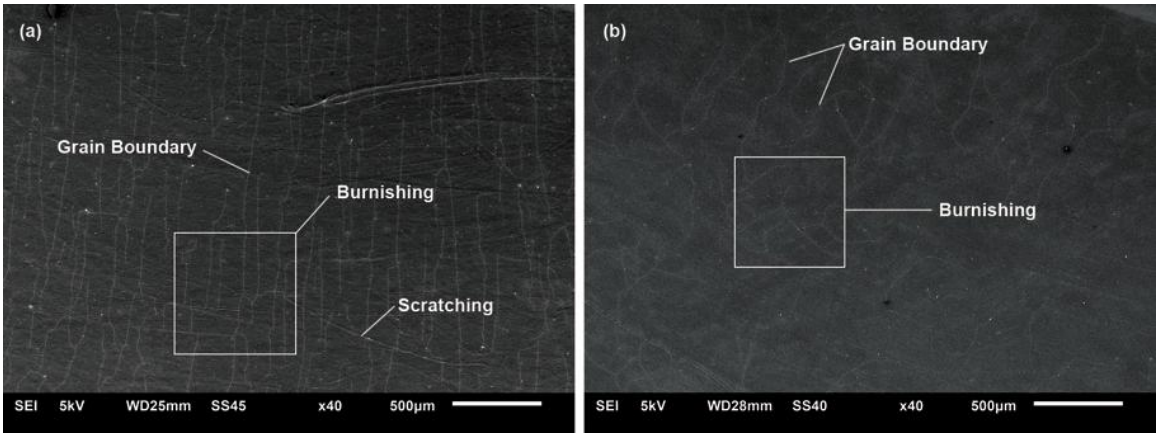


**Figure 5.2: SEM images of retreived Marathon acetabular cups at x40 magnification. (a) Case 1575 (implantation period: 8 years) shows burnishing and light scratching. (b) Case 1312 (implantation period: 5 years) shows pure burnishing. Both cases show the smearing of PE grains and distinct grain boundaries.**

#### 5.3.4.2 Surface Roughness

A rough counterface can often be an indicator of increased wear, although as explained in Section 2.4.2.2, other factors can affect the influence of surface roughness on wear. Lubricant composition can define the presence of clinically relevant damage on a CoCr counterface, as seen by Cooper et al. [146], where protein-rich lubricants can eliminate the presence of PE transfer films on CoCr surfaces; these PE transfer films are not observed on retrieved TJRs, and can produce wear rates that are not seen clinically [146]. At a macroscopic level, these PE transfer films were also absent from the worn CoCr discs used in the present thesis due to the use of bovine serum-based lubricants.

The wear path shape can also influence the impact of surface roughness on wear. Differences between the unworn and worn counterface roughness are greater for rectangular wear paths than for square wear paths [148]. This may partially explain the lack of significant differences in certain roughness parameters obtained from the validation and lubricant investigation wear tests, where a square wear path was implemented; however, examining the roughness parameters more closely may provide additional insight.

Ra is the average roughness of a surface, and while it is the most commonly reported roughness parameter, it provides no information on the topography of the surface, i.e. the presence of peaks and valleys. These surface features have a significant effect on the wear of TJRs, where the presence of peaks can increase adhesive and abrasive wear, and the presence of valleys can improve the wettability of a surface [151]. Therefore, in addition to Ra, the present thesis reported Rz (difference in height between the five

highest peaks and the five lowest valleys) and $Rp_{max}$ (maximum peak height). $Rv_{max}$, which is the maximum valley depth, was not reported since it has been found that the presence of peaks is more prevalent on CoCr surfaces, and has a greater effect on PE wear [151].

In validation Test 1 (CoCr-CPE), there was no significant difference between the unworn and worn Ra parameters; however, Rz and $Rp_{max}$ reveal that significant changes to the surface topography did occur, where Rz and $Rp_{max}$ increased after 1.98 Mc ($p < 0.001$, paired t-test; Figure 4.14). This suggests that some extent of material removal occurred in order to increase the valley depth; SEM images show that carbide particles may have been removed during the wear process, and this may explain the increase in Rz and $Rp_{max}$ (Figure 4.17d). In addition, surface damage may have also been caused by corrosion byproducts due to tribocorrosion processes [82, 180].

Roughness parameters obtained in validation Test 2 (CoCr-XLK) showed the opposite effect, where Ra and Rz significantly decreased after 1.98 Mc of wear testing ($p < 0.001$, paired t-test; Figure 4.15). $Rp_{max}$, however, appeared to increase after 1.98 Mc ($p = 0.047$, paired t-test; Figure 4.15). This may be due to the presence of vibrations when the initial roughness parameters were being obtained from the unworn CoCr surface. Although the profilometer was situated on an air table, at the time of the present thesis, its temporary location the third floor of the Orthopaedic Innovation Centre subjected the profilometer to additional vibrations. It is quite possible that the initial roughness parameters would be lower in the absence of these vibrations.

In validation Test 3 (CoCr-Marathon), no significant differences in Ra and Rz were observed after 1.98 Mc of wear testing; however, $Rp_{max}$ increase significantly ($p < 0.001$,

paired t-test; Figure 4.16). While there were non-significant increases in Rz, it appears that $Rp_{max}$ may be sensitive to even the slightest material removal.

Changes in surface roughness parameters obtained from the lubricant investigation wear tests showed some discrepancies between CoCr discs used in Tests 4 and 5. Based on the higher wear rates obtained with the DW lubricant, it was expected that the CoCr surface would have higher peaks, essentially becoming rougher. While this appeared to be true for $Rp_{max}$ in both tests, these changes were not significantly different ($p \geq 0.127$, GLM). In addition, no discernible pattern was observed between Tests 4 and 5 when comparing the surface roughness parameters obtained from each lubricant interval: while Ra appeared to decrease following the 2HA lubricant interval in Test 4, Ra increased in the same lubricant interval during Test 5. Evidently, some aspects of the surface topography changed, although reasons for these differences remain inconclusive, although it is suspected that the method of reporting the surface roughness values in the present thesis may contribute to these uncertainties. The roughness values reported were averaged across all measurements taken from all unworn and worn discs used in each wear test. Because all of these measurements were averaged for each test, any discernible patterns may have been obscured. By comparing the change in roughness of each disc individually, it is possible that the effect of different lubricant compositions on surface roughness can be made more apparent.

#### 5.3.4.3 Re-Melting Polyethylene

Due to the low wear rates observed from the lubricant investigation wear tests (Section 4.4), XLK and Marathon pins were re-melted in order to qualitatively evaluate the extent

of PE wear. Previous studies [167, 168] had shown that burnished surfaces of worn PE components may be due to plastic deformation rather than wear. To confirm this, PE components were slowly heated and re-melted. The reappearance of the original machining marks indicated that the burnished surface was mainly due to plastic deformation and some wear.

In the present thesis, machining marks reappeared on the surface of the worn Marathon pin, which suggests that the wear damage previously observed was partially due to creep and small amounts of wear; however, no machining marks appeared on the surface of the re-melted, worn XLK pin, and so it can be concluded that the initial damage seen on the worn XLK pin was indeed due to wear. Clearly, this re-melting experiment provides additional evidence that XLK wears more than Marathon under adhesive-abrasive wear conditions using ACS lubricants.

### 5.3.5 Microbial Contamination

SA is the most common microbial inhibitor used in wear testing lubricants; however, its efficacy has recently come under debate [171, 176, 181], where efforts were made to find an alternative microbial inhibitor. In the validation tests, 0.2% wt. of SA was used in the BCS lubricant to determine its effectiveness during wear testing. In the lubricant investigation wear tests, AA was used since the AA solution had shown success in eliminating microbial growth to some extent, but was generally more effective than SA [171].

SA appeared to be ineffective at preventing the growth of gram-positive bacteria, such as *S. aureus*, during the first validation test. Surprisingly, subsequent validation tests showed no growth in the unworn and worn BCS lubricants, which may indicate that either SA was effective at preventing microbial growth, or the present microbial analysis may not have detected any growth. The growth found in validation Test 1 may have been eliminated by implementing gradual improvements to the equipment sterilization techniques. The use of alcohol and disinfectant wipes to clean every piece of equipment used during the wear tests, and the development of more thorough laboratory cleaning protocols appeared to contribute to the lack of growth in validation Tests 2 and 3.

Some strains of staphylococci are resistant to methicillin, a penicillin class of narrow-spectrum antibiotic that is effective against certain families of bacteria; these bacteria are known as Methicillin-Resistant *Staphylococcus aureus* (MRSA), which can make treatment difficult for patients with infections. The presence of staphylococci in the lubricants is concerning because of the potential for this bacteria to become resistant to such specific antibiotics and, in the case of the present thesis, SA. The other types of organisms identified in the unworn BCS lubricant were not considered concerning (Table 4.3).

AA is less toxic than SA, and appears to be a more broad-spectrum antibiotic based on its solution containing penicillin, streptomycin, and Fungizone B. Brandt et al. [171] initially had some success with the use of AA in the wear testing lubricants; however, its efficacy diminished with continued wear testing. Similar results were obtained in the present thesis, where no growth was evident in the unworn ACS lubricants; however, when worn samples were obtained after each lubricant interval, AA appeared to be less

effective, suggesting that the organisms grown in the lubricant had quickly become resistant to even a more broad-spectrum antibiotic, as shown in a previous study. This was evident in the growth of a bacillus species, which appeared consistently in every worn ACS lubricant sample despite the type of dilutive media, and the implementation of improved cleaning and sterilization techniques using alcohol and disinfectant wipes.

In the study by Brandt et al.[19], the organism *E. cloacae* JK-1 was identified in the wear testing lubricant; however, the present thesis identified different species of bacteria in the wear testing lubricants, which suggests that the type of organism grown differs between laboratories. Because of this, identifying the organism grown in the wear testing lubricant will allow researchers to use an appropriate, and perhaps less toxic antimicrobial agent in their wear testing lubricants. Previous studies [171, 182] recently found that microbial growth may have an effect on friction and wear rates; however, the mechanism of this effect remains uncertain.

### 5.3.6 Biochemical Analyses of Wear Testing Lubricants

#### 5.3.6.1 Protein Concentration and Degradation

Since the use of human SF in *in vitro* wear testing is not feasible, mainly due to volume and ethical concerns [9, 15, 16], BCS and ACS-based lubricants were used in the present thesis in order to investigate the effect of lubricant composition on wear. A BCS lubricant, which was diluted with DW to a protein concentration of 54 g/L (Figure 5.3), was used in order to validate the POD apparatus to similar test parameters and conditions

as DePuy Synthes; this protein concentration was considered to be an approximation of the 90% BCS lubricant commonly used by DePuy Synthes.

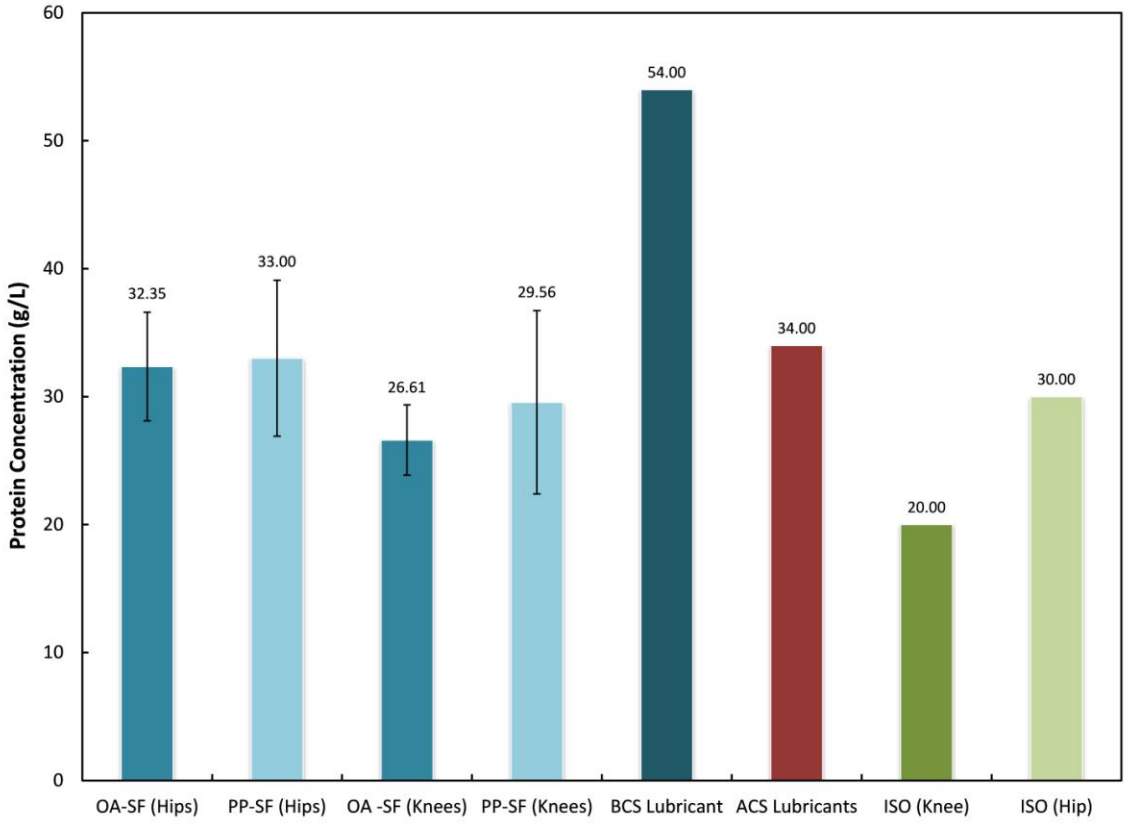


**Figure 5.3: Summary of protein concentrations found in the clinical investigation, and the target protein concentrations used in the wear testing lubricants of the present thesis. Diluted lubricant protein concentrations currently recommended by the ISO [2, 3] have also been included for comparison. ASTM recommendations were excluded due to the wide range of protein concentrations resulting from the specification.**

Protein composition is known to have a significant effect on *in vitro* wear [9, 16, 17, 19, 20, 46, 105, 107], and because of this, it is important to match physiological

conditions as closely as possible [9, 12, 20, 46]; however, recommendations made by some current wear testing standards [2, 6] may not reflect clinical data (Figure 5.3). Because of this discrepancy, these wear testing standards should be adjusted accordingly. ACS was selected as the base for the lubricant investigation wear tests, as it had been found to be more comparable to OA-SF in comparison to other commercially available sera [20]. ACS was diluted with either DW or PBS to a concentration of 34 g/L (Figure 5.3) based on the clinical data obtained by Brandt et al. [20]. Clinical protein concentrations obtained from the present thesis (Section 4.2) were not used, since the clinical study and the wear tests were performed concurrently; however, the 4 g/L difference between the clinical protein concentrations found by Brandt et al. [20] and by the present thesis was not considered to be substantially different.

Current practices for preparing lubricant mixtures were deemed accurate and consistent, where resulting lubricant protein concentrations were within approximately 1% of target protein concentrations. The BCS lubricant appeared to have the greatest protein degradation in comparison to the ACS lubricants used in the present thesis (Figure 5.4). From a wear testing perspective, protein degradation can accelerate wear [106] due to the high temperatures and friction produced at the CoCr-PE interface [83]. Physiologically, protein degradation may also occur, although these degraded proteins may be continually flushed out when SF is replenished by the body [114]. This continuous state of homeostasis implies that excessive protein degradation in *in vitro* wear testing lubricants may not be desirable, or clinically relevant.

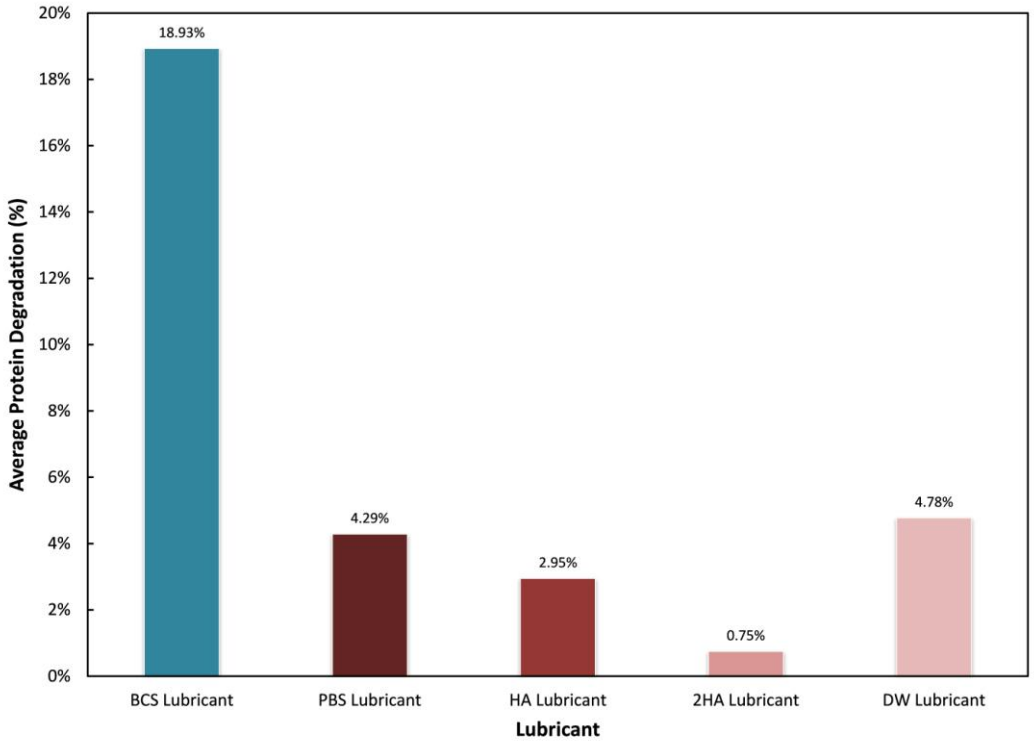


**Figure 5.4: Summary of protein degradation in the wear testing lubricants used in the present thesis. Note that the ACS lubricants generally had lower protein degradation. Of the ACS lubricants, the 2HA lubricant had the least amount of protein degradation.**

Generally, the ACS lubricants used in the present thesis were found to have significantly lower protein degradation than the BCS lubricants used in the validation tests (Figure 5.4). This may be explained by the lower protein concentrations in the ACS lubricants. Liao et al. [105] found that the degradation of lubricants containing lower protein concentrations were less likely to degrade than lubricants at higher protein concentrations at increased temperatures; however, wear rates obtained from a 40% serum-based lubricant were comparable to wear rates from a 90% serum-based lubricant, even though the protein degradation was markedly lower in the 40% lubricant. Evidently, there may be more factors that influence protein degradation.

Different types of commercially available calf sera contain different distributions of proteins which may not match the distribution found in SF [20]. Electrophoretic analysis was performed on the wear testing lubricants used in the present study, and were compared to the average distribution in the OA-SF and PP-SF samples obtained in Section 3.2 (Figure 5.5). The distribution in the BCS and ACS lubricants were found to be quite different, where the distributions found in ACS lubricants were more comparable to OA-SF and PP-SF in comparison to the BCS lubricants (Figure 5.5).

Furthermore, the degradation of these proteins also appeared to be different (Figure 5.6). For the purposes of comparison, the average protein constituent degradation was used, even though there were some differences in degradation existed between Tests 4 and 5. This may be due to how these samples were collected. Differences in degradation between Tests 4 and 5 may be due to the fact that unworn and worn lubricant samples were obtained at the beginning and end of the lubricant interval. Instead, worn lubricant samples should be collected from the same batch of lubricant used to collected the unworn lubricant. Differences between batches of the same lubricant composition may result in inconsistent data.

γ-globulin appeared to consistently have the highest degradation in comparison to the other proteins present in the lubricants (Figure 5.6); therefore, it could be suggested that higher amounts of certain proteins, such as γ-globulin, may also increase protein degradation. Interestingly, the amount of degradation for each protein appeared to be affected by the type of dilutive media used in the lubricant, which was evident in the 2HA lubricant, where there was almost no degradation of certain proteins (Figure 5.6). An explanation for this will be discussed subsequently in the following section.

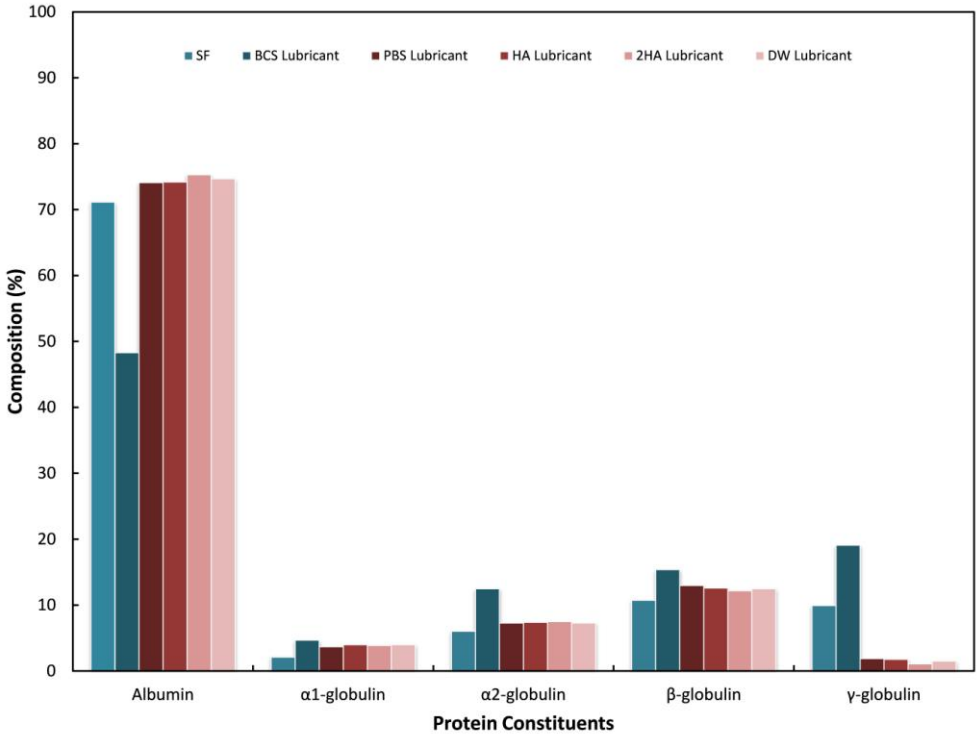


**Figure 5.6: Summary of protein constituent distributions in OA-SF and PP-SF, collectively, and the wear testing lubricants used in the present thesis. Note that the ACS lubricants are more comparable to synovial fluid.**

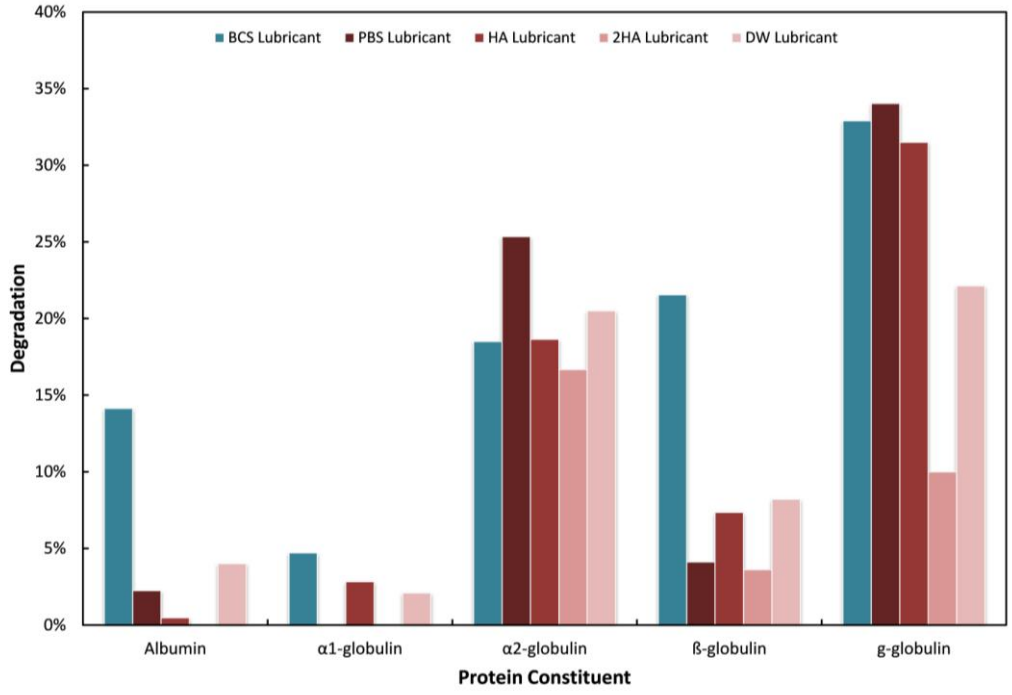


**Figure 5.5: Summary of protein constituent degradation observed after 0.33 Mc of wear testing for each lubricant used in the present thesis. Note that γ-globulins consistently had the highest degradation percentage. Also note that the 2HA lubricant had the least amount of protein constituent degradation.**

From a wear testing perspective, a more clinically relevant distribution of protein constituents had recently been found to decrease PE wear in knee simulator testing [19]. These protein constituents have also been found to affect the friction coefficient and morphology of the wear surface [11]. A previous study had shown that the lack of certain proteins in a wear testing lubricant may affect the boundary lubrication of TJR components [105], which may result in wear rates that are not clinically relevant.

EDTA was not added to the lubricants used in Tests 4 and 5. Historically, EDTA was added to lubricants to prevent the precipitation of metal ions in the bovine serum, which may affect PE wear [103]; however, the use of EDTA was deemed unnecessary due to the low amounts of metal ions present in ACS.

#### 5.3.6.2 Osmolality and Thermal Stability

The ionic strength of a solution had been shown to increase the thermal stability of proteins in a solution [109]. In the present thesis, changing the ionic strength was done in two ways: (1) by changing the dilutive media and (2) by adding HA.

The clinical investigation performed in the present thesis showed that the overall osmolality of OA-SF and PP-SF collectively was 280.84 ± 4.43 mmol/kg. Osmolality measurements were also obtained for the unworn lubricants used in the wear tests. These measurements ranged from 268 – 303 mmol/kg, and appeared to be dependent on the type of calf serum and dilutive media used (Figure 5.7). The BCS lubricant produced the lowest osmolality in comparison to the ACS lubricants. In addition, ACS lubricants diluted with PBS ultimately increased the ionic strength of the solution, and therefore the osmolality. Lubricants diluted with DW, however, had a lower osmolality than that

observed from the clinical investigation (Figure 5.7). Lubricants containing HA, however, had the highest osmolality, which indicates that the addition of HA, and the concentration of HA added to the lubricant has a significant effect on the ionic strength of a solution.

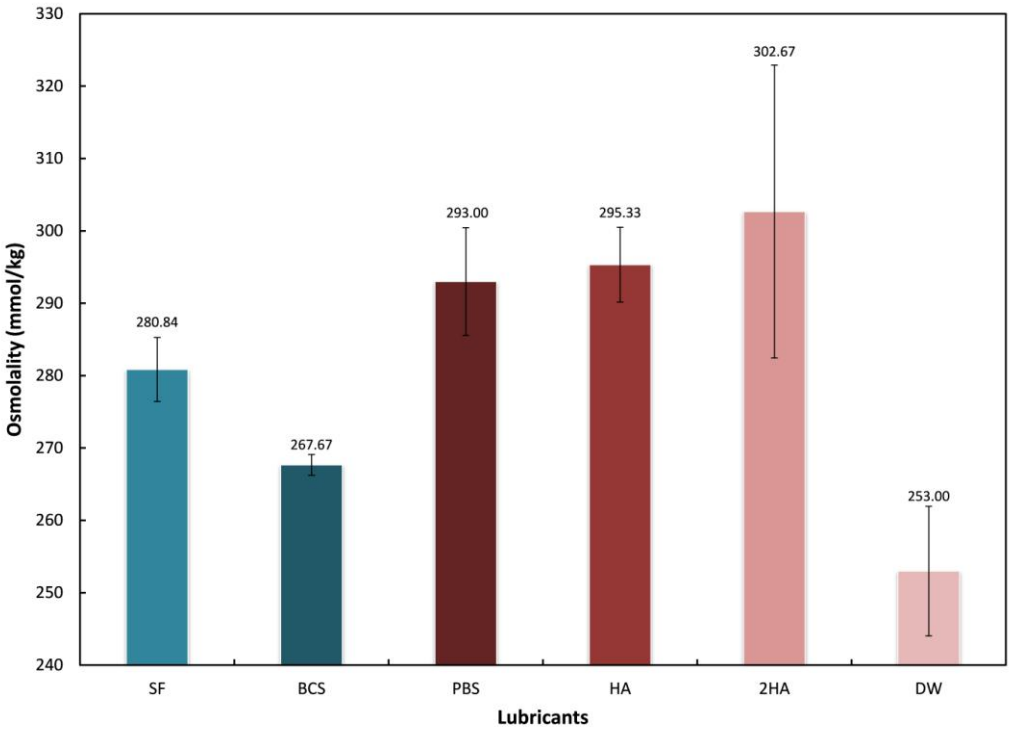


**Figure 5.7: Summary of average osmolality measurements obtained from OA-SF and PP-SF collectively, and the wear testing lubricants used in the present study. Note that the BCS lubricant and the DW lubricant had the lowest osmolality in comparison to the other wear testing lubricants. The 2HA lubricant had the highest osmolality.**

HA has the ability to retain $H_2O$ molecules [183], which essentially dehydrates the solution. This ultimately increases the ionic strength of the solution, and therefore, increases its thermal stability, which can be seen from the clinical investigation, where the energy required to degrade 50% of the proteins in the SF samples was quite high at 1422.5 ± 152.6 kJ/mol. To mimic this effect, a lubricant containing ACS, PBS, and HA was developed.

Tm1 was highest with the PBS lubricant, and lowest with the DW lubricant; however, based on these findings, the lower Tm1 for the HA and 2HA lubricants suggests that adding HA may actually lower the thermal stability of proteins in the lubricant. On the other hand, the 2HA lubricant was found to have the highest enthalpy and entropy in comparison to the PBS, HA, and DW lubricants (Figure 4.41-42), which is consistent with a previous study [20] that had shown that the addition of HA produced the highest and most clinically relevant enthalpy; however, the values obtained for the HA and 2HA lubricants were still lower than the study by Brandt et al. [20]. The results obtained in the study by Brandt et al. [20] determined the enthalpy of ACS lubricants at a protein concentration of 17g/L, which is half of the 34g/L protein concentration used in the present thesis. The lower 17g/L protein concentration may have enhanced the effect of HA on the ACS lubricant thermal stability. In a clinical study, Mazzucco et al. [9] had suggested that a negative correlation exists between protein concentration and HA concentration in OA-SF and PP-SF. This relationship may explain why the presence of HA did not appear to raise the thermal stability of the HA and 2HA lubricants to the higher clinical levels found in the present thesis (Figure 5.8) and in the study by Brandt et al. [20].

The discrepancy between the clinical and HA and 2HA lubricant thermal stability measurements obtained in the present thesis may also be explained by HA molecular weight distribution. In a previous study [184], OA-SF was found to contain HA at various molecular weights ranging from less than 0.5 MDa to 6.1 MDa, where the distribution was highest between 1.1 – 6.1 MDa. The present thesis utilized HA at a molecular weight of 1.56 MDa, which is within the clinical range; however, the absence of HA at the lower

and the higher molecular weights may also explain why the thermal stability measurements were lower than clinical levels (Figure 5.8). The higher molecular weight HA molecules may be able to retain more $H_2O$ molecules, thus increasing the ionic strength and the thermal stability of the solution. Incorporating a clinical distribution of HA into wear testing lubricants may be more representative of OA-SF and PP-SF; however, determining the concentration and molecular weight of HA in the SF samples was considered a limitation of the present thesis.

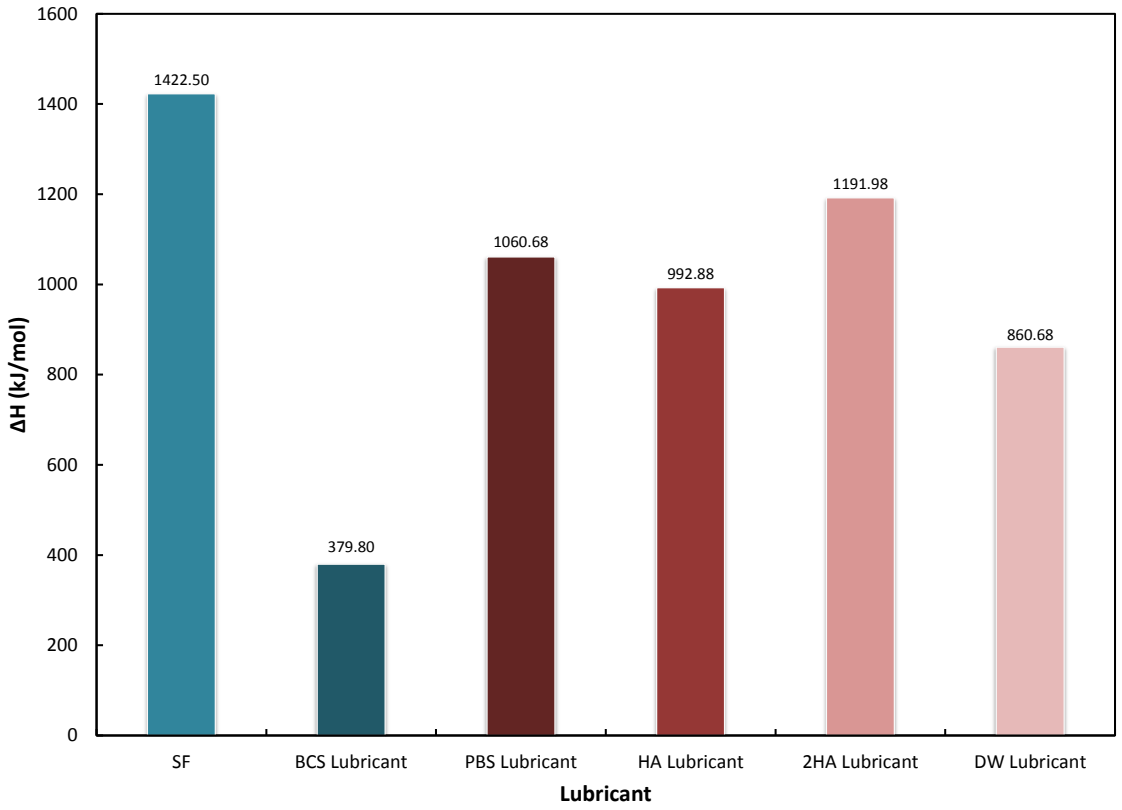


**Figure 5.8: Comparison between the enthalpies of SF and the wear testing lubricants used in the present thesis. Note that the BCS lubricant has the lowest enthalpy, and that the ACS lubricants are closer to the clinical value obtained in the present thesis.**

During the wear tests, the wear rate appeared much lower than wear rates produced from the PBS lubricant, which is inconsistent with the patterns observed by Brandt [23] and Desjardins et al. [22] who used the same HA concentration in their wear testing lubricants as the present thesis; however, this drop in wear was consistent with the findings by Wang et al. [24] and Joyce [25]. Doubling the concentration of HA in the 2HA lubricant also showed low wear rates that were not found to be significantly different from the wear rates produced by the HA lubricant. As explained in Section 5.3.3, failure to see an increase in wear during POD testing may be due to the absence of tractive rolling, which is characteristic of knee joint kinematics.

The dehydrating effect of HA may affect the ductility of the solution. The presence of water is known to increase the ductility of wood and cellulose fibers [185], and it is speculated that the same could be said for lubricants. Tractive rolling produces large shear stresses at the CoCr-PE interface, producing energies that can be dissipated in more ductile lubricants [23]; however, in the presence of HA, lubricants may become less ductile due to the absence of water molecules. Because of this decrease in ductility, less energy can be dissipated. Instead, the energy may be released in the form of heat, resulting in higher temperatures at the articulating surface. These high temperatures may increase protein degradation during wear testing, which may promote adhesive-abrasive wear that ultimately results in the increase of wear rates that were observed by Brandt [23] and Desjardins et al. [22].

Though the addition of HA may thermally stabilize the proteins in wear testing lubricants, it may also have the ability to increase protein degradation and wear rates under certain joint kinematics. In POD tests, however, it is difficult to assess the effect of

HA on wear due to the absence of tractive rolling, even if the addition of HA creates a more clinically relevant lubricant.

# Chapter 6

# Conclusion and Future Work

## 6.1 Synovial Fluid Analysis

To the author's knowledge, the present thesis is the first to quantify clinical values for protein concentration, protein constituent fractions, osmolality, and thermal stability in OA-SF and PP-SF from both hip and knee joints. These findings were deemed crucial to establish a baseline for the development of a lubricant that is more similar to the SF produced following TJA. In addition, these outcomes may encourage changes to the currently used ISO [172] and ASTM [6] test protocols for knee simulator wear testing and pin-on-disc testing, respectively.

The present thesis is an extension of previous studies that characterized and quantified the protein composition [9, 20], osmolality [20, 170], and thermal stability [18, 20] of OA-SF and PP-SF collected from knee joints. In order to better establish the clinical data currently available, OA-SF and PP-SF samples were also collected from hip joints. This comprehensive analysis revealed that the protein concentration and the

thermal stability of the SF samples were not found to be statistically different between OA-SF and PP-SF, further supporting that the pseudo synovial capsule may be able to maintain its SF generating ability following primary TJA [9]. Slight differences in protein constituent fractions present in OA-SF and PP-SF may be due to patient-to-patient variability.

The present thesis also illustrates that there are major differences between clinical protein concentrations, and ISO [172] and ASTM [6] recommendations for wear testing lubricants. While the ability to replicate *in vivo* wear mechanisms is an important aspect of finding an appropriate SF analogue, the thermal properties and biochemical nature of the lubricant should not be ignored, as this has been found to influence the tribology of joint replacement materials [19].

## 6.2 *In Vitro* Wear Testing

In Section 4.3, results from the validation wear tests reported valuable information regarding the pre-test soaking behaviour of various PE materials. Pre-test fluid absorption during wear testing for moderately crosslinked (5Mrad) PE pins was found to be nearly 2-times higher in comparison to non-crosslinked PE. During wear testing, however, fluid absorption decreased substantially; this may be due to changing the soaking fluid from DW to the BCS lubricant. To minimize this effect, it is suggested that the wear testing lubricant be used as the pre-test soaking fluid in addition to being used as the soaking fluid during wear testing.

When comparing the wear rates for crosslinked PE produced by the BCS lubricant and the ACS lubricants, it appeared that the composition of the lubricant had a significant effect on the resulting wear rates. Wear rates were generally much higher when the BCS lubricant was used in comparison to the wear rates produced using the ACS lubricants. In addition, the wear performance ranking for Marathon was much higher than XLK due to the differences in resins and material properties. The differences in ranking, however, were more pronounced when the ACS lubricants were used in comparison to the use of the BCS lubricant (Table 5.3). The present thesis is the first to show the effect of lubricant composition on the ranking of PE materials.

Interestingly, the addition of HA to the ACS lubricants produced the lowest wear rates; it was also found that the wear rates produced by the HA and 2HA lubricants were not significantly different for both XLK and Marathon (Figures 4.26-27), despite the change in viscosity. This suggests that the effects of increased viscosity may not be evident in POD testing.

Upon closer inspection of the PE wear surfaces, it was found that the surface damage on the XLK pins were distinctly different from the surface damage on the Marathon pins. Burnishing appeared to be the dominant surface damage on Marathon, while protrusions appeared consistently on the surfaces of the XLK pins. At higher magnifications, the SEM analysis showed that these protrusions may be a result of GUR 1020 being more ductile, and perhaps less malleable than GUR 1050. At x40 and x2000 magnification, the PE grains in GUR 1020 became extruded during the wear process, whereas the PE grains in GUR 1050 were smeared in appearance. The surface damage on retrieved Marathon acetabular cups were also analyzed in the SEM, and showed that the surface damage was

comparable to the damage observed on the Marathon pins using the ACS lubricant (Figure 4.30d). The damage on the Marathon pins using the BCS lubricant appeared to be different (Figure 4.18i).

Due to the low wear rates observed during the lubricant investigations, worn XLK and Marathon pins were re-melted in order to distinguish plastic deformation from actual wear [167]. Machining marks did not re-appear on the XLK pin; however, some machining marks were apparent on the surface of the worn Marathon pin, which suggests that the initial surface damage observed was partially due to plastic deformation and some wear.

Surface roughness measurements showed that reporting additional values, in addition to Ra, could better characterize the unworn and worn surfaces of CoCr discs. $Rp_{max}$ appeared to increase with the use of the DW lubricant, which could possibly account for the higher wear rates observed; however, there was no significant difference between the Rpmax of the worn and unworn CoCr surfaces. An attempt was made to find a relationship between lubricant composition and surface roughness; however, discernible patterns could not be established. Reporting roughness values averaged across all discs used in each test may have obscured distinct effects of lubricant composition on surface roughness; therefore, it was suggested that comparing the change in roughness between the each disc may alleviate these uncertainties. The location of the profilometer was also found to have a significant impact on the accuracy of the data.

Microbial contamination was observed in both the validation and lubricant investigation tests. SA was found to be ineffective for validation Test 1, but effective for Tests 2 and 3. Improvements to the laboratory cleaning protocols may explain these

inconsistencies. The present microbial analysis also may not have detected any growth. Nonetheless, the presence of staphylococci in the lubricants is concerning due to the organisms' ability to become resistant to both narrow and broad-spectrum antibiotics, including SA. Because of this, sterile wear testing must be guaranteed.

AA was found to be effective initially in lubricant investigation Tests 4 and 5, but microbial growth was evident in all worn lubricant samples. This growth shows that even bacterial contamination can become resistant to broad-spectrum antibiotics, as shown by Brandt et al. [171]. It was suggested that initial microbial identification should be implemented prior to wear testing in order to find an appropriate and less toxic antibiotic.

Biochemical analyses of the wear testing lubricants found that the composition of the BCS lubricant used in the validation tests (Section 4.3.6) was quite different from the composition of the ACS lubricants used in the lubricant investigation tests (Section 4.4.6) and the SF analyzed in Section 4.2 (Figures 5.5-7). The ACS lubricants appeared to be more similar to the SF analyzed in the present thesis. The analyses also showed that some of the wear testing standards [2, 6] were quite different from the clinical data obtained in the present thesis (Figure 5.3). Protein composition has been known to considerably affect *in vitro* wear, which is why some modifications should be made to the lubricant specifications.

The HA lubricants used in the lubricant investigation wear tests were found to have the least amount of protein degradation, which may be explained by the presence of HA. The presence and quantity of HA had been found to significantly affect the osmolality of the lubricant; however, wear rates decreased with the addition of HA, which were inconsistent with knee simulator wear tests [22, 23], but similar to results from a hip

simulator [24] and POD [25] wear test. In addition, doubling the concentration of HA did not produce wear rates that were significantly different from the wear rates obtained using the HA lubricant. It is possible that the absence of tractive rolling in both POD and hip simulator wear tests resulted in a failure to see an increase in wear. Previous studies have shown that HA bonds to free water molecules, which increases the ionic strength and therefore, the thermal stability of a solution. It was suggested that even though HA may create a more thermally stable lubricant, the dehydration of the lubricant may also decrease its ductility under tractive rolling [23]. Because of this, energies produced by the articulation are dissipated as heat rather than being absorbed by the lubricant, which can increase protein degradation. These degraded proteins may affect the adhesive-abrasive wear mechanism by acting as abrasive wear particles. Therefore, the combination of tractive rolling and the use of a more thermally stable lubricant may have caused the increase in wear rates observed in knee simulator studies [22, 23]. Conclusively, the effect of a more clinically relevant lubricant on wear may not be apparent in POD and hip simulator wear tests; however, the present author does not discourage the use of a more clinically relevant lubricant in these types of wear tests, since other factors, such as protein composition, have been found to significantly influence wear rates and surface damage.

Future work may include investigating the relationship between HA and protein concentration and its effect on wear; Mazzucco et al. [9] had found that HA decreased with an increase in protein concentration. If the concentration and molecular weight of HA changes after arthroplasty, it may have a significant effect on *in vivo* PE wear. PE wear particle characterization from POD tests using an ACS lubricant containing HA

may also be done in order to compare wear particles collected from hip and knee simulator tests using the same lubricant. With the recent interest in metal-on-metal bearings [186-189], the effect of a more clinically relevant lubricant on the wear and corrosion of metal-on-metal implants may also provide a greater understanding to a wear mechanisms that is currently not well understood.

In conjunction with the suggestions made by Fisher et al. [5] for wear testing, the present author encourages the modification of current wear testing standards to reflect lubricant compositions that are seen clinically; by doing so, many of the inconsistencies that are present in the implementation of wear tests may be better understood.

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# Appendix A

**A.1**: Summary of Lubricants used in pin-on-disc testing.

| # | Author | Year | Lubricant | Dilutant | % Lubricant | Protein Conc. (g/L) | Bacterial Inhibitor |
|---|---|---|---|---|---|---|---|
| - | ASTM-F732-00 | 2011 | BS | DW | Min. 25% | n/s | SA (0.2-0.3% wt.) |
| 1 | Dressler et al. | 2011 | BS | DW | 90% | n/s | SA (0.2% wt.) |
| 2 | Korduba et al. | 2011 | ACS | DW | 50% | 20 | None |
| 3 | Saikko et al. | 2011 | ACS | DW | 50% | n/s | None |
| 4 | Kilgour et al. | 2009 | NCS | RS | 40% | 22 | SA (0.2% wt.) |
| 5 | Kang et al. | 2008 | BS | DW | 25% | 15.46 | SA (0.1% wt.) |
| - | Wilches et al. | 2007 | BS | n/s | n/s | 30 | None |
| 6 | Saikko et al. | 2006 | ACS | DW | 50% | 21 | None |
| - | Serro et al. | 2006 | BSA | HBSS | n/s | 4<br>20<br>40<br>60 | None |
| - | Heuberger et al. | 2005 | HSA | RS, DW | n/s | 20 | None |
| - | Gevaert et al. | 2005 | BS | n/s | 50% | 40 | SA (0.2% wt.) |
| 7 | Saikko et al. | 2004 | ACS | DW | 50% | 60 | None |
| - | Mazzucco et al. | 2004 | NCS | DW | 40% | n/s | None |
| | | | PBS | None | 100% | n/s | |
| | | | HA | PBS | n/s | n/s | |
| | | | HA | PBS | n/s | n/s | |
| | | | DPPC | PBS | n/s | n/s | |
| | | | DPPC | PBS | n/s | n/s | |
| | | | Proteins | PBS | n/s | 10.2 | |
| | | | Proteins | PBS | n/s | 49 | |
| | | | SF | n/s | n/s | 31.4 | |
| | | | SF | n/s | n/s | 30.5 | |
| | | | BS (digested) | None | 100% | n/s | |
| 8 | Mazzucco et al. | 2003 | NCS | n/s | 40% | 29 | None |
| 9 | Saikko, V. | 2003 | ACS | None | 100% | 45 | None |
| | | | ACS | DW | 6-258% | 2.9-116 | |
| | | | ACS | DW | 50% | 22.5 | |
| 10 | Turell et al. | 2003 | BS | DW | n/s | 23 | SA (0.2% wt.) |
| 11 | Yao et al. | 2003 | BCS | None | n/s | 69 | SA (3g/L) |
| 12 | Bragdon et al. | 2001 | BS | None | 100% | n/s | SA (0.2% wt.) |
| 13 | Saikko et al. | 2001 | ACS | DW | 50% | 21 | None |
| 14 | Joyce et al. | 2000 | BCS | DW | 30% | n/s | SA (1g/L) |
| 15 | Cornwall et al. | 2000 | BSA | None | 100% | n/s | SA (5%) |
| 16 | Marrs et al. | 1999 | BCS | DW | 25% | n/s | SA (0.1% wt.) |
| 17 | Sawae et al. | 1998 | Saline | None | 0% | n/s | None |
| | | | BSA | Saline | n/s | n/s | SA (0.3% wt.) |
| | | | HA | Saline | n/s | n/s | None |
| | | | BS | DW | 30% | 37 | SA (0.3%wt.) |

Abbreviations: BS = bovine serum; ACS = alpha calf serum; NCS = newborn calf serum; BSA = bovine serum albumin; HSA = human serum albumin; PBS = phosphate buffer solution; HA = hyaluronic acid; DPPC = Dipalmitoylphosphatidylcholine (phospholipids); S-SF = synthesized synovial fluid; BCS = bovine calf serum; DW = distilled water; RS = Ringer's Solution; HBSS = Hank's Balanced Salt Solution; SA = sodium azide.

**A.2**: Summary of test parameters used in pin-on-disc testing.

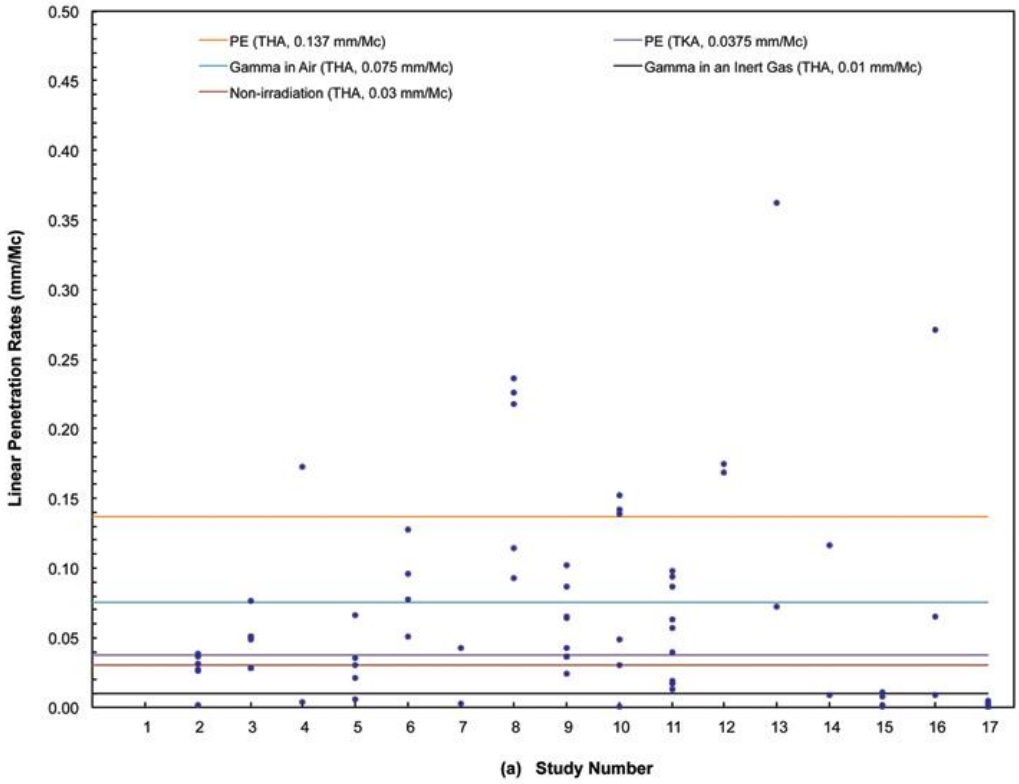
| # | Author | Year | Joint | Pin Material | Disc Material | $Ra_{disc}$ (μm) | Wear Path | Sliding distance/cycle | Loading |
|---|---|---|---|---|---|---|---|---|---|
| - | ASTM-F732-00 | 2011 | n/s | n/s | n/s | n/s | L | 50mm (avg.) | Constant |
| | | | H | n/s | n/s | n/s | 60°-90° CS | 25-150mm | n/s |
| | | | K | CoCr (ASTM F75/F799/F1537) | UHMWPE | n/s | L | 25-150mm | Dynamic |
| 1 | Dressler et al. | 2011 | H, K | GUR 1020 (GVF™) [X], GUR 1020 (XLK™) [X] | CoCr | 0.01 | L+C | 0mm-100mm | Constant |
| 2 | Korduba et al. | 2011 | H | GUR 1020, GUR 1020 [X] | CoCr | 0.01 | S, R, L | 20mm (5x5, 4x6, 3x7, 2x8, 1x9, 0x10) | Constant |
| 3 | Saikko et al. | 2011 | H | GUR 1020 (ISO) | CoCr (Zimmer) | 0.01 | CT, Random | 31.4mm (10mmØ), Random (10mmØ) | Constant, Dynamic |
| 4 | Kilgour et al. | 2009 | H | GUR 1050 (S&N), GUR 1050 (S&N) [X] | CoCr | 0.01 | L, E | 25mm, 49.67mm | Dynamic |
| 5 | Kang et al. | 2008 | H, K | GUR 1050, GUR 1050 [X] | CoCr | 0.01 | L+C | 10-38mm | Constant |
| - | Wilches et al. | 2008 | H | AISI 316L S/S, Ti6Al4V ELI alloy | UHMWPE (TIVAR® 1000) | 0.24 | CT | n/s | Constant |
| 6 | Saikko, V. | 2006 | H | GUR 1020 (Sulene™-PE) | CoCr | n/s | CT | 10mm | Constant, Dynamic |
| - | Serro et al. | 2006 | H | AISI 316L S/S, Co-28Cr-6Mo alloy, α-alumina | UHMWPE (CHIRULEN®) | n/s | CT | 1000m (total) | Constant, Dynamic |
| - | Gevaert et al. | 2005 | K | S/S | GUR 4150 (Poly Hi Solidur) | n/s | 5-pt. star | 100mm (20mmside) | Dynamic |
| 7 | Saikko et al. | 2004 | H | GUR 1020 [X] | CoCr (Protasul-20) | 0.014 | E, L | 20mm-31.4mm | Constant |
| - | Mazzucco et al. | 2004 | H | GUR 1150 | CoCr (S&N), OxZr (S&N) | IGS | CT | 20mm | Constant |
| 8 | Mazzucco et al. | 2003 | H | GUR 1150 (S&N) | CoCr (S&N) | 0.025-0.05 | S | 40mm (10mm/side) | Constant |
| 9 | Saikko, V. | 2003 | H | GUR 1020 (Sulene™-PE) | CoCr (Protasul-20) | 0.012 | CT | 31.4mm (10mmØ) | Constant |
| 10 | Turell et al. | 2003 | H | GUR 1050 (Poly Hi Solidur) | CoCr | 0.015 | S, R, L | 20mm (5x5, 4x6, 3x7, 2x8, 1x9, 0x10) | Constant |
| 11 | Yao et al. | 2003 | H | GUR 1050, GUR 1050 [X], GUR 4150 [X] | Cocr | 0.003 | S | 60mm (15mm/side) | Constant |
| 12 | Bragdon et al. | 2001 | H | GUR 4150 | CoCr | 0.05 | L, R | 3mm (0x1.5, 0.5x1) | Dynamic |
| 13 | Saikko et al. | 2001 | H | GUR 1020 (Sulene™-PE), GUR 1050 (Durasul) [X] | CoCr (Protasul-20) | 0.014-0.237 | CT | 31.4mm (10mmØ) | Constant |
| 14 | Joyce et al. | 2000 | H | UHMWPE | 316 S/S | 0.05 | L, E | 52mm (0x26) | Constant |
| 15 | Cornwall et al. | 2000 | K | UHMWPE (Chirulen®), CoCr | CoCr, UHMWPE (Chirulen®) | 0.05, n/s | L | 21mm | Constant |
| 16 | Marrs et al. | 1999 | H | GUR 120, GUR 120 [X] | CoCr | 0.01-0.09 | L, L+C | 52mm, 64mm, 80mm | Constant |
| 17 | Sawae et al. | 1998 | n/s | GUR 415 | 316 S/S, Alumina | 0.18, 0.14 | L | 34.6km (total) | Constant |

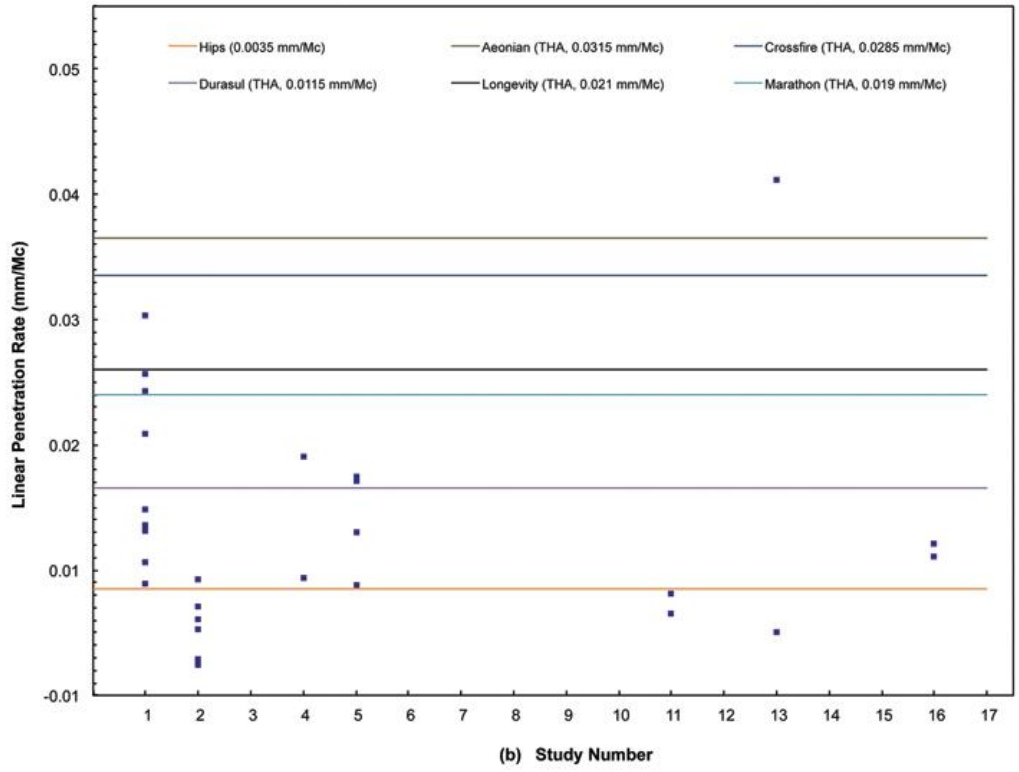
Abbreviations: H = hip; K = knee; n/s = not specified; [X] = cross-linked; S&N = Smith & Nephew; CoCr = Cobalt Chrome alloy; S/S = stainless steel; Ra = average roughness; L = line; CS = cross-shear; C = pin rotation; S = square; R = rectangle; CT = circular translation, E = ellipse

**A.3**: Summary of test parameters and conditions used in pin-on-disc testing.

| # | Author | Year | Pressure (MPa) | Sliding Velocity | Frequency | Mcycles | Temperature | Wear Rate | Wear Factor, k ($x10^{-6}$ $mm^3$/Nm) |
|---|---|---|---|---|---|---|---|---|---|
| - | ASTM-F732-00 | 2011 | 3.54 (avg.) | 50 mm/s (avg.) | 1 Hz | 2 (min.) | n/s | n/s | n/s |
| | | | 2-10 (H) | 12.5-75 mm/s | 0.5-2.0 Hz | 2 (min.) | n/s | ~7$mm^3$/Mc | n/s |
| | | | 19-36 (K) | 12.5-75 mm/s | 0.5-2.0 Hz | 2 (min.) | n/s | n/s | n/s |
| 1 | Dressler et al. | 2011 | 4.7 | 64 mm/s | n/s | 0.3-0.81 | B | 0-5.9 mg/Mc | n/s |
| 2 | Korduba et al. | 2011 | 1.05 | 20 mm/s | 1 Hz | 1 | B | n/s | 0.1-1.8; -0.1-0.2 [X] |
| 3 | Saikko et al. | 2011 | 1.12 (avg.) | 31.4 mm/s (CT);<br>0-32 mm/s (Rand.) | 1 Hz;<br>0.5Hz | 0.4-1.6<br>0.2-0.8 | RT | n/s;<br>n/s | 2.49-4.34;<br>4.57-6.84 |
| 4 | Kilgour et al. | 2009 | 2.4-4.7 | 50.17-50.65 mm/s (avg.) | 1 Hz; 2Hz | 3 | B | 0.1-3.25mg/Mc | 0.03-1.6 |
| 5 | Kang et al. | 2008 | 3.18 | n/s | 1 Hz | 0.66 | n/s | n/s | k(CS) (range: 0.47-0.55; avg. 0.103 [X]) |
| - | Wilches et al. | 2008 | 0.177, 0.531, 1.060, 1.767 | 580 mm/s | 3.2 Hz | ~0.8 | RT | n/s | n/s |
| 6 | Saikko, V. | 2006 | 1.1-11, 20 | 31.4 mm/s | 1 Hz | 0.085 | RT | n/s | 0.23-50 |
| - | Serro et al. | 2006 | 0.88 | 46 mm/s | n/s | n/s | RT | n/s | n/s |
| - | Gevaert et al. | 2005 | 12 (avg.) | 50 mm/s (avg.) | 0.25 Hz | 0.1 | B | n/s | n/s |
| 7 | Saikko et al. | 2004 | 1.1 | 32 mm/s (max.) | 1.02 Hz | 3 | RT | n/s | k(1.0<AR<5.5) (range: 1.0-1.8), E; k(AR>5.5) < 1.0, L |
| - | Mazzucco et al. | 2004 | 59.2 | 20 mm/s | 1 Hz | 0.0008 | n/s | n/s | n/s |
| 8 | Mazzucco et al. | 2003 | 3.1-7.0 | 40 mm/s | 1 Hz | 0.5-1.5 | B | 3-16$mm^3$/Mc | k(σ) (range: 0.33-1.8) |
| 9 | Saikko, V. | 2003 | 1.1 | 32 mm/s | 1.02 Hz | 3 | RT | n/s | k(p.c.) (range: 0.66-2.83) |
| 10 | Turell et al. | 2003 | 3 | 20 mm/s | 1 Hz | 1 | B | n/s | 0.05-2.5 |
| 11 | Yao et al. | 2003 | 7 | 60 mm/s | 1 Hz | 1 | n/s | 6 mg/Mc; <1.0 mg/Mc [X]; 2.75 mg/Mc [X] | n/s |
| 12 | Bragdon et al. | 2001 | 4.8 | 6 mm/s | 2 Hz; 1 Hz | 2 | RT | -0.9 mg/Mc, L; 10-10.4 mg/Mc, R | n/s |
| 13 | Saikko et al. | 2001 | 1.1 | 32 mm/s | 1.02 Hz | 3 | RT | n/s | 1.8-15.8; 0.0019-1.7 [X] |
| 14 | Joyce et al. | 2000 | 2.04 | 52 mm/s | 1 Hz | 3.3 | B | n/s | 0.085, L; 1.1, E |
| 15 | Cornwall et al. | 2000 | 3, 32, 22-32 | 42.84 mm/s | 1.02 Hz | 3 | B | 0.036-1.643 $mm^3$/Mc | 0.000529-0.02515 |
| 16 | Marrs et al. | 1999 | 11.3, 22.6 | 64-104 mm/s | 1 Hz, 2 Hz | 0.3, 0.7 | n/s | n/s | 0.00516-2.71 |
| 17 | Sawae et al. | 1998 | 3 | 20 mm/s | n/s | n/s | B | n/s | 0.014-0.058, S/S; 0.012-0.034, Alumina |

Abbreviations: H = hip; K = knee; n/s = not specified; Mc = million cycle(s); [X] = cross-linked; S/S = stainless steel; L = line; CT = circular translation, E = ellipse; R = rectangle; Rand. = random; B = body temperature (37°C); RT = room temperature (20-28°C); k(CS) = k is a function of cross shear; k(AR) = k is a function of aspect ratio; k(σ) = k is a function of contact stress; k(p.c.) = k is a function of protein concentration.




**A.4:** Linear penetration rates (mm/million cycles [Mc]) of (a) conventional, non-crosslinked polyethylene and (b) crosslinked polyethylene were calculated from pin-on-disc tests reviewed in the present thesis. Clinical penetration rates, indicated by the lines on each graph, were converted from mm/year to mm/Mc by approximating patient activity at 2 Mc/year. Study numbers correspond to those indicated in A.1, 2, and 3.

# Appendix B

Pin-on-Disc Testing: Cleaning Protocol

1. Fill sink 1/8 full of warm water and liquinox.

2. With a soft bristled brush, thoroughly clean each pin. Place cleaned pins in the metal sieve box. Clean plastic spacers, sleeve bearings, and nuts in the same way. Place cleaned components in the metal sieve box.

3. Place metal sieve box in the ultrasonic cleaner for 10 minutes.

4. Rinse with distilled water.

5. Place metal sieve box in the ultrasonic cleaner for 10 minutes.

6. Rinse with distilled water.

7. Remove components from metal sieve box. Fill one of the 250 mL beakers with ethanol (70% v/v). Rinse components in alcohol.

8. Place rinsed components in desiccator for 30 minutes.

Pin-on-Disc Testing: Gravimetric Measurements

1. Internally adjust the balance to the current environmental conditions.

2. Externally adjust the balance using the 10g weight.

3. Check accuracy by obtaining 3 measurements for the 10g weight.

4. Weigh soak station pins first. Each pin should be measured 3 times. Record each measurement in a notebook.

5. Weigh wear station pins 3 times each. Record each measurement in a notebook.

# Appendix C

## Average POD Station Wear Rates

### C.1. Validation Test 1: CoCr-CPE

| Wear Rates (mg/Mc) | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | Average | 95% C.I. |
| 14.26070 | 13.10717 | 13.30861 | 14.83127 | 13.87023 | 11.95969 | 13.55628 | 1.05284 |

### C.2. Validation Test 2: CoCr-XLK

| Wear Rates (mg/Mc) | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | Average | 95% C.I. |
| 6.37306 | 5.93179 | 5.59990 | 6.91419 | 5.66686 | 5.56123 | 6.00784 | 0.56373 |

### C.3. Validation Test 3: CoCr-Marathon

| Wear Rates (mg/Mc) | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | Average | 95% C.I. |
| 5.37778 | 5.35209 | 5.08773 | 5.33737 | 4.33045 | 4.43954 | 4.98749 | 0.50322 |

### C.4. Lubricant Investigaion Test 4: CoCr-XLK

| Wear Rates (mg/Mc) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Lubricant | 1 | 2 | 3 | 4 | 5 | 6 | Average | 95% C.I. |
| PBS | 0.87379 | 0.56700 | 0.88389 | 0.89399 | 0.76729 | 0.89543 | 0.81356 | 0.13657 |
| HA | 0.32458 | 0.16498 | 0.37205 | 0.16902 | 0.14074 | 0.35185 | 0.25387 | 0.11152 |
| 2HA | 0.13704 | 0.32290 | 0.40269 | 0.17744 | 0.25623 | 0.18956 | 0.24764 | 0.10527 |
| DW | 2.90404 | 2.54848 | 3.48485 | 3.83737 | 2.87273 | 3.07374 | 3.12020 | 0.48870 |

### C.4. Lubricant Investigaion Test 5: CoCr-Marathon

| Wear Rates (mg/Mc) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Lubricant | 1 | 2 | 3 | 4 | 5 | 6 | Average | 95% C.I. |
| PBS | 0.28369 | 0.24762 | 0.23925 | 0.25541 | 0.23319 | 0.28139 | 0.25676 | 0.02241 |
| HA | 0.17912 | 0.15589 | 0.14882 | 0.12761 | 0.17003 | 0.11246 | 0.14899 | 0.02651 |
| 2HA | 0.16734 | 0.10774 | 0.16229 | 0.13805 | 0.19057 | 0.11582 | 0.14697 | 0.03364 |
| DW | 1.62525 | 1.64646 | 1.71515 | 1.51010 | 1.50202 | 1.57980 | 1.59646 | 0.08663 |

# Appendix D

## Grants & Awards

| | |
|---|---|
| **Funding:** | MHRC Establishment Grant |
| **Agency:** | Manitoba Health Research Council (MHRC) |
| **Held by:** | Dr. Jan-M. Brandt |
| **Status:** | Granted 2011-2012 |

| | |
|---|---|
| **Funding:** | NSERC Discovery Grant |
| **Agency:** | Natural Sciences and Engineering Research Council of Canada |
| **Held By:** | Dr. Jan-M. Brandt |
| **Status:** | Granted 2012-2013 |

| | |
|---|---|
| **Funding:** | Graduate Enhancement of Tri-Council Stipends |
| **Agency:** | Natural Sciences and Engineering Research Council of Canada |
| **Project Title:** | Engineering a Synovial Fluid Analogue for the Wear Testing of Orthopaedic Biomaterials |
| **Status:** | Granted 2012-2013 |

| | |
|---|---|
| **Competition:** | 3 Minute Thesis, 2013 |
| **Agency:** | University of Manitoba |
| **Award:** | First Place, $5000 |
| **Project Title:** | Engineering a Synovial Fluid Analogue for the Wear Testing of Orthopaedic Biomaterials |
| **Status:** | Awarded 2013 |

| | |
|---|---|
| **Conference:** | 2013 Annual Meeting of the Canadian Orthopaedic Research Society |
| **Agency:** | Canadian Orthopaedic Research Society |
| **Award:** | Founder's Medal |
| **Presentation:** | Biochemical Analyses of Human Osteoarthritic and Periprosthetic Synovial Fluid From Human Hip and Knee Joints |
| **Status:** | Awarded 2013 |

## First Author Publications

| | |
|---|---|
| **Title:** | Biochemical Analyses of Human Osteoarthritic and Periprosthetic Synovial Fluid |
| **Journal:** | Part H: Journal of Engineering in Medicine |
| **Authors:** | L. Guenther[1], T. Turgeon[2], E. Bohm[2], U. Wyss[1], T. Schmidt[3], J-M. Brandt[1,2] |
| **Status:** | Accepted 2013 |

| | |
|---|---|
| **Title:** | Tribological Issues in Pin-on-Disc Testing of Polyethylene for Orthopaedic Applications |
| **Journal:** | Journal of Tribology |
| **Authors:** | L. Guenther[1], U. Wyss[1], E. Bohm[2], J-M. Brandt[1,2] |
| **Status:** | In Progress 2013 |

## Co-Authored Publications

| | |
|---|---|
| **Title:** | Performance assessment of femoral knee components made from cobalt–chromium alloy and oxidized zirconium |
| **Journal:** | The Knee |
| **Authors:** | J-M. Brandt[2], L. Guenther[2], S. O'Brien[2], A. Vecherya[2], T. Turgeon[2,4], E. Bohm[1] |
| **Status:** | Published 2013 |

| | |
|---|---|
| **Title:** | Clinical failure analysis of contemporary ceramic-on-ceramic total hip replacements. |
| **Journal:** | Part H: Journal of Engineering in Medicine |
| **Authors:** | Brandt JM[2], Gascoyne TC[2], Guenther LE[2], Allen A[2], Hedden DR[2], Turgeon TR[2], Bohm ER[2] |
| **Status:** | Accepted; Awaiting publication 2013 |

## Conference Posters and Podium Presentations

| | |
|---|---|
| **Title:** | Biochemical Analyses of Osteoarthritic and Periprosthetic Synovial Fluid |
| **Presentation:** | Poster, Orthopaedic Research Society Annual Meeting 2013, San Antonio, TX |
| **Authors:** | L. Guenther[1], D. Hedden[2], T. Turgeon[2], C. Burnell[2], E. Bohm[2], J-M. Brandt[1,2] |
| **Status:** | Presented |

| | |
|---|---|
| **Title:** | Biochemical Analyses of Human Osteoarthritic and Periprosthetic Synovial Fluid From Human Hip and Knee Joints |
| **Presentation:** | Podium presentation, Joints and Arthritis, Canadian Orthopaedic Association Annual Meeting 2013, Winnipeg, MB |
| **Authors:** | L. Guenther[1], T. Turgeon[2], E. Bohm[2], U. Wyss[1], T. Schmidt[3], J-M. Brandt[1,2] |
| **Status:** | Presented |

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