Comparison of wear against articulating cartilage with different materials

by

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Abstract

Osteoarthritis is one of the most common medical conditions, mainly affecting older adults. The majority of cases are found within the hip or knee joint however, small joints, including the ankle, finger and facet joints are affected as well. The usual treatment for osteoarthritis is surgery including hemi (partial) and total arthroplasties. These operations involve the introduction of foreign materials, predominantly medical grade metals and plastics, into the joint space. Depending upon the operation, these materials are directly or indirectly in contact with surrounding cartilage and soft tissue. The goal of this study is to determine the amount of wear different materials, commonly used in these procedures, cause to the articulating cartilage they are in contact with.

The effects of three different materials were tested against porcine femoral cartilage to see which caused the most damage macroscopically and microscopically. Titanium, ultra-high molecular weight polyethylene (UHMWP) and carbon reinforced polyether ether ketone (CR-PEEK) were all compared to each other and to other cartilage samples. These materials were tested using a uniaxial loading machine that provided variable vertical loads and rotary displacement to emulate movements similar to a human joint. Macroscopic observations in the form of wear patterns, as well as pathologic changes analyzed histologically allowed for the accurate quantification of wear that had taken place for each material.

Cartilage vs cartilage tests acted as the controls for this study. None to minimal wear was seen both grossly and microscopically for these tests. UHMWP, titanium and CR-

PEEK showed increasing amounts of macroscopic wear, respectively. Histologic grading revealed that titanium and UHMWP created similar amounts of cartilage damage while CR-PEEK had statistically significant more wear than both titanium ($p=0.008$) and UHMWP ($p=0.035$).

This study showed that tests conducted on a physiologically similar machine, CR-PEEK contributed to the greatest amount of wear to the cartilage both macroscopically and microscopically. This material should be carefully reviewed prior to its use in further orthopaedic procedures in the future.

Preface

This thesis is an original work by Karan Vats. No part of this paper has been previously published. The use of tissues for this project was approved by the University of Alberta Research Ethics Office under the Exceptions to the Requirement for ACUC Review.

Acknowledgments

This project would not be possible without the support of many important individuals. Firstly, I would like to thank my supervisors, Dr. Samer Adeeb, Dr. Lindsey Westover and Dr. Nadr Jomha for their continued support, guidance and patience for the duration of this project. Without their help, none of this would have been possible.

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Introduction

Osteoarthritis (OA) was ranked the second most common medical condition among older adults in the United States in 2016[1], with the medial compartment of the knee being the most commonly affected[2]. The main joints that are thought of when OA is mentioned in conversation are the hip and the knee joints, however, even smaller joints such as fingers, facet joints within the vertebral column and the ankle are also prone to developing OA.

Osteoarthritis occurs when there is destruction of the articular cartilage and underlying subchondral bone. This can be due to mechanical, structural, genetic and environmental factors. OA is not the only pathology that can cause a deterioration in the native articular cartilage that humans possess. Trauma, congenital or acquired joint diseases can also cause a loss of cartilage for many people worldwide. Since there has been no significant medical breakthroughs in preventing or repairing cartilage loss, joint replacement operations are becoming one of the most commonly performed surgical procedures in Canada and around the world. In Canadian operating theatres between 2016 and 2017, there were approximately 67,000 total knee replacements and 56,000 total hip replacements[3]. The only other elective operation that was performed more was the caesarean section.

These operations involve introducing implants made up of a variety of different materials, including, stainless steel, titanium, cobalt chrome, ceramics and plastics such as polyether ether ketone (PEEK) and ultra-high molecular weight polyethylene

(UHMWP)[4]. These materials may be in contact with each other, or with the surrounding cartilage, depending upon the type of procedure that is taking place. In the event of a hemiarthroplasty, where one half of the joint is replaced by an implant and the other surface is left untouched, the interaction between the implant material and the opposing cartilage is very important to understand. Even with the high numbers of operations, there has not been an abundance of research showing the effects of these surgical materials and the wear they cause to the surrounding cartilage. Further information may be crucial in determining which materials are used in the future if one material is found to cause less damage to the cartilage as compared to others. This would not only increase patient satisfaction but also decrease the revision rates of these surgeries, placing less burden on the health care system as a whole.

The research conducted in this area consists of various models that are unlike the anatomical human joint and do not accurately portray the forces or the loads that are placed upon cartilage on a daily basis. The main purpose of these models is to determine the amount of wear that is possible and in some cases these models do not accurately demonstrate how the implants will interact when placed within a joint. This said, there have been a few breakthroughs in previous research and with advances in laboratory testing, changes to the cartilage have been seen both macroscopically and microscopically.

With our model of mechanical testing against cartilage, we hope to portray accurate and physiologic interactions between different materials and cartilage. This will determine

which material (that common orthopedic implants comprise of) cause the most amount of damage to articulating cartilage and will provide surgeons and implant developers a greater insight before further research and progression takes place.

Human Joints

Classification of Joints

There are two types of joints within the human body, the solid and synovial joints[5]. The solid joint is found between two adjacent skeletal surfaces that are connected by cartilage without a cavity, in which movement is restricted. For example, the sutures in the cranium, the pubic symphysis, and between the head and the shaft of a long bones at the growth plate. Synovial joints, on the other hand, are connections between two skeletal surfaces separated by a cavity[5]. Here, two bones, covered with hyaline cartilage on each articulating surface meet within a joint capsule, which is made up of a synovial membrane, a fibrous membrane and is full of synovial fluid. The synovial membrane is highly vascular and produces synovial fluid which acts as lubrication for the articulating surfaces. The fibrous membrane is found outside of the synovial membrane and acts to stabilize the joint. It is usually made up of ligaments which provide reinforcement to further stabilize the joint. Examples of synovial joints include but are not limited to, the hip joint, the knee joint and the ankle joint.

Hip Anatomy

The hip is a synovial joint that is also known as a ‘ball and socket’ joint (Figure 1). It is one of the most mobile joints in the body and is capable of movement in three degrees of freedom. Movements include flexion/extension, adduction/abduction as well as full circumduction. It is made up of two components. The ‘ball’ is the femoral head, which is found on the proximal (top) aspect of the femur and the ‘socket’ is the acetabulum, which is part of the pelvis. The surfaces of both the femoral head and the acetabulum are covered with articular cartilage and the joint is filled with synovial fluid allowing for smooth movement. Surrounding the acetabulum is a fibrocartilaginous ring known as the labrum, which allows the femoral head to articulate with the pelvis. Encasing the bones are a thick set of connective tissue structures known as ligaments, forming a capsule. Ligaments are fibrous connective tissues that hold two bones together.

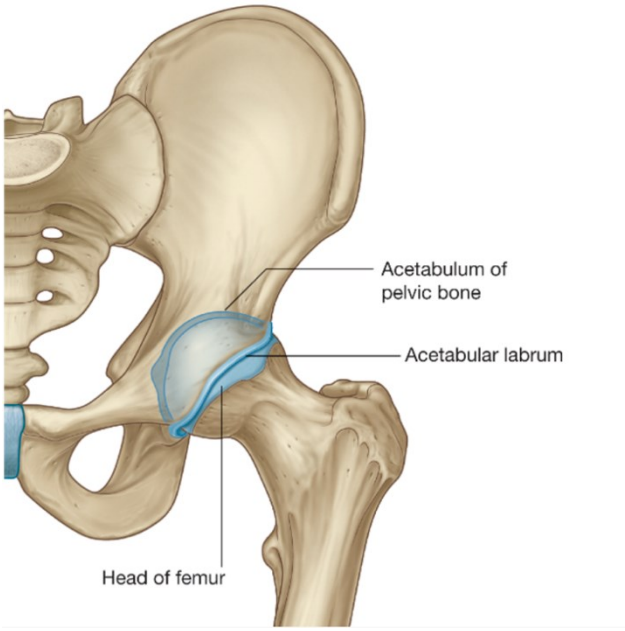

Figure 1: Hip Joint[5]

Around the hip joint, there are numerous muscles which not only act to stabilize it but also allow for movements that are essential to the human gait. The first set of muscles are the gluteal muscles found in the buttocks. These three muscles, the gluteus maximus, medius and minimus are responsible for extension and abduction of the thigh. The next set of muscles are the iliopsoas muscles made up of two separate muscles,

the iliacus and the psoas. They originate at the vertebral bodies of T12 and L1-3 and are the strongest flexors of the thigh at the hip. Next are the quadriceps muscles, made up of four different muscles including the vastus lateralis, vastus intermedius, vastus medialis and rectus femoris. The quadriceps are all responsible for extending the knee, however the rectus femoris muscle also plays a role in flexing the thigh at the hip. The groin muscles, which are made up of six different muscles (gracilis, adductor longus, adductor brevis, adductor magnus, pectineus and obturator internus) are responsible for adduction, flexion, medial and lateral rotation of the thigh at the hip. Finally, the hamstring muscles are a set of three muscles, made up of semitendinosus, semimembranosus and biceps femoris, found on the back of the leg which mainly act on the knee, but do provide support and some extension movement to the hip, particularly during walking.

Knee Anatomy

This knee is also a synovial joint that is classified to be a modified hinge joint (Figure 2). Where hinge joints only perform movements in a uniaxial plane, the knee can perform those movements (i.e. flexion/extension) as well as having slight rotational capabilities, making it a modified hinge joint. It is made up of three bones, the femur, the tibia and the patella.

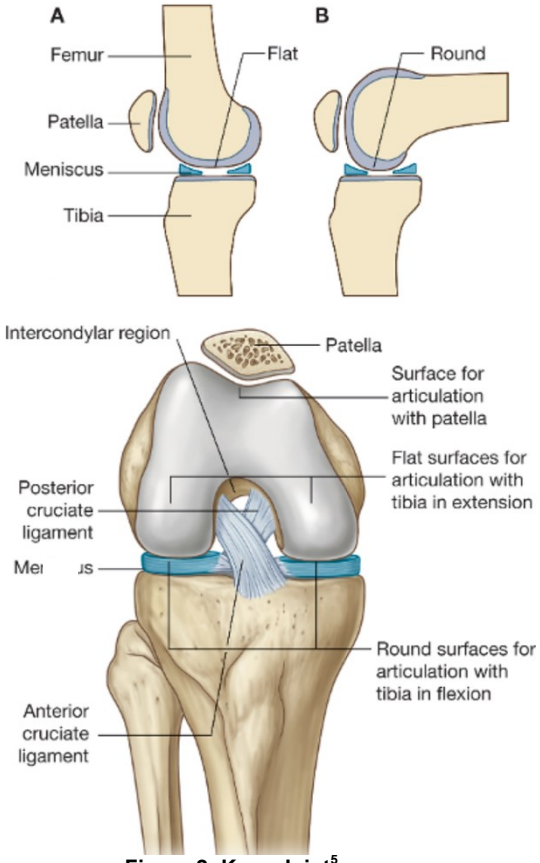

Figure 2: Knee Joint[5]

Upon the distal (lower) end of the femur are two convex condyles, the medial and lateral condyle, which are met by two concave condyles of the same name on the proximal (top) portion of the tibia. Both of these surfaces are covered with articular cartilage and the joint is filled with synovial fluid. Between these are two crescent shaped fibrocartilaginous structures known as the menisci, which act as reinforcement to the compressive forces felt at the knee joint.

The femur and tibia are held together by two sets of ligaments that are found in and around the knee joint. The first set are known as the cruciate ligaments, made up of the anterior cruciate ligament (ACL) and posterior cruciate ligament (PCL). These ligaments are found on the inside of the joint. They cross over each other creating an 'X' shape, where the ACL is in front and the PCL is found in the back. They are responsible for resisting the forward and backward movement of the knee. More specifically, the ACL prevents the tibia from sliding forward, in relation to the femur, and the PCL prevents the tibia from moving backward in the same relation.

The other set of ligaments are known as the collateral ligaments and are made up of the medial collateral ligament and the lateral collateral ligament. These two are found on the outside of the joint itself, medial being on the inside and lateral facing the outside. They are responsible for resisting the sideways motion of the knee and prevent abnormal movements from taking place.

Above the knee joint are the quadriceps muscles which extend the knee. This set of four muscles converge to form the quadriceps tendon. A tendon is another form of

connective tissue that joins muscles to bones. In this case, the patella, or the kneecap, which is a sesamoid bone, is attached to the quadriceps tendon from above and to the patellar ligament from below, holding it in place. The main function of the patella is to aid knee extension by providing leverage for the quadriceps muscle. The hamstrings found on the back of the thigh, allow for knee flexion. The gracilis muscle and a muscle known as popliteus (which wraps behind the knee) act on the knee and provide the slight rotational movement which allows this joint to be classified as a modified hinge joint.

Ankle Anatomy

The ankle joint is a synovial hinge joint, meaning that it only moves in a uniaxial plane (Figure 3). There are other joints found within the foot that allow for inversion/eversion movements to take place. The ankle joint is made up three bones, the distal (bottom) aspect of the tibia, the distal aspect of the fibula and the talus. Similar to the hip and knee joints, the surfaces of each bone are covered with articular cartilage and they are all lubricated with synovial fluid. Around the ankle joint, ligaments are found that support the ankle as a whole. On the medial (inside) surface is the deltoid ligament which is made up of

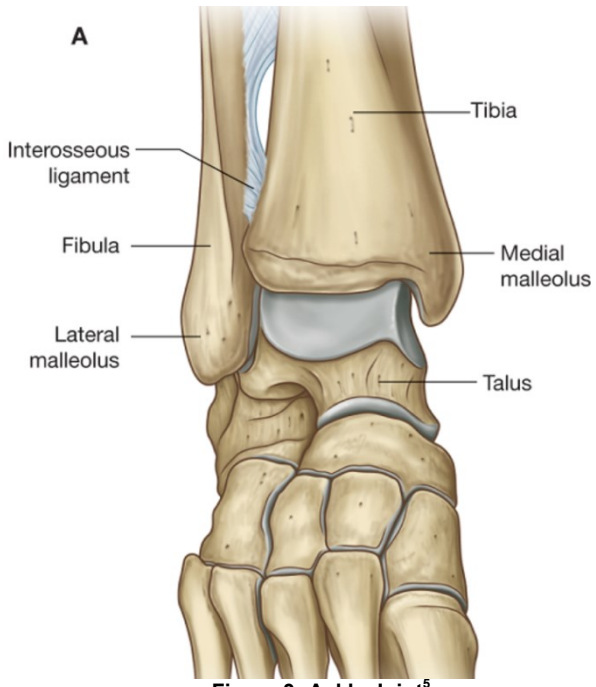

Figure 3: Ankle Joint[5]

four separate ligaments. And on the lateral (outside) surface there are three more ligaments including, the anterior talofibular, the posterior talofibular and the calcaneofibular ligament. The anterior talofibular ligament runs from the top of the foot to the ankle, preventing the foot from sliding forward in relation to the tibia and the posterior talofibular ligament does the opposite, running across the back of the foot to the ankle.

The muscles of the leg are the driving factors that move the foot during the gait cycle. On the back of the leg, also known as the calf, are three muscles known as the gastrocnemius, the soleus and the plantaris muscles. These muscles converge to form the Achilles' tendon and are involved in plantarflexing (moving the foot towards the ground). Deep to these muscles are three more muscles, flexor hallucis longus, flexor digitorum longus and tibialis posterior. The first two are responsible for flexing the toes while the latter inverts the foot, plantarflexes and supports the foot's arch during walking. On the anterior aspect, or front of the leg, are three muscles including, extensor hallucis longus, extensor digitorum longus and tibialis anterior. The first two play opposite roles to muscles described earlier in that they extend the toes and all three of these muscles function to dorsiflex (moving the foot in an upward direction).

Cartilage

Structure and Function

Cartilage is an avascular viscoelastic tissue found throughout the human body and serves a variety of different functions. It can be found as hyaline cartilage in joints,

protecting the ends of long bones; elastic cartilage, which forms the outer ear, Eustachain tubes, parts of the larynx and epiglottis[6]; and also as fibrocartilage located in areas such as the menisci, intervertebral discs, tendons, ligaments and the temporomandibular joint[7].

Articular cartilage in particular, is hyaline cartilage found on the ends of long bones within joints that are filled with synovial fluid to provide lubrication. The articular cartilage is approximately 2 to 4 mm thick, dependent upon the location and the amount of load that is normally transmitted through a particular joint[8]. All three types of cartilage are made up of specialized cells called chondrocytes. These cells create and maintain the cartilaginous matrix made mostly of proteins, known as proteoglycans and collagen. Articular cartilage in particular is made up of a dense extracellular matrix (ECM) with a sparse distribution of these cells[8]. The ECM is made up of mostly water, collagen II, which are arranged as fibres to provide tensile strength and the proteoglycan, aggrecan, which provides the elasticity[9]. When the cartilage is placed under a compressive load (during an activity), the water within cartilage flows out, however, the negatively charged proteoglycans create an osmotic effect, drawing the water back into the cartilage. This balance within the cartilage between compressive forces and osmotic pressure allows it to handle the forces that it is put through on a daily basis for many years[10].

Articular cartilage is divided up into zones which include, the superficial zone, the middle zone, the deep zone and the calcified zone (Figure 4). The superficial zone protects the deeper layers from sheer stress forces. The middle zone and deep zones

provide resistance to the compressive forces[8]. The calcified zone acts in securing the cartilage to the bone. In addition to providing resistance against compressive forces, cartilage also serves to provide a smooth surface with a low coefficient of friction to allow for an effective gliding motion during joint movement[11]. The synovial fluid helps maintain lubrication within the joint and reduces the friction and wear amongst two articulating surfaces[12].

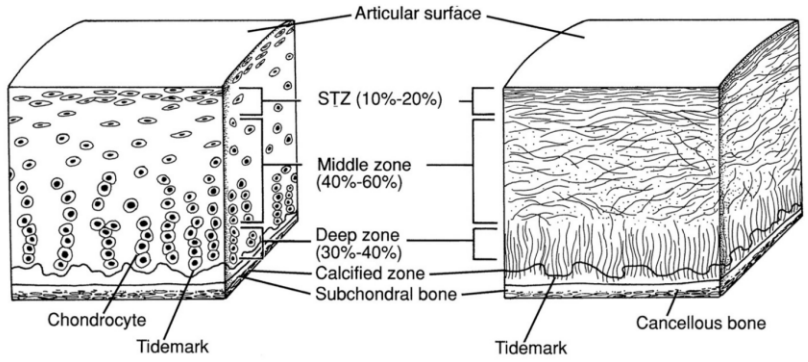

Figure 4: Zones of Cartilage[13]

Osteoarthritis

Pathophysiology

The main pathology that affects cartilage is called osteoarthritis (OA) and is associated with disabling pain and a reduced range of motion in the affected joints. The main risk factors of OA include old age, joint injury, obesity and genetics. The hallmark pathological changes that are found in joints affected by OA include loss of cartilage, joint space narrowing, hypertrophic changes to the subchondral bone, osteophyte formation, synovial inflammation, degeneration of ligaments and menisci (in the knee) and hypertrophy of the joint capsule[11].

These pathologic changes occur due to the lack of articular cartilage causing bone to bone contact. Since the periosteum, or outer layer of bone, is highly vascularized and has many nerve endings the pain associated with OA is tremendous. Subchondral cysts are caused after the subchondral bone stiffens and undergoes infarction due to lack of blood supply[13]. Osteophytes form on joint margins in attempts to stabilize the joint. As cartilage is avascular, its ability to repair itself is very poor.

Osteoarthritis has been considered to be a 'wear and tear' process for many years. Cartilage wear is defined as the result of one of four major mechanisms: abrasion, adhesion, surface fatigue and tribochemical reactions[14]. With age, the cartilage goes through years of degeneration, with many different forces being placed upon it. However, contrary to popular believe, cartilage destruction in conditions such as osteoarthritis is not only a 'wear and tear' process, but also a complex biochemical one as well.

Risks of Aging

Aging is the strongest risk factor known to cause OA and a study published by Verzigl et al. stated that advanced glycation end products, which are proteins that become glycated when exposed to sugars, increase within cartilage with increasing age. This causes the cartilage to become more brittle and stiff all the while decreasing the synthesis and degradation of the ECM and its constituents, making it more prone to damage[15]. They have also shown that aging results in reduced repair of the ECM by the chondrocytes. This repair induces an increased production of matrix proteins as well as

matrix degrading enzymes including members of the matrix metalloproteinase family, such as aggrecanases and collagenases and also serine and cysteine proteinases[11]. Other studies show that a disorganized ECM and decreased proteoglycan content no longer have a strong osmotic pressure, and therefore the overall water content that is found within the cartilage, decreases with OA[10]. This leads to the hallmark signs that are found with osteoarthritis.

Risk of Obesity

Obesity is a risk factor for osteoarthritis as it causes increased or abnormal loading to joints, particularly the hip and the knee joints. In addition, overall muscle weakness leads to an increased amount of wear on cartilage. By losing this weight, the progression of OA can be reduced and studies have shown that by increasing the functional loading of cartilage through moderate exercise, there can be an increase in articulating cartilage thickness, proteoglycan content and mechanical stiffness of the tissue[16]. In contrast to that, joint immobilization can lead to cartilage thinning and decrease in proteoglycan content[16].

Human Gait

Gait Cycle

The normal human gait consists of two phases, the stance phase and the swing phase, hence it commonly described as a biphasic cycle (Figure 5). The gait cycle is defined as the time between successive foot contacts of the same limb[17]. The stance phase accounts for ~60% of the cycle, while the swing phase accounts for the remaining 40%.

The stance phase can be divided into the heel-strike, loading, mid-stance, terminal stance and pre-swing phases. And the swing phase can be divided into the initial, mid and terminal swing phases[18].

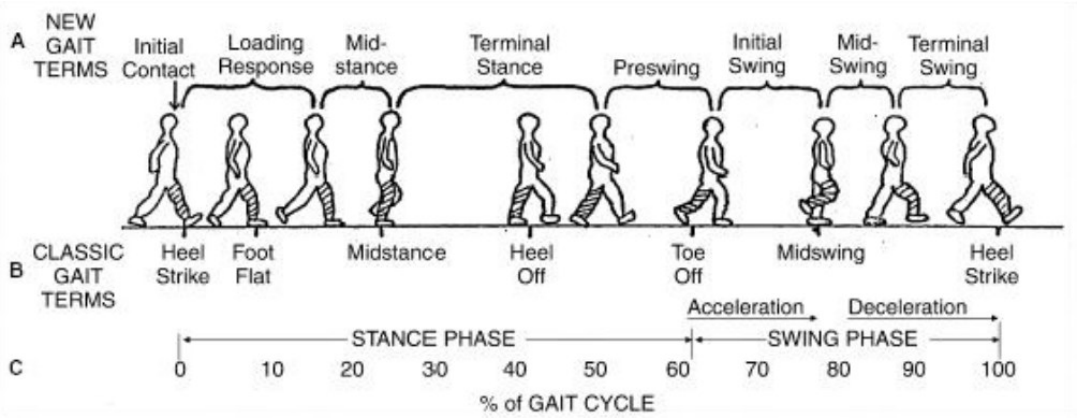

Figure 5: Gait Cycle[19]

During the heel-strike phase, the gluteus muscles contract to stabilize the hip, while the quadriceps and ankle dorsiflexors contract eccentrically. Next, during the support phase the bodyweight is transferred onto the supporting limb. Here the hip extensors stabilize the hip, the tibialis anterior contracts to control plantar flexion and the quadriceps contract to stabilize the knee and counteract flexion of the knee. Mid-stance marks the moment when there is single leg support and involves the contraction of the hip extensors and quadriceps to contract. Terminal stance begins when the supporting heel rises from the ground and continues until the opposite heel touches the ground. During this time, the gastrocnemius, soleus, the toe extensors and tibialis posterior all contract. Finally, in the pre-swing phase, the hip flexors as well as gastrocnemius and soleus propel the advancing limb[18].

The swing phase begins with the initial swing or 'toe-off' and is defined as elevation of the limb to a point of maximal flexion. Here the hip flexors contract to advance the swinging leg. Next, the mid-swing's aim is to allow for foot clearance of the ground or flexion of the knee to a point where the tibia is vertical. To achieve this the ankle dorsiflexors ensure sufficient ground clearance of the foot. Finally, in terminal swing, the hamstrings are involved in decelerating the forward motion of the thigh[18]. During the gait cycle, the hip has a range of motion of 30° of flexion to 10° of hyperextension[19], The knee ranges between 15° and 60° of flexion before reaching full extension[19]; and at the ankle the range of motion varies from 12-15° of dorsiflexion to 9-11° of plantarflexion[20].

Studies have shown that over a typical human lifespan, an individual takes approximately 2 million steps per year[21]. This means that each leg undergoes 1 million cycles per year at a rate of 1 Hertz or one cycle per second[21,22].

Gait Forces

To generate movement in the gait cycle, both internal and external forces act upon the human body. The internal forces are made up of the muscle and ligaments to contract and relax causing movement of the lower limbs. These internal forces are the driving force behind human movement. When a person is still, they are at equilibrium as gravity is causing a vertical force downwards. This force is caused by the mass of the individual and the force of gravity, also known as body weight. As per Newton's Third Law, 'For every action, there is an equal and opposite reaction', when a person is standing still a

force is reflected back onto the body as a ground reaction force (GRF). When standing still, the GRF will be equal to the person's bodyweight.

When a person begins to move, a frictional force is acting upon them in the opposite direction to their momentum. This not only acts against the individual to slow the movement, but also propels them in the direction they wish to advance in. If there was no frictional force, then the foot would tend to slip (e.g. walking upon ice) and there would be no forward movement. This frictional force can change depending upon the coefficient of friction (μ) that is present between the foot or shoe and the surface that is being walked upon. The friction can therefore be calculated by the equation $F = \mu G$, where F is the friction force, μ is the coefficient of friction and G is the normal force at the point of contact.

The GRF is a vector, which means that it is a quantity that is dependent upon magnitude and direction. Therefore, when an individual is in motion, the GRF is dependent upon which direction the force is being applied into the ground by the person. The GRF contains a component normal to the ground surface and a component parallel to the ground surface (the frictional component). The magnitude of the GRF is dependent on the acceleration of the person.

The centre of gravity of the individual is another important element of the gait cycle. The centre of gravity by definition is where the weight of a person is concentrated so that if supported at this point, the body would remain in equilibrium at any position[23]. During

the gait cycle, we need to place one foot in front of the other, and during this a person needs to balance themselves and their centre of gravity may change to do so.

Loading of Hip Joint

The hip joint experiences a range of forces placed upon it during typical daily activities. These forces are usually quantified by estimating the amount of bodyweight (BW) going through the joint at a particular time. The loads range between 1.0 and 10.0 BW depending upon the activity[24,25]. For example, when standing still upon two legs there is a load of 0.5 BW upon the hip joint. When walking at a speed between 3 and 6 km/h, the load upon the hip increases to 4.2 and 5.1 BW, respectively[24]. When running between a speed of 6 and 12 km/h, the load further increases to 7.5 and 10.0 BW, respectively[24]. When going up and down stairs, the load placed upon the hip joint has been reported as 2.5 and 2.6 BW, respectively[26].

Loading of Knee Joint

Similar to the hip, the knee joint also experiences a range of forces throughout activities that are normally carried out during the typical day. The variation in forces through the tibiofemoral joint are anywhere between 0.5 and 7.6 BW[27,28]. For example, when a person is standing still, on two feet, there is exactly 0.5 BW going through the knee. When they begin walking on a level surface, that force increases to 2.5 BW[27]. If they are ascending stairs or descending stairs the load on the knee increases to 3.0 and 3.5 BW, respectively[27]. If a person is standing on one leg, the load is approximately 2.5 BW[27]. When a person jumps in the air and lands, the load on the knee joint is 6.9 and 7.6 BW,

respectively[28]. These loads that are described are those that are being placed upon the tibiofemoral joint.

Loading of Ankle Joint

In the hip joint, there is a contact area 1100 mm^2 between the acetabulum and the femoral head[29]. In the knee, there is 1120 mm^2 of contact area but ankle only has a 350 mm^2 area to transmit all the forces that are being placed on it[29]. As the contact area of the ankle is smaller and since it would have the same amount of force applied to it as the other two joints, the amount of pressure that is applied at the ankle is increased. While standing still, similar to the hip and the knee joints, there will be 0.5 BW placed upon each ankle joint. Models have been created to study the loading of ankle joints during the gait cycle. They have found that the range of loading is between 0 and 5.4 BW when walking and 0 and 11.1 BW when running[30]. By learning the anatomy and understanding differences between the three main joints involved in the gait cycle, it is important to recognise the difference between contact areas of each of the joints.

Literature Review

The purpose of this literature review is to understand the fundamentals behind osteoarthritis and to critically assess the research that looks at cartilage loss in relation to other materials and to determine the strengths and weaknesses of different studies on this subject. This will allow us to compare the results from our model of mechanical testing, to others that have been completed, to investigate the amount of wear on articulating cartilage when interacting with different materials used in many orthopaedic operations today.

Introduction

For this literature review, seven papers were analyzed to provide context and background relating to the topic of orthopaedic implant materials and the wear the cause to cartilage. These papers are listed and summarized in Tables 1 and 2. Table 1 focuses on studies that included cartilage on cartilage testing as part of their research, using them as controls or as the main aspect of their study. Table 2 includes papers that did not include any cartilage on cartilage testing throughout their studies and focused solely upon testing cartilage with different materials.

Table of Papers Reviewed

Table 1: Cartilage on Cartilage Testing Included

Paper	Sample	Articular Cartilage	Materials Used	Mechanical Testing	Other Tests	Results
Vanlommel et al. (2016)[31]	n=11	Porcine patellae	• Cartilage • Oxidized Zirconium (OxZr) • Cobalt-Chrome (CoCr)	Pin-on-Disk immersed in 40% newborn calf bovine serum, diluted water, ethylenediaminetetraacetic acid and sodium azide	• Histological Staining • Cartilage Thickness • Mod. Mankin Score	• Cartilage vs Cartilage exhibited least damage • Cartilage vs OxZr showed less damage than CoCr
Trevino et al. (2016)[14]	n = 20	Steer knee joints	• Cartilage • CoCr	Custom Tribological Pin-on-Ball Machine immersed in 1% Mini-ITS (insulin, transferrin, selenous acid, ascorbic acid, and bovine serum albumin/linoleic acid) and antibiotic solution	• Histology • Topography (CT) • Metabolism • GAG content • HYP content • Aggrecan Detection	• Cartilage vs Cartilage showed no damage • CoCr vs Cartilage caused increased wear

Paper	Sample	Articular Cartilage	Materials Used	Mechanical Testing	Other Tests	Results
Verberne et al. (2009)[12]		Human femoral head	• Cartilage	Custom device providing a reciprocating sliding motion immersed in PBS	• Surface Morphology with optical profilometer and optical microscope • Proteoglycan concentration • Collagen and PG weight	• Changes in surface morphology visible but no definite conclusion • Decrease in PG concentration when compared to unworn cartilage • Scaled weight of collagen and PG increased with higher loads and longer duration of tests

Table 2: No Cartilage on Cartilage Testing Included

Paper	Sample	Articular Cartilage	Materials Used	Mechanical Testing	Other Tests	Results
Oungoulian et al. (2015)[32]	n=8	Calf knee joints	• Glass • CoCr low carbon • CoCr low carbon - Ra 25 nm • CoCr high carbon • 316 Low Vacuum Melt Stainless Steel (SS)	Custom two-axis device with reciprocal sliding motion immersed in PBS	• Particulate Testing • Biochemical Analysis • Histological Staining	• 316 SS and CoCrLC-Ra25 nm exhibited greater damage than all other materials
Chan et al. (2011)[33]	n = 28	Calf knee joint	• Aluminum Oxide (Al_2O_3) • CoCr • SS • UHMWPE	Pin-on-Disk immersed in PBS	• Nanoscale Friction • Protein Wear Assay • Histology	• CoCr had largest μ, most protein loss, greatest change in nanoscale friction. • UHMWPE had lowest μ, least protein loss, insignificant changes in nanoscale friction

Paper	Sample	Articular Cartilage	Materials Used	Mechanical Testing	Other Tests	Results
Patel el al. (1997)[34]	n = 10	Calf knee joints	• OxZr • CoCr	Pin-on-Disk immersed in bovine calf serum	• Cartilage thickness • Histology	• OxZr caused 30% less wear on cartilage compared to CoCr
Schwartz et al. (2007)[35]	n = 12	Bovine hip joints	• SS • 'Compliant counter face' – vinyl lab tubing stretched over SS	Dual Axis Wear Simulator immersed in bovine serum solution	• HYP Concentration • Cartilage wear via weighing dehydrated mass of worn cartilage • Microscopic analysis of cartilage and subchondral bone	• Wear significantly reduced with the use of compliant counter face

Discussion

Mechanical Testing

One aspect of these papers that sets them apart was the manner in which they conducted their experiments. Six of the seven studies that were reviewed used a 'pin-on-disk' or similar sliding method while one of the studies (Trevino et al.) used a custom device that provided a dual-axis rotation.

Pin-on-Disk Configuration

The pin-on-disk configuration has been used in many studies to provide data regarding cartilage wear against other materials, whether they be plastic or metal (Figure 6). In this model, cartilage samples or other controls would act as the disk on the inferior aspect of the testing device. These discs ranged between 4 mm and 12 mm in size. The researchers lowered the pin, made up of the material that they wished to test, until it was in contact with the disk below. The disk would then begin to slide with a translational speed, along a particular track radius with a predetermined stroke length and loading force applied. They would determine the values of each parameter to accurately exhibit human physiological movement and forces applied during the gait cycle. For example, Chan et al. carried out their experiments at 0.5 mm/s sliding speed along a 5 mm wear track radius, with 7.85 mm

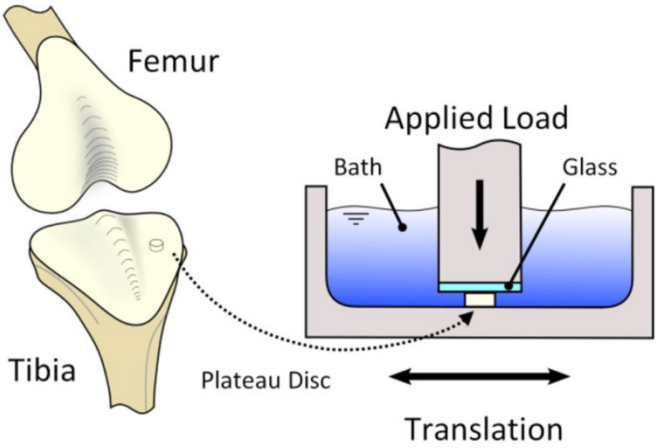

Figure 6: Pin-on-Disk Configuration[31]

stroke length under a 1.8 N load[33]. Other studies carried out experiments under different conditions to determine the effects of cartilage in different aspects, whether that was increasing or decreasing the load, changing the sliding speed or having the experiments going on for a longer period of time.

The main benefit of this configuration is the amount of control that the researchers have over the variables that they wish to test. The parameters for the experiments previously mentioned will not change unless the researchers wish to test their materials in different ways or if there is mechanical malfunction. In all of the studies that were reviewed. there were uniform loading pressures and sliding speeds at all times. These models are useful when evaluating friction and wear between two materials. Saying this however, these experiments poorly account for the physiological biomechanics that are present in the day to day life of a human. For example, there is not a uniform loading force on a joint like the hip or the knee. Along with this, the typical human synovial joint does not perform sliding movements that these researchers are testing. Even though these configurations allow for the understanding of how cartilage wears against metals or plastics, it does not accurately represent how cartilage would react when forced to interact with the same materials after they are implanted into the human body.

Custom Configuration

In the study by Trevino et al., the researchers used a custom apparatus where they created a bi-axial pin-on-ball design to create both compressive loading and complex shear force that is similar to the forces felt on cartilage *in vivo*[14]. Here, the researchers

pressed a cartilage pin extracted from a bovine knee joint onto a polyethylene ball. This ball was then placed upon a disc made up of metal or cartilage and a constant force of 40 N was applied. The ball rotated at a frequency of 0.5 Hz and a stroke of 30°, while the disc underneath rotated at a frequency of 0.1 Hz and a stroke of 15°.

The benefits of this configuration are that they represent the rolling and gliding movements that are present in most synovial joints in the human body. This would more accurately describe what would happen to the cartilage *in vivo* when it is placed in contact with the materials the researchers tested. However, once again, this study used a constant force throughout its experiments. As mentioned previously, there is not always a constant force going through joints and therefore a model with variable or cyclic forces would be more accurate.

Materials Used

Orthopaedic implants used in joint replacement surgeries are made up of a variety of different materials. They include metals such as stainless steel (SS), titanium and cobalt-chromium (CoCr); ceramics such as oxidized zirconium (OxZr), aluminum oxide, silicon oxide and calcium phosphates; and polymers such as ultra-high-molecular-weight polyethelene (UHMWP), high density polyethylene (HDP) and polyether ether ketone (PEEK).

When considering which materials are suitable to use as orthopaedic implants, testing needs to be conducted on that particular substance to determine how it will react when

placed inside the body. Properties such as density, specific heat, thermal conductivity, thermal expansion, malleability and corrosion all need to be carefully considered prior to beginning the design. In addition the stiffness of a material needs to be considered as a material that is much stiffer than the bone it is placed next to can cause stress shielding resulting in bone loss[36]. Also, knowing how the material will react when placed in bodily fluids such as blood is essential. Many metallic materials are susceptible to corrosion when placed in aqueous environments or if there are changes in the acidity or pH, highlighting another reason that careful consideration needs to be taken before utilizing a certain substance[36].

The ideal implant should have a low elastic modulus and be chemically inert, biocompatible, strong, highly resistant to fatigue, corrosion-proof, resistant to wear and inexpensive[37].

Solution Used

Another aspect that differentiates all the papers reviewed was the type of solution they used to act as a lubricant when mechanical testing was taking place. Three of the seven papers used phosphate buffered saline (PBS) while the other papers either used bovine serum solution or a mixture of bovine serum with other components added to it. PBS is a water-based salt solution that can maintain a pH between 7.1 and 7.3 and is able to match the osmolality (280-320 mOsm/kg) and ion concentrations of the human body[38]. Whereas bovine serum solution is taken from the blood of cattle, centrifuged and filtered. Its pH is between 6.5 and 8.5 and has a similar osmolality (280-360 mOsm/kg) as PBS. A key aspect to note is that bovine serum solution contains a high protein

content (3.0-4.5g/dl)[39]. Each of these solutions were used to simulate human synovial fluid by providing lubrication to all aspects of the respective configurations that were used. Both of these solutions were meant to act in a similar way to human synovial fluid. As mentioned previously, this fluid not only provides lubrication to the joint and but also provides a relatively friction free environment which allows for smooth movement of the joints.

Although none of the papers that were reviewed gave an exact explanation as to the reasoning behind the usage of their respective solutions, cost may have played a role in this decision. For example, the cost of PBS is approximately $6.00 CAD for 500 mL solution, whereas bovine serum solution can cost between $400-800 CAD for the same volume of solution[40]. Another possible reason may be due to the high protein content that is found within the bovine solution. This may contribute to a more protective role when testing materials other than cartilage. A study by Russell, 2010, stated that they used PBS when testing materials in the presence of articulating cartilage and used bovine serum solution in the absence of the cartilage due to the lack of proteins that aid the lubrication[41].

The use of either PBS or bovine serum solution did not seem to change the results that were found in each paper, and therefore do not seem to play a significant role in the testing of the wear of articular cartilage against different materials. Both seemed to provide lubrication and reduce the friction between the two surfaces, enabling the researchers to simulate a human joint.

Histological Analysis

Histology is the study of tissue by using a microscope. To enhance the image that is seen through the microscope and to highlight certain elements that the researchers wish to study, staining with dyes or chemicals are commonly used. In five of the seven studies that were reviewed for this paper, histology was used to assess not only the cartilage structure to quantify the amount of wear that took place but also the researchers used stains to assess if there was a deficiency of any of the different components that make up cartilage after testing.

The researchers of these five papers used a variety of different stains to assess different parts of the cartilage. Some of the stains that were used include Safranin-O/Fast green to assess for collagen integrity, proteoglycan content and chondrocyte appearance[14,32,34]. Along with this, Patel et al. used Masson Trichome staining to specifically assess the collagen and also used haematoxylin and eosin, more commonly known as an H&E stain, used to visualize the cellular structures in more detail[34]. Chan et al. wanted to assess wear by assaying for total protein and amount of superficial zone protein by using Bouin's fixative[33].

The histology results varied between each paper. Patel et al. found that degraded cartilage due to wear had a decreased proteoglycan content. Oungoulian et al. noticed delamination of the superficial zone of the cartilage, which was not apparent upon gross macroscopic examination[32]. This area also had collagen fibrils which exhibited a random orientation. Chan et al. reported that the superficial zone proteins were

removed by friction and that there was a lower content of these proteins when articulating against CoCr compared to Al_2O_3[33]. Finally, Trevino et al. stated that samples that were articulating with metals had an increase in surface damage and decreased amount of staining in the middle zone when compared to cartilage on cartilage testing[14]. They also noted that the superficial zone had a displacement of chondrocytes and that there was little disruption of the ECM[14].

Overall, histology proved to be a strong tool when assessing the amount of wear that cartilage goes through when articulating with different materials. When assessing the results from these studies, although differences were found, the overall result is that worn cartilage has a decreased protein level and a disruption of the chondrocytes is present. These two findings have been supported by previous evidence to be found in osteoarthritis and can be an effective way to determine wear of cartilage in further experiments.

Biochemical Analysis

Another way that studies have shown how much cartilage wear is occurring is through biochemical analysis of the cartilage. This type of analysis is measured by a few different techniques. The main technique that is seen in many papers is by weighing specific components that make up the cartilage. After testing is complete, researchers attempt to collect all the components that are found within the solution that they are testing in, dry them and weigh them[14,35]. The components that researchers are testing for by this method are glycosaminoglycans (GAG) and hydroxyproline (HYP). GAGs are

polysaccharides, or carbohydrates, that are attached to the proteoglycan structures in cartilage. HYP is an amino acid, which is the building block for any protein, that is a major constituent of collagen.

The alternative way to analyze cartilage wear biochemically is by assessing the concentration of proteoglycans and/or HYP. This is done by slicing the cartilage after mechanical testing is complete using a microtome, which is a machine with the ability to slice materials extremely thinly. A microtome is able to cut slices of cartilage to a thickness of 50 μm[12]. This slice is then analyzed under a spectrophotometer. A spectrophotometer provides the ability to view tissues at different wavelengths of light. In two of the studies reviewed, wavelengths of 540 to 550 nm were used to assess concentrations of proteoglycans and HYP[12,35].

By using these techniques researchers can assess exactly how much wear has taken place within the cartilage. For example, Trevino et al. were able to elicit that cartilage that had been articulating with metal released more GAG and HYP (28.18μg/ml) into their solution when compared to their control sample that was in contact with cartilage (16.14 μg/ml)[14]. And Verberne et al. found that the scaled weight of collagen and proteoglycans within their medium increased with an increasing number of loading cycles and with increasing amounts of force[12].

Since collagen, proteoglycans, GAG and HYP all make up cartilage, measuring the loss of these contents by different scientific techniques gives researchers the ability to

quantify the amount of cartilage loss. This can prove especially important when assessing the variances in cartilage wear, especially minute differences, that different materials can have.

Experimentation

Cartilage on Cartilage

As mentioned previously, cartilage naturally wears throughout our lifetime. Certain risk factors increase this wear leading to OA. Understanding how cartilage naturally wears *in vivo* can explain how it may interact with other materials and substances as well. By understanding the loads and different movements that cartilage can be placed under will allow for the proper research and development of implants so that their impact on the native cartilage is as minimal as possible.

Only three of the seven papers reviewed performed cartilage on cartilage (CoC) testing. Oungoulian et al. used cartilage vs glass as an alternative control group however this method will not be able to accurately describe how cartilage responds when it is loaded and mechanically tested. Cartilage was tested under the same conditions that metals were placed under and as expected, wear was found in all of the tests however, much less as compared to metal vs. cartilage testing.

Vanlommel et al. found macroscopically that there was a thinner cartilage in the metal vs. cartilage testing than in the CoC testing[31]. Also, after measuring the thickness of the

cartilage, the control group had a residual cartilage thickness that was 1 mm greater than those tested with OxZr and CoCr[31].

Trevino et al. found that 'articulation against cartilage did not cause additional cell death', whereas when cartilage was articulating with CoCr there was a statistically significant loss of dead cells found[14]. Also, when measuring GAG release into their medium of mini-ITS, they found that there was a significantly higher release in the CoCr tests as compared to the cartilage tests[14]. HYP also followed with similar results.

The study by Verberne et al. was to determine the most accurate techniques to quantify cartilage wear and was the only study to use human cartilage instead of cattle or porcine tissue[12]. The researchers found that the proteoglycan content of unworn cartilage was higher than that of the worn tissue after mechanical testing was completed. They also found that when they increased the number of cycles of testing, under the same load, the weight of both collagen and proteoglycans within their medium increased. They tested to see if this would happen if the cartilage was under 'no load' or 0 N of force. There was loss of collagen and proteoglycans, which they attribute to the agitation with the lubricating medium that they used however the weight of both components stayed the same, no matter how many cycles of testing were run[12].

The researchers came to the conclusion that the most accurate way of measuring cartilage wear was by their methods of measuring proteoglycan content within the

cartilage, by weighing the proteoglycan and collagen content within their medium after wear testing and with macroscopic and microscopic imaging of the surface.

These three studies all showed that cartilage wears when in contact and loaded against other samples of cartilage as we know from learning the pathophysiology of osteoarthritis. By understanding these methods that they used to quantify cartilage wear, further research can be done using the techniques described when testing to see which material causes the least amount of the wear with articulating cartilage.

Materials v Cartilage

Understanding which materials cause the most wear on articular cartilage, whether they are metals or polymers, can be very significant to the orthopaedic surgery community. Materials that have been used for decades in particular operations, may have to be replaced or may have to go through further research and development to make them more compatible for implantation.

After reviewing all the papers listed in both tables above, a few conclusions can be made. First, cobalt-chromium appears to be a very harmful material when articulating against cartilage as compared to other commonly used implants. Four papers compared CoCr to other materials, and they all concluded that it causes more cartilage wear than other materials that were tested[31–34]. It is one of the most common materials used in hip, knee and shoulder replacements[33]. Even in 1997 when Patel et al. conducted their study, their conclusions were that there may be a more suitable material than cobalt

chrome and, in their study, it was oxidized zirconium[34]. Vanlommel et al. agreed with this conclusion made two decades earlier after their testing produced the same results[31].

Chan et al. described how CoCr had the least desirable tribological properties, having the largest coefficient of friction when compared to stainless steel, aluminum oxide and UHMWP[33]. Oungoulian et al. were able to show that CoCr with a higher surface roughness (25 nm vs. 10 nm) caused the same amount of wear on cartilage as stainless steel[32]. This finding may lead researchers to further understand the effect of surface roughness and its effect on cartilage wear. The surface roughness of materials used for implants is currently regulated to be less than 50 nm. However, Oungoulian et al. stated that this should be reconsidered as the CoCr with a surface roughness of 25 nm had quite significant wear against cartilage that they tested[32]. It should be noted that these studies were performed using a pin-on-disk configuration and therefore may not elicit the same results when placed under normal physiologic conditions; however, the results are still significant, and CoCr may need to be re-evaluated if an implant of this material will be articulating directly with cartilage.

Next, a material that performed well in many tests were ceramics, more specifically in these studies, aluminum oxide and oxidized zirconium. Vanlommel et al. stated that ceramics as a whole have gained interest in terms of orthopaedic implants as they are more scratch resistant, and have less surface roughness[31]. Patel et al. found that OxZr caused 30% less wear than CoCr on articulating cartilage[34]. And Chan et al. noted that

even though Al_2O_3 had a higher coefficient of friction than CoCr, it still had less wear than its counterpart[33].

Finally, two studies tested the polymers, ultra-high-molecular-weight polyethylene and vinyl lab tubing. Chan et al. measured that the coefficient of friction of UHMWP was significantly lower than all the other metals they tested with[33]. However, this coefficient of friction between the polymer and cartilage was still two to ten times higher than that of cartilage on cartilage testing[33]. It also had the least amount of superior zone protein lost indicating that it caused the least amount of wear to the cartilage[33]. Similarly, Schwartz et al. used a vinyl laboratory tubing and stretched it over a SS shaft and they found that this caused less wear on the cartilage compared to SS[35]. They attribute this to lower contact pressures between the cartilage and the material[35]. Both of these studies conclude that polymers interact in positive ways with cartilage. Along with that, they recommend that more research and development should be directed into the use of these materials when creating implants that will be interactive directly with the articulating cartilage[33,35].

Conclusion

Overall from these studies, we can understand exactly how different materials articulate with cartilage. By using the correct configuration, ideal lubricating solutions and by using the correct assessment techniques of cartilage wear, an accurate understanding of the cartilage wear can be inferred. This can provide more insights into treating patients

suffering from such a common condition and preventing them from having major complication or revision surgery in the future.

Methods

Material Acquisition & Preparation

The materials that were tested for this project included titanium, UHMWP and carbon reinforced polyether ether ketone (CR-PEEK). The titanium samples were acquired previously through Southern Medical in South Africa and were cut to the specifications needed. The UHMWP and CR-PEEK samples were obtained from Johnston Industrial Plastics in Edmonton, Alberta. These materials were bought as 1-foot rods and the UHMWP and CR-PEEK samples were 1½ inches and 40 mm in diameter, respectively. These rods were then cut into several 40 mm discs (Figure 7) so that they could be

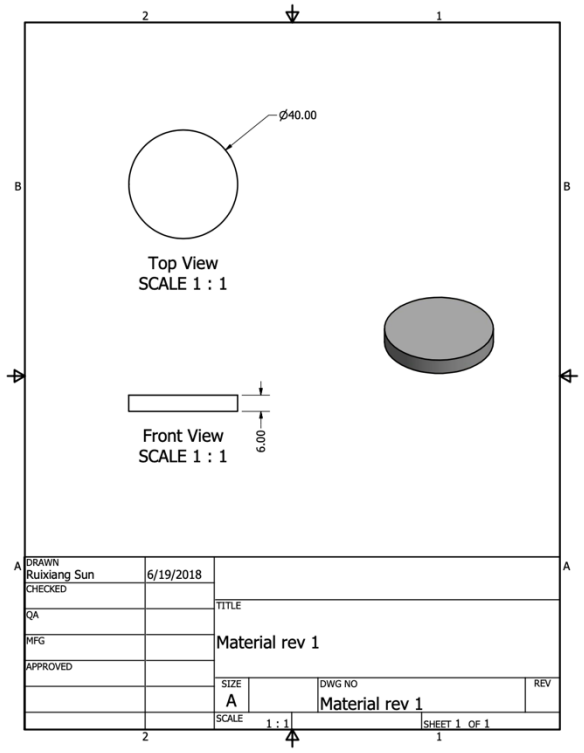

Figure 7: Specifications of Disc

placed in the previously designed holder that fit into the upper jig of the testing machine (Figure 8). These discs needed to be 40 mm in diameter and no more than 6 mm in height to fit into the top jig holder. All materials were cut to the required specifications by the staff in the Machine Shop, in the Faculty of Engineering at the University of Alberta.

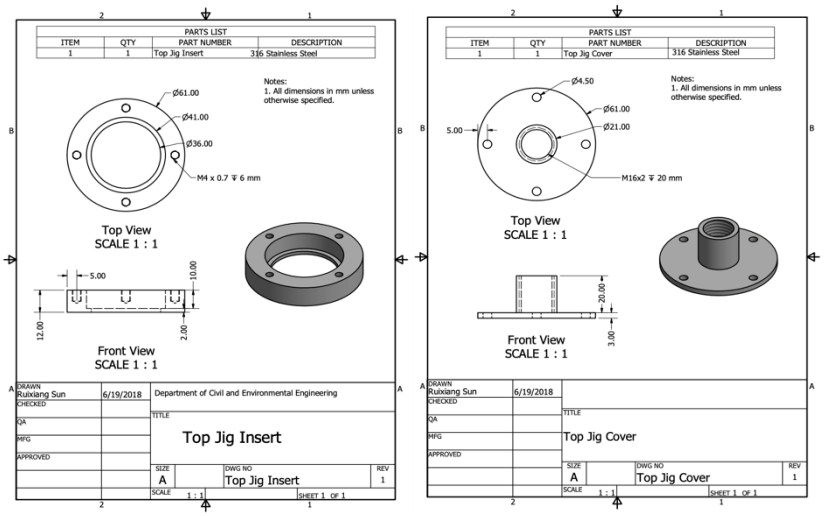

Figure 8: Top Jig Design

Sample Acquisition & Preparation

For testing, porcine cartilage was used to emulate human cartilage. Porcine legs were acquired from Delton Sausage and Deli House, a local butcher in Edmonton, Alberta. All specimens that were used for this project were obtained fresh from the butcher. The femoral condyles and patellae were dissected from the knee joints. First the patellar ligament was cut using a scalpel, releasing the patella from the joint. Following this, the soft tissue surrounding joint was removed with a scalpel. Next, a cut was made through the medial and lateral collateral ligaments, allowing for greater movement in the joint to continue to clear soft tissue. When visible, both the anterior and posterior cruciate ligaments were cut, separating the femur from the tibia. Then, using a saw, the distal

femur was cut just above the cartilage. Finally, the saw was used to separate the medial and lateral condyles so that they could be tested individually.

For the cartilage vs cartilage tests, the porcine patellae were used. The reason this bone was used was because its layer of articulating cartilage on the posterior aspect and also because of its shape. They are relatively flat bones, allowing them to fit within the holder attached to the upper jig of the testing machine. These were cut from the tissue using a blade. If a patella's diameter was too large (> 40 mm), some of the bone on the lateral and medial aspect would be trimmed using saws so that it could fit within the upper holder. The height would also be measured, and the anterior aspect would be trimmed using a saw to fit the required dimension (< 6 mm). After dissection, all cartilage samples were kept in phosphate buffered saline (PBS) at 4°C. Unused samples were appropriately discarded after 7 days.

Mechanical Testing

Mechanical testing took place on a uniaxial loading machine that was modified for these experiments by previous students (Figure 9). The femoral condyle, upon which wear was being observed, was placed on the previously designed lower metal jig (Figure 10).

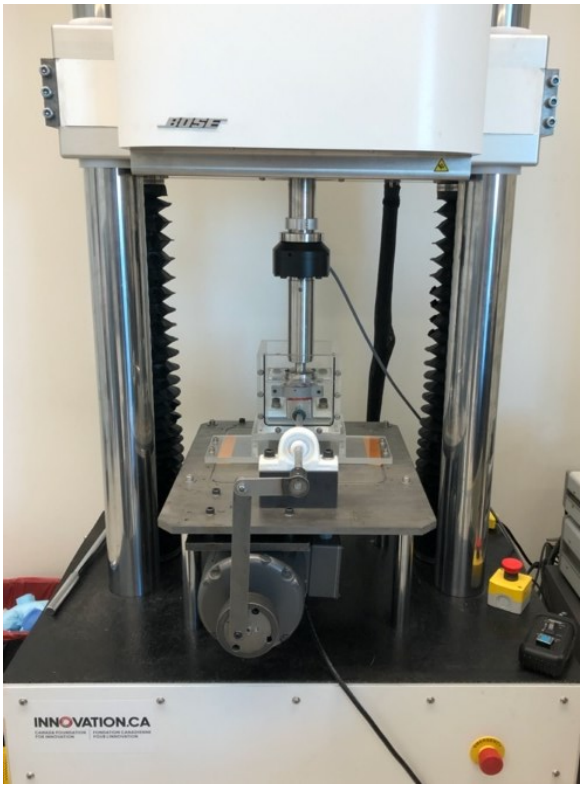

Figure 9: Uniaxial Loading Machine

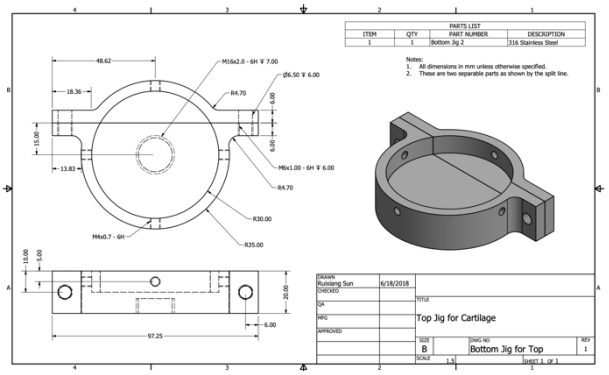

Figure 10: Lower jig configuration

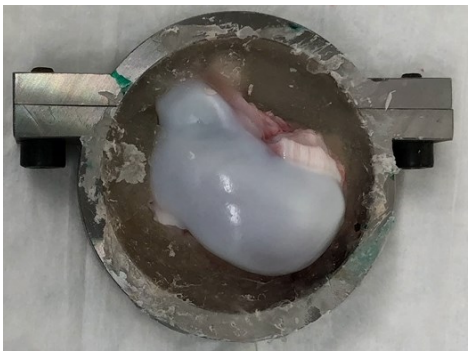

Figure 11: Porcine femoral condyle within resin

The condyle was kept in place using a non-dental resin (Central Dental, Scarborough, ON) (Figure 11).

The material that was being tested, was placed within an enclosed compartment which was then attached to the upper arm of the machine. The edges of this enclosed compartment were chamfered to ensure that the femoral condyle would only be in contact with the material it was being tested against.

This upper arm was capable of providing a compressive load to the femoral condyle. For our experiments, we used a sinusoidal load from 30-160 N, which was calculated to achieve an active contact stress of 1MPa. It has been shown previously that the average range of stresses on a joint during the gait cycle is between 0.5 to 5.0 MPa[42]. A motor was used to deliver rotary motion to the plate so that the femoral condyle could oscillate, simulating the physiologic joint movement. A rotary displacement of 10° at 3 Hz was configured (Figure 10). The rotary displacement of +/- 10° was chosen since the range of motion of the ankle throughout the gait cycle is 12-15° during dorsiflexion and 9-11° during plantarflexion[20]. The frequency of 3 Hz was used since a human's stride frequency ranges between 0.41-3.57 Hz[43]. The condyle and the material that it was being tested against were fully immersed in PBS to provide lubrication and act as a synthetic synovial fluid. An illustrated image showing the interaction of all the components of the machine is shown in Appendix A. The duration of each test was set to 4 hours during which the machine ran a total of 43,200 cycles. One cycle was defined as two vertical loads with the upper arm for one oscillation of the lower jig.

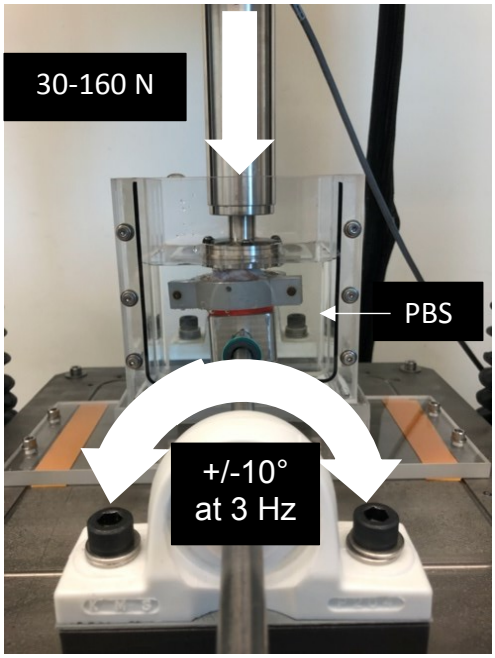

Figure 12: Labelled photo of experiment setup

Post-Testing

After completing a test, two cores were taken from each worn condyle. One core was taken from the apex, where the majority of the impact was placed during testing. Another core was taken from the side of the condyle, where there was minimal or no impact (Figure 13). The layers of cartilage were then separated from the bone using a blade and bone rongeurs. The cartilage was then cut into three equal pieces and placed in formalin 10% for fixation for a minimum of 24 hours.

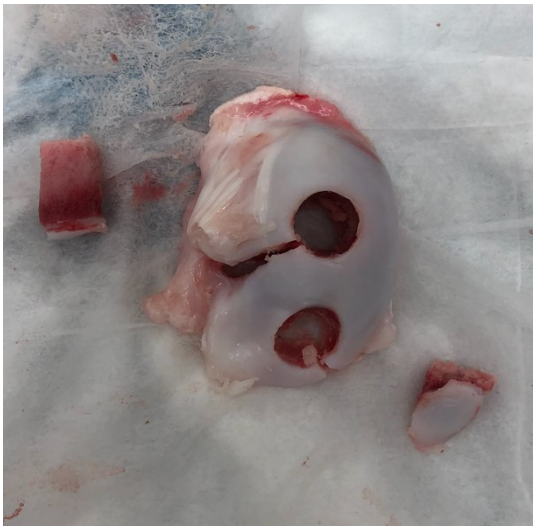

Figure 13: Two cores taken from porcine femoral condyle

Each piece of cartilage was then put into separate slotted cassettes in order to process the samples. Processing took place in a “dip and dunk” processor. These machines prepare tissue samples for embedding with paraffin wax. Since paraffin wax is hydrophobic, the first step in processing is to dehydrate specimens by using different concentrations of alcohol, which replace all the cells with alcohol instead of water. Since alcohol is immiscible with the paraffin wax, xylene or other clearing agents, remove the alcohol from the specimens. The final step uses paraffin wax to infiltrate the tissue, preparing it for embedding[44].

Embedding creates ‘blocks’ which are used on the microtome to cut very thin slices of tissue. The tissue sample is oriented accordingly and the container holding it is then filled with paraffin wax and immediately cooled, creating a block. For our experiment, two of the three pieces of cartilage were placed on their sides, in order to appreciate a cross-section of the cartilage histologically. The last piece was embedded face down, in order to appreciate only the surface of the cartilage.

Table 3: Safranin O Staining Protocol

Safranin O Staining Protocol	
Solution	**Time**
Haematoxylin Mayer and Eosin	
Ultra Clear	10 minutes
Ultra Clear	10 minutes
Ethanol 100%	1 minute
Ethanol 100%	1 minute
Ethanol 96%	1 minute
Ethanol 70%	1 minute
Ethanol 50%	1 minute
H20 Distilled	1 minute
HE Mayer	3 minutes
HE Mayer	2 minutes
H20 Tap	5 minutes
	Total = 15 minutes
Safranin O	
Running Tap Water	5 minutes
0.02% Fast Green FCF	4 minutes
1% Acetic Acid	3 dips
0.1% Safranin O	20 minutes
95% EtOH	10 dips
100% EtOH	20 dips
100% EtOH	1 minute
Ultra Clear	1 minute
Ultra Clear	1 minute

Following this process, each paraffin block was cut using a microtome to produce two, 5-micrometer sections of the sample. These paraffin scrolls were then placed on a glass slide. Two sections were taken to histologically asses the condition of the cartilage at two different levels. Each glass slide was placed in a room temperature incubator for 24 hours. Following incubation, the slides were stained with safranin O, as per lab protocols (Table 3).

Histology

Histological analysis was conducted on each slide. For histological grading, the osteoarthritis cartilage histopathology grade assessment was used, based on the criteria created by the Osteoarthritis Research Society International (OARSI) group[45] (table 4). This grading system took into account the condition of the surface of cartilage, the viability of the cells within the cartilage and the condition of the underlying bone as well. The grades from both levels (superficial and deep) of the two cross-sectional pieces of cartilage were averaged. The grading was conducted by two separate researchers and their respective grades were also averaged to determine the overall grade that was given to each sample. Virtual images were taken using the Nikon Elements software.

Table 4: Osteoarthritis Cartilage Histopathology Grade Assessment

Osteoarthritis Cartilage Histopathology Grade Assessment			
Grade		**Subgrade**	
Grade 0	Surface intact, cartilage intact	**No Subgrade**	
Grade 1	Surface intact	**Subgrade 1.0**	Cells intact
		Subgrade 1.5	Cell death
Grade 2	Surface discontinuity	**Subgrade 2.0**	Fibrillation through superficial zone
		Subgrade 2.5	Surface abrasion with matrix loss within superficial zone
Grade 3	Vertical fissures	**Subgrade 3.0**	Simple fissures
		Subgrade 3.5	Branched/complex fissures
Grade 4	Erosion	**Subgrade 4.0**	Superficial zone delamination
		Subgrade 4.5	Mid zone excavation
Grade 5	Denudation	**Subgrade 5.0**	Bone surface intact
		Subgrade 5.5	Reparative tissue surface present
Grade 6	Deformation	**Subgrade 6.0**	Joint margin osteophytes
		Subgrade 6.5	Joint margin and central osteophytes

Statistics

The statistical analysis was completed using IBM SPSS software. As this project had more than two groups, with independent observations that were following an ordinal scale and these observations would not follow normal distribution, a Kruskal-Wallis test was used. This test is the non-parametric equivalent of the one-way ANOVA test but the measurement variable does not meet the normality assumption[46]. The assumptions of this test include: the observations in each group come from populations with the shape of distribution, the observations are on an ordinal scale and that the observations are independent. The null hypothesis of this test is that the population medians are equal. The alternative hypothesis is that the population medians are not equal. The Kruskal-Wallis test would be able to show if there are differences among the groups being compared, however, it would not explain which groups are different[47]. Therefore, a post-hoc test was conducted to determine which groups are different from the others. For this, the Dunn's Pairwise test was used. A p-value of less than 0.05 was considered significant.

Results

Gross Observations

Following the completion of each test, the femoral condyle was examined grossly for areas of wear (Figure 14). The cartilage vs cartilage tests acted as the control test. Grossly, these tests showed none to very minimal wear. In contrast to this, the titanium, UHMWP and CR-PEEK displayed quite significant wear on the apex of the femoral

condyle. Of these three materials, UHMWP showed the least amount of wear grossly. Titanium had a deeper wear pattern, compared to UHMWP, but the area of the wear was very similar with some delamination seen grossly. The CR-PEEK exhibited the most significant wear. The area of wear was larger than both the titanium and the UHMWP and the depth of wear also appeared deeper. However, one anomaly was observed; one of the four tests conducted with CR-PEEK showed very little wear, and the area of this wear was off centre in relation to the apex of the femoral condyle. This result was most likely due to poor alignment of the femoral condyle on the lower plate.

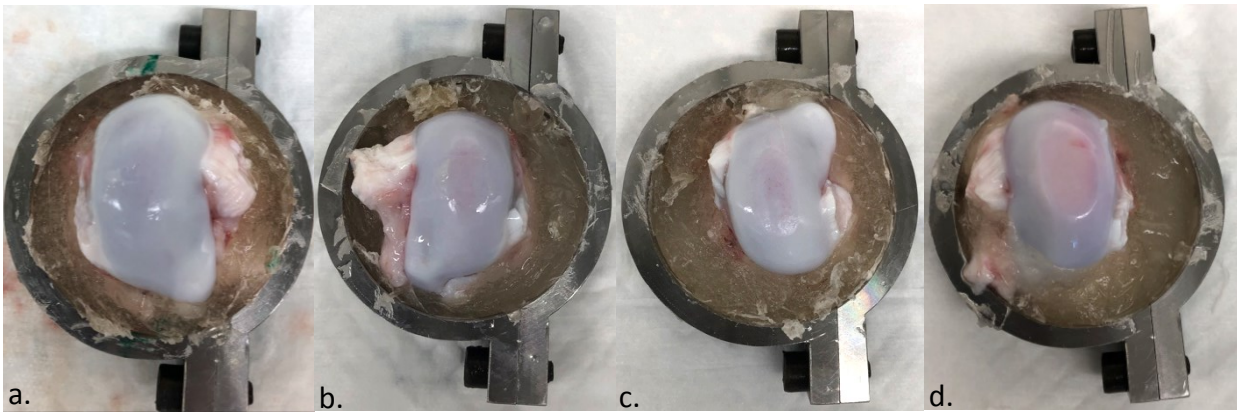

Figure 14: Gross images of the femoral condyles taken immediately after testing: a) Cartilage v Cartilage, b) Titanium v Cartilage, c) UHMWP v Cartilage, d) CR-PEEK v Cartilage.

Histological Results

Grading of the cartilage samples (n=14) was undertaken by two, independent researchers. The secondary scoring indicates the grades given by the second researcher. Both sets of grades were then averaged to determine the overall grade given to the cartilage. The damage of the apex (A) and the side (S) of the porcine femoral cartilage specimens were analyzed. Each material was divided into subgroups. For example, W1A1 was the first cartilage vs cartilage test. W1A1-1 was a more

superficial layer of cartilage that was histologically examined. W1A1-2 was a deeper layer of cartilage that was examined. W1A2-1 would be the superficial layer of the second cross-sectional piece examined from the apex of that condyle. This was done for samples taken at the apex and at the side of the cartilage specimen. Healthy cartilage was not tested against any material.

Table 5: Healthy Cartilage Grading

Healthy	Subgroup	Score	Subgroup	Score
	H1A1-1	0	H1S1-1	1
	H1A1-2	0	H1S1-2	0
	H1A2-1	0	H1S2-1	0
	H1A2-2	0	H1S2-2	0
Secondary Scoring	H1A1-1	0	H1S1-1	0
	H1A1-2	0	H1S1-2	0
	H1A2-1	0	H1S2-1	0
	H1A2-2	0	H1S2-2	0
Average		0		0.125

Table 6: Cartilage vs Cartilage Grading

Cartilage	Subgroup	Score	Subgroup	Score	Subgroup	Score	Subgroup	Score	Subgroup	Score	Subgroup	Score
	W1A1-1	1.5	W1S1-1	1	W2A1-1	1.5	W2S1-1	0	W3A1-1	1	W3S1-1	0
	W1A1-2	1.5	W1S1-2	1	W2A1-2	1.5	W2S1-2	0	W3A1-2	1	W3S1-2	0
	W1A2-1	1.5	W1S2-1	0	W2A2-1	1.5	W2S2-1	0	W3A2-1	1	W3S2-1	0
	W1A2-2	1.5	W1S2-2	0	W2A2-2	1.5	W2S2-2	0	W3A2-2	1	W3S2-2	0
Secondary Scoring	W1A1-1	0	W1S1-1	1	W2A1-1	0	W2S1-1	0	W3A1-1	1	W3S1-1	0
	W1A1-2	0	W1S1-2	1	W2A1-2	0	W2S1-2	0	W3A1-2	0	W3S1-2	0
	W1A2-1	1	W1S2-1	0	W2A2-1	1	W2S2-1	0	W3A2-1	1	W3S2-1	1
	W1A2-2	1	W1S2-2	1	W2A2-2	1	W2S2-2	0	W3A2-2	1	W3S2-2	0
Average		1		0.625		1		0		0.875		0.125

Table 7: Titanium vs Cartilage Grading

Titanium	Subgroup	Score	Subgroup	Score	Subgroup	Score	Subgroup	Score	Subgroup	Score	Subgroup	Score	Subgroup	Score	Subgroup	Score
	T1A1-1	3	T1S1-1	0	T2A1-1	2	T2S1-1	0	T3A1-1	3	T3S1-1	0	T4A1-1	3	T4S1-1	0
	T1A1-2	3	T1S1-2	0	T2A1-2	2.5	T2S1-2	0	T3A1-2	3	T3S1-2	0	T4A1-2	3	T4S1-2	0
	T1A2-1	2	T1S2-1	0	T2A2-1	1.5	T2S2-1	0	T3A2-1	2.5	T3S2-1	0	T4A2-1	2.5	T4S2-1	0
	T1A2-2	2	T1S2-2	0	T2A2-2	2	T2S2-2	0	T3A2-2	2.5	T3S2-2	0	T4A2-2	2.5	T4S2-2	0
Secondary Scoring	T1A1-1	1	T1S1-1	0	T2A1-1	1	T2S1-1	0	T3A1-1	3	T3S1-1	0	T4A1-1	0	T4S1-1	0
	T1A1-2	2	T1S1-2	0	T2A1-2	2	T2S1-2	0	T3A1-2	1	T3S1-2	0	T4A1-2	1	T4S1-2	0
	T1A2-1	1	T1S2-1	0	T2A2-1	2	T2S2-1	0	T3A2-1	1	T3S2-1	0	T4A2-1	1	T4S2-1	0
	T1A2-2	2	T1S2-2	0	T2A2-2	2	T2S2-2	0	T3A2-2	3	T3S2-2	0	T4A2-2	1	T4S2-2	0
Average		2		0		1.875		0		2.375		0		1.75		0

Table 8: UHMWP vs Cartilage Grading

UHWMP	Subgroup	Score	Subgroup	Score	Subgroup	Score	Subgroup	Score	Subgroup	Score	Subgroup	Score
	U1A1-1	2.5	U1S1-1	1.5	U2A1-1	2.5	U2S1-1	0	U3A1-1	1.5	U3S1-1	0
	U1A1-2	2.5	U1S1-2	0	U2A1-2	2	U2S1-2	0	U3A1-2	1.5	U3S1-2	0
	U1A2-1	1.5	U1S2-1	0	U2A2-1	2	U2S2-1	0	U3A2-1	1.5	U3S2-1	0
	U1A2-2	2	U1S2-2	0	U2A2-2	2	U2S2-2	0	U3A2-2	1.5	U3S2-2	0
Secondary Scoring	U1A1-1	2	U1S1-1	2	U2A1-1	2	U2S1-1	1	U3A1-1	1	U3S1-1	2
	U1A1-2	3	U1S1-2	1	U2A1-2	2	U2S1-2	0	U3A1-2	3	U3S1-2	1
	U1A2-1	2	U1S2-1	2	U2A2-1	2	U2S2-1	1	U3A2-1	1	U3S2-1	1
	U1A2-2	2	U1S2-2	1	U2A2-2	2	U2S2-2	1	U3A2-2	3	U3S2-2	1
Average		2.188		0.938		2.063		0.375		1.75		0.625

Table 9: CR-PEEK vs Cartilage Grading

CR-PEEK	Subgroup	Score	Subgroup	Score	Subgroup	Score	Subgroup	Score	Subgroup	Score	Subgroup	Score	Subgroup	Score	Subgroup	Score
	P1A1-1	5	P1S1-1	0	P2A1-1	5	P2S1-1	0	P3A1-1	4	P3S1-1	0	P4A1-1	1.5	P4S1-1	0
	P1A1-2	5	P1S1-2	1	P2A1-2	5	P2S1-2	0	P3A1-2	4	P3S1-2	1.5	P4A1-2	1.5	P4S1-2	0
	P1A2-1	5	P1S2-1	0	P2A2-1	5	P2S2-1	0	P3A2-1	4.5	P3S2-1	0	P4A2-1	1.5	P4S2-1	1
	P1A2-2	5	P1S2-2	0	P2A2-2	5	P2S2-2	0	P3A2-2	4.5	P3S2-2	0	P4A2-2	1.5	P4S2-2	0
Secondary Scoring	P1A1-1	3	P1S1-1	0	P2A1-1	3	P2S1-1	0	P3A1-1	3	P3S1-1	0	P4A1-1	2	P4S1-1	0
	P1A1-2	3	P1S1-2	1	P2A1-2	3	P2S1-2	0	P3A1-2	3	P3S1-2	2	P4A1-2	1	P4S1-2	0
	P1A2-1	3	P1S2-1	0	P2A2-1	3	P2S2-1	1	P3A2-1	3	P3S2-1	0	P4A2-1	1	P4S2-1	1
	P1A2-2	2	P1S2-2	1	P2A2-2	3	P2S2-2	0	P3A2-2	3	P3S2-2	0	P4A2-2	1	P4S2-2	0
Average		3.875		0.375		4		0.125		3.625		0.438		1.375		0.25

The healthy cartilage (H) showed little to no damage on the surface of the cartilage with cells intact (Figure 15a.). Overall, an OARSI grade of zero was given to all samples analyzed at the apex (Table 5). Most cartilage vs cartilage specimens (W) received a grade between 0 and 1.5 (Table 6). They appeared to have an intact surface with some superficial fibrillation in areas. Most cells were intact, however some cell death was seen. (Figure 15b.). Both the titanium vs cartilage (T) and the UHMWP vs cartilage (U) specimens developed some vertical fissures and widespread cell death (Figures 15c. and 15d.). A higher proportion of titanium tests received higher grades, e.g. 3, but when averaged, both titanium and UHMWP tests received equivalent grades (Tables 7 & 8). After grading and histological analysis, the results showed that CR-PEEK (P) caused the most damage to the cartilage samples. The grades given to these tests ranged between 2 and 5 (Table 9). Histologically, there were widespread vertical fissures seen in most samples. Some delamination and erosion of the superficial layer was found, as well (Figure 15e.). In accordance with our gross findings, one CR-PEEK test had significantly less wear and a lower OARSI grade than the other samples that were tested against the same material (P4A). The overall grades of each given to cartilage in tests conducted against each material were then compared to each other (Figure 16). Unlike the gross examination, UHMWP and titanium had similar OARSI grades.

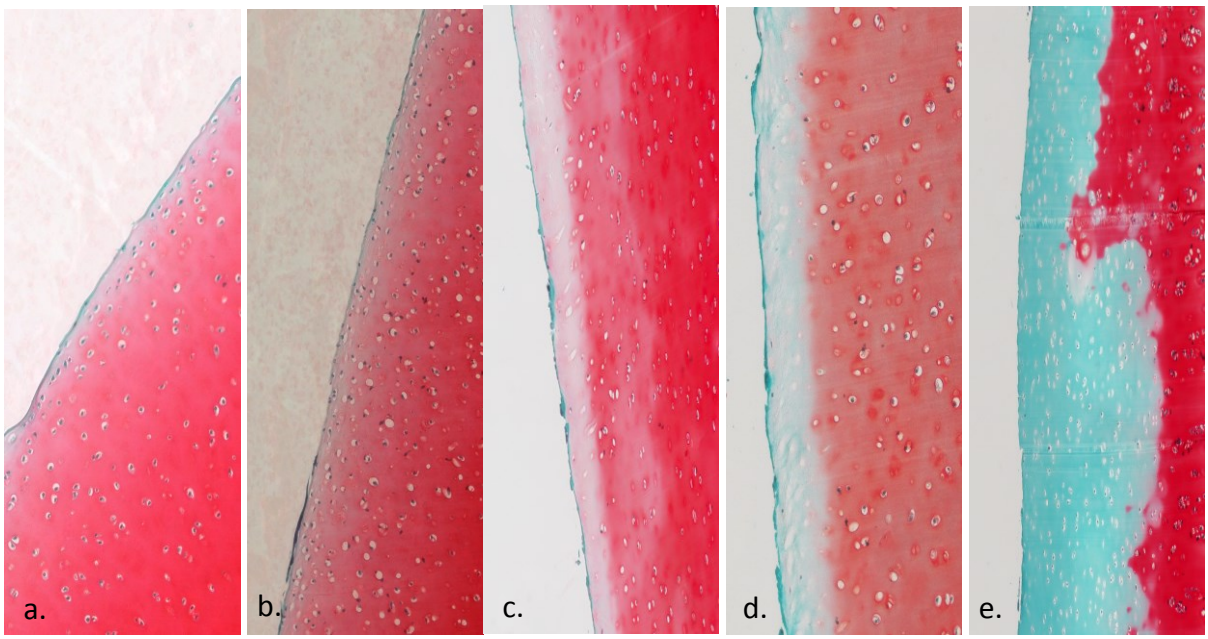

Figure 15: Representative Sections of Damaged Cartilage: a. Healthy Cartilage, b. Cartilage vs Cartilage, c. Titanium vs Cartilage, d. UHMWP vs Cartilage, e. CR-PEEK vs Cartilage

The average grade for the cartilage v cartilage test was 0.96, which shows that there was some microscopic damage occurring to the cartilage during our tests. Titanium, UHMWP and CR-PEEK had OARSI grades of 2, 2 and 3.22, respectively (Figure 17). Although titanium and UHMWP had the same average grades, UHMWP had a greater range of the grades it received.

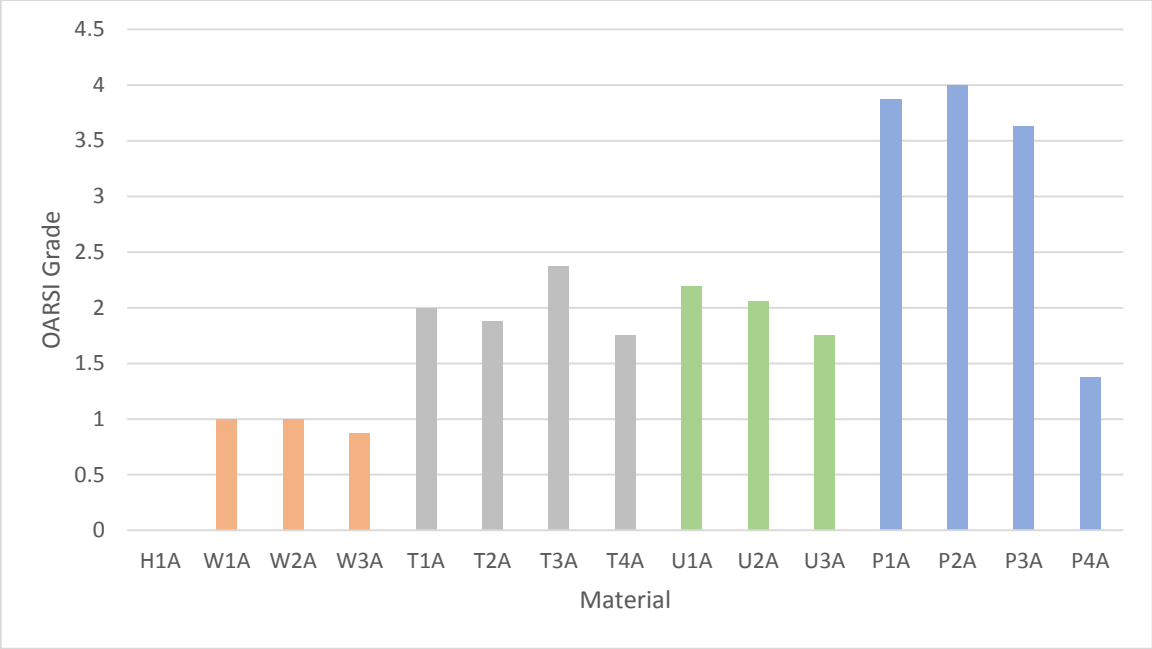

Figure 16: OARSI Grading. Healthy (H), Cartilage (W), Titanium (T), UHMWP (U), CR-PEEK (P)

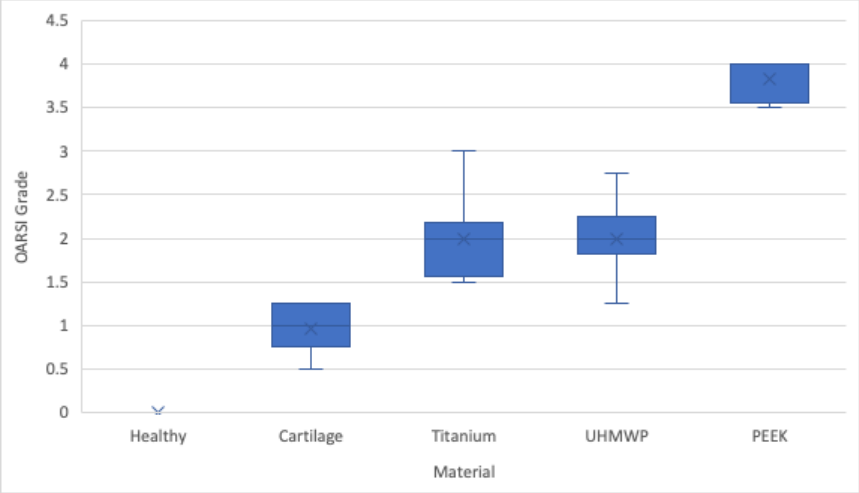

Figure 17: Average OARSI grading for each material (n=14)

Statistical Analysis

A Kruskal-Wallis test with Bonferroni's correction (which protects from Type I error) was performed on the OARSI grading (Figure 18). The outlier (P4A), which had a much lower OARSI grade as compared to the other tests in the CR-PEEK group, was excluded for the statistical analysis. The Kruskal-Wallis test showed that there was strong evidence ($p<0.001$) that the mean rank of all the materials were different. A post hoc test (Dunn's pairwise test) was then conducted to determine where the differences were found (Figure 19). There were statistically significant differences between the grades given to healthy cartilage compared to those of cartilage tested against titanium, UHWMP and CR-PEEK ($p=0.027$, $p=0.022$ and $p<0.001$, respectively). There were significant differences between cartilage vs cartilage tests compared to titanium, UHWMP and CR-PEEK tests ($p= 0.023$, $p=0.022$, $p<0.001$). And finally, statistically significant differences were also noted between titanium and CR-PEEK ($p=0.008$) and between UHMWP and CR-PEEK ($p=0.035$). There was no significant difference between titanium and UHMWP ($p=1.000$). The image taken from the IBS SPSS software for the Kruskal-Wallis test is shown in Appendix B and the image taken for the Dunn's Pairwise test in shown in Appendix C.

Discussion

The purpose of this research was to compare the wear caused to articulating cartilage against different materials which are commonly used in the development of orthopedic implants. These implants are used in operations such as hip and knee arthroplasties to treat a variety of conditions, including osteoarthritis. By understanding the effect that these materials have to the articulating cartilage and surrounding tissues, and

determining which material causes the least amount of wear and damage could positively affect the future of one of the most common operations in orthopedic surgery. By using the most optimal material in future orthopedic implants, will not only improve patient satisfaction, decrease revision rates but it may also increase the longevity of the implants as well. We compared 3 materials during the course of this project, titanium, UHMWP and CR-PEEK.

Cores of both the apex and the side of the porcine femoral condyle cartilage were taken from the tested specimen and processed to be examined histologically. As expected, the healthy cartilage received a grade of 0 on the apex. There was some slight wear on the side of the healthy cartilage that observed, however, normal physiologic wear can be expected as the condyles were received from living animals.

In the cartilage vs cartilage specimens, no to minimal wear was seen on the surface. Similar results were obtained during the histological grading of these specimens, with the group as a whole receiving an average grade of 0.96, demonstrating some surface damage and some cell death. These results show that our control test caused some damage to the articulating cartilage. It is difficult to say how many human years it would take to cause this amount of minimal wear on cartilage, however these are normal changes.

Interestingly, although macroscopically UHMWP appeared to cause less wear than titanium, histologically both of these materials incurred similar amounts of damage to

the articulating cartilage. In the literature that was reviewed for this project, no other researchers studied the effects of titanium against cartilage. Chan et al. did use UHMWP and they showed similar results, as this material caused the least amount of damage compared to Al_2O_3, CoCr and stainless steel[33]. They also found that UHMWP had the least amount of protein loss compared to tests against those same materials. These results, however, were based on a pin-on-disk model. The results may have been different if our physiologically similar model was used. More testing should be conducted between these UHMWP and titanium to determine if the similar results continue.

The material that caused the most damage to cartilage macroscopically and microscopically was the CR-PEEK. It had significantly higher OARSI grades and appeared to have much more wear when examined grossly.

One possible explanation for the CR-PEEK causing much more wear could be attributed to the reinforcement of the material with the carbon fibres. Although they provide structural support to the implant, the fibres may be causing more damage to the surrounding tissues. It would be interesting to see the effects of non-carbon reinforced PEEK on articulating cartilage and also the comparison of the two materials under compression testing. When choosing the material used for an orthopedic implant, it is important to determine how the material will react when placed inside a human body; it's interaction with the surrounding tissues and its ability to maintain its structure under the typical load place on a lower limb on a daily basis.

A model that was used to test cartilage wear and that was also able to recreate a movement similar to a human joint proved novel upon literature review. Most other projects were carried out using pin-on-disk tests which do not allow for the exact comparison between test scenarios and clinical applications. Trevino et al. used a custom apparatus to test materials against cartilage, however, they used a constant force of 40N throughout the testing[14]. In our experiments, a sinusoidal load provided a more accurate picture of the loads that are placed on a human joint on a daily basis along with a rotary displacement recreating a movement seen in a typical hinge joint. This new method allowed for direct comparison of how human articular cartilage would fair against a variety of different materials. In future testing, an increased number of cycles should be undertaken. This would allow us to understand the effects of these materials have against articulating cartilage over the life course of a joint replacement. This could last anywhere from a few years to a few decades.

For our project, we submersed all our experimental femoral condyles in PBS based on the designs of similar studies[12,32,33]. This was used as lubricant during the testing cycles, but also acted as a synthetic synovial fluid. The other lubricating option we had was a type of fetal bovine serum. This solution was more viscous and may have provided a more accurate imitation of human synovial fluid, however, it was more expensive and therefore not feasible to use for the scope of this project. Although a direct comparison between PBS and bovine serum was not done in our project, it may be a noteworthy aspect to consider in future tests. The differences in viscosity may play

a role in protecting the surface of the articulating cartilage and therefore the results of the wear tests may differ from the ones that were found in the current project.

The materials that were used in this project are commonly used in the development of current and future orthopedic implants. Another material that was used in similar studies to ours was cobalt chrome. Although we were unable to test cartilage against CoCr, for future tests it would be valuable to use this material as well. Other studies saw that CoCr caused greater amounts of wear compared to the materials that they used[14,31,32,33,34]. These materials included cremains (OxZr), other metals (Al_2O_3 and SS) and UHMWP. It would be important to test this material on our model to determine the amount of wear it causes compared to a pin-on-disk model or other testing methods. It would also be interesting to compare CoCr to CR-PEEK, using our model to see if any differences in wear exist as this comparison was not found in the literature.

There were a number of limitations in our project that may have impacted the efficacy of our results. Firstly, the alignment of the condyles against the material proved to be an issue. The use of non-dental resin ensured that the condyles were unable to shift during testing. However, it was difficult to place each condyle in precisely the same location for every test that was conducted. For this reason, it was difficult to replicate tests, causing a wider variability in results. For example, test P4A had a much lower grade of wear as compared to the other tests against CR-PEEK. This could in part be attributed to an imprecise alignment of the femoral condyle. However, the difference in the sizes and shapes of the condyles could be another contributing factor to the variability in the

results. Lastly, decalcifying agent should have been used during the processing of specimens for microscopy. A decalcifying agent softens the bone attached to the cartilage samples. This would have improved the quality of slices cut by on the microtome. This would have avoided the many incomplete slices that were obtained and may have led to better histological analysis.

For further evaluation of wear against articulating cartilage, more tests should be conducted to increase the sample size which could provide more significant results. Additionally, a wider use of materials, including cobalt chrome and ceramics, will be able to provide a broader comparison of the options available on the orthopedic implant market and their impact on patient cartilage health post-surgery.

Conclusion

In conclusion, CR-PEEK proved to cause the most amount of wear to cartilage, both macroscopically and microscopically, when compared to titanium and UHMWP ($p=0.008$ and $p=0.035$, respectively). UHMWP and titanium caused similar amounts of wear to cartilage. The utilization of CR-PEEK in the development of orthopedic implants should be carefully evaluated due to the damage it can cause to its surrounding tissues. UHMWP and titanium should undergo further wear tests using our model to determine which of these materials would be best suited for future orthopedic implant development.

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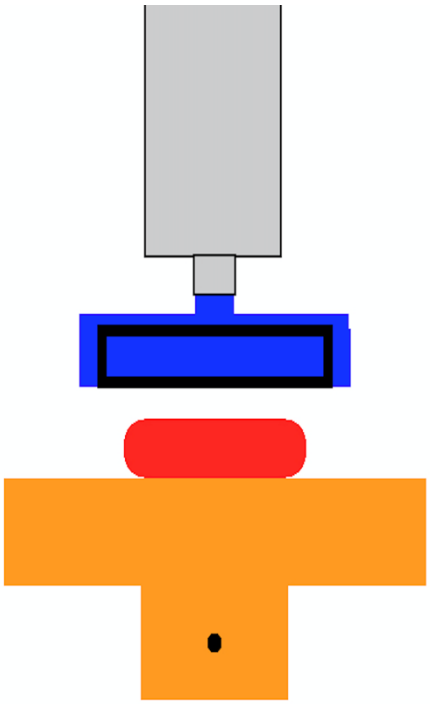
Appendix A

An illustrated version of the experimental setup shows how all components interact with each other. The figure in grey is the upper arm of the uniaxial loading machine. This is attached to the upper jig (blue) by a screw. When open, the upper jig contains a compartment that fits the disc of material that is being tested (black outline). The femoral condyle (red) is placed upon the lower disk (orange), which is then undergoes oscillation during the testing.



Appendix B

Kruskall-Wallis Test – IBM SPSS

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of Score is the same across categories of Materials.	Independent-Samples Kruskal-Wallis Test	.000	Reject the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

Total N	56
Test Statistic	46.946
Degrees of Freedom	4
Asymptotic Sig. (2-sided test)	.000

Appendix C

Dunn's Pairwise Test – IBM SPSS

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
Healthy-Cartilage	8.333	9.371	.889	.374	1.000
Healthy-Titanium	-27.250	9.073	-3.003	.003	.027
Healthy-UHMWP	-28.667	9.371	-3.059	.002	.022
Healthy-PEEK	-48.000	9.371	-5.122	.000	.000
Cartilage-Titanium	-18.917	6.198	-3.052	.002	.023
Cartilage-UHMWP	-20.333	6.626	-3.069	.002	.022
Cartilage-PEEK	-39.667	6.626	-5.986	.000	.000
Titanium-UHMWP	-1.417	6.198	-.229	.819	1.000
Titanium-PEEK	20.750	6.198	3.348	.001	.008
UHMWP-PEEK	19.333	6.626	2.918	.004	.035