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Retrieval Analysis of Necropsy Total Hip Replacements: Considerations Beyond the Implant

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Abstract

Introduction. Total hip arthroplasty (THA) surgery is one of the most commonly performed and successful orthopedic procedures in the United States. More than 300,000 primary THAs and 40,000 revision THAs performed in the United States every year. While the need for revision surgeries can stem from a variety of causes, there have been, to the author's knowledge, no studies attempting to correlate the concentrations of certain inflammatory cytokines to metal ion concentrations found in the tissue surrounding the implant, amount of polyethylene wear, or strength of the interface of the modular taper. The purpose of this study was to begin to look at those factors to see if any were indicative of implant survivorship, as well as to see if metal ion content contributes to implant longevity. The testing for this group of well-functioning implants will be useful as a baseline when comparing the same types of testing for failed implants.

Methods. A total of nineteen cadaveric total hip implants were obtained from two sources, the Medical Education and Research Institute (Memphis, TN) and RestoreLifeUSA (Elizabethton, TN). The bearings for these implants were either metal on polyethylene or ceramic on polyethylene. Synovial fluid and tissue samples were taken from the joint for testing. Head dissociation was performed, in which an Instron 4505 was used in accordance with ASTM Standard F2009-00 to remove the head from the stem of the implant, recording force. Corrosion scoring was performed on taper surfaces by three scorers. The polyethylene acetabular liner was measured on the superior side with a micrometer to determine how much material loss was evident compared to the inferior side. These three values were then correlated to the testing performed using the synovial fluid and tissue. The synovial fluid was analyzed for inflammatory cytokines IL-6, MCP-1, IL-1β, MIP-3α, M-CSF, IL-8, IL-2, and TNF-α using a premixed Luminex screening assay. These results were given in picograms per milliliter. An anterior and posterior synovial tissue sample was analyzed for the presence of metal ions cobalt, chromium, and titanium using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). All of these results were compiled and analyzed together to search for potential correlations.

Results. There were no significant differences in dissociation forces between the groups of implants with head corrosion score 1 and head corrosion score 2. The comparison of MCP-1 to the dissociation force produced a correlation coefficient of 0.64 (p-value 0.05) and the comparison of MIP-3α to the dissociation force produced a correlation coefficient of 0.67 (p-value 0.03). However, when the graphs of these correlations were observed, it seemed likely that this correlation was due to one sample pulling the graph in a positive direction which is demonstrated by the 95% confidence interval (CI) of the correlation coefficient (0.011 to 0.90 for MCP-1, and 0.069 to 0.91 for MIP-3α). When comparing polyethylene wear to the inflammatory cytokine concentrations, no significant correlations were seen. There was a positive correlation between cobalt and chromium levels and dissociation force (r=0.56 for cobalt, r=0.66 for chromium), and a negative correlation between titanium levels and dissociation force (r=-0.30). The positive relationship was opposite of what was expected, as more metal debris should mean the implant surfaces are losing material, which should therefore decrease the strength of the taper connection. The 95% confidence interval for the correlation coefficients included zero for cobalt and titanium, and was fairly wide for chromium (0.11 to 0.90). When observing cytokines and metal ion presence, most relationships were very scattered with low correlation coefficients. However, for cobalt, strong positive relationships were seen for IL-6 (r=0.67, CI: 0.19 to 0.89), MCP-1 (r=0.76, CI: 0.33 to 0.93), and MIP-3α (r=0.60, CI: 0.066 to 0.86). When looking at confidence intervals, there seemed to be a mild correlation between cobalt and IL-6 and a moderate correlation between cobalt and MCP-1. No meaningful relationships were seen for any cytokines with chromium or titanium, so it may be useful to select

cytokines known to be responsive to those two metals in particular for future studies. When comparing metal levels between the two corrosion levels seen in the heads, there were no statistically significant differences in any of the metals between implants with a corrosion score of one and those with a corrosion score of two.

Discussion. This study was limited by the fact that the sample size for this study was very low. With only nineteen total implants, it was difficult to draw meaningful conclusions. Additional implants are being recruited in order to increase this sample size for future studies. Additionally, it was difficult for meaningful correlations to be seen when comparing any factor to the inflammatory cytokine concentrations, as these values were clustered around the lower limit of detection. However, this was expected with well-functioning implants. While it is difficult to draw meaningful conclusions when used as a correlation, this data will be useful when comparing cytokine concentrations of a group of failed implants. This group is able to serve as a baseline value for each type of testing performed, and will help to make sense of the same testing of failed implants in the future.

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By
Julie Alyse Lowell
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DEDICATION

I would like to thank my family for their unconditional love and support, especially my parents, Rich and Lisa. They have always encouraged me to do my very best and to dream big, and reminded me that I can accomplish anything with a positive attitude and hard work. I would also like to thank my brother and sister, Brent and Caroline, for always being able to cheer me up when I was frustrated or stressed. Life would be way less fun without you two. Finally, thank you to Memi, who always thinks I'm way smarter than I am and gives me a confidence boost right when I need it. The last two years wouldn't have been possible without each of you.

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ABSTRACT

Introduction. Total hip arthroplasty (THA) surgery is one of the most commonly performed and successful orthopedic procedures in the United States. More than 300,000 primary THAs and 40,000 revision THAs performed in the United States every year. While the need for revision surgeries can stem from a variety of causes, there have been, to the author's knowledge, no studies attempting to correlate the concentrations of certain inflammatory cytokines to metal ion concentrations found in the tissue surrounding the implant, amount of polyethylene wear, or strength of the interface of the modular taper. The purpose of this study was to begin to look at those factors to see if any were indicative of implant survivorship, as well as to see if metal ion content contributes to implant longevity. The testing for this group of well-functioning implants will be useful as a baseline when comparing the same types of testing for failed implants.

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Discussion. This study was limited by the fact that the sample size for this study was very low. With only nineteen total implants, it was difficult to draw meaningful conclusions. Additional implants are being recruited in order to increase this sample size for future studies. Additionally, it was difficult for meaningful correlations to be seen when comparing any factor to the inflammatory cytokine concentrations, as these values were clustered around the lower limit of detection. However, this was expected with well-functioning implants. While it is difficult to draw meaningful conclusions when used as a correlation, this data will be useful when comparing cytokine concentrations of a group of failed implants. This group is able to serve as a baseline value for each type of testing performed, and will help to make sense of the same testing of failed implants in the future.

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LIST OF ABBREVIATIONS

ASTM	American Society for Testing and Materials
CI	Confidence interval
CoCrMo	Cobalt chromium molybdenum
GM-CSF	Granulocyte macrophage colony-stimulating factor
ICP-MS	Inductively coupled plasma mass spectrometry
IFN	Interferon
IL	Interleukin
MACC	Mechanically assisted crevice corrosion
MCP	Monocyte chemotactic protein
M-CSF	Macrophage colony stimulating factor
MIP	Macrophage inflammatory protein
pg	Picograms
PMMA	Polymethylmethacrylate
ppb	Parts per billion
THA	Total hip arthroplasty
TNF	Tumor necrosis factor
UHMWPE	Ultra high molecular weight polyethylene

CHAPTER 1. INTRODUCTION

Significance of Research

Total hip arthroplasty (THA) surgery is one of the most commonly performed and successful orthopedic procedures in the United States [1]. The first THA in the United States was performed in 1960, and currently more than 300,000 THAs are performed in the United States every year [2]. While many of these operations are successful, there are almost 40,000 revision THAs performed in the United States annually as well. This number has been steadily rising over the last twenty years, and is predicted to continue to do so, making it a growing problem. The average hospital charge for a revision THA is $54,600 [3]. These revision surgeries can be due to a variety of causes, but so far there have been no studies attempting to correlate the concentrations of certain inflammatory cytokines to metal ion concentrations found in the tissue surrounding the implant, amount of polyethylene wear, or strength of the interface of the modular taper. The purpose of this study was to begin to look at those factors to see if any potential biomarkers could be identified as indicative of implant survivorship, as well as if metal ion content contributes to implant longevity. The testing for this group of well-functioning implants (implanted at time of death) will be useful as a baseline when comparing the same types of testing for failed implants.

Relevant Anatomy

The hip joint is one of the largest joints in the human body, comprised of the femur and acetabulum. It is considered to be a ball-and-socket joint, in which the ball is the femoral head and the socket is the acetabulum of the pelvis. The femur is the only bone in the upper leg, while the acetabulum is a deep, semispherical socket cavity in the hip located at the convergence of the ilium, ischium, and pubis **(Figure 1-1).** The acetabulum is where the femoral head articulates during hip motion.

A THA may be necessary because of a variety of causes, but one of the most common causes is osteoarthritis, where the cartilage that typically cushions the joint is worn away. When the cartilage is worn away, the femoral head and acetabulum directly articulate against one another, causing pain and stiffness. Osteoarthritis accounts for 70% of THA cases [1]. Another frequent cause of hip pain can be post-traumatic arthritis, which occurs when an injury to the hip causes the cartilage to become damaged, and therefore, stiffness and pain occur. Trauma can lead to articular cartilage loss, incongruency, or cartilage damage which can then lead to painful joint articulation, resulting in the need for a total hip arthroplasty. Rheumatoid arthritis, aseptic necrosis, or the presence of a tumor are other factors that can be indicative of the need for a total hip replacement [4].

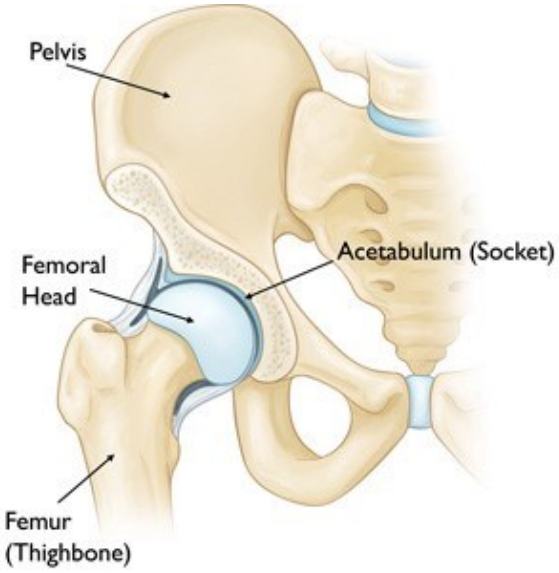

Figure 1-1. Ball and socket joint of the hip

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Foran, J.R.H. *Total Hip Replacement*. OrthoInfo 2015 8/2015 [cited 2016 8/18/16].

Total Hip Arthroplasty

Surgical Procedure

A THA is performed by an orthopedic surgeon, and can be done with either an anterior or posterior approach. The implant used for a THA has several components: a femoral stem which goes into the femur, a femoral head which is placed onto the stem via an impaction, the acetabular shell which sits in the reamed acetabulum, and the polyethylene liner which fits in the shell and is the articulating surface for the femoral head **(Figure 1-2).**

The two most popular surgical approaches used in THA are the posterolateral approach and the direct anterior approach [5]. For the posterolateral approach, the patient is first anchored in a lateral decubitus position. An incision is then made that is centered over the greater trochanter and is curved posteriorly, beginning at a point level with the anterior superior iliac spine. The incision is then extended distally to the center of the greater trochanter along the femoral shaft, and to a point ten centimeters distally from the greater trochanter. The subcutaneous tissues are dissected and the gluteus maximus is split in the direction of its fibers. The fascia is dissected away from the fibers of the gluteus medius, and a Charnley or other self-retaining retractor is inserted beneath the fascia lata at the level of the trochanter, ensuring the sciatic nerve is not entrapped beneath the retractor. The external rotators are divided as closely to the femur as possible, and the rotators are reflected posteriorly to protect the sciatic nerve. The interval between the gluteus minimus and superior capsule are bluntly dissected. Hohmann retractors are placed superiorly and inferiorly to obtain exposure of the entire capsule. The capsule is divided adjacent to its femoral attachment and preserved for later repair. A Steinmann pin is inserted into the ilium superiorly to the acetabulum to determine leg length. The hip is then dislocated posteriorly by flexing, adducting, and gently internally rotating the hip, and the head is lifted out of the acetabulum with a bone hook. The bony margins of the rim of the acetabulum are exposed to facilitate proper placement of the acetabular component, and any osteophytes that protrude beyond the bony limits of the acetabulum are removed. The acetabular component, and later, the femoral component, are then implanted as described in the following paragraphs [5].

The direct anterior approach requires less muscular dissection than the posterior approach, and is done with the patient in the supine position. A skin incision is placed lateral to the interval between the tensor fascia latae and sartorius to avoid injury to the fibers of the lateral femoral cutaneous nerve, which may be variable in its course. The fascia is divided over the muscle belly of the tensor fascia latae fibers to stay lateral to the lateral femoral cutaneous nerve. The interval between the tensor fascia latae and the sartorius is bluntly dissected with an index finger so that the femoral neck can be palpated through a thin layer of fat overlying the anterior capsule. Blunt curved retractors are placed superior and inferior to the femoral neck, taking care in the placement of the retractor beneath the rectus femoris to avoid injury to the femoral nerve and vessels. The anterior hip capsule is divided into a T or H shape for later repair. An in situ osteotomy is

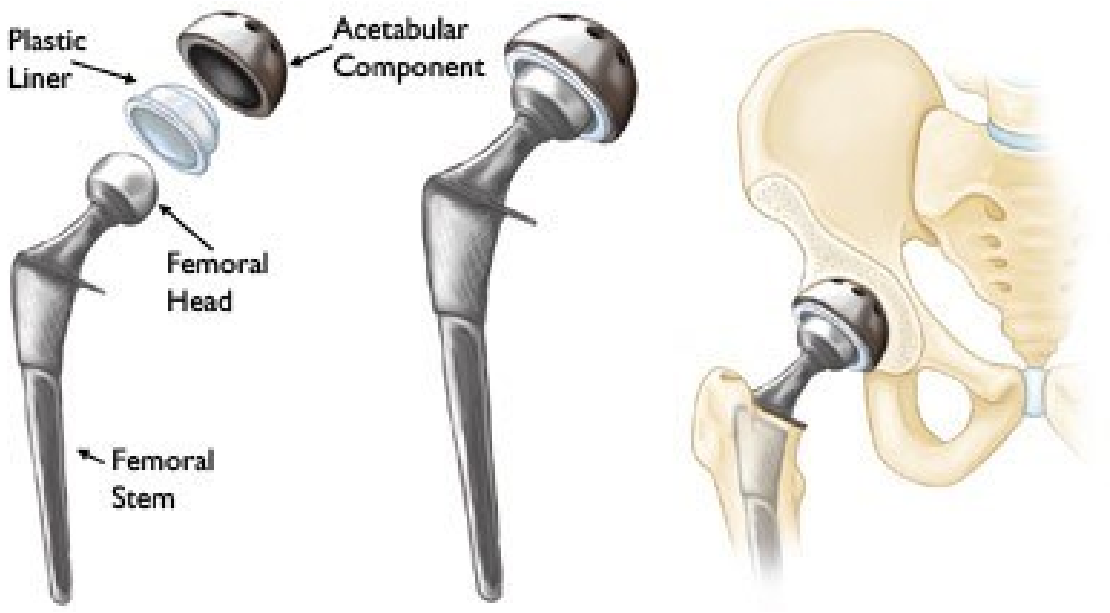

Figure 1-2. Components of total hip prosthesis

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Foran, J.R.H. *Total Hip Replacement*. OrthoInfo 2015 8/2015 [cited 2016 8/18/16].

performed of the femoral neck and the femoral head is extracted with a corkscrew. The acetabulum is then exposed by placing curved retractors distal to the transverse acetabular ligament and along the posterior rim of the acetabulum to displace the femur posteriorly. The proximal femur is exposed by placing the operated limb in a figure-of-four position and adducting the femur slightly while externally rotating 90 degrees. The femur is elevated laterally and upward with a bone hook, taking care not to trap the femur behind the acetabulum. Often, additional soft-tissue release is needed at this stage to avoid excess retraction force. The acetabulum and femur are then prepared and components are implanted as described in the next paragraph [5].

The acetabulum is prepared by excising the ligamentum teres and any remaining soft tissue. The floor of the acetabulum is palpated, and the acetabulum is prepared with reamers, going from smaller to larger reamers in the direction of the opening face of the acetabulum. Reaming is complete when all cartilage has been removed, the reamers have cut bone out to the periphery of the acetabulum, and a hemispherical shape has been produced. When inserting the acetabular cup, the surgeon ensures the patient is in a true lateral position to avoid the cup being placed in a retroverted position. The acetabular component that is the same size as the last reamer can then be implanted with fixation, or a slightly oversized component (1 to 2 mm) can be press-fit for more initial stability. The acetabular component is attached to the positioning device which is used to ensure the proper angle of inclination and anteversion is obtained. Once the correct position has been determined, the acetabular component can be impacted until there is intimate contact between the implant and the bone. Screws may be used for ancillary fixation, preferably in the posterosuperior quadrant. Once screws are implanted, the stability of the component is tested, looking for no detectable movement between the implant and bone. Once this is complete, the polyethylene liner can be implanted, ensuring that no soft tissue is interposed between the liner and its metal backing, as this can interfere with the locking mechanism. Next, to prepare the femur, a laparotomy sponge is placed in the acetabulum to protect the component. The proximal femur is exposed by internally rotating the femur so the tibia is perpendicular to the floor. A retractor is used to deliver the proximal femur, and any remaining soft tissue is excised from the neck. A box osteotome can be used to remove any remaining portions of the lateral aspect of the femoral neck and medial portion of the greater trochanter to allow access to the center of the femoral canal. Once the femur is exposed, a small reamer is inserted slightly posterior and lateral on the cut surface of the femoral neck. The reamer is aimed down toward the medial femoral condyle and is progressed to the appropriate depth. This continues with progressively larger reamers until diaphyseal cortical reaming is felt. The proximal portion of the femur is prepared by removing residual cancellous bone along the medial aspect of the neck with precision broaches. The broach is placed in the same alignment as the axial reamers and the handle is pushed laterally during insertion to ensure enough lateral bone is removed and to avoid varus positioning of the stem. The broach can be rotated to control anteversion. From the posterior approach, the medial aspect of the broach must be rotated toward the floor. Progressively larger broaches are used, maintaining identical alignment and rotation. The final broach is seated where it is axially stable within the canal with the cutting teeth at or below the level of the preliminary neck cut. The fit of the broach within the canal is assessed, and when adequate stability has

been obtained the final adjustment of the neck cut is made. The final level of the neck cut should correspond with the measured distance above the lesser trochanter established in preoperative templating. The trial neck component is selected and impacted, and the center of the femoral head is evaluated with radiographs. The hip is moved through a range of motion, noting any areas of impingement between the femur and pelvis or between the prosthetic components. If the stability is acceptable, the hip is redislocated and the head is lifted out of the acetabulum. The trial components and broach can then be removed, and the appropriately sized femoral component can be gently impacted down the canal. An audible change in pitch can be detected as the stem nears final seating. The stability of the stem is assessed when exposed to rotational and extraction forces. If satisfactory, the debris are wiped from the Morse taper and the segment is dried and the prosthetic head is affixed to the neck with a single blow over a plastic-capped head impactor. The stability is again confirmed through a full range of motion, and if satisfactory, the patient can be closed [5].

Design History

The earliest recorded attempt at a hip replacement surgery was in Germany in 1891 [6]. The methodology and results were presented at the 10th International Medical Conference by Professor Themistocles Glück. In his method, ivory was used to replace the femoral heads for patients in which tuberculosis had destroyed their hip joints. Later, in the late 19th and early 20th centuries, surgeons tried interpositional arthroplasty in which various tissues (fascia lata, skin) were placed between articulating hip surfaces for arthritic hips [6]. In 1925, Marius Smith-Petersen created the first mold arthroplasty made of glass. His design featured a hollow hemisphere to fit over the femoral head to provide a smooth surface for movement. Although glass is biocompatible, it was not able to withstand the forces the hip joint experiences and shattered. The same surgeon later went on to try stainless steel and created the first total hip replacement that was fitted to bone with bolts and screws [9]. In 1953, George McKee was the first to regularly use a metal-on-metal prosthesis [7]. He began by using a modified Thompson stem, which was a cemented hemiarthroplasty used in femur neck fracture treatment, with a cobalt-chrome socket for the acetabulum. One study showed a 28 year survival rate of 74% for this method, but by the mid-1970s this method became unpopular due to local effects of metal particles seen in revision surgeries for failure [9]. Sir John Charnley is considered to be the father of the modern THA [8]. He created a low friction arthroplasty in the early 1960s which consisted of three parts: a metal femoral stem, a polyethylene acetabular component, and acrylic bone cement. This is very similar in principle to many of the designs being used today [9].

Current Design

During typical gait, the human hip is placed under cyclic loading in which it is subjected to forces that are three to five times the force of body weight [1]. This force can increase up to twelve times body weight for more strenuous activities, such as running or

climbing [1]. Therefore, the design of a hip implant must be able to be subjected to this excess loading over many cycles without wearing down, while still approximating the normal motion of the natural hip joint. Most of the acetabular cup portions of these implants consist of a metal alloy lined with ultrahigh molecular weight polyethylene. These acetabular components are not typically cemented, but they can be. The acetabular cups can also be created with an outer porous metal shell in which bone can grow into. Previous designs did not have the polyethylene component between the acetabular cup and the femoral head, but the two metal components articulating with one another created high friction, resulting in metallic wear debris and then loosening of the implants as well as pseudotumors, therefore, a polyethylene liner is now typically used between the acetabular shell and femoral head [10]. As for the femoral component, it is typically made of metal such as stainless steel, titanium, or cobalt-chromium in order to ensure long-term resistance to breakdown from loading [1]. Titanium alloys are frequently used in hip stems and other bone-contacting components because of their good bone-ingrowth qualities, and high strength. Titanium alloys may not be as useful in bearing components, because they have a higher wear rate. Cobalt-chromium-molybdenum (CoCrMo) alloys are frequently used in these articulating components because of their high wear resistance, but they do not have as good osteointegration qualities so they are less-frequently used in the bone contacting components [10].

A design feature that is common in all modern total hip replacements is a modular, Morse-like taper between the neck and head. The neck component is typically part of the stem, unless a second modular taper is used between the stem and neck, therefore making the neck its own separate piece. Designs have moved away from the dual-taper system, however, due to higher risk of wear and metallic debris. The taper consists of the male portion, called the trunnion, and the female portion, called the bore [11]. With the head-neck modular taper, the head is fixed to the neck potion by an interference fit, where the surgeon applies one impaction to the head in order to secure it. This modularity allows the surgeon to have many options in regards to designing an implant specific to the patient. It allows for different materials to be combined, specific head sizes to be used, and allows the surgeon to control leg length through neck offset. The strength of the connection between the neck and head is dependent on the taper design, the impaction force, and the condition of the taper surfaces [12].

In the trunnion portion of the taper, there are varying design parameters that differ depending on the type of implant being used. The bore portion of the taper must be designed to fit the trunnion, so it is mostly dependent on the trunnion design used. These two parts are not interchangeable between different manufacturers or implant designs, they are created to be placed together. The characteristics that can vary with the trunnion are the taper angle (2α), the diameters of the proximal and distal portions, the engagement length, and the head-neck offset **(Figure 1-3).** Typical top (D1) and bottom (D2) diameters used are 12/14 mm, 11/13 mm, 9/10 mm, and so on. These dimensions are disclosed by companies, however, most consider the taper angles to be proprietary and will not disclose the specific angles. The taper angles are typically only a few degrees. The engagement length, L, is the axial length of the taper that makes contact across the junction. While each of these factors can play a role in implant design, there

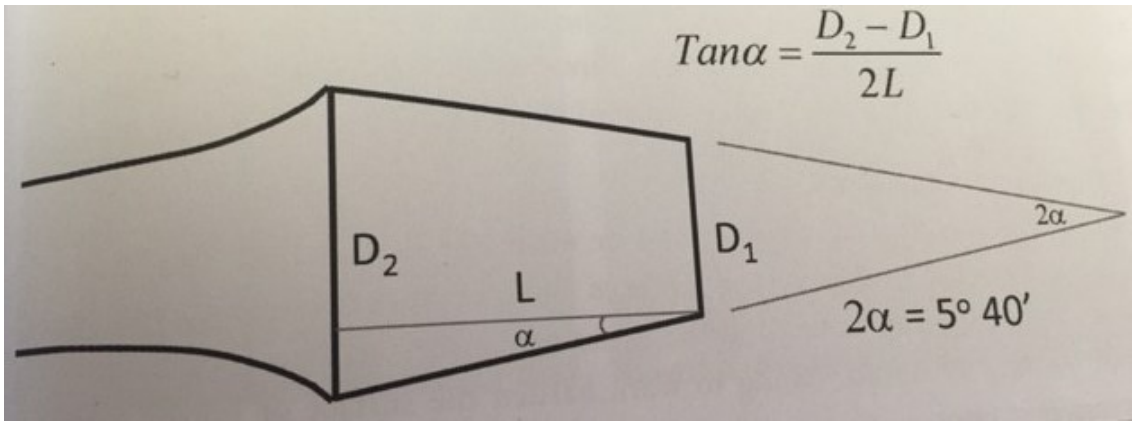

Figure 1-3. Schematic drawing of taper geometry

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Gilbert, J., S. Mali, and S. Sivan, *Corrosion of Modular Tapers in Total Joint Replacements: A Critical Assessment of Design, Materials, Surface Structure, Mechanics, Electrochemistry, and Biology*, in *Modularity and Tapers in Total Joint Replacement Devices*. 2015, ASTM International.

are also differences in how the materials are processed. For example, manufacturers may vary the roughness of the surfaces using purposefully machined microgrooves or micro-ridges. These ridged or microgrooved geometries are typically used to accommodate ceramic heads in order to avoid overloading the ceramic and thus decreasing the potential for burst fracture. Another variation in taper design is the head-neck offset. These can change depending on what best fits the needs of the patient, and refers to the position of the center of the head component and the most proximal taper trunnion position. The ability to vary head-neck offset is necessary in order to limit joint laxity, restore leg length, and for acetabular and femoral rotation center alignment [10].

Current Materials Used in Bearing Articulation

Metal on Polyethylene

Metal-on-polyethylene bearings are the most commonly used today, and provide a safe, predictable, and cost-effective bearing for the majority of patients [13]. This particular design features a metal stem and metal acetabular cup, with a polyethylene liner on the inside of the acetabular cup **(Figure 1-4).** The main concern with this type of material is polyethylene debris, as when the body encounters this debris the macrophages secrete inflammatory cytokines which can be mediators of bone lysis and can lead to aseptic loosening and eventually, implant failure. The presence of these debris can be minimized with the irradiation of polyethylene with gamma particles [9].

Metal on Metal

Metal-on-metal bearings fell out of favor in the 1970s due to concerns of the bearings producing metal ions, leading to metallosis and in severe cases, pseudotumors, but came back into production in the late 1980s through the early 2000s. A problem that may occur from metal on metal bearings is possible hypersensitivity reactions and loosening of the implant [7]. Patients receiving this type of implant also tend to have cobalt and chromium ion blood levels that are three to five times higher than those seen in patients with metal on polyethylene prostheses **(Figure 1-5)** [9]. However, this may not be true if there is excessive mechanically assisted crevice corrosion between the cobalt chromium head and stem of a metal on polyethylene bearing. In these instances, the metal on polyethylene bearing may exhibit similar increased metal levels to metal on metal [10].

Ceramic on Ceramic

Half of the THAs performed in central Europe use ceramic heads, but in the UK and USA this rate drops to less than ten percent. Implants using these materials were

Figure 1-4. Cup portion of metal-on-polyethylene prosthesis

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Knight, S.R., R. Aujla, and S.P. Biswas, *Total hip arthroplasty–over 100 years of operative history.* Orthopedic reviews, 2011. **3**(2): p. 16.

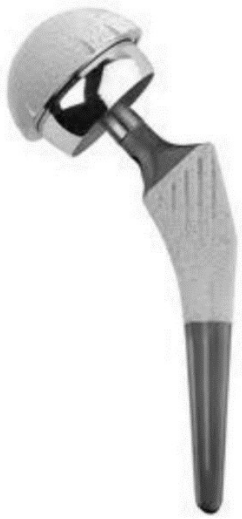

Figure 1-5. Metal on metal total hip prosthesis

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Knight, S.R., R. Aujla, and S.P. Biswas, *Total hip arthroplasty–over 100 years of operative history.* Orthopedic reviews, 2011. **3**(2): p. 16.

designed to address to problems of friction and wear reported with other materials [13]. This design is typically a ceramic head on a metal stem, and a ceramic lining in the acetabular cup **(Figure 1-6).** The ceramic used typically consists of either alumina or zirconia. The benefits of ceramic-on-ceramic bearings include the high level of hardness, scratch resistance, and the inert nature of debris. These prostheses have also been shown to have improved lubrication, lowering the coefficient of friction and improving wear resistance. These are often a good choice for young, active patients. Excellent surgical technique is needed with this type of implant, because chipping of the contact surface with insertion of the prosthesis or dislocation are possible and can lead to third body wear [9].

Ceramic on Polyethylene

Ceramic-on-polyethylene bearings are one of the most popular bearing types used today[14, 15]. This particular design features a metal femoral stem with a ceramic femoral head and a polyethylene liner on the inside of the acetabular cup. Similar to metal on polyethylene, one major concern with these materials is the generation of polyethylene debris. When the body encounters this debris, the macrophages secrete inflammatory cytokines which can be mediators of bone lysis and can lead to aseptic loosening and eventually, implant failure. The presence of these debris can be minimized with the irradiation of polyethylene with gamma particles [9]. Another risk with using ceramics in total joint replacements is the risk of a burst fracture occurring. If there is any defect in the material upon implantation, that can lead to failure of the femoral head. The femoral head can also experience a burst fracture if it receives a strong impact, such as experienced in car accidents. In cases of burst fracture, the implant must be revised. However, regardless of this potential failure mechanism, this type of bearing is frequently used in THAs because of its strong performance history. This type of bearing also eliminates the production of metallic debris from the taper connection, as this connection is now a ceramic with a metal instead of a metal on a metal.

American Society for Testing and Materials (ASTM) Standards for Testing of Bearing Materials

Each of the bearing materials mentioned above goes through a series of testing before being implemented as a material used in total joint replacements. ASTM Standard F732-00 provides a test method for evaluating the wear properties of combinations of materials that are being considered for use in bearing surfaces of total joint prostheses. It describes various tests to quickly and reliably screen material combinations for wear performance in different orthopedic wear applications prior to beginning joint simulator testing. The recommendations from this standard describe test methods to evaluate the friction and wear properties of materials being considered in bearing surfaces for total hip replacement. The standard provides a baseline of wear quantities per year clinically for the results of the testing to be compared to (69 ± 33 mm^3 per year for 22 mm heads, 85 ± 33 mm^3 per year for 28 mm heads, and 90 ± 44 mm^3 per year for 32 mm heads). It also

Figure 1-6. Ceramic on ceramic total hip prosthesis

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Knight, S.R., R. Aujla, and S.P. Biswas, *Total hip arthroplasty–over 100 years of operative history.* Orthopedic reviews, 2011. **3**(2): p. 16.

provides a wear method of 7 mm^3 per million cycles for ultra high molecular weight polyethylene (UHMWPE). The standard also defines how to prepare the polymer specimen and the counterface, as well as the specifications for the wear machine. The standard allows for load to be variable as long as it correlates to existing contact stresses. It also specifies that motion between the specimen and counterface must be multidirectional to achieve wear rates and wear mechanisms that are representative of those in a fixed-bearing ball-cup application, and recommends the system includes a cycle counter and strain gauge to measure friction. This standard is consistently used in the development and testing for new bearing materials, and has already been completed for the bearing materials used in total hip replacements discussed previously [16].

Taper Wear

One problem that is evident at the taper component of hip implants is Mechanically Assisted Crevice Corrosion (MACC). MACC is a process in which mechanical wear or deformation affects the alloy surface electrochemically. The contributing factors to MACC are varied and include material and mechanical factors, transport factors, solution chemistry inside and outside of the taper, electrochemical factors, and biological factors [10]. This type of corrosion is also frequently called fretting corrosion and is seen very commonly in retrieval analysis of devices using the Morse- type taper. A study done by Gilbert showed that 16 to 35 percent of 148 retrieved total hip implants had signs of moderate to severe corrosion at the head-neck taper connection [10]. Some of these implants consisted of a Ti-6Al-4V-alloy stem and a cobalt-alloy head, and some consisted of both a cobalt-alloy stem and neck. Based on a literature review, the prevalence of MACC ranges from 10 to 100% of retrieval specimens, each with varying degrees of damage. The damage amount is dependent on alloy composition, femoral head diameter, implantation time, and physical and mechanical factors [11].

The alloys used in the taper connection have oxide films that form on them that are a few nanometers thick. These oxide films give the alloys their corrosion resistance and serve as kinetic barriers to help keep corrosion rates low [17]. They have the ability to repassivate, or self-heal, in milliseconds if the conditions are favorable. However, the crevices found in the taper portion of the implant are typically at a higher risk of stress and micromotion which necessitates constant repassivation of the oxide layer, causing loss of oxygen and leading to a lower pH, high chloride content, and more negative potentials. These conditions can prevent the oxide films from self-healing, which can allow for the release of cobalt and chromium ions. Additionally, the formation of oxides (Cr_2O_3 and CoO) leads to a continuation of the oxide layer being unable to heal and can cause more oxides and ions to be released [10].

Disassembly testing has been done to determine how assembly procedure and material combination can affect the disassembly force of a modular total hip implant. A study by Rehmer found that disassembly forces were directly related to the assembly forces [18]. It also found that multiple impactions during assembly did not increase the

taper strength [18]. Although this finding is interesting, the study discussed in this thesis did not know the impaction force of the received implants, so therefore one cannot say whether the disassembly forces are due to the taper conditions or assembly forces and thus, must just be taken as a stand-alone force value.

ASTM Standard for Determining the Axial Disassembly Force of Taper Connections

ASTM Standard F2009-00 was created in order to establish a standard methodology for determining the force required to disassemble tapers of implants that are otherwise not intended to release. This method is used primarily for evaluation of metal and ceramic head designs and provides a means to measure the axial locking strength of the taper connections. For this testing, the cone portion of the assembly should be constrained by suitable features, and the modular head should be disassembled with a cage that provides even contact around the inferior edge of the head. The testing machine should deliver a tensile force at a constant displacement, and should have load monitoring and recording. For the disassembly, special care should be taken to ensure no artificial hoop stresses or bending moments are placed on the taper assembly, and a displacement rate of 0.05 mm/s should be used. The load and displacement should be recorded continuously until the test is complete [19].

Inflammatory Cytokines

With THA being as widely performed an operation as it is, a focus has been placed on periprosthetic osteolysis and aseptic loosening, because both of these factors can cause loosening of the implant, and therefore, failure. Metal debris from THAs can stimulate the production of polymorphonuclear leukocytes and macrophages locally, which can lead to a foreign body chronic inflammatory reaction [20]. When looking at the soft tissue around loose prostheses, there is often a foreign body reaction because of polyethylene, metal, or polymethylmethacrylate (PMMA) particles. Macrophages, lymphocytes, and other immune cells are activated when wear debris are introduced into the tissue, and these can secrete inflammatory cytokines such as interleukins, chemokines, interferons, and tumor necrosis factors in response to these debris.

There are several cytokines known to be involved in macrophage activation via T-cells. Macrophages are phagocytic cells that are found in tissues or as mobile white blood cells, especially at infection sites. The cytokines that are known to do this are interferon-gamma (IFN-gamma) and interleukin-2 (IL-2) [21]. These have each been shown in the literature to be present during macrophage activation. Another category of cytokines is those present in inflammation. These have been well-documented in literature and include tumor necrosis factor alpha (TNF-α), macrophage colony stimulating factor (M-CSF), granulocyte macrophage colony stimulating factor (GM-CSF), interleukin-1-beta (IL-1β), interleukin-6 (IL-6), and monocyte chemotactic protein-1 (MCP-1) [22, 23]. These have each been shown to be present at times of inflammation, specifically

associated with orthopedic implants. Chemokines are a final category of cytokines present and relevant in the loosening of THA devices. These function in attracting white blood cells to sites of infection. The well-known chemokines found in literature to be relevant in metal orthopedic implants with loosening are interleukin-8 (IL-8), macrophage inflammatory protein-1-alpha (MIP-1α), eotaxin, and macrophage inflammatory protein-3-alpha (MIP-3α) [24].

Each of these classes of cytokines play a role in the inflammation and potentially result in aseptic loosening, which is why the concentrations of each of these will be measured in the synovial fluid of each retrieved device. The kits used for the inflammatory cytokine testing were purchased from R&D Systems (Minneapolis, MN). The kits purchased allowed us to test for TNF- α, M-CSF, IL-1 β, IL-6, CCL2/MCP-1, IL-2, CXCL8/IL-8, and MIP-3 α. These eight cytokines were targeted because they are known to play a role in the inflammatory process. TNF-α and IL-1β were the first cytokines shown to stimulate bone resorption in vitro, so these cytokines were identified early as cytokines of interest when studying total joint replacements. One study compared the synovial levels of TNF-α and IL-1β in patients with loosened prostheses, fixed prostheses, and osteoarthritis controls [25]. A difference was found in these levels between loosened and osteoarthritis groups, but not loosened and fixed implants. IL-6 and IL-8 were selected because of their roles in the inflammatory process as well. IL-6 is secreted by osteoblasts to induce osteoclast formation and can act as either a pro-inflammatory or anti-inflammatory cytokine. IL-8 is a chemokine released by peri-implant cells such as macrophages, epithelial cells, mesenchymal stem cells, mast cells, and endothelial cells. Lassus et al. reported elevated levels of IL-8 in the pseudocapsular tissue and synovial-like interface membrane in loosened THAs compared to controls [26]. Clarke et al. found statistically significant increases in the levels of IL-1β, IL-6, and IL-8 in synovial fluid from TJRs requiring revision due to aseptic loosening compared to patients undergoing primary TJR for OA [27]. IL-6 has also been identified as a potential biomarker in periprosthetic joint infection. One study noted significantly elevated levels of serum IL-6 in infected prosthetic joints when compared to aseptic joints undergoing revision surgery [28]. MCP-1 and MIP-3α have been identified as chemokines that are involved in the implant aseptic loosening pathology. Nakashima et al. observed MCP-1 and MIP-3α expression in all tissue samples from failed arthroplasties, establishing their presence in the inflammatory cascade of arthroplasty failure [29]. They were also able to induce expression of MCP-1 by macrophages in cell culture after exposure to different types of wear particles. Because of the results identified in these studies, these eight cytokines were selected as cytokines of interest in the inflammatory process that may contribute to implant complications such as infection or aseptic loosening.

Metal Ion Concentrations

Levels of cobalt, chromium, molybdenum, and titanium ions in the blood are frequently used as an indication of if there are metal components of an implant articulating against one another and causing pain and potentially other systemic problems in patients with a THA. In a study done by Savarino et al, cobalt, chromium, and

molybdenum ion levels were measured in patients in four groups: those with a metal on metal bearing, those with a metal on polyethylene bearing, those with osteoarthritis before implantation of a THA, and those with no systemic problems [30]. The values seen for cobalt levels in each of these groups, respectively, were 1.33 ng/ml, 0.64 ng/ml, 0.36 ng/ml, and 0.24 ng/ml. For chromium, the values were 1.72 ng/ml, 0.60 ng/ml, 0.26 ng/ml, and 0.25 ng/ml. Finally, for molybdenum, these values were 0.62 ng/ml, 0.62 ng/ml, 0.42 ng/ml, and below detection limits for the group with no pathology. These values were consistent with other findings in literature. The same ions were tested for in this study and were compared to these values. In this study, the samples were analyzed at Brooks Applied Labs (Bothel, WA) and the results were sent in micrograms per liter (μg/L).

Toxicology of Metal Ions

As mentioned previously, the ions of interest when studying orthopedic implants are mainly cobalt, chromium, and molybdenum, each of which have the capacity of affect the body in different ways. Cobalt has been shown to be cytotoxic and induce apoptosis at lower doses, and cause necrosis with an inflammatory response at higher doses in mammalian in vitro test systems. It is primarily accumulated in the liver, kidney, pancreas, and heart. The excretion of cobalt is initially rapid through the renal system over the first few days, but then slows and leads to significant long-term retention in the tissues for several years. In serum, the cobalt ions bind to binding sites on albumin, so therefore, the concentration of free cobalt ions is estimated to be only 5-12 percent of the total cobalt concentration in the body [31]. Because cobalt ions can bind to albumin, it can affect how it is distributed in the body. If the albumin concentrations in the body are low, less cobalt ions are able to bind to the binding sites on the albumin, leaving these cobalt ions free to interact with specific protein carriers with other cellular targets. These free cobalt ions can cause cobalt ion buildup in other tissues [32]. Studies have shown chromium to be cytotoxic as well, especially hexavalent chromium, which is considered grossly cytotoxic. Chromium can cause inhibited osteoblast-like cell metabolism, reduced phagocytic ability of polymorphonuclear leukocytes and murine macrophages, and can increase release of inflammatory mediators and cell death in macrophages. It has also been found that if more toxic elements such as chromium are selectively leached, this can lead to an increase in the toxicity of degradation products from the cobalt-chromium-molybdenum alloy [33]. Additionally, in a study to observe how metal ions affect bone marrow stromal cells, chromium ions were found to be grossly cytotoxic, while cobalt, molybdenum, iron, and nickel ions were found to be moderately cytotoxic, and titanium, aluminum, vanadium, and manganese ions were found to be minimally cytotoxic [34]. There has been less research done on molybdenum's cytotoxicity, however, one study that tested particles of pure metals in a mouse fibroblast cell line. While they found toxic effects of cobalt and chromium at several levels, toxicity of molybdenum was only seen at the highest concentration tested, 500 micrograms per milliliter [35].

Objective and Hypothesis

While total hip arthroplasty is one of the most common orthopaedic procedures performed today, there are very few implant retrieval studies being performed to assess these implants after implantation. Of the implant retrieval studies in existence, most are of failed implants retrieved at time of revision surgery, and very few are of well-functioning implants at time of necropsy. The objective of this work was to study a few key factors such as taper dissociation force, polyethylene wear, taper corrosion, inflammatory cytokine content in synovial fluid, and metal ion content in the tissue to see if any of these factors may be indicative of implant survivorship. Another objective was to compare these parameters to one another and see what relationship existed between them, if any. A final objective is to use these as "baseline values" when completing the same tests on a group of failed implants in the future. To this end, the following hypotheses were tested:

(a) A negative relationship will exist between dissociation force and cytokine concentration. A higher dissociation force means that the taper connection is more intact, therefore, there is less corrosion and material loss in the taper connection and therefore less of an inflammatory response due to debris. However, it should be noted that cytokine concentrations are affected not only by metallic debris, but also by polyethylene debris, so an increase in cytokine concentrations could be due to either type of debris. Nonetheless, a negative association between dissociation force and cytokine concentration is expected in this study.

(b) A negative relationship will exist between dissociation force and corrosion. The tapers that are more highly-corroded will likely be experiencing more material loss on the trunnion and bore, and therefore, will have a lower dissociation force. The implants with less corrosion (minimal to mild) should have higher dissociation forces than those with more corrosion (moderate to severe), and the contacting surfaces have less corrosion and therefore material loss.

(c) A negative relationship will exist between metal ion content and dissociation force. As mentioned previously, a higher dissociation force means the taper connection is more intact, so there should be less corrosion and material loss at this site. Therefore, if there is less material being lost, the metal ion content will be lower.

(d) A positive relationship will exist between polyethylene wear and cytokine concentration. As the femoral head articulates against the polyethylene liner, debris are generated. In order to combat these debris, macrophages attempt to engulf the particles and often secrete inflammatory cytokines as a response. Therefore, a higher amount of wear on the polyethylene liner should result in a higher concentration of inflammatory cytokines.

(e) A positive relationship will exist between metal ion content and cytokine concentrations, specifically cobalt and titanium ion content. Similar to the

polyethylene and cytokine comparison, if there is more metal debris in the tissue, inflammatory cytokines will be released as macrophages are recruited to manage this debris. Cobalt has been found to increase IL-6 from osteoblast like cells[36, 37]. Cobalt ions have also been shown to rapidly induced the protein secretion of IL-8 and MCP-1 in primary human osteoblasts[38]. Titanium has been shown to induce the differentiation of osteoclast precursors toward mature osteoclasts in about twenty percent of individuals[39]. While cobalt and titanium have been shown to increase cytokine activity, there have been no studies demonstrating this same phenomenon with chromium ion. Therefore, it is likely that no relationship will exist between chromium ion content and cytokine concentrations.

(f) Higher metal ion content will be seen in implants with higher corrosion due to material loss as mechanically assisted crevice corrosion occurs. Therefore, the implants with higher corrosion scores (moderate, severe) will have an increased concentration of metal ions compared to the implants with lower corrosion scores (minimal, mild).

Equipment Used

Instron 4505 Load Frame

An Instron 4505 Load Frame (Instron, Norwood, MA) was used for the mechanical head dissociation testing. This allows the stem of the implant to be pulled from the head of the implant at a precise rate and the resulting force to be measured. A custom mechanical test frame was created for this testing in the Implant Research Center at Drexel University (Philadelphia, PA), where the testing was completed **(Figure 1-7).**

Fluoroscopy

Fluoroscopy was used prior to implant retrievals in order to assess the fixation of the implant, check for osteolysis, or bone loss around the implant surface thus verifying it is in fact a well-functioning implant, and to see if there were any screws or other parts in place to be aware of before beginning retrieval. The model used was the OrthoScan HD Model 1000-0001 (OrthoScan, Scottsdale, AZ).

Calibrated Micrometer

A calibrated digital micrometer was used for linear polyethylene wear measurements. One side of the micrometer was placed on the back of the polyethylene and the other side was placed on the front in order to determine thickness at a particular location. The micrometer used is a Mitotoyo Digimatic Micrometer Series 293 MDC-MX Lite and measures to 0.001 millimeters (Mitotoyo, Aurora, IL).

Figure 1-7. Custom test frame for head dissociation testing, Drexel University

(Personal communication from Genymphas Higgs on May 16, 2016)

Luminex Multiplexer

Multiplex assays were performed using the Luminex system. This platform enables simultaneous measurement of multiple proteins per well on a ninety-six well plate using very little sample (approximately thirty microliters per sample). This technology produces results comparable to ELISA assays but with higher efficiency and speed. It is also less expensive per target than ELISA. The multiplexer used was the Luminex MAGPIX (Luminex Corporation, Austin, TX).

CHAPTER 2. METHODOLOGY

Retrieval Methods

The total hip implants used in this study came from one of two sources: the Medical Education and Research Institute (Memphis, TN) or RestoreLifeUSA (Elizabethton, TN). Cadaver specimens of the hip and proximal femur were obtained from both institutes, and were frozen until retrieval could take place. Before retrieval, images were taken using fluoroscopy to see if there was any obvious osteolysis. During the retrieval, incisions were made in order to expose the tissue surrounding the bone where the implant was located. Tissue samples were obtained anterior, posterior, inferior, and superior to the acetabular cup, as well as from the taper itself. The tissue-implant interface was assessed as recommended by ASTM Standard F561-13. **Figure 2-1** shows pictorially where the samples were obtained. Synovial fluid samples were aspirated from the joint and centrifuged at 1600 rpm for twenty minutes to remove cell particles, then kept frozen in a -80 degrees Celsius freezer. The implant was then removed from the bone as recommended by ASTM Standard F561-13, placed in biohazard bags, and shipped to Drexel University (Philadelphia, PA) for cleaning, wear scoring, and mechanical testing [40].

Implant Cleaning

The implants were cleaned using a method designed by Drexel University Implant Research Center in accordance with ASTM Standard F561-13 [40]. The implants were removed from packaging and biohazard bags and examined to ensure all parts were present. Inventory pictures were taken of the bag and all implant components, ensuring the implant number was visible. Each component was rinsed in cold water in a biohazard sink in order to remove any loose tissue. A 1:10 Discide:water solution was mixed in a mixing cup and the implants were placed in the solution for a twenty minute soak. Brushes were then used to remove remaining tissue from the implant while being careful not to scratch or damage the implant surface in any way. The Discide:water solution was then disposed of and a 1:10 bleach:water solution was created. The implants then completed another 20 minute soak, and brushes were again used to remove loose debris. At this point, the implant components were no longer considered biohazard and could be placed in a clean mixing cup. For ceramic components, the cleaning process ended here.

If the components were metallic, they were placed in a clean mixing cup and the cup was filled with water. These cups were then placed in an ultrasonicator for 25 minutes, keeping the water level in the ultrasonicator the same as the water level in the mixing cups. The mixing cups and implants were then removed from the ultrasonicator, the water was drained and the cups were refilled and placed back in the ultrasonicator for another 25 minutes. The implants were then laid out on Versidry sheets overnight to air dry within the fume hood. The implants were then packaged in separate bags to avoid scratching one another and labeled to await testing and wear scoring.

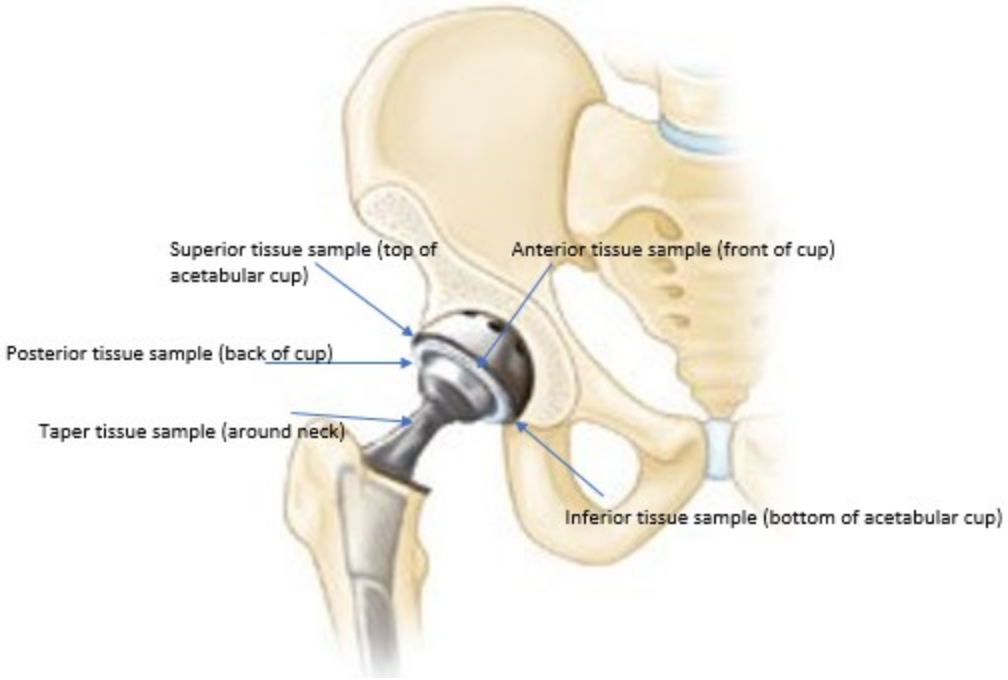

Figure 2-1. Tissue sample locations

Modified with permission.
Foran, J.R.H. *Total Hip Replacement*. OrthoInfo 2015 8/2015 [cited 2016 8/18/16].

Head Dissociation Testing

This testing was completed in accordance with ASTM Standard F2009-00, discussed previously. An Instron 4505 was used for the entirety of this testing procedure, following a Standard Operating Procedures document created by Drexel University. Load frame verification should be performed before any testing to ensure all components are working properly. A custom testing frame (Drexel University) was used for this testing to allow the head to be separated from the stem of the implant. This frame included specially designed head and stem plates that allowed the taper to be oriented vertically for the entirety of the testing. Before testing began, a 30 kN load cell was installed in the Instron, and calibration and balance of the load cell was completed on the Instron machine.

After calibration and balance was completed, the custom femoral stem and head fixtures were assembled into the crosshead of the Instron. The specimen was placed in the head fixture with the stem hanging downward. The crosshead was then raised until the stem entered the stem fixture. The crosshead was then raised slowly until there was only enough space between the head fixture and stem fixture for attachment of the stem plate. The load channel was set to zero to create a unique set point for each test sample, as samples masses may vary. The stem plate was then connected to the fixture using four bolts, ensuring no preload was exerted on the implant, and the crosshead was slowly lowered until the sample was oriented with the taper connection vertical. A small gap was left between the connection and stem plate to allow for a toe region in the data and to ensure no preload was present. The Instron then began moving the stem fixture down, away from the head fixture, at 0.05 mm/s, recording the resulting force continually. When the load suddenly dropped, the Instron recognized that the connection between the stem and head was broken and stopped displacement. The peak load was recorded as the force needed to dissociate the head and stem.

Corrosion Scoring

For head corrosion scoring, a Goldberg Corrosion Classification was used [41]. The components of the implants were cleaned to ensure the damage that was seen was damage to the implant itself and not residue of any kind. Three scorers examined the implant components under a microscope and independently viewed the male and female taper components and scored them from 1 to 4, following the criteria shown in **Table 2-1** and looking for all signs of fretting or corrosive attacks. After each scorer had completed scoring of both components, the scorers reviewed the results together and discussed any discrepancies. The components with discrepancies were studied under microscopy again, and a final score was agreed upon by all three scorers.

Table 2-1. Criteria for taper corrosion scoring

Damage	Score	Criteria
Minimal	1	Fretting on < 10% of surface and no corrosion damage
Mild	2	Fretting on > 10% of surface and/or corrosion attack confined to one or more small areas
Moderate	3	Fretting > 30% and/or aggressive local corrosion attack with corrosion debris
Severe	4	Damage over majority (> 50%) of mating surface with severe corrosion attack and abundant corrosion debris

Metal Ion Testing

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is an analytical technique used to make elemental determinations of a material. This method combines high-temperature ICP with a mass spectrometer. The ICP source converts the atoms of the elements in the associated sample to ions, which are then separated and able to be detected by a mass spectrometer. For this testing, tissue samples were taken from two locations: anterior and posterior to the acetabular cup. These samples were placed in fixative and sent to Drexel University for sample preparation and analysis.

To prepare for the acid digestion of the tissue samples, the laboratory space and equipment was prepared by rinsing and soaking all tools in an acid solution consisting of five to ten percent trace metal nitric acid and eighteen mega-ohm ultrapure water overnight. This process included sample containers, the digest vessel, and any other equipment that would be in direct contact with the samples. Any minor tools involved in the process were washed twice and dried with Kimtech wipes. Once the laboratory space and equipment were prepared, the samples were cut to a 25 milligram size using a ceramic knife and plastic tweezers. Care was taken to ensure the samples did not contact any form of metal. A water bath was set to ninety-five degrees Celsius, and centrifuge tubes were labeled to identify the samples. The samples were then washed with eighteen mega-ohm ultrapure water to remove ethanol, and were placed in the correctly labeled tube. The acid solution was created by adding two milliliters of seventy percent trace metal grade nitric acid to each tube under a fume hood. Next, one milliliter of hydrogen peroxide was added to each tube followed by three milliliters of thirty-seven percent trace metal grade hydrochloric acid, also under the fume hood. Three samples of only acid solution were also prepared in three tubes with no tissue samples as blank samples. The hydrogen peroxide was allowed to react as the water bath heated to the correct temperature. Once heated, the tubes were placed in the water bath for a two-hour incubation period. After the two-hour period, the tubes were removed and placed in the fume hood for thirty minutes to cool. After the cooling period, an addition one milliliter of hydrogen peroxide was added to each tube and given thirty minutes to react. The tubes were then placed back into the water bath for an additional two-to-three-hour incubation period.

The samples were then placed into labeled two milliliter micro-centrifuge tubes to be sent to Brooks Applied Labs (Bothell, WA). Each tube received one milliliter of the sample and acid mixture and one milliliter of eighteen mega-ohm ultrapure water. The remaining sample was diluted to a fifteen milliliter volume with the eighteen mega-ohm ultrapure water. The method for analysis used by Brooks Applied Labs was validated using the Luts-1 certified reference material from the National Research Council of Canada. This material is a solid biological matrix that contains certified quantities of cobalt and chromium.

Once Brooks Applied Labs completes the analysis, the data was first checked to ensure the blank samples had low levels of metal. Any amount of metal in the blank sample was considered to be a contamination. If the contaminants were low in value and

consistent between the blanks, they were averaged and the average was subtracted from the remaining samples. This allows the final data to be represented without the background noise present due to uncontrollable contamination. The data was then reported in either micrograms per liter or parts per billion.

Polyethylene Degradation

A Standard Operating Procedure from Drexel University was used for these measurements. The polyethylene liners were removed from the acetabular cups and rinsed to remove any debris present. Next, these liners were studied under a microscope to see if any machining lines could be seen. A marker was used to draw a border of where machining lines were present, and where they tended to be worn down. The area where the machining lines were worn was considered to be the superior side. A few measurements were taken using a Mitotoyo Digimatic Micrometer Series 293 MDC-MX Lite (Mitotoyo, Aurora, IL) to determine the thinnest area of the liner. This was marked with an "S" for superior and the opposite was marked with an "I" for inferior. Three measurements were taken from the superior side halfway up the polyethylene liner, and three measurements were taken from the inferior side, ensuring they were also made at the halfway point of the liner. These three measurements were averaged and the superior measurements were subtracted from the inferior, giving the total polyethylene linear wear. Although this measurement was called the wear, it should be noted that some of the decrease in thickness of the superior side could be due to creep, or deformation of the material due to high stresses. Unfortunately, this was not something that could necessarily be directly calculated so it was a noted area of weakness in our measurement.

Inflammatory Cytokine Testing

The cadaveric cytokine samples were tested using the Luminex Multiplex Assay. A group of sixteen osteoarthritic samples were obtained with IRB approval from patients undergoing primary total hip arthroplasty to use as a comparison to the fourteen cadaveric samples. For the assay, the standards, calibrator diluent RD6-52, samples, and diluent RD2-1 were brought to room temperature. The Certificate of Analysis provided with the kit was followed in order to reconstitute each standard cocktail with RD6-52 diluent. These were left under gentle agitation for 15 minutes prior to making dilutions. The RD6-52 diluent was used to dilute each sample by 2, using 80 microliters (µL) of sample and 80 µL of diluent, giving 160 µl of diluted sample in each tube. The standard cocktails were created according to directions in the booklet to create standards 1-6. The lights were turned off, and the microparticle cocktail was centrifuged for 30 seconds at 1000 G. The cocktails were gently vortexed and then diluted with RD2-1. The standards and samples were then added to their corresponding wells and covered with a foil sealer, and incubated for two hours at room temperature on a microplate shaker. During this time, the wash buffer was created by adding 20 milliliters (mL) wash buffer concentrate to 480 mL deionized water. With the lights still off, a magnet was placed on the bottom of the microplate and the plate was shaken over sink to remove liquid. Each well was

filled with 100 µL of wash buffer which was then shaken out, and this was repeated two more times. The biotin antibody cocktail was then centrifuged and diluted with RD2-1. Each well received 50 µL of the diluted biotin cocktail and was incubated for 1 hour at 800 rotations per minute (rpm) under a foil plate sealer. The streptavinin cocktail was then created by mixing 220 µL with 5.35 mL of wash buffer. The wells were washed with wash buffer three times, and then 50 µL of the streptavinin cocktail was added to each well. This was incubated for 30 minutes at room temperature at 800 rpm. The wells were then washed three times with wash buffer again. Finally, 100 µL of wash buffer was added to each well and incubated for two minutes at 800 rpm, and the plate was read on the magpix reader, with concentrations given in picograms per milliliter (pg/mL).

CHAPTER 3. RESULTS

Implant Information

There were a total of nineteen implants retrieved for this testing, but not all of the implants were able to undergo each type of testing, and the full implant information was not able to be obtained for all implants. In these nineteen implants, there were five with 28 mm heads, five with 32 mm heads, four with 36 mm heads, and two with 40 mm heads. Fourteen of the acetabular liners were highly crosslinked polyethylene, and four were not highly crosslinked. Of the metal on polyethylene implants, nine had a cobalt chromium alloy head on a titanium alloy stem, and four had a cobalt chromium alloy head and stem. Two implants were ceramic heads on a titanium alloy stem. One of these heads was zirconia and the other was a zirconia alumina combination. Eight of the implants had a head taper angle of 12/14 and one had an angle of 16/18. None of the implants had cemented shells, and two had cemented stems. The manufacturer, design, and other implant characteristics can be seen in **Table 3-1.**

Head Dissociation Testing

The dissociation forces in this study ranged from 1428 to 5368 Newtons, with a mean and standard deviation of 2790±1200 Newtons. The sample size for this testing was 15 implants, with two of these having ceramic heads (16-03-730R and RLU0519169R). These results can be seen in **Figure 3-1**.

Corrosion Scoring

As mentioned previously, the male and female tapers were each scored from 1 to 4 based on the Goldberg Corrosion Classification. The scores for each portion of these implants can be seen in **Table 3-2**.

Inflammatory Cytokine Testing

For the group of sixteen osteoarthritis control samples, eight cytokines were tested. They were IL-6, MCP-1, IL-1β, MIP-3α, M-CSF, IL-8, IL-2, and TNF-α. The values for IL-8, MCP-1, MIP-3α, and M-CSF were mostly in range for each sample, but most were very close to the lower limit of detection so were obviously not very active in the body. For TNF-α, only six of the sixteen samples were in the detectable range, and these samples were just above the lower limit of detection. For IL-6, seven of the sixteen samples were in the detectable range, with the other nine samples being above the limits of detection. For IL-2, nine of the sixteen samples were in the detectable range, with the other seven being below the lower limit of detection. For IL-1β, all sixteen samples were

Table 3-1. Femoral component information

Sample ID	Design	Manufacturer	Head Material	Head Size (mm)	Taper Angle	HXLPE	Stem Material
14-11-788R	Duraloc	Depuy	CoCrMo	28	--	N	--
16-03-730L	Trilogy	Zimmer	CoCrMo	32	12/14	Y	Ti_6Al_4V
14-08-614L	Reflection	Smith and Nephew	CoCrMo	--	--	Y	--
14-08-580L	Reflection	Smith and Nephew	--	--	--	Y	--
14-05-425L	Trilogy	Zimmer	Metal	32	12/14	Y	Ti_6Al_4V
16-03-730R	Richard Reflection	Smith and Nephew	White Zirconia	28	--	N	Ti_6Al_4V
16-08-983R	Trident	Stryker	CoCrMo	36	12/14	Y	Ti_6Al_4V
RLU1114149R	Pinnacle	Depuy	CoCrMo	40	12/14	Y	Ti_6Al_4V
RLU0315169 L	Trilogy	Zimmer	CoCrMo	28	12/14	Y	CoCrMo
15-10-288L	Pinnacle	Depuy	CoCrMo	36		Y	CoCrMo
RLU0519169L	Trilogy	Zimmer	CoCrMo	36	--	Y	Ti_6Al_4V
15-10-491R	Trilogy	Zimmer	CoCrMo	36	12/14	Y	CoCr
14-12-835L	Continuum	Zimmer	CoCrMo	32	12/14	Y	Ti_6Al_4V
15-08-338L	Ringloc Constrained	Biomet	CoCrMo	--	--	--	Ti_6Al_4V
15-10-466L	Pinnacle	Depuy	CoCrMo	32	--	Y	CoCrMo
RLU1029149R	Richard Reflection	Smith and Nephew	CoCrMo	28	--	N	Ti_6Al_4V
14-12-835R	Trilogy	Zimmer	CoCrMo	32	12/14	Y	Ti_6Al_4V / TA
RLU0519169R	Trident	Stryker	Zirconia-Toughened Alumina	40	16/18	Y	Ti_6Al_4V

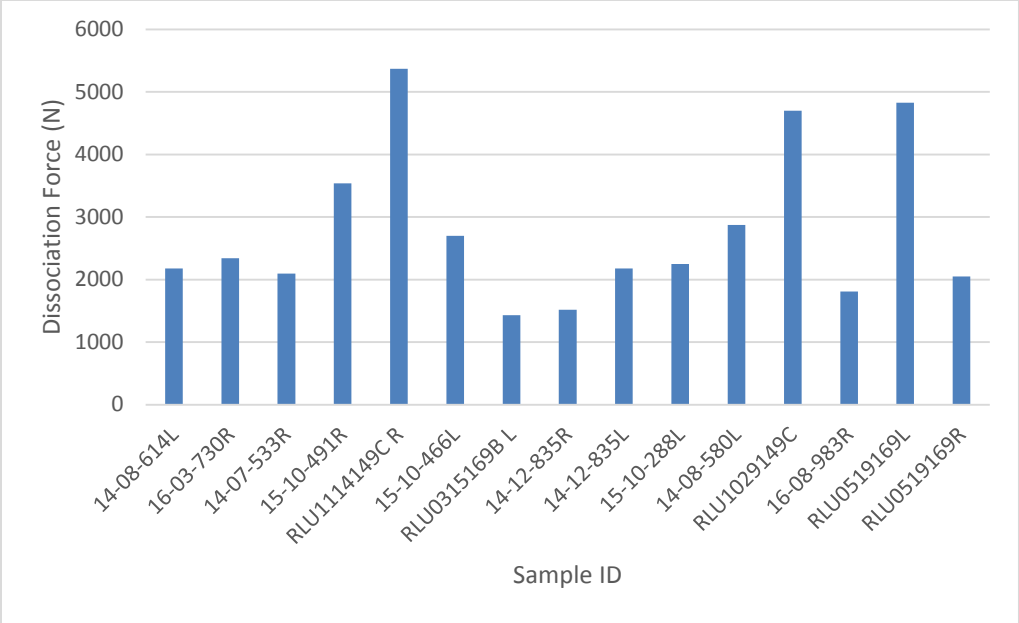

Figure 3-1. Graph of the dissociation force for each tested implant

Table 3-2. Corrosion scores for male and female taper components

Sample ID	Corrosion Score	
	Female	Male
14-08-614L	2	1
16-03-730R	Ceramic	1
15-10-491R	1	1
RLU1114149C R	2	1
15-10-466L	2	1
RLU0315169B L	2	3
14-12-835R	1	1
14-12-835L	2	1
15-10-288L	1	1
14-08-580L	2	1
RLU1029149C	1	1
14-05-425L	2	1
16-08-983R	1	1
RLU0519169L	1	1
RLU0519169R	Ceramic	1

below the limits of detection. The cytokine concentrations for each sample can be seen in **Table 3-3.**

For the group of fourteen cadaveric implants, the same eight cytokines were tested, but there were only five main cytokines of interest. These were IL-6, MCP-1, IL-1β, MIP-3α, and M-CSF. The cytokine results for IL-8 were above the detection limits for nine out of the fourteen samples tested, meaning that there is likely IL-8 present in high amounts, though it is not quantifiable. For TNF-α, only five of the fourteen samples were in range, with the rest falling below the limits of detection. The five that were in range were very close to the lower limit of detection, making them essentially irrelevant. For IL-2, only four of the fourteen samples tested were in range, the rest falling below the limits of detection. Similarly to TNF-α, even the samples from these four samples that were in range were very close to the lower limit of detection. The cytokine concentrations for each sample can be seen in **Table 3-4**.

Mann Whitney tests were done between the two groups for each cytokine, and significant differences were seen between the groups for IL-8 ($p < 0.001$), IL-1β ($p<0.001$), IL-1 ($p=0.002$), and M-CSF ($p<0.001$). However, this could be due to the fact that many of the values were on the very low end of detection for these groups, with one sample being elevated which could cause the differences to be seen.

Linear Polyethylene Wear Measurements

Values for polyethylene wear measurements ranged from 0.040 to 1.867 millimeters (mm). However, the value of 1.867 mm was found to be an outlier. The mean and standard deviation were 0.296±0.468 mm including this outlier, but decreased to 0.184±0.215 mm excluding the outlier. These values can be seen in **Table 3-5.**

ICP-MS

The samples were analyzed by Brooks Applied Labs (Bothell, WA) and results for cobalt, chromium, and titanium levels were obtained from the anterior and posterior tissue capsule in micrograms per liter (µg/L). These were averaged and are reported in **Table 3-6** for every implant except 16-08-983R, as tissue samples were not obtained for this components. This table also includes the head and stem material for the implants to help interpret these results.

Table 3-3. Concentrations of inflammatory cytokines for osteoarthritis control specimens (pg/mL)

Sample ID	IL-6	MCP-1	IL-1B	MIP-3a	M-CSF	TNF-α	IL-8	IL-2
002	>1180	350.84	<16.3	11.64	563.13	<8.9	8.53	35.86
003	17.73	232.96	<16.3	8.85	<514	<8.9	6.80	<35
004	804.15	391.40	<16.3	10.70	1104.00	<8.9	12.78	<35
005	>1180	250.69	<16.3	13.56	1168.03	<8.9	11.18	<35
006	44.60	342.23	<16.3	<7.9	742.55	<8.9	11.24	<35
007	16.22	455.35	<16.3	9.77	1042.74	9.93	19.77	<35
008	>1180	1613.10	<16.3	20.79	1050.52	9.43	137.66	47.89
009	>1180	720.25	<16.3	14.04	1698.83	<8.9	58.71	37.24
010	88.60	515.90	<16.3	44.08	1184.25	9.43	25.34	<35
011	>1180	3210.13	<16.3	47.26	1018.99	9.18	>1140	43.48
012	>1180	1254.41	<16.3	45.43	673.61	9.56	56.51	36.55
013	>1180	923.25	<16.3	20.04	1578.72	<8.9	191.22	40.01
014	174.07	389.67	<16.3	9.76	6050.69	<8.9	14.39	<35
015	>1180	758.41	<16.3	14.55	<514	<8.9	33.72	36.55
016	>1180	819.11	<16.3	12.61	703.08	<8.9	26.71	36.78
017	1164.60	1605.89	<16.3	37.93	1234.53	<8.9	244.69	46.03
Mean	**808.12**	**864.60**	<16.3	**21.40**	**1415.26**	**9.37**	**124.95**	**40.04**
St. Dev.	**524.48**	**768.205**	**-**	**14.42**	**1372.90**	**0.42**	**279.93**	**4.60**

Table 3-4. Concentrations of inflammatory cytokines for cadaveric specimens (pg/mL)

Sample ID	IL-6	MCP-1	IL-1B	MIP-3a	M-CSF	TNF-α	IL-8	IL-2
14-08-614L	702.97	<98	3724.40	11.73	73237.05	<8.9	>1140	<35
16-03-730L	829.69	511.02	20.16	9.55	51918.99	<8.9	>1140	<35
16-03-730R	735.44	2177.16	53.18	15.34	46051.50	<8.9	>1140	35.64
15-10-491R	81.66	271.68	143.90	14.22	54057.49	9.78	>1140	<35
RLU1114149C R	>1180	>7940	485.56	>1920	32312.03	16.05	>1140	76.29
15-10-466L	27.77	113.35	129.72	<7.9	52170.45	<8.9	284.82	<35
15-08-338L	885.25	4243.78	45.43	122.22	33171.82	<8.9	>1140	<35
RLU0315169B L	20.18	411.68	178.07	28.96	14963.88	<8.9	694.16	<35
14-12-835R	91.31	286.65	70.96	8.53	62937.91	<8.9	>1140	<35
14-12-835L	87.13	414.07	115.95	18.69	69956.68	<8.9	>1140	<35
15-10-288L	>1180	649.77	128.98	100.84	77613.82	9.78	>1140	<35
RLU0519169L	102.86	489.24	63.06	20.55	34835.14	9.43	>1140	<35
RLU0519169R	93.87	409.96	51.12	23.90	54334.86	8.93	>1140	<35
Mean	**433.71**	**1292.27**	**376.67**	**164.51**	**52054.13**	**<8.9**	**977.70**	**<35**
St. Dev.	**456.11**	**2219.31**	**970.36**	**506.54**	**18336.89**	**-**	**340.18**	**-**

Table 3-5. Linear polyethylene wear measurements for each sample (mm)

Sample ID	Polyethylene Linear Wear (mm)
14-08-614L	0.040
16-03-730L	0.151
16-03-730R	1.867
15-10-491R	0.224
RLU1114149C R	0.097
15-10-466L	0.099
RLU0315169B L	0.086
14-12-835R	0.087
15-10-288L	0.351
14-08-580L	0.089
RLU1029149C	0.909
14-05-425L	0.111
16-08-983R	0.071
RLU0519169L	0.131
RLU0519169R	0.126

Table 3-6. Cobalt, chromium, and titanium levels for implant (ppb or µg/L) and component material for each

Sample ID	Cobalt (ppb)	Chromium (ppb)	Titanium (ppb)	Stem Material	Head Material
14-08-614L	0.2875	3.3730	0.0000	CoCr	-
16-03-730L	0.8030	8.0955	0.7548	CoCr	Ti
16-03-730R	2.3013	5.2407	0.0000	Zirconia	Ti
15-10-491R	1.3335	1.2918	0.0000	CoCr	CoCr
RLU1114149CR	4.4546	51.2542	1.9681	CoCr	Ti
15-10-466L	0.5363	24.5765	21.2185	CoCr	CoCr
15-08-338L	3.8725	4.8174	0.0000	CoCr	Ti
RLU0315169BL	1.1816	1.4999	0.0000	CoCr	CoCr
14-12-835R	0.3901	1.9043	269.5773	CoCr	Ti
14-12-835L	2.2751	44.4078	3.0290	CoCr	Ti
15-10-288L	3.6876	32.4262	0.0000	CoCr	CoCr
14-08-580L	0.5759	10.4695	2.2960	-	-
RLU1029149C	0.7221	1.4135	33.7643	CoCr	Ti
14-05-425L	7.8357	29.7613	0.0000	Metal	Ti
14-11-788R	576.0150	1960.2175	142.6086	CoCr	Ti
16-08-983R	-	-	-	CoCr	Ti
RLU0519169L	2.0000	185.5829	13.1055	CoCr	Ti
RLU0519169R	0.2637	4.4603	1.9699	Alumina	Ti
Mean	**35.7962**	**139.4584**	**28.8407**	**-**	**-**
St. Dev.	**139.2254**	**471.2798**	**70.9989**	**-**	**-**

Testing Comparisons

Dissociation Force versus Cytokine Concentrations (a)

The concentrations of each of the relevant inflammatory cytokines were compared to the dissociation forces for each sample. Cytokine levels that were below the limits of detection were not included in these comparisons, however, those that were above the limits of detection were included, with their value being the maximum detectable limit. Direct comparisons were done between the two variables and a linear trendline was applied to determine how significant this correlation was. However, since no one in literature has used any specific trend to assign to this type of data, the variables were also ranked (one rank for dissociation force and one rank for cytokine concentrations) for each sample, and those ranks were plotted against one another to determine if there was any significance in these comparisons. The direct comparison and the ranked comparison for the five relevant inflammatory cytokines can be seen in **Figures 3-2** through **3-11.** The correlation coefficients, confidence interval for the correlation, and Pearson's rank p-values for each comparison can be seen in **Tables 3-7** and **3-8,** and the correlation coefficients and Pearson rank p-values once outliers were removed can be seen in **Tables 3-9** and **3-10.**

Dissociation Force versus Head Corrosion Scores (b)

The stem components of the implants, as shown previously in **Table 3-1**, were almost all given a score of 1 with one exception, being scored a 3. However, the head scores were fairly equally distributed between 1 and 2. Therefore, these were separated into two groups based on corrosion scores, and normality was assessed with a Shapiro-Wilk test. The two groups were found to be normally distributed with equal variances, so a t-test was performed to see if there was a difference in dissociation force between the two groups. The p-value for this t-test was 0.6 with a power of 8%, meaning that while no significant difference could be detected, a low power makes us less likely to detect a difference when one does in fact exist.

Dissociation Force versus Metal Ion Concentrations (c)

The dissociation force of each implant was compared to the cobalt, chromium, and titanium levels for each implant. There were eleven total samples for which dissociation forces as well as metal ion concentrations were able to be obtained. Direct comparisons were done between the two variables and a linear trend line was applied to assess the significance of the relationship. However, since nothing was found in literature applying any specific trend to assess this type of data, the variables were also ranked (one rank for the dissociation force and one rank for the metal ion levels) for each sample, and those ranks were plotted against each other to determine if there was any significance in these comparisons. The direct comparison and ranked comparison for each of the three

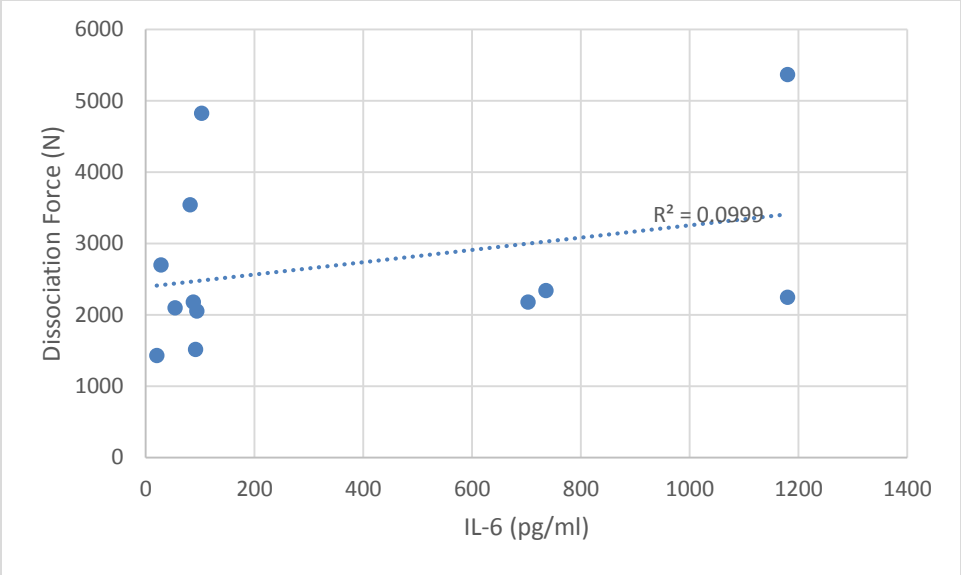

Figure 3-2. IL-6 concentration compared to the dissociation force

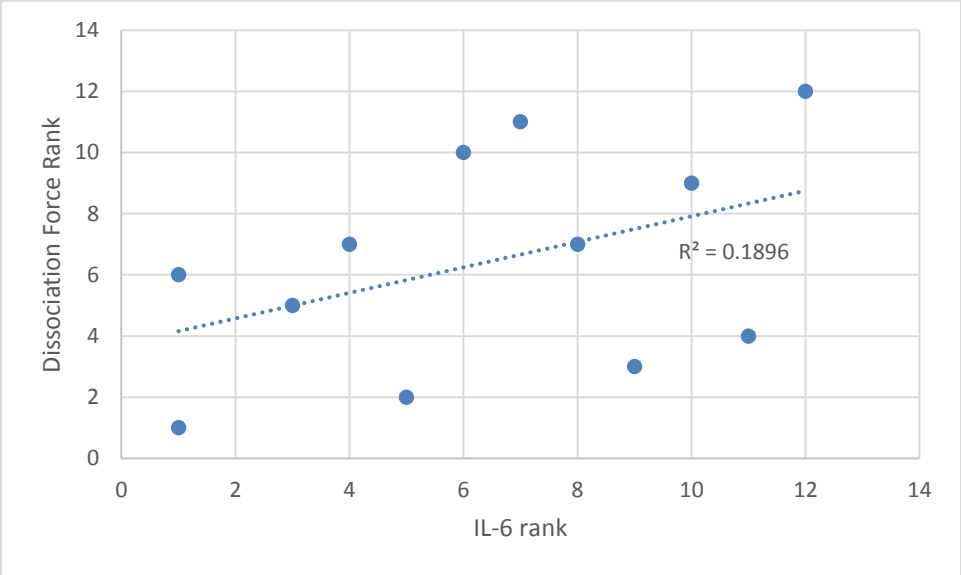

Figure 3-3. IL-6 rank among all samples compared to the dissociation force rank

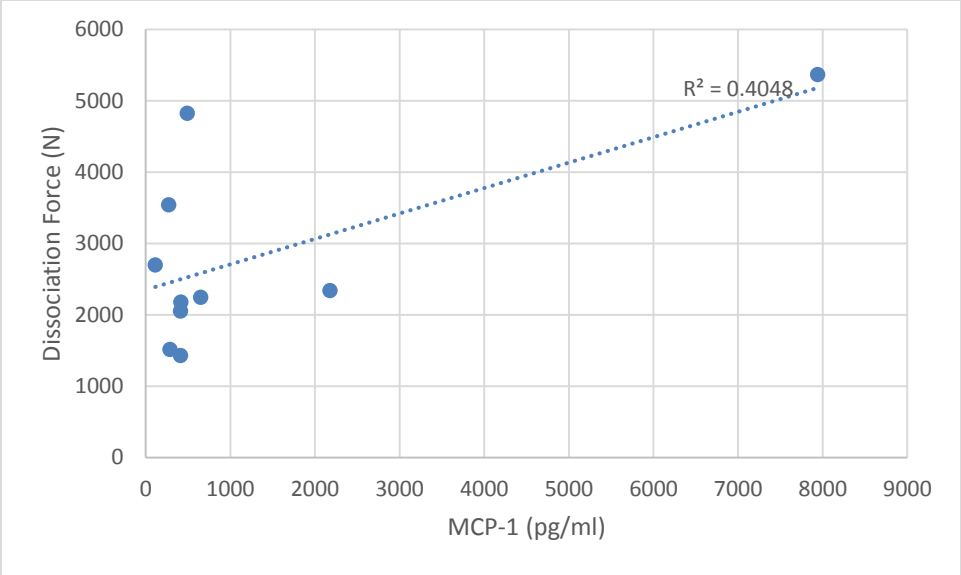

Figure 3-4. MCP-1 concentration compared to the dissociation force

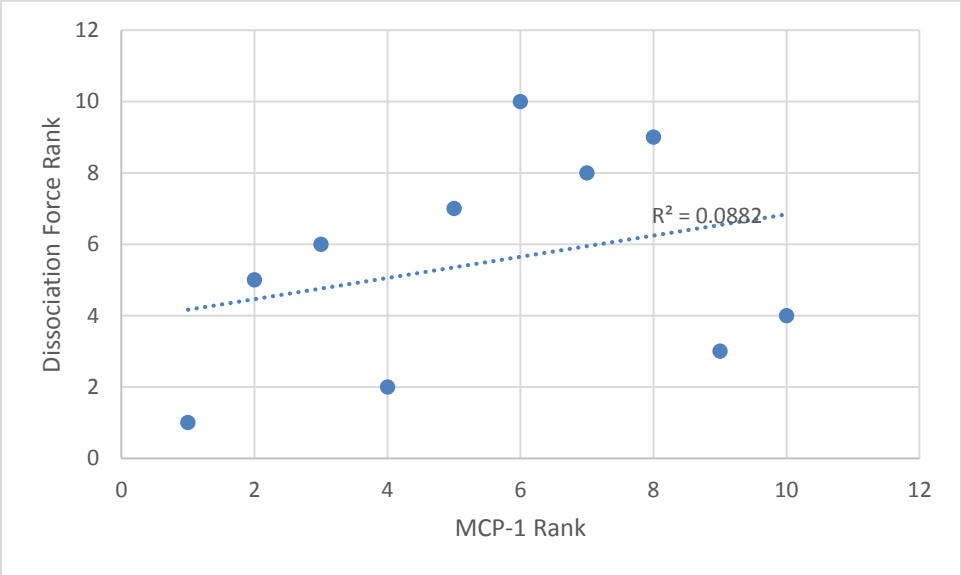

Figure 3-5. MCP-1 rank among all samples compared to the dissociation force rank

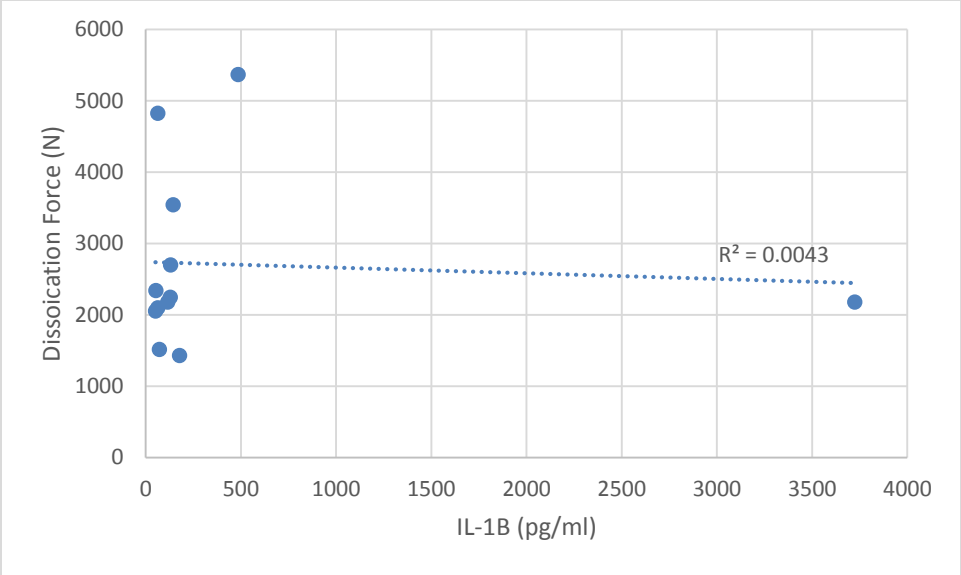

Figure 3-6. IL-1β concentration compared to the dissociation force

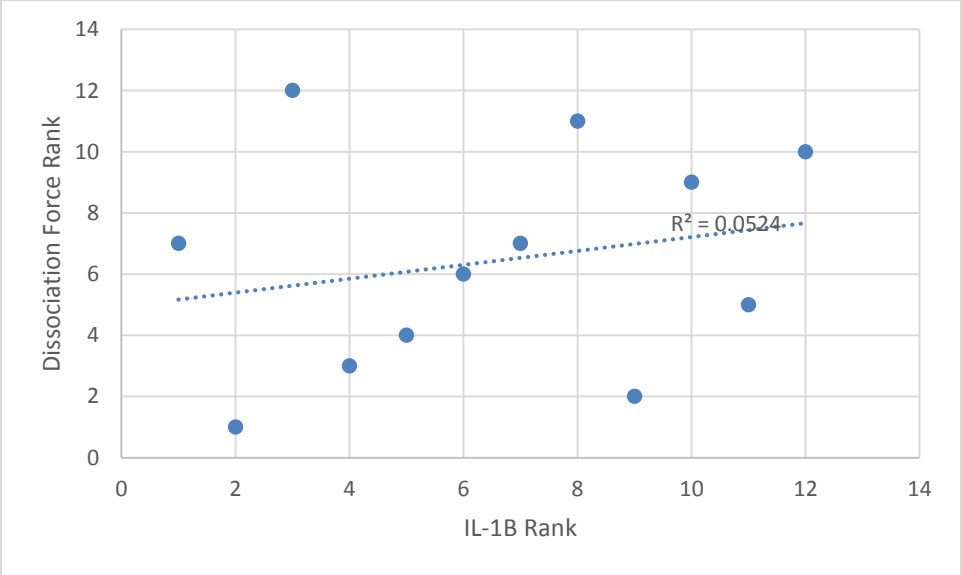

Figure 3-7. IL-1β rank among all samples compared to the dissociation force rank

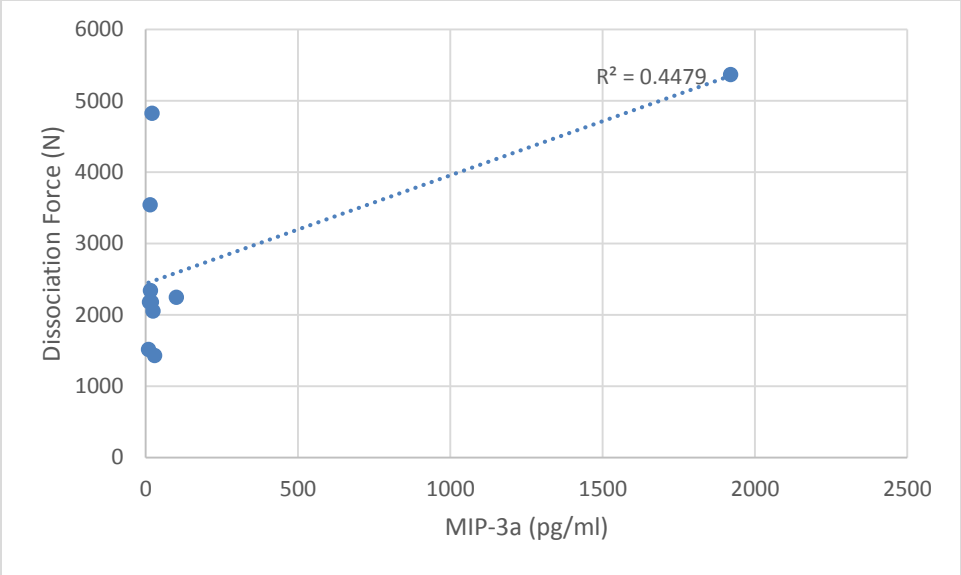

Figure 3-8. MIP-3α concentration compared to the dissociation force

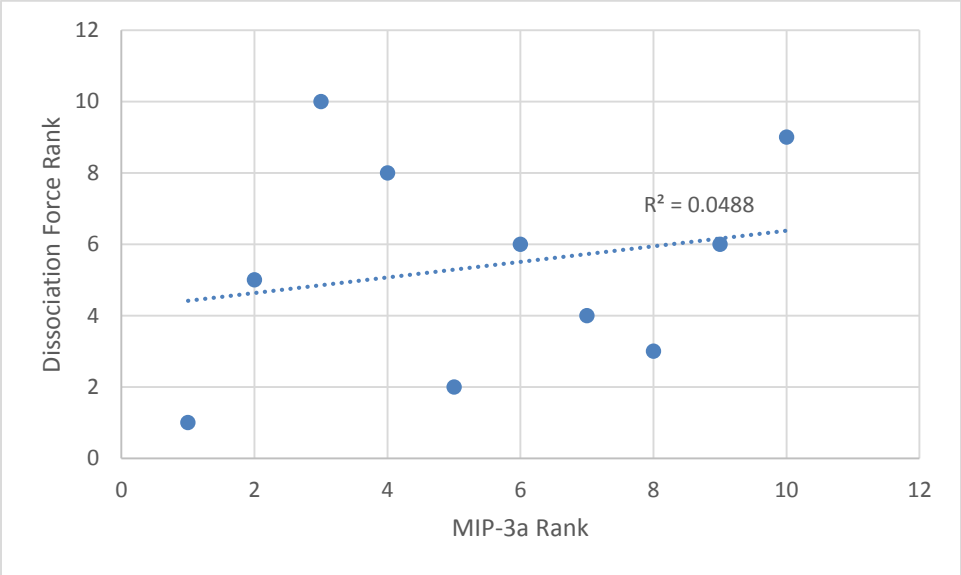

Figure 3-9. MIP-3α rank among all samples compared to the dissociation force rank

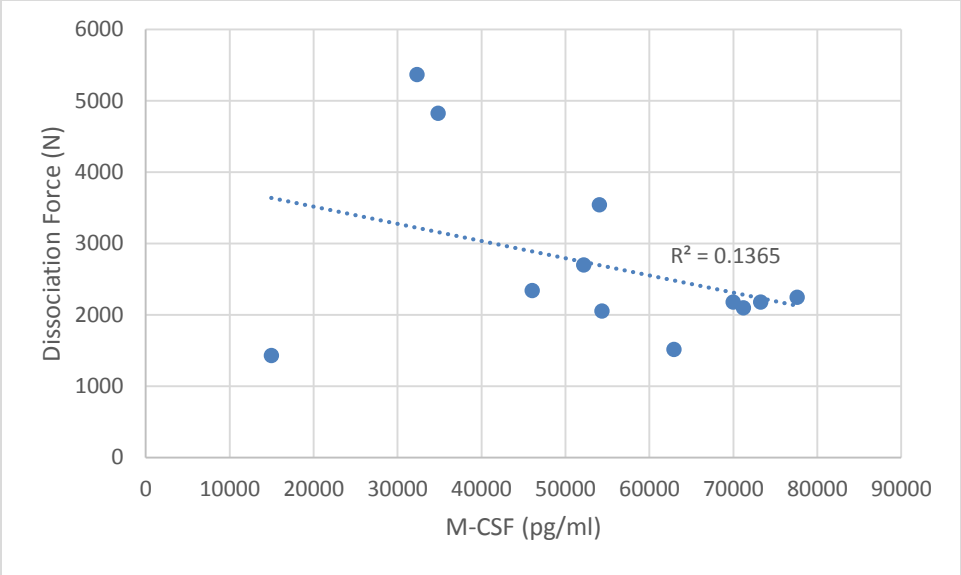

Figure 3-10. M-CSF concentration compared to the dissociation force

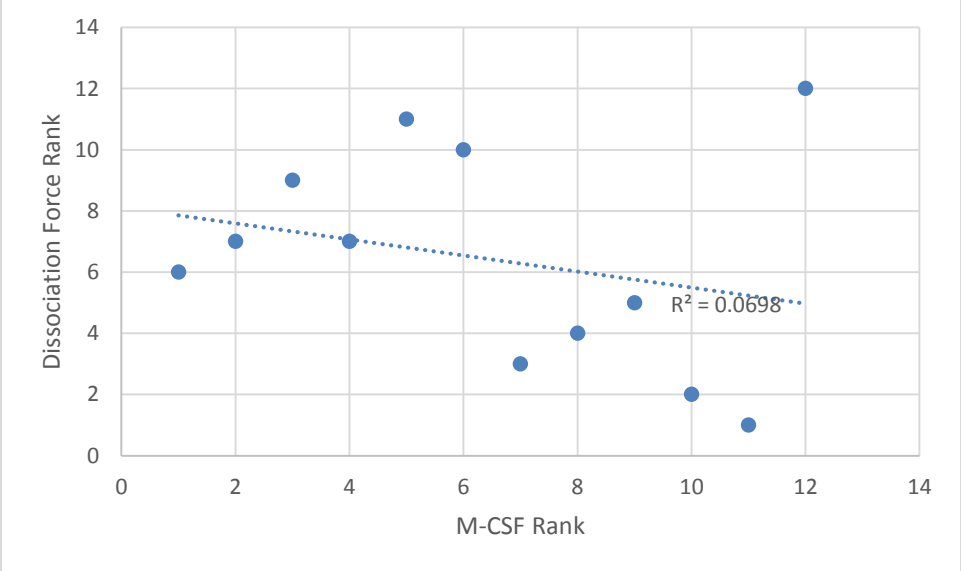

Figure 3-11. M-CSF rank among all samples compared to the dissociation force rank

Table 3-7. Correlation coefficients and p-values for the direct comparison of each cytokine to its dissociation force

Cytokine	Sample Size	Correlation Coefficient (r)	95% Confidence Interval of r	P-Value
IL-6	12	0.32	-0.32 to 0.75	0.3
MCP-1	10	0.64	0.01 to 0.90	0.05
IL-1β	12	-.066	-0.62 to 0.53	0.8
MIP-3α	10	0.67	0.069 to 0.91	0.03
M-CSF	12	-0.37	-0.78 to 0.26	0.2

Table 3-8. Correlation coefficients and p-values for the ranked comparison of each cytokine to its dissociation force

Cytokine	Sample Size	Correlation Coefficient (r)	95% Confidence Interval of r	P-Value
IL-6	12	0.44	-0.19 to 0.81	0.2
MCP-1	10	0.30	-0.41 to 0.78	0.4
IL-1β	12	0.23	-0.40 to 0.71	0.5
MIP-3α	10	0.22	-0.47 to 0.75	0.5
M-CSF	12	-0.26	-0.73 to 0.37	0.4

Table 3-9. Correlation coefficients and p-values for the direct comparison of each cytokine to its dissociation force with outliers removed

Cytokine	Sample Size	Correlation Coefficient (r)	95% Confidence Interval of r	P-Value
IL-6	12	0.32	-0.32 to 0.75	0.3
MCP-1	8	0.041	-0.68 to 0.72	0.9
IL-1β	10	-0.14	-0.71 to 0.54	0.7
MIP-3α	10	0.014	-0.62 to 0.64	0.9
M-CSF	12	-0.37	-0.78 to 0.26	0.2

Table 3-10. Correlation coefficients and p-values for the ranked comparison of each cytokine to its dissociation force with outliers removed

Cytokine	Sample Size	Correlation Coefficient (r)	95% Confidence Interval of r	P-Value
IL-6	12	0.44	-0.19 to 0.81	0.2
MCP-1	8	0.050	-0.68 to 0.73	0.9
IL-1β	10	0.069	-0.59 to 0.67	0.9
MIP-3α	10	-0.16	-0.72 to 0.52	0.7
M-CSF	12	-0.26	-0.73 to 0.37	0.4

metal ions assessed can be seen in **Figures 3-12** through **3-17**. The correlation coefficients, confidence interval for the correlations, and Pearson rank p-values for each comparison can be seen in **Tables 3-11** and **3-12.**

Linear Polyethylene Wear versus Cytokine Concentrations (d)

The concentrations of each of the relevant inflammatory cytokines was compared to the linear polyethylene wear measurements for each sample. Cytokine levels that were below the limits of detection were not included in these comparisons, however, those that were above the limits of detection were included, with their value being the maximum detectable limit. Direct comparisons were done between the two variables and a linear trendline was applied to determine how significant this correlation was. However, since nothing was found in literature applying any specific trend to assess this type of data, the variables were also ranked (one rank for the polyethylene wear and one rank for cytokine concentrations) for each sample, and those ranks were plotted against one another to determine if there was any significance in these comparisons. The direct comparison and the ranked comparison for the five relevant inflammatory cytokines can be seen in **Figures 3-18** through **3-27.** The correlation coefficients, confidence interval for the correlation, and Pearson rank p-values for each comparison can be seen in **Tables 3-13** and **3-14,** and the correlation coefficients, confidence interval for the correlation, and Pearson Rank p-values once outliers were removed can be seen in **Tables 3-15** and **3-16.**

Metal Ion Concentrations versus Cytokine Concentrations (e)

The cytokine concentrations for the cytokines that had values within the limits of detection (IL-6, MCP-1, IL-1β, MIP-3α, and M-CSF) were compared to the cobalt, chromium, and titanium levels for each implant. There were thirteen samples for which the cytokine values and metal levels were available, except for MCP-1 in which there were only twelve available, due to one of the samples being below limits of detection for the cytokine. Direct comparisons were done between the two variables and a linear trendline was applied. The comparison of these two variables can be seen in **Figures 3-28** through **3-42**, and the correlation coefficients, confidence interval for the correlation, and Pearson rank p-values for each comparison can be seen in **Tables 3-17** through **3-19.**

Metal Ion Concentrations versus Corrosion Scores (f)

Because the levels of corrosion were so low in these samples, graphical representation did not give much insight into what relationships, if any, were evident. The samples were divided into two groups, those with a head corrosion of 1 and those with a head corrosion of 2, and a t-tests was completed between these two groups for each of the three metals. There were five samples in the group with corrosion scores of 1, and seven samples in the group with corrosion scores of 2. For cobalt, the Shapiro-Wilk normality

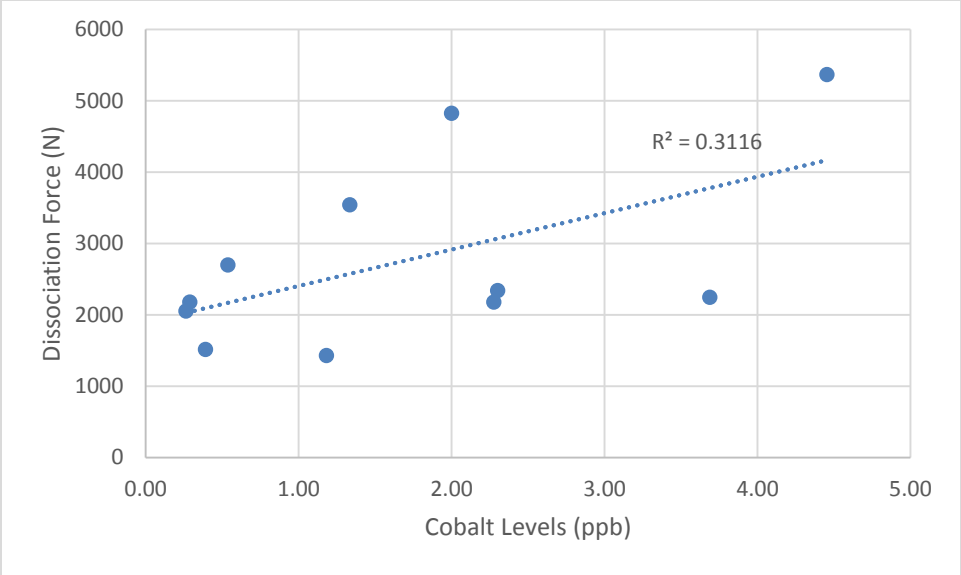

Figure 3-12. Dissociation force compared cobalt levels in tissue

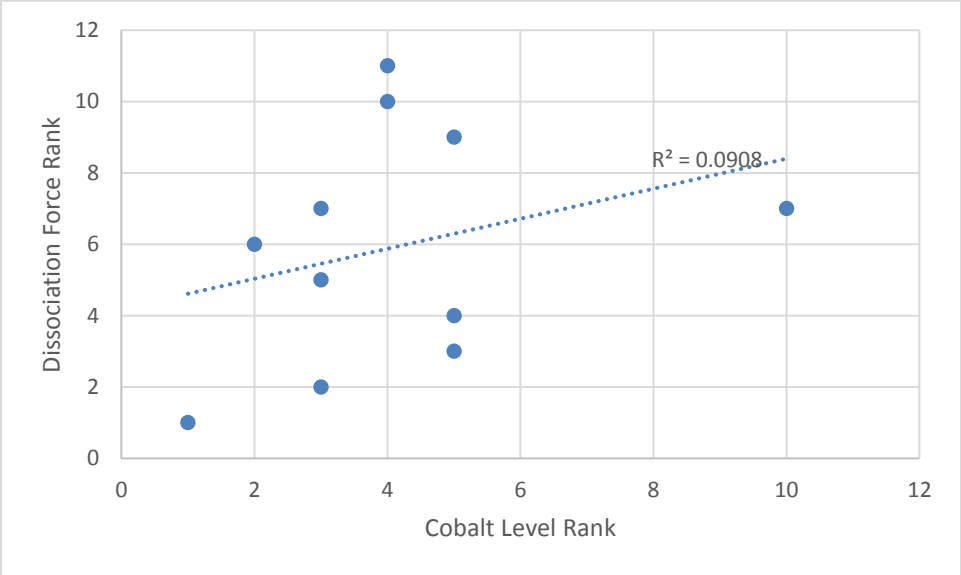

Figure 3-13. Dissociation force rank compared cobalt level in tissue rank

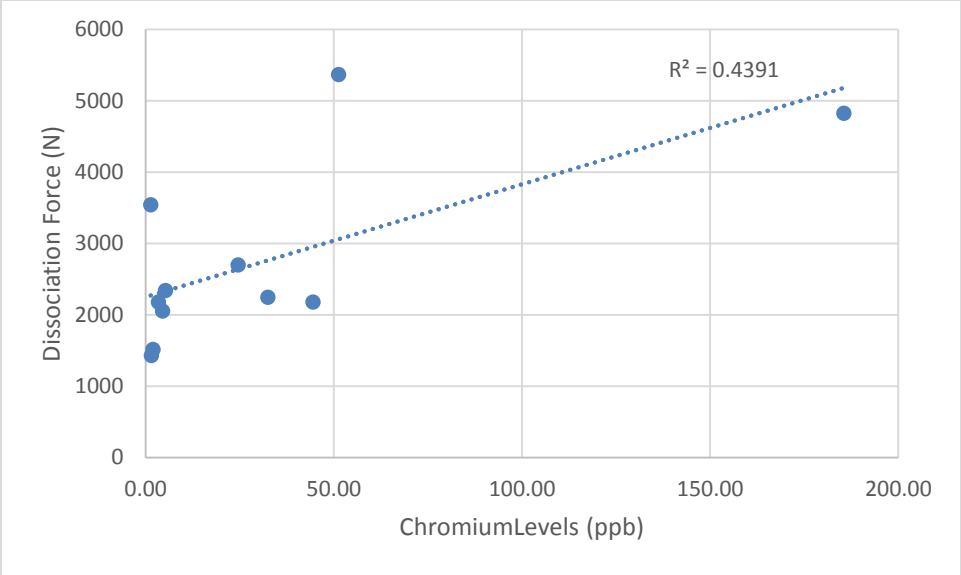

Figure 3-14. Dissociation force compared to chromium levels in tissue

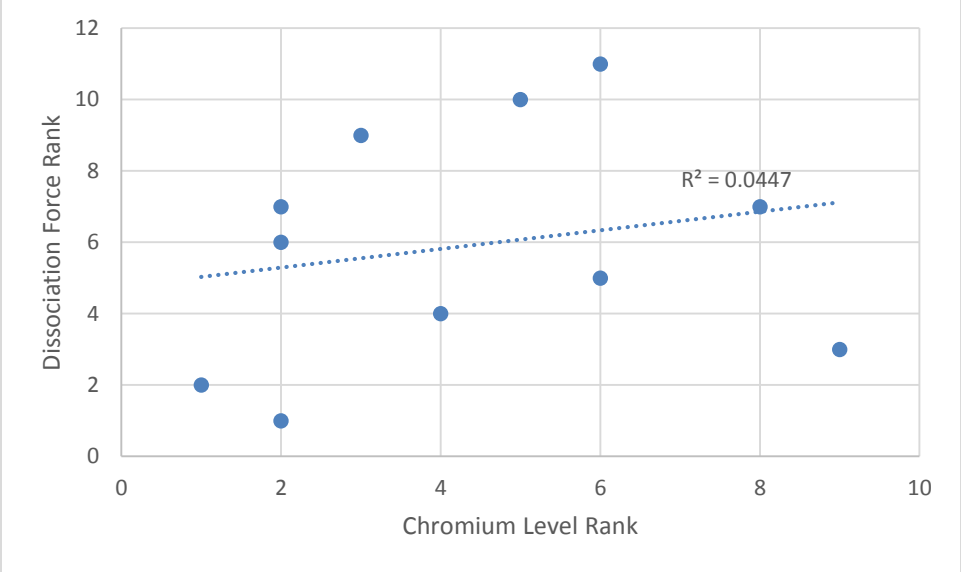

Figure 3-15. Dissociation force rank compared to chromium levels in tissue rank

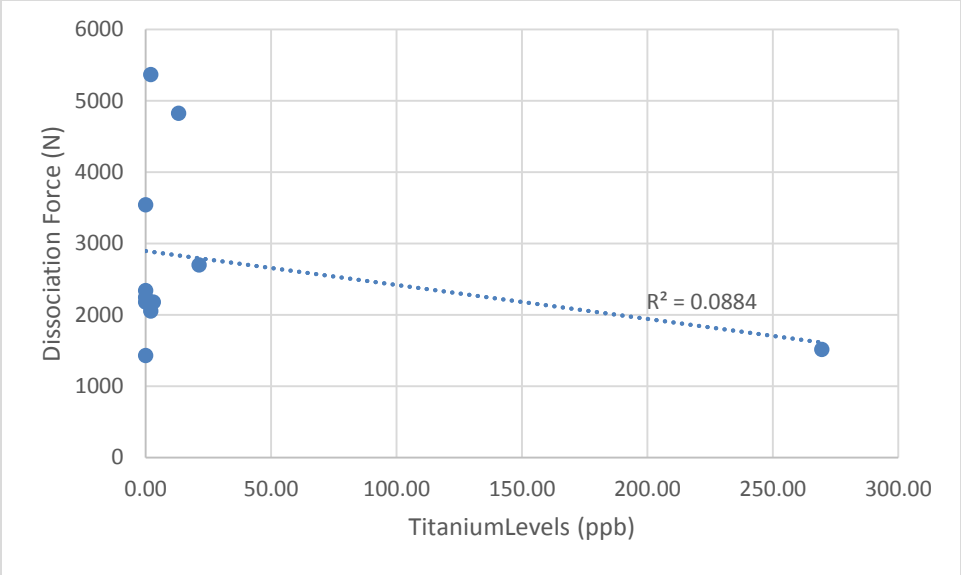

Figure 3-16. Dissociation force compared to titanium levels in tissue

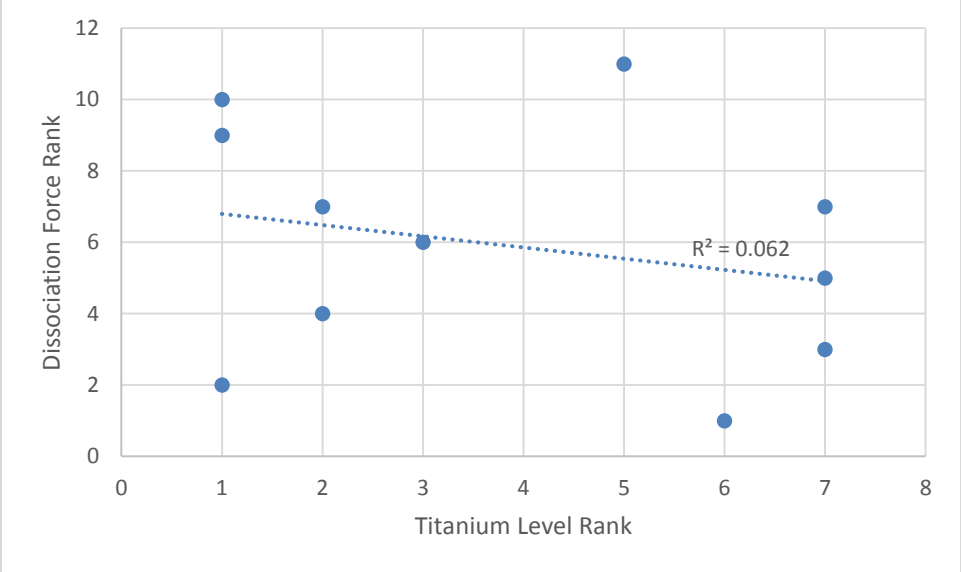

Figure 3-17. Dissociation force rank compared to titanium levels in tissue rank

Table 3-11. Correlation coefficients and p-values for the direct comparison of each metal ion type to dissociation force

Metal	Sample Size	Correlation Coefficient (r)	95% Confidence Interval of r	P-Value
Cobalt	11	0.56	-0.062 to 0.87	0.07
Chromium	11	0.66	0.11 to 0.90	0.03
Titanium	11	-0.30	-0.76 to 0.37	0.4

Table 3-12. Correlation coefficients and p-values for the ranked comparison of each metal ion type to its dissociation force

Metal	Sample Size	Correlation Coefficient (r)	95% Confidence Interval of r	P-Value
Cobalt	11	0.30	-0.36 to 0.76	0.4
Chromium	11	0.21	-0.45 to 0.72	0.5
Titanium	11	-0.14	-0.68 to 0.50	0.7

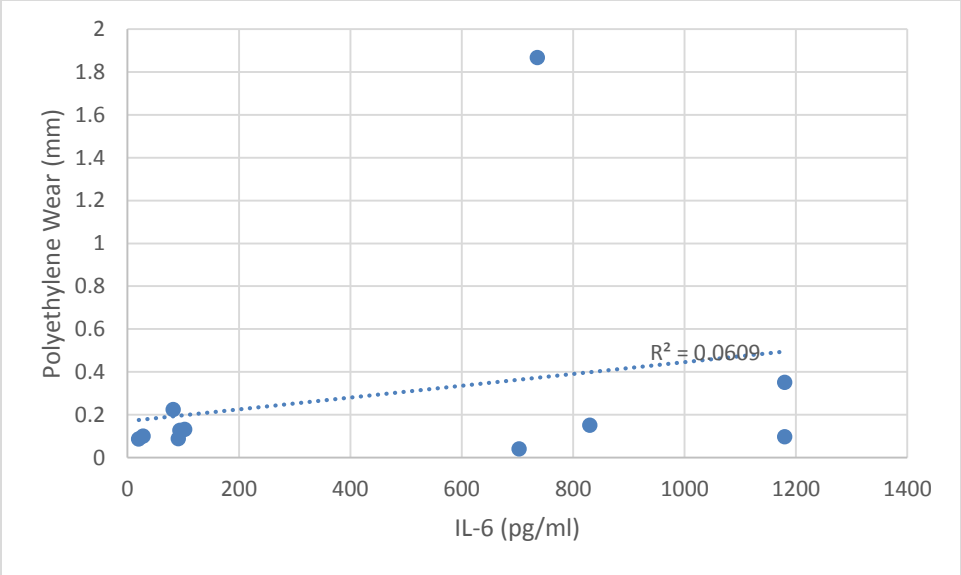

Figure 3-18. IL-6 concentration compared to the polyethylene wear

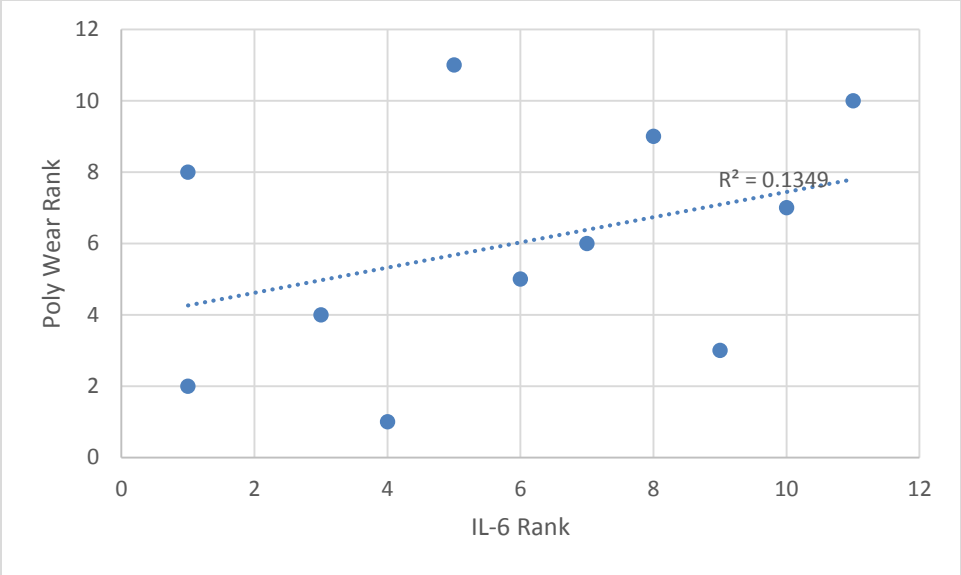

Figure 3-19. IL-6 rank among all samples compared to the polyethylene wear rank

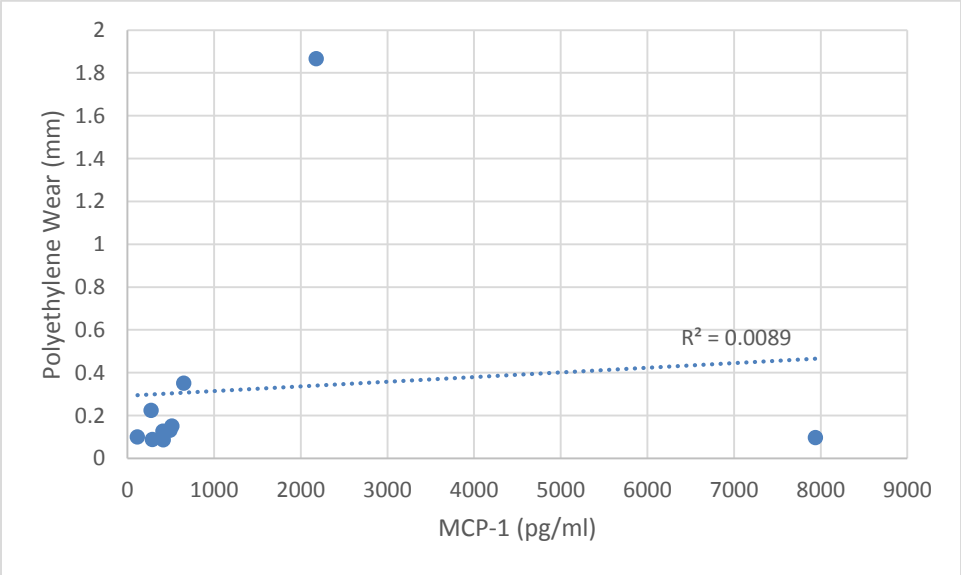

Figure 3-20. MCP-1 concentration compared to the polyethylene wear

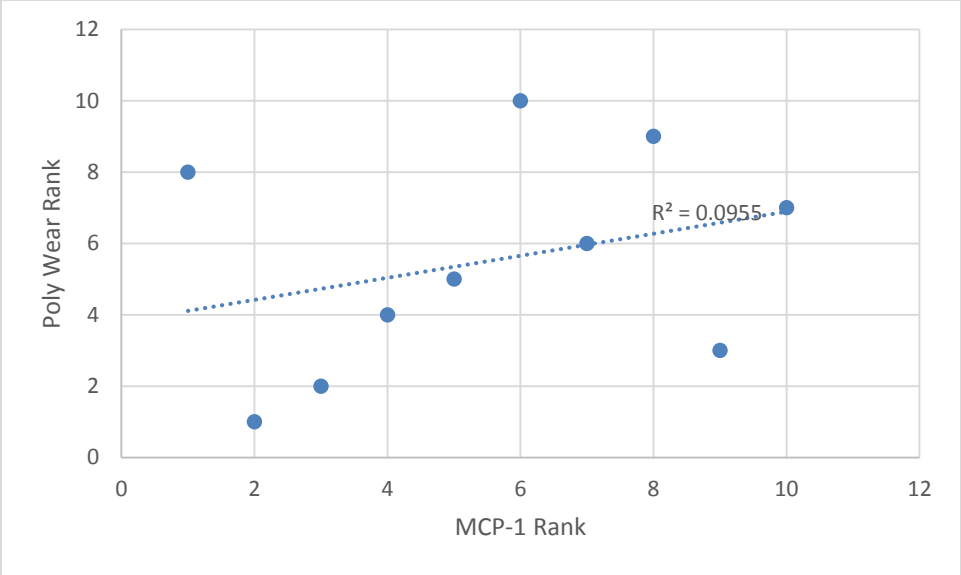

Figure 3-21. MCP-1 rank among all samples compared to the polyethylene wear rank

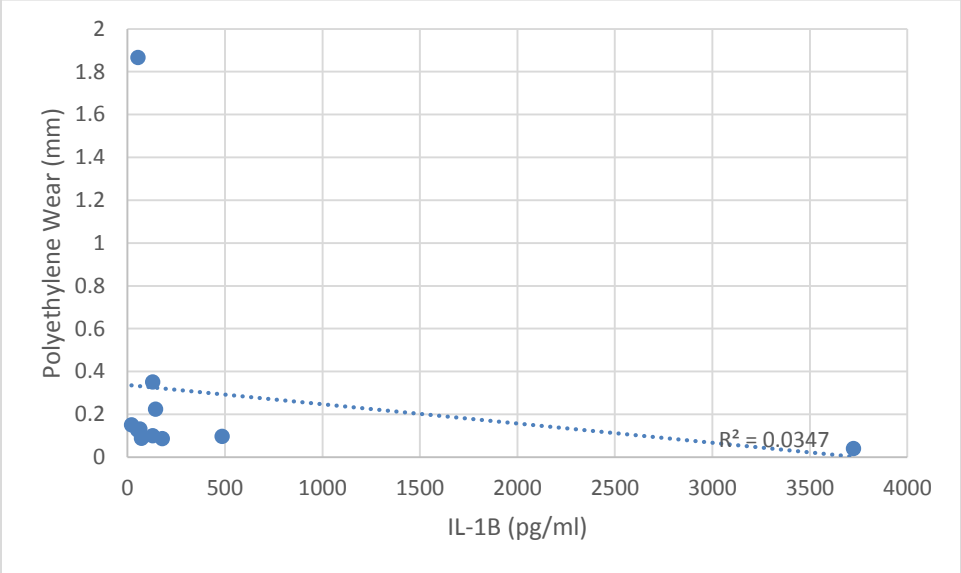

Figure 3-22. IL-1β concentration compared to the polyethylene wear

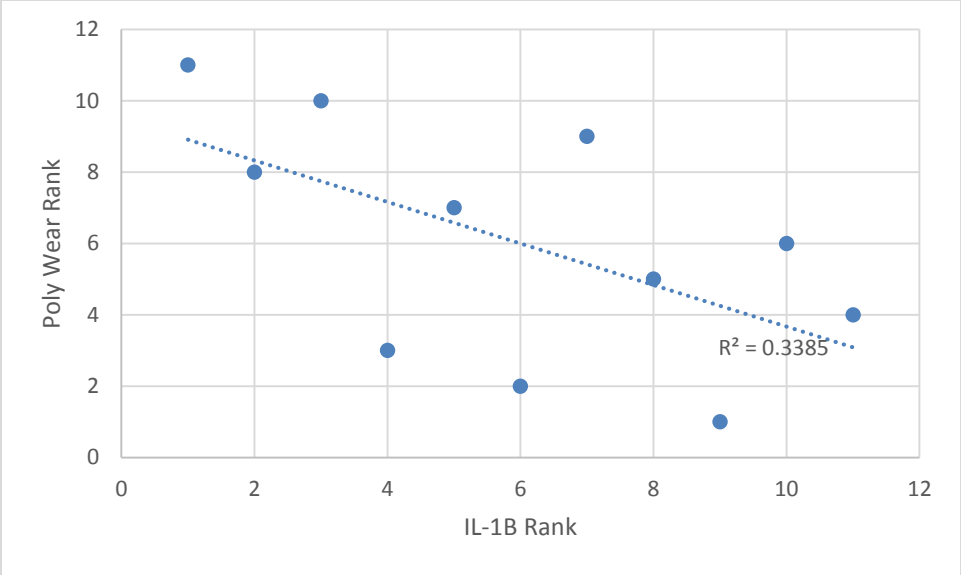

Figure 3-23. IL-1β rank among all samples compared to the polyethylene wear rank

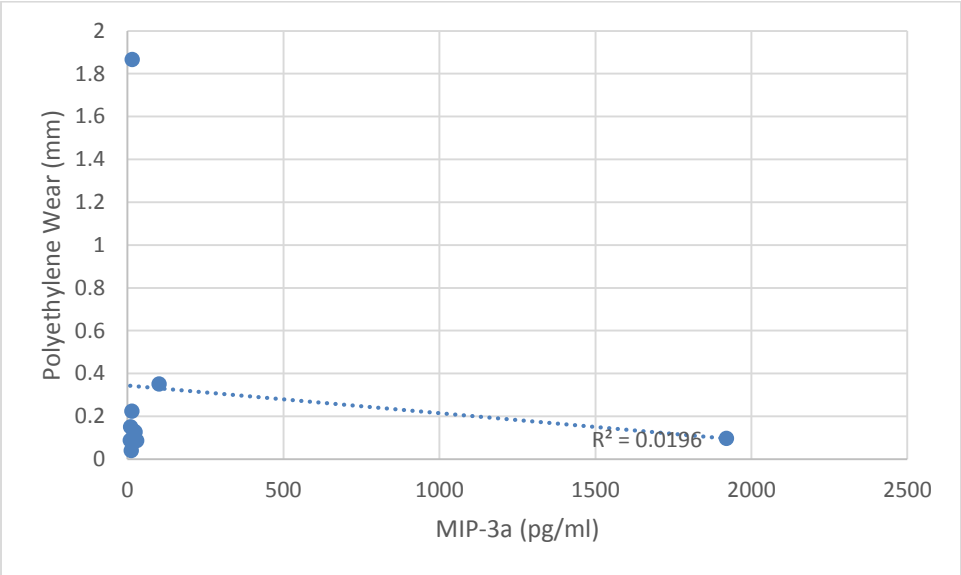

Figure 3-24. MIP-3α concentration compared to the polyethylene wear

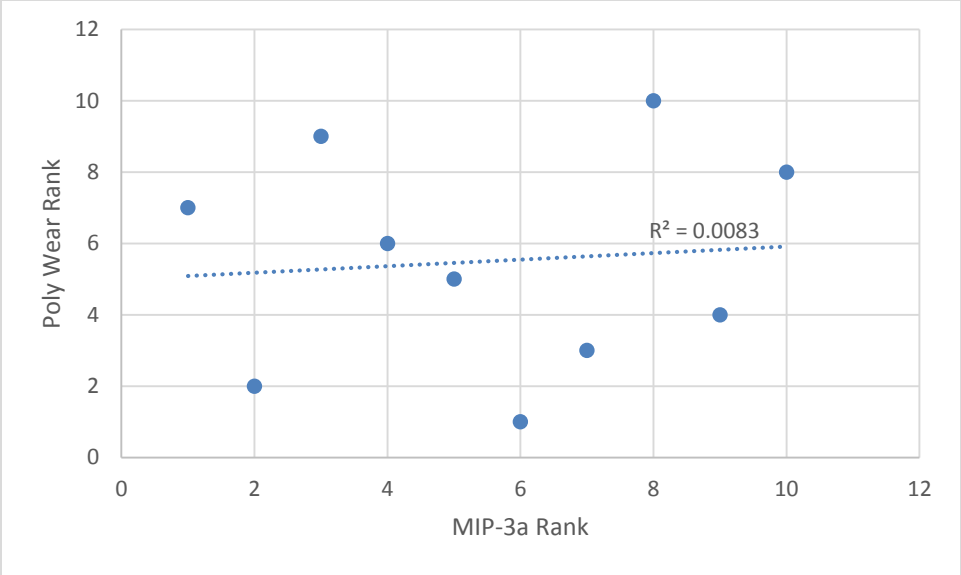

Figure 3-25. MIP-3α rank among all samples compared to the polyethylene wear rank

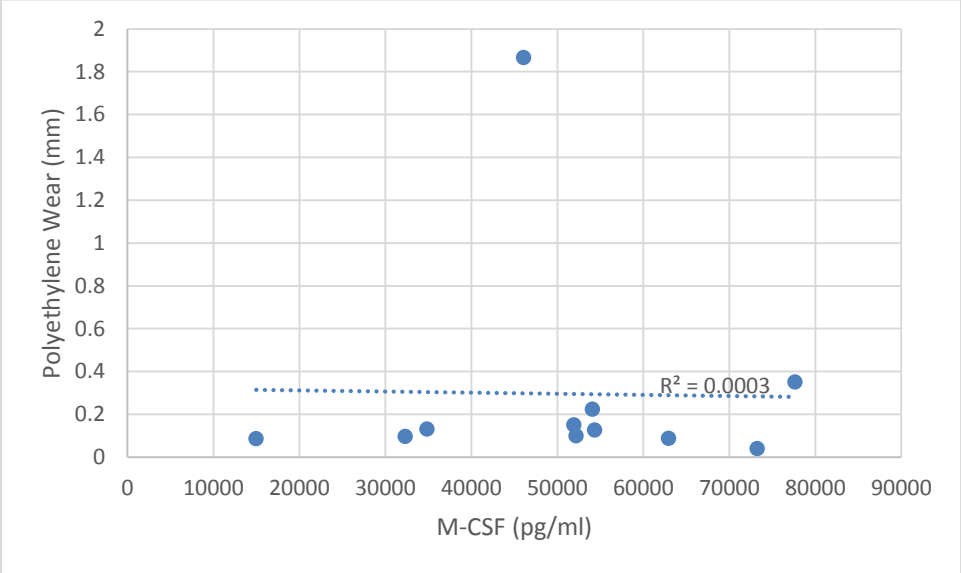

Figure 3-26. M-CSF concentration compared to the polyethylene wear

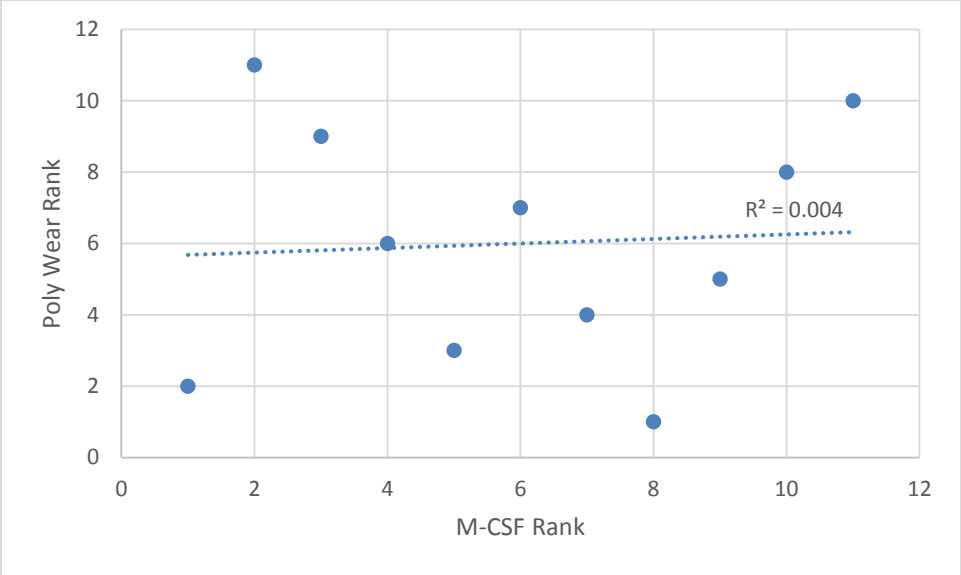

Figure 3-27. M-CSF rank among all samples compared to the polyethylene wear rank

Table 3-13. Correlation coefficients and p-values for the direct comparison of each cytokine to its polyethylene wear

Cytokine	Sample Size	Correlation Coefficient (r)	95% Confidence Interval of r	P-Value
IL-6	11	0.25	-0.42 to 0.74	0.5
MCP-1	10	0.094	-0.57 to 0.68	0.8
IL-1β	11	-0.19	-0.71 to 0.47	0.6
MIP-3α	10	-0.14	-0.71 to 0.54	0.7
M-CSF	11	-0.017	-0.61 to 0.59	0.9

Table 3-14. Correlation coefficients and p-values for the ranked comparison of each cytokine to its polyethylene wear

Cytokine	Sample Size	Correlation Coefficient (r)	95% Confidence Interval of r	P-Value
IL-6	11	0.37	-0.30 to 0.79	0.3
MCP-1	10	0.31	-0.40 to 0.79	0.4
IL-1β	11	-0.58	-0.88 to 0.027	0.06
MIP-3α	10	0.091	-0.57 to 0.68	0.8
M-CSF	11	0.063	-0.56 to 0.64	0.9

Table 3-15. Correlation coefficients and p-values for the direct comparison of each cytokine to its polyethylene wear with outliers removed

Cytokine	Sample Size	Correlation Coefficient (r)	95% Confidence Interval of r	P-Value
IL-6	11	0.25	-0.42 to 0.74	0.5
MCP-1	8	0.57	-0.22 to 0.91	0.1
IL-1β	9	-0.25	-0.79 to 0.49	0.5
MIP-3α	8	-0.074	-0.74 to 0.67	0.9
M-CSF	11	0.017	-0.59 to 0.61	0.9

Table 3-16. Correlation coefficients and p-values for the ranked comparison of each cytokine to its polyethylene wear with outliers removed

Cytokine	Sample Size	Correlation Coefficient (r)	95% Confidence Interval of r	P-Value
IL-6	11	0.37	-0.30 to 0.79	0.3
MCP-1	8	0.39	-0.43 to 0.86	0.3
IL-1β	9	-0.36	-0.83 to 0.40	0.3
MIP-3α	8	0	-0.70 to 0.70	1.00
M-CSF	11	0.063	-0.56 to 0.64	0.9

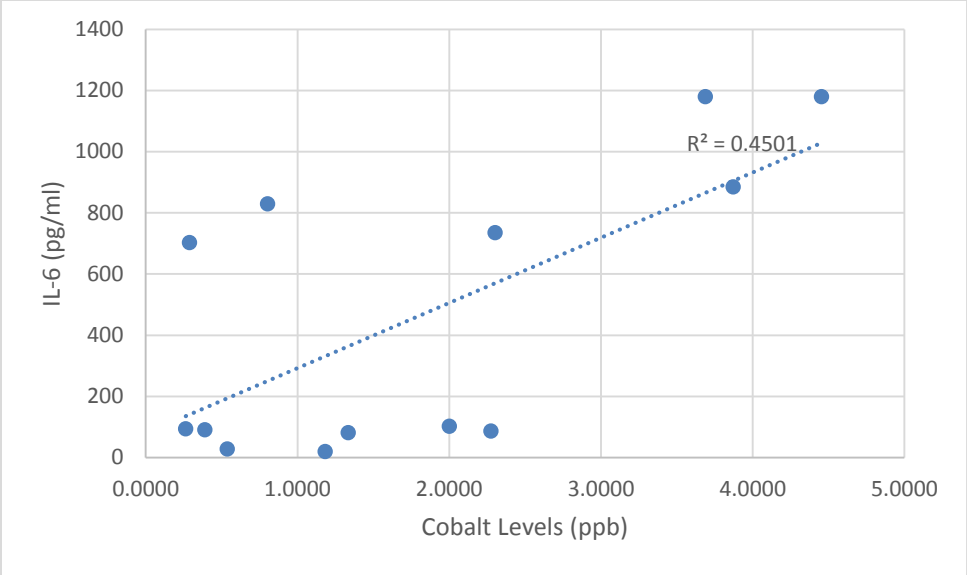

Figure 3-28. Cobalt levels in tissue compared to IL-6 levels in synovial fluid

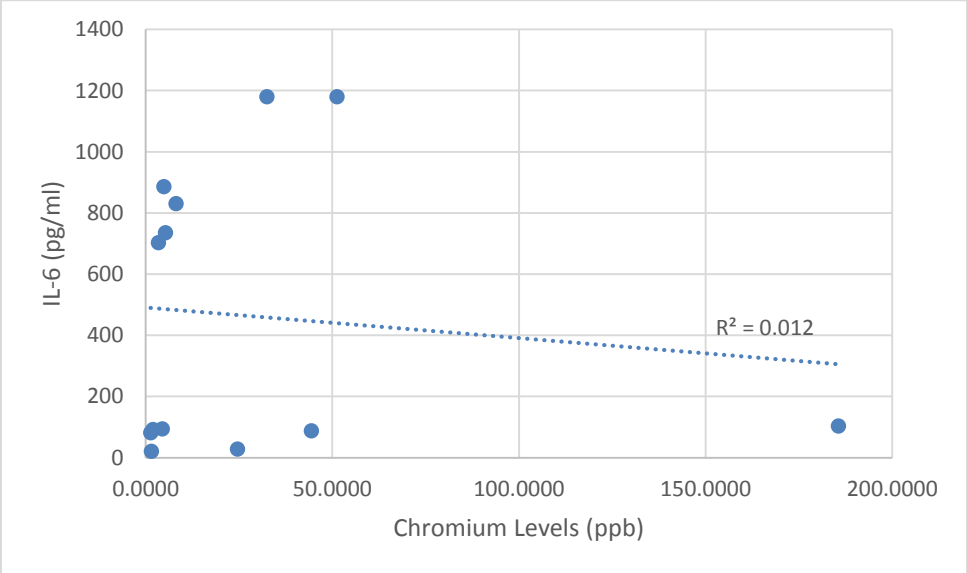

Figure 3-29. Chromium levels in tissue compared to IL-6 levels in synovial fluid

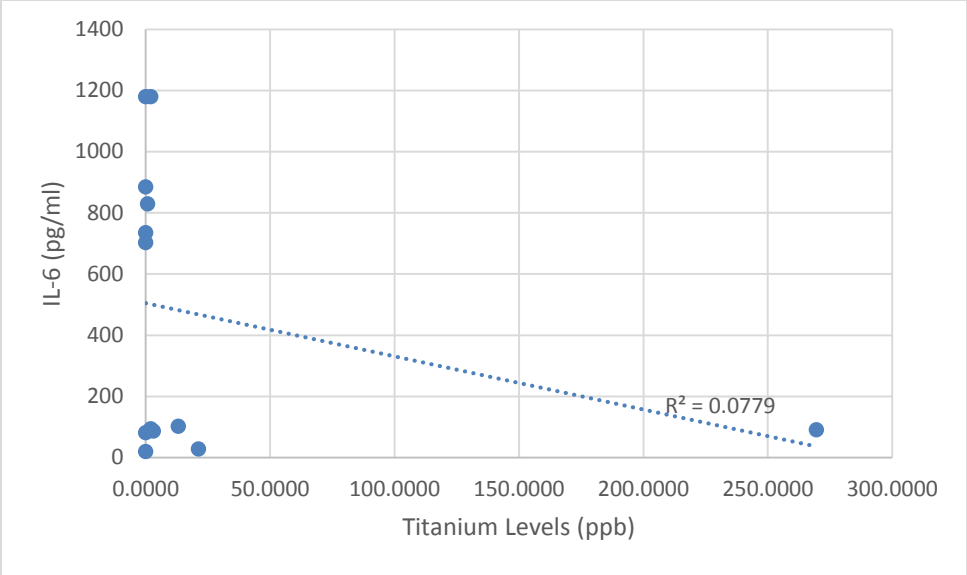

Figure 3-30. Titanium levels in tissue compared to IL-6 levels in synovial fluid

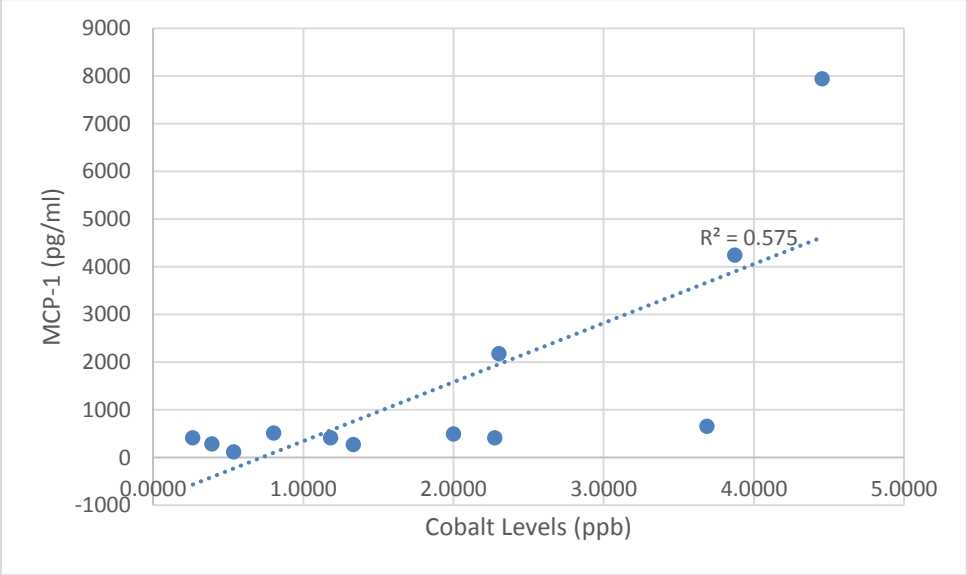

Figure 3-31. Cobalt levels in tissue compared to MCP-1 levels in synovial fluid

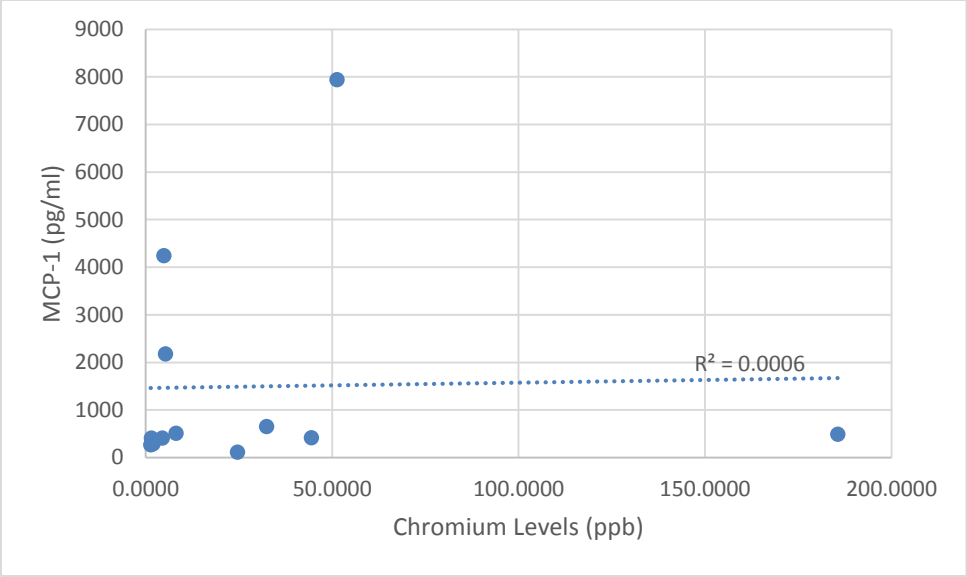

Figure 3-32. Chromium levels in tissue compared to MCP-1 levels in synovial fluid

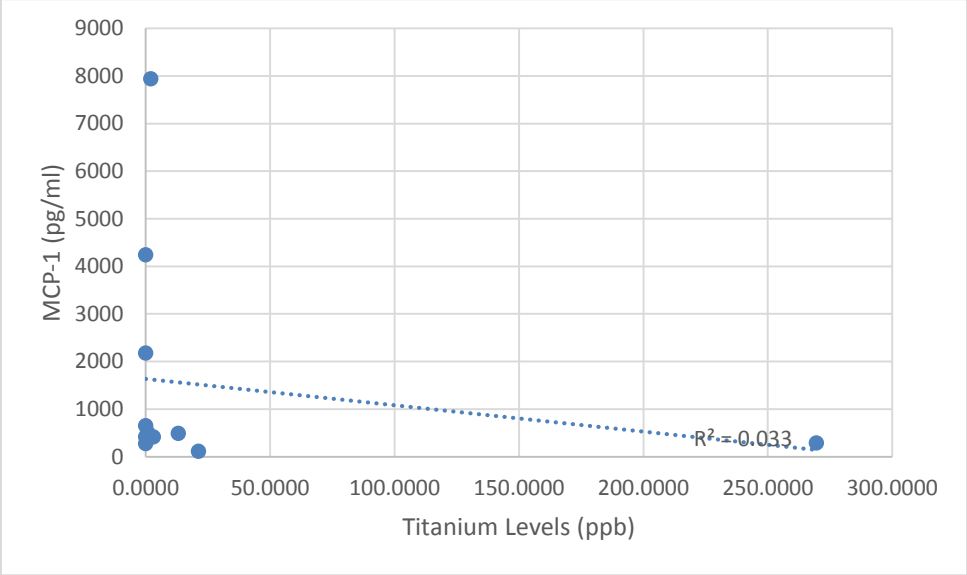

Figure 3-33. Titanium levels in tissue compared to MCP-1 levels in synovial fluid

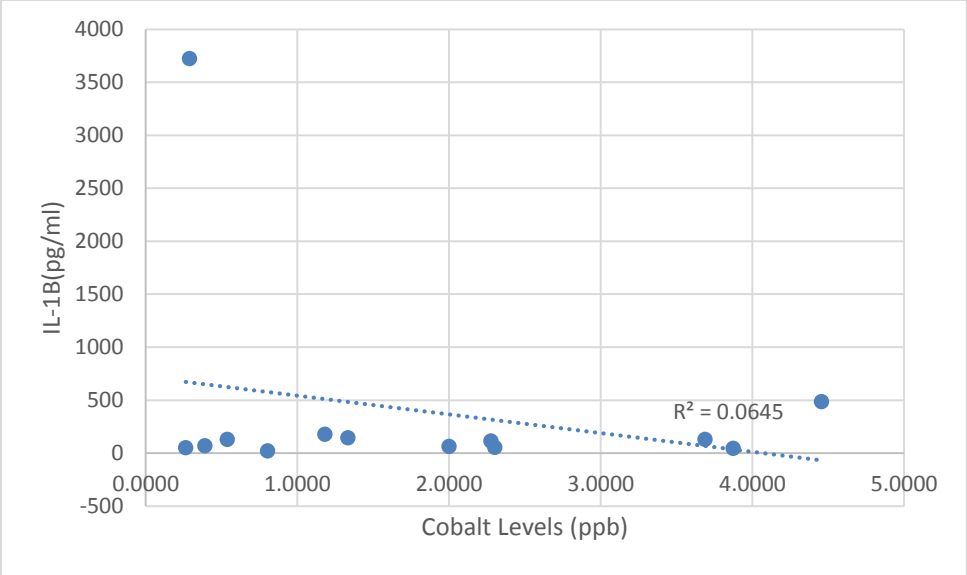

Figure 3-34. Cobalt levels in tissue compared to IL-1β levels in synovial fluid

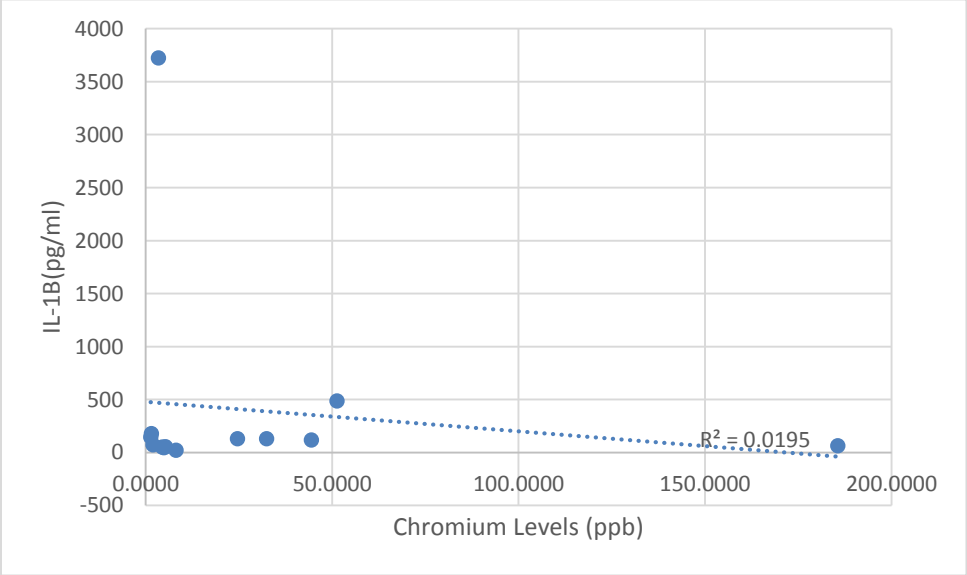

Figure 3-35. Chromium levels in tissue compared to IL-1β levels in synovial fluid

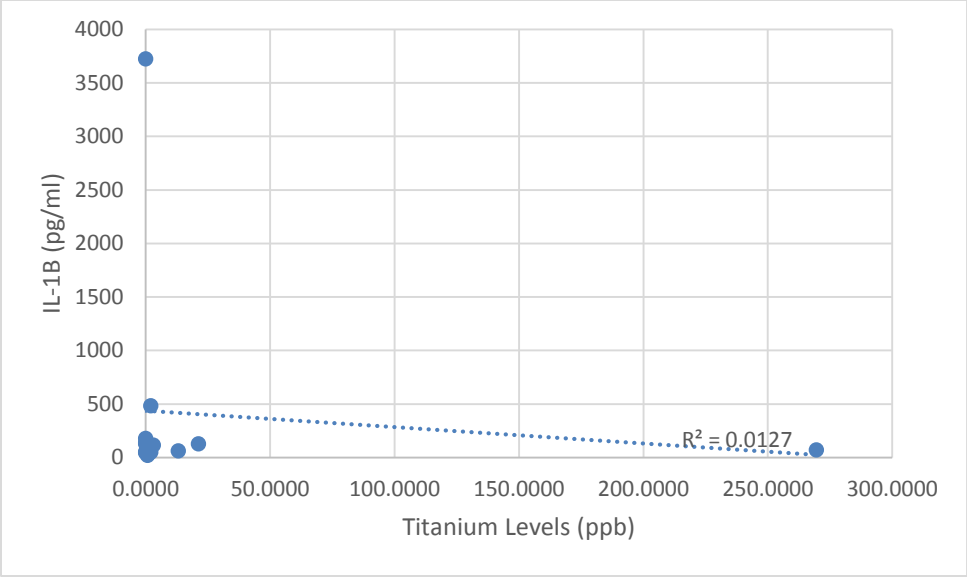

Figure 3-36. Titanium levels in tissue compared to IL-1β levels in synovial fluid

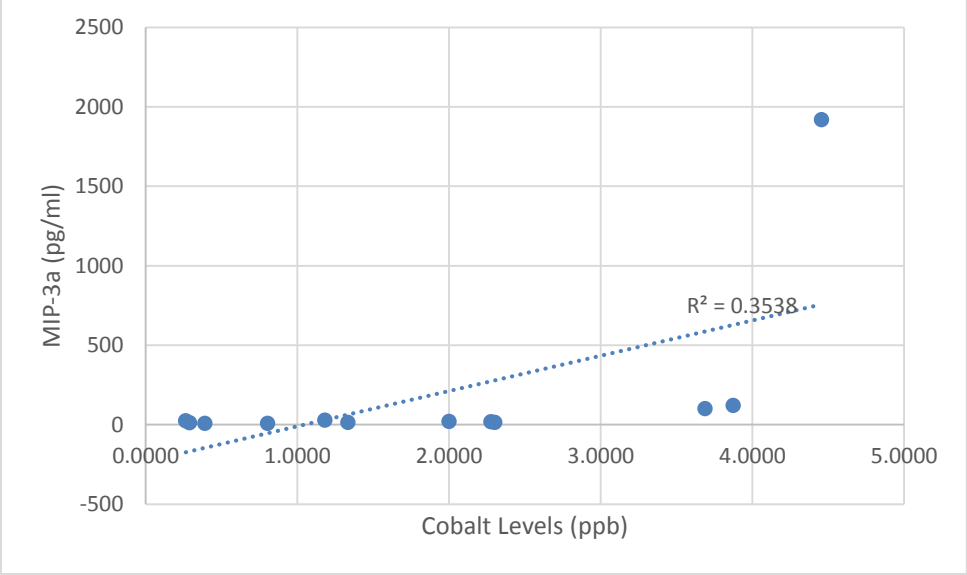

Figure 3-37. Cobalt levels in tissue compared to MIP-3α levels in synovial fluid

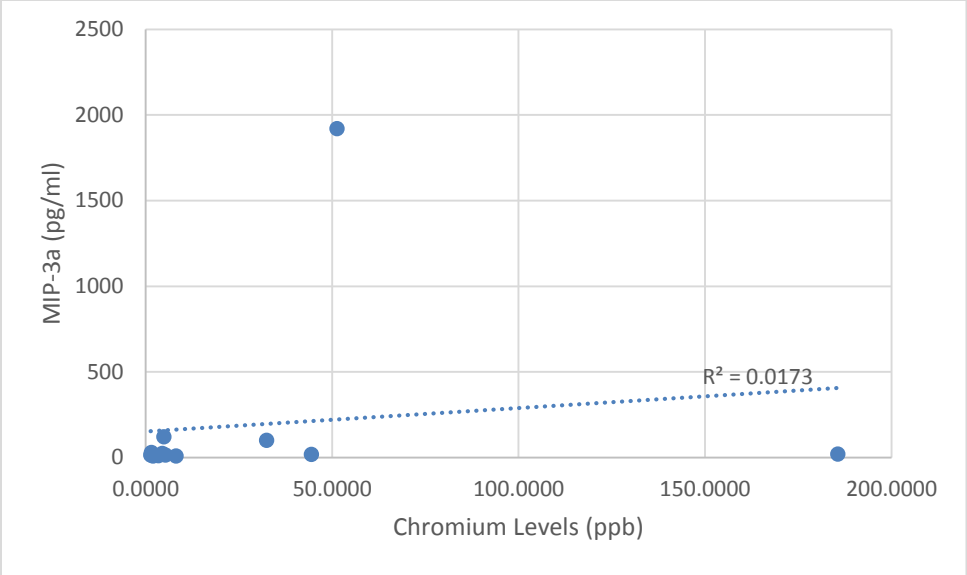

Figure 3-38. Chromium levels in tissue compared to MIP-3α levels in synovial fluid

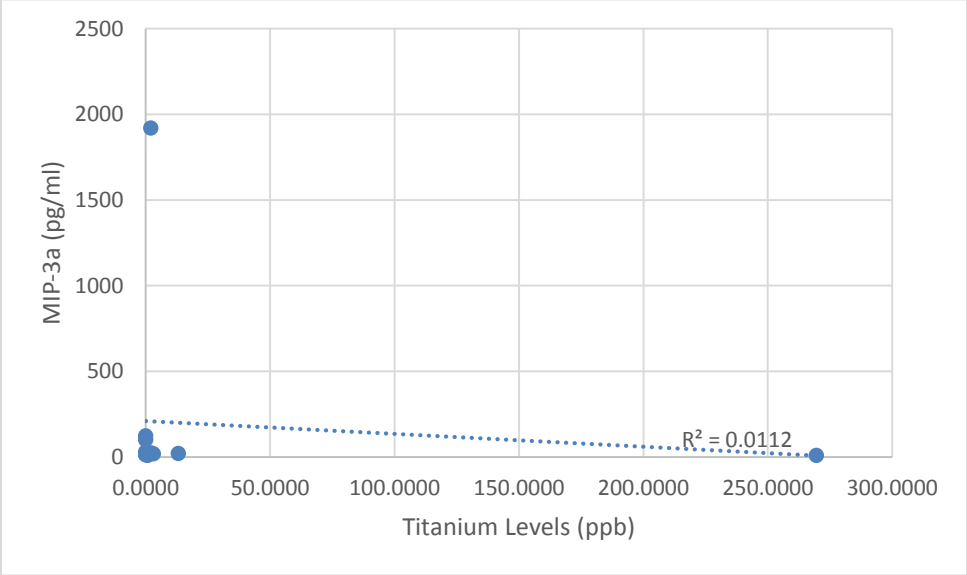

Figure 3-39. Titanium levels in tissue compared to MIP-3α levels in synovial fluid

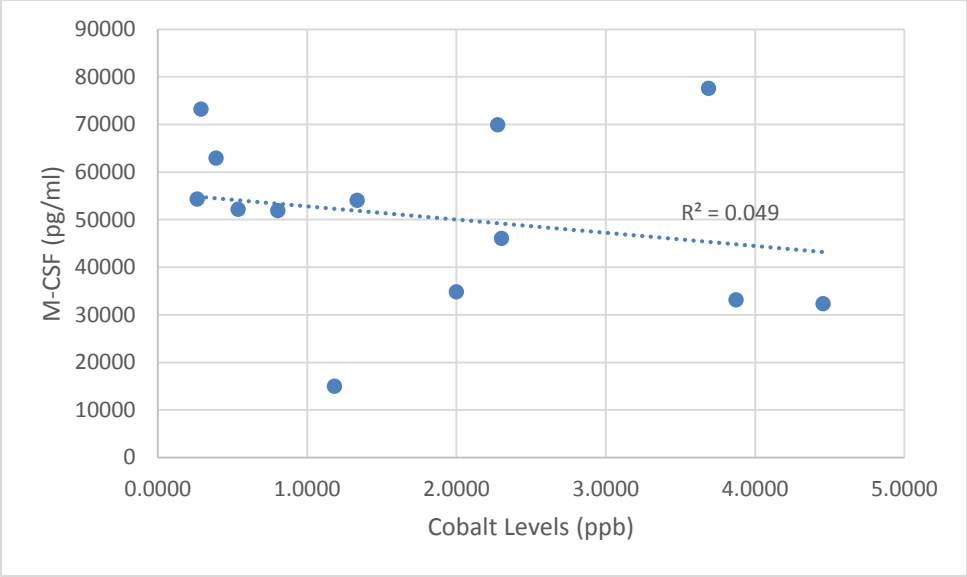

Figure 3-40. Cobalt levels in tissue compared to M-CSF levels in synovial fluid

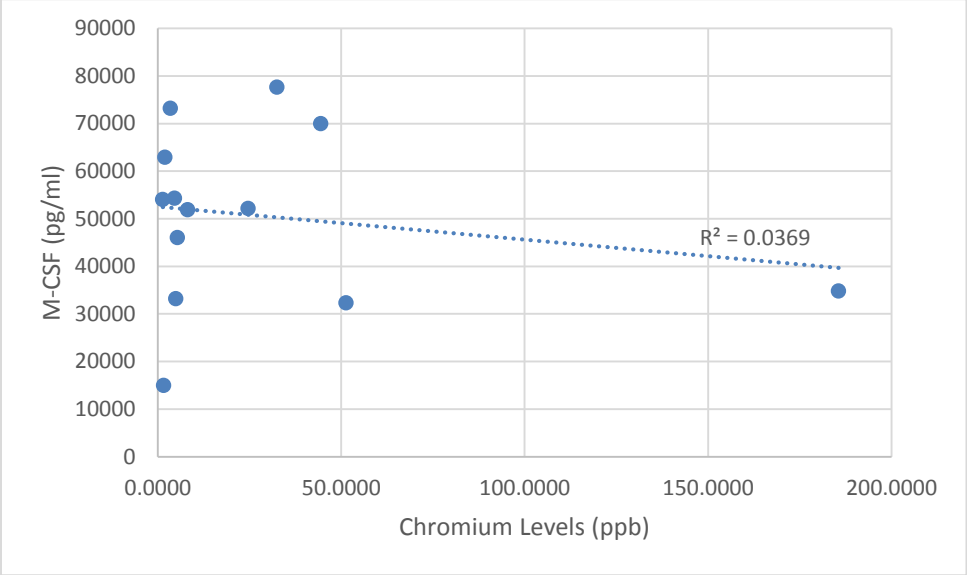

Figure 3-41. Chromium levels in tissue compared to M-CSF levels in synovial fluid

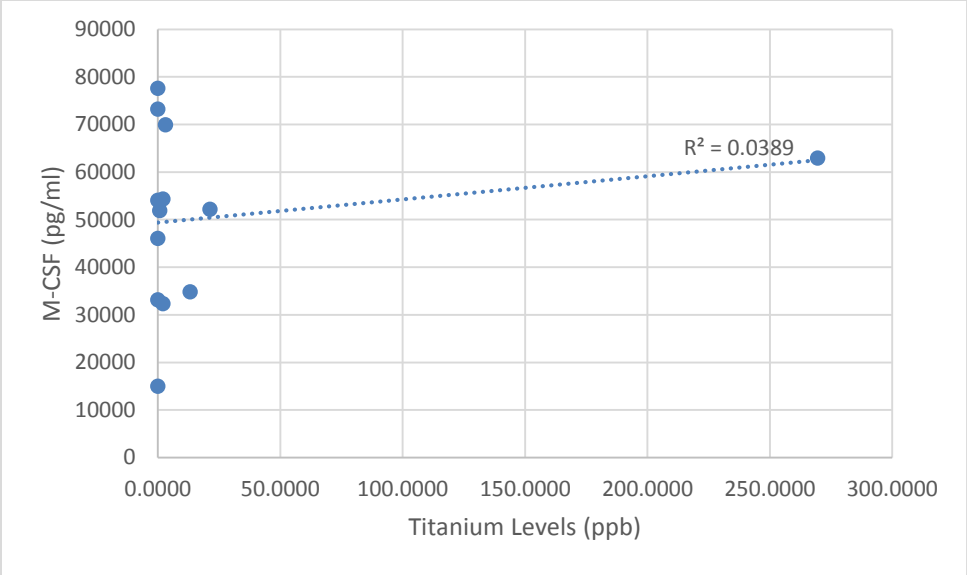

Figure 3-42. Titanium levels in tissue compared to M-CSF levels in synovial fluid

Table 3-17. Correlation coefficients and p-values for comparison between cytokines and cobalt levels

Cytokine	Sample Size	Correlation Coefficient (r)	95% Confidence Interval of r	P-Value
IL-6	13	0.67	0.19 to 0.89	0.01
MCP-1	12	0.76	0.33 to 0.93	0.004
IL-1B	13	-0.25	-0.71 to 0.35	0.4
MIP-3a	13	0.60	0.031 to 0.87	0.04
M-CSF	13	-0.22	-0.69 to 0.38	0.5

Table 3-18. Correlation coefficients and p-values for comparison between cytokines and chromium levels

Cytokine	Sample Size	Correlation Coefficient (r)	95% Confidence Interval of r	P-Value
IL-6	13	-0.11	-0.62 to 0.47	0.7
MCP-1	12	0.025	-0.56 to 0.59	0.9
IL-1B	13	-0.14	-0.64 to 0.45	0.7
MIP-3a	13	0.13	-0.45 to 0.64	0.7
M-CSF	13	-0.19	-0.67 to 0.40	0.5

Table 3-19. Correlation coefficients and p-values for comparison between cytokines and titanium levels

Cytokine	Sample Size	Correlation Coefficient (r)	95% Confidence Interval of r	P-Value
IL-6	13	-0.28	-0.72 to 0.32	0.4
MCP-1	12	-0.18	-0.68 to 0.44	0.6
IL-1B	13	-0.11	-0.63 to 0.47	0.7
MIP-3a	13	-0.11	-0.62 to 0.47	0.7
M-CSF	13	0.20	-0.40 to 0.67	0.5

test did not pass, so a Mann-Whitney Rank Sum test was performed to determine if there was a significant difference between the two groups. The p-value for this test was 0.6, meaning that a statistically significant difference was not able to be determined. For chromium, the normality test also failed, and the Mann-Whitney Rank Sum test showed similar results, a p-value of 0.5. Therefore, a significant relationship could not be confirmed. A Mann-Whitney Rank Sum test was also performed for titanium, as it was also non-normal. This test gave a p-value of 0.8, so there was no statistically significant relationship between these groups either.

CHAPTER 4. DISCUSSION

Summary

Because the implants studied in these groups were known to be well-functioning with no loosening present, the hypothesis was that there would be low amounts of wear and damage, along with low inflammatory cytokine values and metal ion levels. This hypothesis was confirmed, as these implants all had minimal or mild corrosion, low inflammatory cytokine concentrations overall, and relatively low polyethylene wear, although this study did not allow a wear rate to be calculated. It is difficult to say whether metal ion content was on the lower end or not, as there are very few studies looking at metal ion levels in tissue through ICP-MS (most of the known levels are in serum). Although the levels of most of these factors were expected to be low, the hope was a relationship could be established between dissociation force and cytokine concentrations, dissociation force and metal ion concentrations, polyethylene wear and cytokine concentrations, metal ion concentrations and cytokine concentrations. There was also expected to be a difference in dissociation force and metal ion content at different levels of corrosion, with dissociation force decreasing as corrosion increased, and metal ion content increasing as corrosion increased. One hypothesis was that a negative relationship would exist between dissociation forces and inflammatory cytokine concentrations, because a higher dissociation force should mean the connection is more intact, therefore, there should be less metallic debris and less of an inflammatory response. Two cytokines had moderate positive correlations to the dissociation force (MCP-1: r=0.64, CI: 0.011 to 0.90; MIP-3α: r=0.67, CI: 0.069 to 0.91), however, these were more due to one data point pulling the trend in a positive direction, as seen by the wide confidence intervals. A second hypothesis was that a positive relationship would exist between polyethylene wear and cytokine values because as polyethylene debris are released, monocytes and macrophages secrete cytokines to help manage the polyethylene debris. However, in this study, there were no strong correlations for any of the comparisons between cytokines and polyethylene wear. Another hypothesis was that a positive relationship would exist between cobalt and titanium and some inflammatory cytokines (specifically IL-6, MCP-1, TNF-α, IL-8). The concentrations of TNF-α and IL-8 were not within range to allow for comparison of these cytokines to any other factors. Nothing in literature suggested a relationship between chromium and inflammatory cytokines. In this study, there was no meaningful correlation between titanium or chromium and any cytokines, as these comparisons all had very low correlation coefficients. However, when looking at cobalt, meaningful relationships seemed to emerge for IL-6 (r=0.67, CI: 0.19 to 0.89), MCP-1 (r=0.758, CI: 0.326 to 0.928), and MIP-3α (r=0.60, CI: 0.066 to 0.86). The relationship between cobalt and IL-6 and MCP-1 was not surprising based on other findings in literature, but it is typically not associated with a change in MIP-3α. The confidence intervals seem to show some promise for a positive relationship between cobalt and MCP-1 and IL-6, but the lower end of the confidence interval for MIP-3α is almost zero, meaning a relationship between these two factors seems less likely. A fourth hypothesis was that the relationship between the metal ions and dissociation forces would be negatively related, but a moderate positive correlation was seen for cobalt (r=0.59, CI: -

0.062 to 0.87) and chromium (r=0.66, CI: 0.11 to 0.90). Although this is opposite of what was hypothesized, there seems to be one data point in each of these comparisons that is pulling the trend line to be as moderately positive as it is. The confidence interval for cobalt and dissociation force includes zero. More research should be done to include implants with varying degrees of corrosion and varying dissociation forces in order to make any meaningful conclusions about these comparisons. Finally, there was no detectable difference in metal ion content or dissociation forces between minimally and mildly corroded implants. A difference may have been able to be detected with a broader spread of corrosion, but with such low corrosion being seen in these implants, no difference was able to be detected.

The fact that this study contained well-functioning implants with low levels of damage and inflammatory cytokine content made it difficult to identify potential relationships, as the cytokine values were mostly the same for each sample, while the other factors such as dissociation force and polyethylene wear were more variable. While this factor made it difficult to identify potential relationships, these values were also very preliminary with only fourteen samples. As more samples are added, the hope is that more defined relationships will emerge for each of these comparisons. While this study did not necessarily provide meaningful information about how these values are related to one another, it is a step in the right direction for understanding what makes certain implants work well. There is a lack of retrieval studies on well-functioning implants, and therefore it is difficult to establish baseline values for comparison when looking at failed implants. This study will be continued with the addition of these failed implants in the future, and this will hopefully help to solve some of the mystery around what factors lead to implant failures, and what factors are crucial for their success.

Inflammatory Cytokine Concentrations

The two groups assessed for the inflammatory cytokine testing were an osteoarthritis control group (sample size of 16) and a well-functioning cadaveric implant group (sample size of 14). Values were expected to be on the lower end of the detectable range of the cytokine concentrations for each of these groups. These low levels were expected for the osteoarthritis control group because the cytokines selected were mostly associated with the body's response to wear debris that may contribute to bone resorption, and since the samples in this group did not have an implant yet in the joint of interest, the concentrations of many of these were expected to be low. In the cadaveric implant group, the values were expected to be low because, again, the cytokines selected were cytokines that are known to contribute to the RANK/RANKL pathway that can lead to aseptic loosening, and fluoroscopic images of these implants showed there was no aseptic loosening present in the joint. Bone cement particles can also cause an inflammatory response leading to higher concentrations of these cytokines, but none of these implants had cemented acetabular cups, and only two implants had cemented femoral stems, so the bone cement debris is not considered to play a major role in the cytokine concentrations. While there were a few samples in each group with slightly elevated values of one particular cytokine, as a whole, these values were very close to the

lower limit of detection. This makes it difficult to establish any meaningful correlations with these values, as there is not a good spread of the data across the entire detection range, however, it does create a good "baseline value" for use with comparing with groups of revision or failed total hip arthroplasty.

For TNF-α, the osteoarthritis group had only five out of the sixteen within range. The mean of these five was 9.37 pg/ml. The cadaveric implant group had only five of the fourteen within range, and the mean of these five was 10.8 pg/ml. The detection limits for TNF-α ranges from 8.9-2170 pg/ml, so the means of these two groups are only barely within detection limits, and therefore it can be concluded that TNF-α was not present in meaningful concentration in either group. This is not surprising for a couple of reasons. For one, as mentioned earlier, elevated levels of any of these cytokines were not expected. Secondly, the half-life of TNF-α is very low, only around five to eight minutes [42]. There have been studies addressing TNF-α in revision arthroplasties, however, most of these are obtaining a number of cells containing TNF-α in the tissue surrounding the implant, not assessing the levels of TNF-α in synovial fluid. Therefore, a direct comparison of the levels obtained in this study to levels of failed implants in literature is difficult.

For IL-6, the osteoarthritis group had seven of the sixteen samples within range, with the remaining nine being above the limit of detection. The mean for the concentrations for this group was 808 pg/ml. For the cadaveric implant group, twelve of the fourteen samples were within range with the remaining two samples being above the limits of detection. The mean of the group was 433 pg/ml. The detectable range of IL-6 is 4.9-1180 pg/ml. This particular cytokine was more elevated in the osteoarthritis group than it was in the cadaveric group, but this difference was not found to be significant when using a Mann Whitney test. Values for the half-life of IL-6 are found to have a range in literature, with one study reporting a value of about 103 minutes [43]. The levels of this particular cytokine are intriguing, because unlike the majority of the cytokines, this one is actually relatively elevated. IL-6 is known to be secreted by osteoblasts to induce osteoclast formation, so it is interesting that it would be slightly elevated in the groups in which there was no implant present (osteoarthritis) or in which there is an implant with no sign of aseptic loosening (well-functioning implant group). This cytokine has also been shown in literature to be related to periprosthetic joint infections, in which there would be inflammation present to try to remove the infection [28]. This cytokine was shown to be elevated in the serum of total joint revisions, but an exact level was not given [44]. Because the cytokine was more elevated in the osteoarthritis group, it may be interesting to further study whether or not this cytokine may play a role in the body's response to osteoarthritis in a joint.

For IL-8, the osteoarthritis group had fifteen out of the sixteen samples in range, with one above the limits of detection. This one sample drove the mean of the group up to 124 pg/ml, but without this sample, the mean of the group was only 57 pg/ml. However, for the cadaveric implant group, eleven out of the fourteen were above the limits of detection, with the mean of this group being 977 pg/ml. The detectable range of this group is 4.7-1140 pg/ml. For this cytokine, the cadaveric group was significantly more

elevated than seen in the osteoarthritis control group, with a p-value of less than 0.001 when using a Mann Whitney test to compare. While this cytokine was almost non-existent in the osteoarthritis group, it was very close to the upper limit of detection for the cadaveric group. This cytokine, similarly to IL-6 is known to play a role in osteolysis and aseptic loosening. Studies found elevated levels of IL-8 in total joint replacements undergoing revision when compared to primary replacements for OA [27]. However, this study did not include well-functioning implants at time of death, so it is difficult to say whether these results of elevated IL-8 in the cadaver group are abnormal. Because these joints have been checked with fluoroscopy for aseptic loosening and no signs of loosening were seen, this cytokine may play a role in the healing mechanisms that is unrelated to aseptic loosening. The production of IL-8 is known to be enhanced by TNF-α, IL-1, and IL-3 [45], however, the levels of IL-1 and TNF-α in this study was relatively low. More work should be done to determine potential causes for elevated IL-8 that are unrelated to aseptic loosening.

For MCP-1, all sixteen of the osteoarthritis samples were within range with a mean of 864 pg/ml. For the cadaver samples, eleven out of fourteen of the samples were within range with a mean of 1493 pg/ml. The range of this cytokine is 98-7940 pg/ml, so these means are on the lower end of this range. There was no detectable difference between these two groups. While there is no recorded half-life for MCP-1 in literature, MCP-1's role is to recruit macrophages to a site when needed, as monocytes, which mature into macrophages, are thought to leave circulation by about 340 million each day [46]. Because the detectable limit of this cytokine goes up to 7940 pg/ml, a mean value of 1493 pg/ml is considered to still be relatively low. While it is not statistically different than the mean of the levels in the osteoarthritis group, a reason it could have a slightly higher mean is that, while these are well-functioning implants, wear debris are still being produced in the joint daily. As these are being produced, monocytes are recruited to the joint to engulf and phagocytize these debris. Therefore, it would be expected for some level of MCP-1 to be present in any joint that has a joint replacement. This cytokine may be related to aseptic loosening in cases where there is so much wear debris present, the macrophages are unable to keep up with the demand and bone resorption begins to take place. However, that was not the case in the groups tested in this study.

For IL-1β, all sixteen of the osteoarthritis samples were below detectable limits, so no mean could be obtained, but all fourteen of the cadaveric samples were in range with a mean of 376. Although the samples were within range, the detectable range for IL-1β is 16.3-3950 pg/ml, so a mean of 376 is, again, very low on the detection range. IL-1β is a key mediator of the inflammatory response, and is essential for host response to pathogens. It is known to exacerbate damage during chronic disease and acute tissue injury. IL-1β is also known to have a very short half-life, although an exact value could not be found in literature [47]. Because of this short half-life, it is difficult to say whether or not the levels seen in the cadaveric group may have been higher initially. However, because these implants were known to be well-functioning, IL-1β would not need to be secreted in high amounts because there was no acute tissue injury. This cytokine would be expected to be high at the time of implantation, as there is severe tissue injury and

healing that must take place, but as the body adapts to the implant being present in the joint, the need for IL-1β secretion would be low.

For MIP-3α, the osteoarthritis group had fifteen out of sixteen samples within range, with a mean of 21 pg/ml. The cadaveric group had eleven out of fourteen within range. One of these was above detection, while the other two were below detection. The mean of this group was 191, but if the sample above detection is excluded, the mean drops to 34. With a detectable range for MIP-3α of 7.9-1920 pg/ml, neither group had significant concentrations of this cytokine in the synovial fluid. There was no significant difference found between these two groups. Similarly to MCP-1, literature cites a short half-life for chemokines such as MIP-3α, but an exact number is not given [48]. Literature does not specifically address how MIP-3α relates to aseptic loosening, but it is known to be a cytokine that is strongly chemotactic to lymphocytes and is produced by osteoclast cells. It may potentially play a role in aseptic loosening by recruiting lymphocytes in response to polyethylene debris, and contributing to the RANKL pathway leading to osteoclastogenesis and bone loss. However, because it has not been studied in this particular application before, there are no values in literature about levels of MIP-3α in total joint replacements. More work should be done to determine what type of role this cytokine plays in aseptic loosening.

For IL-2, the osteoarthritis group had seven out of sixteen samples below detection, with a mean of 40 pg/ml. The cadaver group had ten out of the fourteen samples below the limit of detection, with a mean of 42 pg/ml. The limits of detection for IL-2 are 35-8510 pg/ml, so these means are extremely low, so IL-2 is essentially negligible in these two sample groups. There is no statistical difference between the two groups. These values are very low, which is not unexpected because IL-2 has a half-life of 3.7 minutes [49]. Because this cytokine has such a short half-life, the values obtained in this study do not give much information. In order to get valuable and accurate concentrations for this cytokine, synovial fluid would need to be aspirated and frozen almost immediately after death for the cadaveric groups. The synovial fluid is spun for twenty minutes after obtaining it and before freezing it, which gives time for the levels to decrease dramatically. Therefore, from a practicality standpoint, this cytokine may not be the most useful for this application.

Finally, for M-CSF, the osteoarthritis group had two out of sixteen samples below the limit of detection with a mean of 1372 pg/ml. The cadaver group had all fourteen samples within range with a mean of 52054 pg/ml. The limits of detection for M-CSF are 514-124810 pg/ml, so these are still fairly low considering the full range of the cytokine. The differences between these two groups were found to be significant, with a p-value of <0.001 after completing a Mann-Whitney test. While studies have shown that this cytokine is present in revision cases and contributes to the RANKL pathway for aseptic loosening, this observation has typically been made by observing that there is M-CSF present in the cells of the tissue, so these results cannot be directly compared to the results of this study [50]. A study by Takei reported higher M-CSF levels in the fluid of loose hip joints when compared to mild OA, but this study did not test M-CSF levels in joints that had no problems evident [51]. While this cytokine was significantly higher in

the cadaver group compared to the OA group, it is still only at a level that is one-fifth the upper limit of detection. This cytokine is responsible for influencing hematopoietic stem cells to differentiate into macrophages or similar cell types, so it would be expected to be at least slightly elevated in the cadaver group, as some wear debris is always being produced and macrophages need to be recruited to assist with that. However, in a group of revision failed implants, these values would be expected to be much higher, as the larger amount of wear debris would necessitate more macrophages to be present. M-CSF is critical for osteoclast differentiation and is known to enhance osteoclast survival, so in joints where aseptic loosening is present, this cytokine may play a crucial role [52].

Although there were significant differences seen between the two groups for IL-8, IL-1β, IL-2, and M-CSF, the limitations of these need to be taken into considerations. Each of these groups had low sample sizes, fourteen in the cadaver group and sixteen in the osteoarthritis group. In some cases, one sample in a group has elevated concentrations of a particular cytokine not seen in the other samples. As a result, the mean is driven slightly upward causing a difference to be seen between the groups. If **Tables 3-2** and **3-3** are studied, one can observe that most of the cytokines concentrations are fairly close to one another on the low end of detection, and therefore, are not considered to be contributing to inflammation in a meaningful way. However, the numbers will be useful moving forward into revision and failure studies for hip implants as a baseline value for comparison.

Corrosion Scores

As mentioned previously, the corrosion scores were very low for the retrieved implants. There were sixteen retrieved implants that the corrosion scoring was completed for. For the male portion of the taper, fifteen out of the sixteen implants received a corrosion score of 1, with the other implant receiving a score of 3. Therefore, these particular numbers were not useful in creating a correlation from male taper damage to any of the other factors studied. However, for the female portion of the taper, eight of the sixteen implants were scored a 2, six of the sixteen implants were scored a 1, and the remaining two were ceramic heads. Therefore, these were split into two groups to see if a difference could be detected in the dissociation forces between the implants with a head score of 1 and the implants with a head score of 2. However, the Shapiro Wilk test showed no detectable difference. This is understandable, because even though the implants were characterized with two different corrosion scores, the scores are representative of minimal (1) and mild (2) damage, and the scoring system continues up to moderate (3) and severe (4). The implants in this group were well-functioning implants in which problems were not seen in the patient before death, and therefore, these low damage scores are detected. A difference in the dissociation forces may have been seen between groups with a broader range of corrosion scores, for example, comparing minimal and severe, however, with these components being so slightly damaged, it is not unexpected that there was no detectable difference in dissociation forces here. It should be noted that there were different taper designs in this study. There are eight tapers that are known to be a 12/14 type taper, and one that was a 16/18 type taper. Because there

were not significant samples with different taper types, no conclusions can be drawn about which taper designs may be more prone to corrosion than others.

Polyethylene Wear Measurements

The polyethylene wear measurements were only able to take into account how much wear was present in linear thickness loss, and could not consider the causes of the loss or produce a rate. The average polyethylene wear was 0.296 mm when including the outlier of 1.867 mm, but dropped to 0.184 mm when excluding this outlier. Unfortunately, one weakness in this measurement is that a wear rate cannot be determined since there is no year of implantation for these implants. Therefore, the measurements must be taken as standalone values of how much polyethylene has been worn down, and cannot conclude anything about the frequency or mechanism that led to the wear. The reported wear rate for highly cross-lined polyethylene is reported to be very low, between 0.00 and 0.01 mm/year in the first three years, with no wear rates higher than 0.1 mm/year in one study [53]. Another study reported a mean linear polyethylene rate of 0.11 mm/year, with a range of 0-0.86 mm/year [54]. The average polyethylene wear was 0.184 mm for this study, and although it is unknown how long the implants were in place before death, this seems to be a relatively low amount of wear considering averages reported around 0-0.1 mm/year. Therefore, as assumed with these well-functioning prostheses, relatively low wear is present. Apart from knowing a specific wear rate, one factor that could aid in better understanding these results would be to know the mechanism of wear. This factor will be assessed in the future by noting damage scores on the femoral heads, as well as seeing if particular modes of damage are evident in the polyethylene liners. Another thing to consider when studying polyethylene wear is the head size of the implant. In this study, five implants had 28 mm heads, five implants had 32 mm heads, four implants had 36 mm heads, and two implants had 40 mm heads. Of these implants, linear wear measurements were obtained for three liners of implants with 28 mm heads, four liners of implants with 32 mm heads, four liners of implants with 36 mm heads, and two liners of implants with 40 mm heads. The mean of each of these groups were 0.954 mm (28 mm), 0.112 mm (32 mm), 0.193 mm (36 mm), and 0.114 mm (40 mm). This shows the highest wear was seen in the implants with the smallest head size, which is conflicting with what is shown in literature. However, the fact remains that the implantation time for these implants is unknown, and it is difficult to compare means of groups with such small sample sizes, between two and four implants per group. However, it is important to note that the head sizes of these implants can affect the polyethylene wear rates, and this should be tracked moving forward.

ICP-MS Analysis

The values obtained in this study were much higher than what was seen in literature, however, these values are difficult to find in literature, and the values that are found are typically reported from serum. To this date, no values were found in literature studying the cobalt, chromium, and titanium levels in the tissue surrounding total hip

implants. In the study by Savarino et al mentioned in the introduction, the levels for cobalt and chromium in patients with a metal on polyethylene bearings were 0.64 µg/L and 0.60 µg/L, respectively, however, these were the values from the patient's serum. In this study, because the values were obtained by analysis of the anterior and posterior capsular tissue, the results were expected to be higher because the metal debris remains in the localized area instead of being diluted throughout the body, as in serum. In this study, the average value seen for cobalt was 35.80 µg/L, for chromium was 139.46 µg/L, and for titanium was 28.84 µg/L. The full set of results can be seen in **Table 3-6.** Out of the seventeen samples metal ion levels were able to be obtained for, nine had a cobalt chromium/titanium taper combination, four had a cobalt chromium/cobalt chromium taper combination, two were a ceramic/titanium taper combination, and two did not have the metal bearing types available at this time. While only four of the implants in this study did not include titanium as a metal, seven of the implants had a titanium level of 0. While the cobalt and chromium levels were low for several of the samples, they did not have any values of 0, while seven of the samples had titanium values of 0. One potential reason for this is the "blank" samples used in the analysis actually had an average titanium value of 8.4 µg/L. The levels of each ion for the blanks was subtracted from the reported value, so this could mean there were samples with very low values of titanium that may have been overlooked due to background noise in the samples. Another reason could be, as mentioned before, since these are well-functioning implants, values could be low. The corrosion scores for the stems of almost all the implants were 1, except for one implant which was scored a 3. This low corrosion seen in the stems of the samples could also explain the low titanium ion levels seen in the samples. This relationship will be further explored in the sections comparing metal ion levels to other factors.

Dissociation Force versus Cytokine Concentrations (a)

The hypothesis for this comparison was that as dissociation force increased, cytokine concentration would decrease, as a higher dissociation force should mean the taper connection is more intact. Therefore, there is less corrosion and material loss in the taper connection and hopefully less of an inflammatory response to debris. However, it should be noted that cytokine concentrations may go up due to polyethylene debris as well. When comparing the dissociation force for each implant to the various cytokine concentrations and trying to determine if any correlations exist, two different methods were used. These were simply compared number-to-number (the cytokine concentrations to the dissociation force) to look for correlations, as well as rank-to-rank, in which the rank for each individual implant's dissociation force and cytokine concentration amongst the group was used. This was done in an attempt to standardize the numbers. However, because these were well-functioning implants and the cytokines were mostly clustered around the lower limit of detection, it was difficult to ascertain any meaningful correlations. With a broader spread of cytokine concentrations, a better conclusion may have been able to be drawn about how these factors relate. As it is, there were only two p-values considered to be significant (<0.05) when looking at these comparisons. The first was found when the concentration of MCP-1 was directly compared to the dissociation force of the implants, and this produced a p-value of 0.05. MCP-1 is the main chemokine

responsible for recruiting monocytes. These monocytes can then mature into macrophages, which are the main scavenger cells of the immune system that attempt to find and phagocytize foreign bodies. This comparison gave a positive correlation of 0.64, which is contrary to what was hypothesized. It is believed that a higher dissociation force means that the taper connection should be more intact, meaning less wear debris is being produced and therefore the need for monocytes to assist in removal of wear debris is lower. However, this belief will be further explored when directly comparing the cytokine concentrations to the metal ion content in the tissue. One reason this positive correlation could be seen is that when looking at the graph in **Figure 3-4**, there is one data point that seems to be pulling the graph in a positive direction. The confidence interval for the correlation coefficient for this comparison was 0.011 to 0.90. Therefore, while the correlation between these two variables was moderate (0.64), the 95% confidence interval states that the actual correlation coefficient could fall anywhere from 0.011 to 0.90 with 95% confidence. This is likely due to the fact that there is a low sample size in this comparison, with one value that is much different than the others pulling the trend in a more positive direction. This data point was considered to be an outlier for the MCP-1 concentrations, and when it was removed, the p-value for this comparison increased to 0.9. Based on the 95% confidence interval and low p-value when outliers are removed, it is not possible to say with certainty what the relationship between these two factors is. In order to see a relevant comparison between these two factors, the sample size of the group would need to be increased, and a wider spread of cytokines would be necessary. The second significant correlation was the direct linear comparison of the concentration of MIP-3α to the dissociation force of the implants, producing a p-value of 0.03. MIP-3α is a cytokine that is strongly chemotactic to lymphocytes. These lymphocytes can play a role in the RANK/RANKL pathway which can lead to bone resorption, which is why it is relevant in this application. Similarly to MCP-1, a negative correlation was hypothesized to be seen for this, but instead a positive correlation of 0.67 was seen. However, when a 95% confidence interval was completed for the correlation coefficient, a range of 0.069 to 0.91 was found to be the interval. Similarly to MCP-1, there were two data points that pulled the trend line in the strong positive direction, which is likely why there was a higher correlation coefficient between these factors. Because the confidence interval again goes from almost 0 to almost 1, it is difficult to confidently state any relationship between these two variables based on the data, as there is 95% confidence that the true correlation coefficient could fall anywhere within that range. There were two outliers in this comparison as well that, when removed, increased the p-value to 0.9. Therefore, again, a larger sample size would be needed with a better spread of data points in order to say anything conclusive about this correlation. With outliers removed, this correlation does not appear to have any significance. The remainder of the correlation coefficients for the comparisons ranged from 0.066 to 0.44, which did not produce any significant p-values and had wide confidence intervals for the correlation coefficients. This was not surprising, because, as mentioned previously, the cytokine values were so low on the limits of detection, the spread of the data makes it difficult to see any correlations.

Dissociation Force versus Corrosion Scoring (b)

The hypothesis for this comparison was that implants that were more highly corroded would have a lower dissociation force due to material loss than those implants with less corrosion. Unfortunately, for this study, this hypothesis was not able to be tested thoroughly, as the implants in this study were minimally corroded. For the trunnion, all implants except one had a corrosion score of 1, or minimal. For the bores, all implants were scored either a 1, minimal, or 2, mild. There was no significant difference in dissociation forces between these two groups. In order to better explore this hypothesis, a broader spread of corrosion scores would be needed.

Dissociation Force versus Metal Ion Concentrations (c)

Positive relationships were seen for the comparisons of cobalt and chromium levels in tissue to the dissociation forces of the implants, and a very slight negative relationship was seen for the comparison of titanium levels in the tissue to the dissociation forces of the implants. The titanium relationship is affected by the fact that five out of the eleven samples had a titanium value of zero, so a relationship may be difficult to determine, as well as the fact that there is one titanium value significantly more elevated than the rest. Upon further analysis, this value was found to be an outlier. When directly comparing the cobalt levels to the dissociation forces, a p-value of 0.07 was seen, and when directly comparing the chromium levels to the dissociation forces, a p-value of 0.03 was seen. While this seems to suggest a promising relationship between these factors, it must also be noted that these values are obtained by using a Pearson -rank coefficient, which assumes a linear relationship between the two variables. Because this relationship has not been explored in literature, it is difficult to definitively say that this relationship would be linear, however, it is a step in the right direction for future exploration of this relationship down the road. A positive relationship was contrary to what was hypothesized, because as micromotion at the articulation of the head and neck of the implant occurs, the oxide layer can be broken down which leads to the release of these metal ions. This can also lead to the corrosion of the surfaces, which can decrease the strength of the taper connection. Therefore, higher concentrations of these metals were expected to lead to a decrease in the dissociation force. However, when the confidence interval of the correlation coefficient of each of these comparisons is calculated, the positive relationship seems less likely. The confidence interval for the correlation coefficient of the comparison of cobalt and dissociation force includes zero (-0.062 to 0.87), which means that there is 95% confidence that the true correlation coefficient falls anywhere within that range. The confidence interval for the correlation coefficient of the comparison of chromium and dissociation force is 0.11 to 0.90. Therefore, it cannot be confidently concluded that there is a positive relationship between the presence of these metal ions and dissociation force, but it does seem to have slightly more of a relationship than cobalt. There have been some studies with preliminary data showing that more metal debris and therefore more corrosion can lead to a higher dissociation force because of the increase in friction between the two surfaces, however, this is for severely corroded implants and does not necessarily apply to this data set that

only experienced minimal and mild corrosion. A broader set of implants with more varying dissociation forces and corrosion forces may give more insight into this relationship. One flaw in this comparison other than the wide confidence intervals that could explain why the relationship is contrary to what is hypothesized could be that there are other factors that contribute to the dissociation force that are unable to be controlled in this study. For one, the assembly of the taper has been shown to greatly influence the strength of the taper connection. For example, the force of the impaction of the head onto the stem, the number of impactions, and whether the surfaces were wet or dry has been shown to impact this taper strength. Because these cannot be controlled for, the dissociation force must simply be taken as-is.

Linear Polyethylene Wear versus Cytokine Concentrations (d)

For this comparison, the linear polyethylene and cytokine values were both directly compared, as well as ranked amongst all the samples and compared. For these comparisons, no significant p-values were seen. There was one value approaching significance (considered to be 0.05) when comparing the rank of IL-1β to the rank of the linear polyethylene wear. The correlation was -0.58 with a p-value of 0.06. Similarly to the two correlations seen previously when comparing the dissociation forces, this correlation is opposite of what was hypothesized. A positive correlation was expected to be seen when comparing polyethylene wear an IL-1β, because as polyethylene debris is generated, macrophages attempting to engulf the debris secrete IL-1β in response. However, the cytokine concentrations for IL-1β were all very close to the lower limit of detection. The mean for these cytokines was 16.3-3950 pg/ml, and the average of these samples was 376 pg/ml. Therefore, without a larger spread of cytokine values and with such a small sample size, it is uncertain whether this negative trend would continue as the sample size expands. This significance was also only seen in the ranked comparison between the two groups. The direct comparison had a p-value of 0.6, so it is difficult to say confidently that this negative correlation exists and is significant. In addition, there was one outlier in the IL-1β concentrations, and when this outlier was removed, the p-value for this comparison increased to 0.3, so the closeness to significance was, as expected, most likely due to an outlier. Finally, when looking at the confidence interval for the correlation coefficient for this data, it ranges from -0.88 to 0.027. Because this confidence interval includes 0, it is difficult to confidently state a relationship between these two variables based on this data. Based on this data alone, the correlation coefficient could fall anywhere between -0.88 and 0.027 with 95% confidence. As with the other comparisons in this study, more samples are needed in order to begin to solidify if any of these relationships do actually exist.

Metal Ion Concentrations versus Cytokine Concentrations (e)

There were very few significant correlation between the cytokine concentrations and metal ion levels. Chromium and titanium did not have any p-values below 0.4 for any of the cytokines tested in this study. This was not surprising for chromium, as there is

very little discussion in literature about a relationship between the presence of chromium ions and an increase in the concentration of any inflammatory cytokines, however, literature does show titanium may increase concentrations of IL-6, IL-8, MCP-1, and TNF-α. While there were no promising results in this study suggesting a relationship between chromium and titanium with the inflammatory cytokines tested, there were several cytokines that seemed to show some promise in their relationships when compared to cobalt. There was a positive relationship between cobalt and IL-6, with a correlation coefficient of 0.67 and a p-value of 0.01. The 95% confidence interval for the correlation coefficient of this comparison was 0.19 to 0.89. There was also a positive correlation between cobalt and MCP-1, with a correlation coefficient of 0.76 and a p-value of 0.004. The 95% confidence interval for the correlation coefficient of this comparison was 0.33 to 0.93. Finally, there was a positive relationship between cobalt and MIP-3α with a correlation coefficient of 0.60 and p-value of 0.04. The 95% confidence interval for the correlation coefficient of this comparison was 0.031 to 0.87. The remaining two cytokines had p-values of 0.4 and 0.5 for cobalt. It is interesting that some distinct relationships emerge when observing cobalt with several of the cytokines, but not titanium or chromium. In this study, the titanium levels were zero in almost half of the samples, which could explain the lack of relationships able to be detected. However, the mean level of chromium was 139 ppb while cobalt's was 36 ppb, which means there should have been enough chromium present to detect the emergence of relationships between it and the cytokines. Cobalt and chromium also had the same number of samples plotted against the cytokine values, so the sample size should not be the main impactor of the lack of relationship. Because cobalt and chromium are both found together in a CoCrMo alloy, more research should be done to observe why cobalt seems to be having a stronger impact on the presence of inflammatory cytokines than chromium does. This does match with what is found in literature, as there are very few studies describing chromium impacting the levels of any inflammatory cytokines. It may also be interesting to intentionally seek out cytokines that are known to be affected by cobalt specifically, or chromium specifically, and note if relationships emerge with those cytokines. However, based on these results, it seems that cobalt is the metal that contributes more to the release of inflammatory cytokines, therefore may be the metal that contributes more to the inflammatory response to metallic debris. There is information in literature supporting the positive relationship seen between cobalt and IL- and MCP-1, but there is not anything showing it to have a relationship to MIP-3α. When looking at the confidence intervals for these three comparisons, the lower end of the confidence interval of cobalt with IL-6 was a correlation coefficient of 0.19, which is a mild correlation. The lower end of the confidence interval for the comparison of cobalt and MCP-1 was 0.33, suggesting a moderate correlation even at the worst-case end of the 95% confidence interval. However, the confidence interval for the correlation coefficient of the comparison between cobalt and MIP-3α had a very wide range, with its lower end being almost zero (0.031). This is not surprising, as when looking at the graph of this comparison, **(Figure 3-37),** there seems to be one data point pulling the trend line in the positive direction, while the rest of the points seem to lie relatively flat. Based on this, the wide range for the confidence interval is not surprising. Based on the data compiled from this study, it does seem likely that the presence of cobalt ions has an effect on the

concentrations of IL-6 and MCP-1 in the body. More research should be done to further explore the extent of this relationship.

Metal Ion Concentrations versus Corrosion Scores (f)

When comparing the metal ion levels between the minimally corroded and mildly corroded head groups, there were no statistically significant differences between the groups. This lack of significance is not surprising, because there were so few samples in each group, and the corrosion levels between groups considered a one and those considered a two would be fairly close. Similarly to the results between dissociation forces and corrosion scores, it would be interesting to compare these levels in a group considered minimally corroded against a severely corroded group to see if differences in the metal levels of the tissues emerge.

Limitations

The first limitation that has already been mentioned is the fact that the sample size for this study is very low. While there were a total of nineteen implants in the study, they were not all used for each portion of the study. Only fourteen of these contained enough synovial fluid to test for inflammatory cytokines. Eighteen were able to be analyzed through ICP-MS for metal ion content. Fifteen were able to be disassembled in order to obtain a dissociation force for the implant. Sixteen were able to be scored for corrosion. Fifteen were able to have the linear polyethylene wear tested. Therefore, most of the comparisons between types of testing are limited to a sample size of fourteen due to the inflammatory cytokine sample limitations. While everything was done to ensure as many samples as possible were obtained, the study was limited by the number of cadavers containing hip implants that could be obtained from our partner organizations (MERI and Restore Life USA).

A second limitation, which has also already been mentioned in the discussion for inflammatory cytokines, is that because these implants were well-functioning with no signs of osteolysis, the cytokine values were very low. While not unexpected, this limitation makes it difficult to draw meaningful conclusions when comparing with other types of testing. A similar limitation was also seen with corrosion scoring, because again, since the implants were well functioning and not damaged, the corrosion seen in the groups was low. Therefore, the corrosion values were not very revealing about how levels of corrosion relate to the other factors measured because only minimal and mild corrosion values were seen. A similar issue was observed in the detection of metal ion levels. While cobalt and chromium levels were able to be detected, the titanium levels observed were very low, with nine samples having titanium levels of zero and most of the other samples fairly close to zero. Only five samples had titanium levels higher than 10 ppb. This could link back to the low corrosion on the stems of the implants, and also the fact that some of the implants had a cobalt chromium-cobalt chromium taper connection. While this is a noted limitation, it is also a necessary one. This study is functioning on

well-functioning implants, and as such, the implants in the group will be minimally damaged with a minimal negative reaction in the body. This study will be beneficial moving forward into the testing of failed and damaged implants, because it gives a baseline of values for a group of implants that will be functioning as expected. However, it is important to continue to build on the number of implants in this group in order to have a more meaningful sample size, which will hopefully allow for significant conclusions to be drawn moving forward.

Thirdly, the polyethylene wear measurements are just that, a singular measurement. As the duration of implantation is not known for this group of implants, a specific wear rate was unable to be calculated. Therefore, this number must be taken as is. A better polyethylene measurement as far as correlation goes would be to compare the wear rate to whatever factor it is being compared with, as it somewhat standardizes the polyethylene wear values. As it stands now, the values are all being treated as equal, when in reality, some of the implants with higher polyethylene values could be from twenty years ago, while some of the implants with lower values could have been fairly recent. This factor adds an element of uncertainty into any comparisons we make using polyethylene. This could also explain why no significance was shown when comparing the cytokine values to the amount of polyethylene wear. When adding a group of damaged implants in the future, wear rates should be used if possible.

Finally, a significant limitation in this study is the differences in the types of implants. Because there are many different designs, alloy combinations, and taper designs, it is not possible to state anything conclusively about which types may perform better than others, as the sample sizes of each group are too small. In this study, there were six Zimmer Trilogy, one Depuy Duraloc, four Smith & Nephew Reflection, two Stryker Trident, one Zimmer Continuum, one Biomet Ringloc Constrained, and three Depuy Pinnacle implants. There were also eight implants with a CoCrMo/Ti_6AlV_4 taper combination, four with a CoCrMo/CoCrMo taper combination, and two with a ceramic/Ti_6AlV_4 taper combination. There were eight implants with a 12/14 taper and one implant with a 16/18 taper. Finally, there were four implants with a 28 mm head, five with a 32 mm head, four with a 36 mm head, and two with a 40 mm head. With so many different variables present, and such small sample sizes of each type, these must just be taken as one large group of implants as they cannot be broken down into groups. Therefore, it must be noted, that the results from this study are results from a variety of designs and metal combinations. In order for conclusions to be drawn in the future about how each of these factors may impact implant function, large sample sizes in each of the groups would need to be gathered and results analyzed for each group.

Future Work

As mentioned previously, the focus moving forward will be to continue the same types of testing explained in this study with a group of failed implants from patients undergoing a total hip revision surgery. Institutional Review Board approval has been obtained to begin collection of such devices, but there are not yet enough samples to

begin analysis of this type of data. The implants studied for the purposes of this thesis will hopefully aid in understanding the values obtained through the testing of the failed devices by providing a baseline of values seen in successful devices. The end goal is to see if there are any biological factors that may be indicative of problems with total hip implants that may lead to failure. While nothing definitive about the relationships between the numerous variables affecting the success of total hip implants can be concluded from this study, it is a step in the right direction for better understanding what causes implants to fail.

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