1-1-2019

Knee Balance Assessment During Cementation Is Detrimental to Initial TKA Tibial Tray Fixation

Yashar Ali Behnam
University of Denver

Knee Balance Assessment During Cementation Is Detrimental to Initial TKA Tibial Tray Fixation

Abstract

Despite recent advancements in implant design and cement technique, aseptic tibial loosening remains the primary cause of revision after cemented total knee arthroplasty (TKA) [1]. While the mechanisms of tibial loosening are multifactorial, previous research has shown that third-generation cement techniques, including the use of jet lavage and cement pressurization, can reduce contamination of the implant surface by lipids endogenous to the surrounding cancellous bone and thereby enhance fixation strength between the implant and bone [2]. The purpose of this thesis is to understand the role contaminating lipids play in the mechanism of aseptic loosening and to perform a cadaveric study on modern commercially available TKA systems to evaluate their ability to mitigate the risks of poor fixation. The results from this study indicate that tibial components with no cement mantle around the cone or keel structure provide greater fixation and enhanced robustness to intraoperative knee balance assessment manipulations.

Document Type

Thesis

Degree Name

M.S.

Department

Mechanical Engineering

First Advisor

Chadd W. Clary, Ph.D.

Keywords

Fixation strength, Knee balance assessment, Lipid contamination, Lipid marrow infiltration, Total knee arthroplasty

Subject Categories

Engineering | Mechanical Engineering

Knee Balance Assessment during Cementation is Detrimental to Initial TKA Tibial Tray Fixation

---

A Thesis

Presented to

the Faculty of the Daniel Felix Ritchie School of Engineering and Computer Science

University of Denver

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

---

by

Yashar Ali Behnam

June 2019

Advisor: Dr. Chadd W. Clary, PhD

Author: Yashar Behnam
Title: Knee Balance Assessment is Detrimental to Initial Tibial Tray Fixation
Advisor: Dr. Chadd W. Clary, PhD
Degree Date: June 2019

Abstract

Despite recent advancements in implant design and cement technique, aseptic tibial loosening remains the primary cause of revision after cemented total knee arthroplasty (TKA) [1]. While the mechanisms of tibial loosening are multifactorial, previous research has shown that third-generation cement techniques, including the use of jet lavage and cement pressurization, can reduce contamination of the implant surface by lipids endogenous to the surrounding cancellous bone and thereby enhance fixation strength between the implant and bone [2]. The purpose of this thesis is to understand the role contaminating lipids play in the mechanism of aseptic loosening and to perform a cadaveric study on modern commercially available TKA systems to evaluate their ability to mitigate the risks of poor fixation. The results from this study indicate that tibial components with no cement mantle around the cone or keel structure provide greater fixation and enhanced robustness to intraoperative knee balance assessment manipulations.

Acknowledgements

First, I would like to take this opportunity to first thank my advisor Dr. Chadd Clary for introducing me to the engineering world of orthopedic medical devices. I am incredibly thankful for his mentorship and guidance both in and outside the academic setting.

I am thankful for the members of my defense committee, Dr. Fogleman and Dr. Rullkoetter for their support and counsel during my pursuit of my master's degree.

I would like to thank Hayden Wilson, for his tireless contribution of early mornings and late nights full of knee surgeries, dissections, and mechanical testing of the specimens throughout the roughly two-year duration of this study.

I would also like to thank surgeons Dr. Bohannon Mason, MD and Dr. Brian Haas, MD for their guidance, counseling, and combined 150+ performed knee surgeries.

I would like to thank DePuy Synthes, for their sponsorship of this study. I would specifically like to thank Mark Heldreth for providing us with the equipment, supplies, and directing this study.

Lastly, I would like to thank all members of the DU Center of Orthopaedic Biomechanics. Whether it be their aid in debugging code or their time holding tissue retractors during a surgery, their contributions are greatly appreciated.

Table of Contents

List of Figures

Chapter 1: Introductory Remarks

1.1 Introduction

Annually, millions of various medical devices are surgically implanted across the world, for a multitude of reasons, and yet early failures and the risk of revision is still seriously addressed by orthopedic surgeons and patients when contemplating the implications of arthroplasty surgery today. Although advancements in Total Knee Arthroplasty (TKA) components and surgical instruments are tirelessly made, the risk of a revision surgery is still persistent, among the inherent risks of surgery. While many focus on optimizing the materials necessary for a TKA, a shift in focus to surgical method is necessary.

Behind infection, aseptic loosening is the leading indication for a revision surgery within the first two years of a primary knee replacement operation (Sharkey 2014, Dalury 2013). The complexity of implant failures and current literature scarcity on endogenous contaminating agents implies that the failure mechanism for aseptic loosening must be multifactorial. The primary focus of this thesis is to explore and understand the effect of surgeon behavior and surgical method on the outcome or success of a total knee replacement, as well as its contributions to aseptic loosening. It will also explore the

effect of contaminating endogenous lipids and the degree in which tibial base plate geometries contribute to the robustness of the detrimental effects of these contaminants.

Furthermore, we present an experimental study aimed to replicate the surgical setting to understand what factors contribute greatest to aseptic loosening and the failure mechanisms of initial tray fixation failure. The results of this study aim to define the retention strength of commercially available tibial components to illustrate the deleterious effects of assessing knee balance prior to the full curing of the bone cement. The goal of this study is to provide surgeons with an evidence-based recommendation that knee balance manipulation of the joint during cement polymerization should be absolutely avoided to maximize the implant-cement fixation strength.

1.2 Objectives

The objectives of this thesis are to:

1. Understand the failure mechanism of aseptic tibial base plate loosening
2. Compare tibial base tray fixation feature geometries
3. Design an experiment to replicate a surgical setting to focus on the effects of knee balance assessment on tibial tray fixation strength
4. Understand the role endogenous contaminating lipids play in aseptic tibial base plate loosening
5. Understand the influence of tibial plateau bone resection preparation on tray fixation force

6. Provide surgeons with an evidence-based recommendation of changes to surgical methods to ensure a successful TKA procedure

1.3 Thesis Overview

Chapter 1: Introductory remarks, Thesis Objective and Motivation

Chapter 2: Review of the Literature, Implant Geometry Review

Chapter 3: Experimental Study

Chapter 4: Recommendations and Concluding Remarks

Chapter 2: Review of Literature

2.1 The Modern TKA and its Surgical Method

Modern primary TKA systems typically consist of four components: a metal-backed tibial implant, a metal-backed femoral implant, a polyethylene spacer, and a polyethylene patella implant. The combination of these components has been prevalent in most primary TKA systems for the last three decades (Ranawat, 2012). Of the millions of knee replacement surgeries performed around the world annually, aseptic loosening of the tibial component remains as the leading indication for implant failure and revision surgery behind infection, after the first two years following operation (AJRR, 2018). Cement bonding features such as metallic surface coatings and backside cement pockets are not singularly responsible for weak tibial implant fixation (Sharkey, 2014). Some argue that endogenous contaminants such as blood, bone debris, and bone marrow lipids may prevent adequate component-bone cement adhesion, and its failure mechanism should be explored (Peters, 2001).

Loosening is characterized by the disassociation of the tibial component from the PMMA or tibial plateau interface (Khan, 2016). The debonding can be seen in stereo-radiographic images as a dark banded region below the tibial plateau and the clouded PMMA-cancellous bone interface (Figure 2, Source: Sumino).

Despite the variations in implant design and surgical instrumentation, cases of aseptic loosening have been reported in every major commercially available knee system around the world (AJRR, 2018). Factors such as variations in orthopedic residency or surgery trainings, country regulations, and implant manufacturer's recommendations likely influence intra-operative surgeon behavior, and therefore intra-operative surgeon behavior should be explored due to the lack of focus in the literature.

Examinations and assessments executed at a surgeon's discretion may introduce dissimilarities between operations and different surgeons. Variance in cementation and lower extremity manipulation techniques and timing are just a few examples among many potential mechanisms of introducing fixation contaminating agents into arthroplasty procedures. Current surgical cementation protocol varies among competitive systems, such as in the method of bone cement application. Cawley et al reports gun cementation provides consistent cement penetration with minimal tibial component movement, whereas his colleagues indicate factors such as spatula or hand-packing, pulsatile lavage, high or medium viscosity PMMA, and plateau or stem geometries contribute to adequate penetration of PMMA into the cancellous tibial bone contributing to tibial tray fixation (Cawley, 2013). Despite PMMAs great ability to penetrate cancellous bone, its inability to be impermeable to contaminating agents and allowing them infiltrate and compromise the bone cement's ability properly adhere to the implant's underside surface.

Lipid Marrow Infiltration (LMI) is a phenomenon in which lipids within bone morrow or surrounding cancellous bone pockets contaminate the tibial component

surface following implantation. Surface contamination is believed to be a mechanism for aseptic loosening (McTighe, 2009). To mitigate contamination, surgical procedure calls for pulsatile jet-lavage methods to remove and clear debris from the prepared bone (Helwig, 2013). Helwig et al have indicated although pulsatile jet-lavage led to significantly better cement penetration and cement-to-bone contact as compared to syringe irrigation or brush cleaning techniques, but inconsistencies in such methods could lead to a greater prevalence of arthroplasty failure. Some argue that precautions to lipid exposure should be taken due to its ability to inhibit PMMA component binding (Rand, 2003).

Intra-operative manual manipulations are routinely performed to assess the balance of the knee (Nagel, 2017). Such assessments are used to determine if ligament release or polyethylene insert thickness adjustments need to be made prior to closing the incision. Often, the manipulation consists of passively ranging the knee from full extension to deep flexion, with varus and valgus joint laxity examinations done in between each range of Motion (ROM) cycle. Manipulations performed by the surgeon before the curing time specified in the cement manufacturer's documentation may displace the tibial component from its original position. Micromotion of the tray during cementation is hypothesized to open up the tray-cement interface, reducing implant-cement adhesion, and possibly creating and providing cavities for the lipids to infiltrate to. It is believed that lipids from the surrounding marrow within the cancellous bone travel through the PMMA and onto the implant surface, but the mechanism is still not fully understood. One hypothesized mechanism in which marrow lipids travel is through

the introduction of lower extremity manipulations executed prior to complete cement polymerization.

Despite the recent advancement towards cementless TKA systems, cemented systems are still most commonly implanted today. Polymethyl methacrylate (PMMA), or "bone cement," is a biologically inert adhesion compound that is used to lock polymeric metal prosthesis to human bone. Its ability to both penetrate cancellous bone and adhere to metal backed or polyethylene components makes it the ideal adhesion compound for implantation of orthopedic medical devices. Two common variants of PMMA are commercially available: high-viscosity and low-viscosity cements. The choice of cement often varies and is likely to be dependent on the surgeon's surgical training. Within the orthopedic community, the preference of high-viscosity over low viscosity cement is often debated and the focus of many research articles. Many credit high viscosity's popularity to its quick settling time, usually between 2 to 5 minutes (DePuy Synthes, 2013), and high cancellous bone penetration. Greater cancellous bone penetration is correlated to greater fixation strength (Schlegel, 2014). High Viscosity's quicker settling times results in shorter operations, reducing the time a patient spends under anesthesia, while also allowing the surgeon to perform more surgeries in a day. Furthermore, Schlegal et al explored the effect of cement penetration on pull-out force and have found a positive correlation between penetration and pull-out force.

Cementation techniques continue to vary as medical devices advance. Rational behind applying bone cement to certain regions of the implant only, to cover the implant completely, or apply to both the implant and the prepared bone are still widely debated

today. Measures to prevent, remove, and clear contaminants from the bone are routinely performed in the operating room, yet the methods used are not consistent among providers. Helwig et al argues that pulsatile lavage cleaning methods must become standard over outdated syringe cleaning methods. In addition, being able to remove bone remnants and marrow lipids from the cancellous bone provides a greater ability for bone cement to penetrate the bone cavities and greatly contributing to an increase in tibial base tray retention strength. The pulsatile lavage system works by flooding the cancellous bone with water in a jet-like fashion while simultaneously suctioning the water, bone-debris, blood, and lipids out of the intramedullary cavity (Figure 1, source: McGraw Hill). Unfortunately, the use of power and/or disposable pulse lavage systems is not globally universal, which is not acceptable when considering the persistently high rate of revision surgeries today, and the vast literature reporting its benefits (Helwig, 2013).

Third generation cementation techniques consist of a pulsatile lavage cleaning system, syringe suction, and the use of an intramedullary plug. The purpose of the plug is to ensure no pressurization of the intramedullary canal is lost during cementation (Saari, 2009). Industry standard third-generation cementation techniques coupled with the wide array of fixation features all aim to prevent the deleterious effects of endogenous contaminating agents.

Understanding where the lipids originate and the mechanism in which they migrate to the surface of the implant may prove useful when considering changes in cement technique. Previous studies replicate *in vivo* conditions through the use of artificial contaminant applications, confirming the need for a similar cadaveric study. The

benefit of using cadaveric specimens is the degree of similarity to true live patients as compared to experiments done in artificial environments (Billi, 2014). We hypothesized three mechanisms in which lipids travel to the surface of the implant: 1) lipid pools at the bottom of the intramedullary canal resection and travels upward, 2) lipids exist around the rim of the tibial plateau due to the surrounding fatty tissue, 3) marrow lipids exist in the area surrounding all bone cuts, upon cement pressurization, lipids travel upward and collect on the tibial plateau surface.

The purpose of this study is to explore the effect of surgeon behavior and TKA systems on tibial tray pull-off force, and to what degree LMI occurs during implantation. To our knowledge, very little biomechanical literature regarding LMI exists, yet it is commonly understood as a source of TKA cementation contamination. We hypothesize that ROM manipulation at the knee prior to adequate PMMA polymerization provides an avenue for LMI to contaminate proper tibial cement interface. We believe understanding the effects of cementation technique and intra-operation manipulations of the lower extremity will provide useful information to the members of the orthopaedic community.

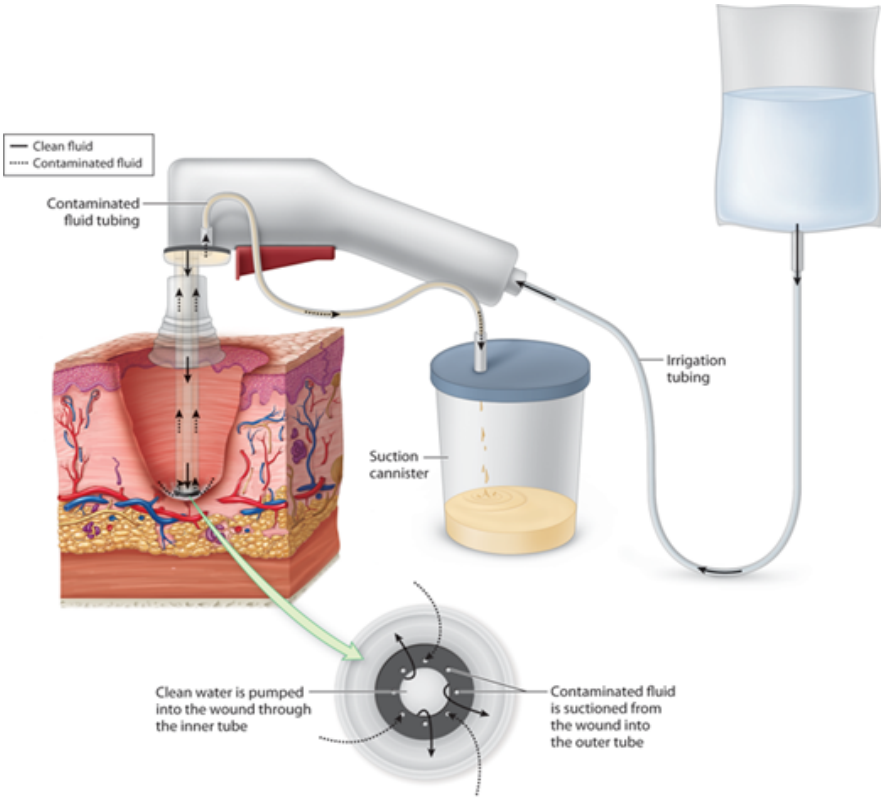

Figure 1: Illustration of generic jet pulsatile lavage cleaning system and its mechanism. Source: McGraw Hill

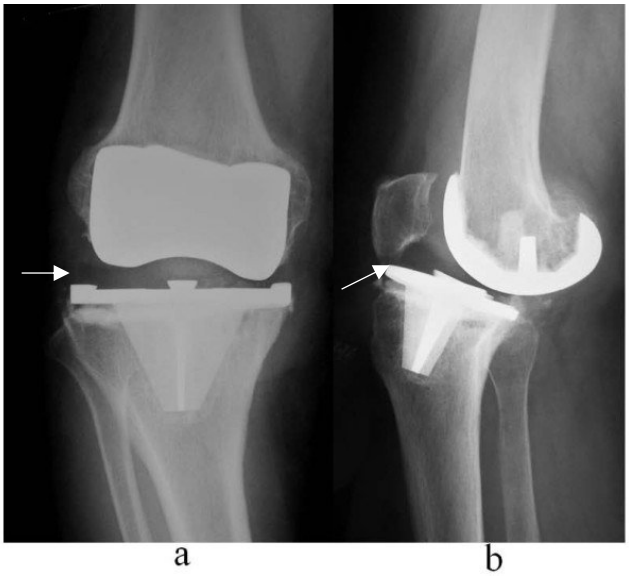

Figure 2: Aseptic loosening case. a: Anteroposterior view; b: Lateral view. Loosening occurred 12.5 years after TKA with the FNK CR type. This patient was female and 70 years old at primary TKA. She underwent a revision of TKA with the FNK PS. (Source: Sumino)

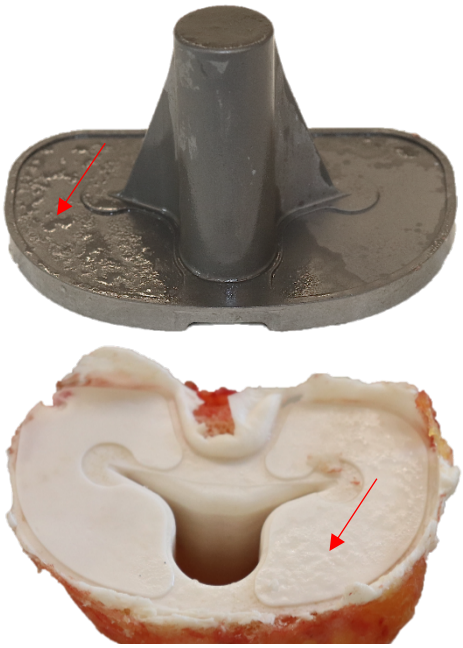

Figure 3: Case specimen which received the "Motion" knee balance manipulation protocol exhibiting significant lipid pooling after successful pull-out testing.

2.2 Tibial Component Implant Geometry Review

Comparison of nine leading contemporary and commercially available metal backed, fixed bearing, tibial base tray components and their plateau-cement interface geometries

TKA components of modern knee replacement systems typically consist of metal-backed tibial and femoral components. Loosening of the tibial component is the leading indication for revision, therefore it is often the focus of early failure mechanisms in the literature. Designers of these components have developed fixation features to maximize the fixation between the component and the bone. Historically, the use of PMMA bone in combination of unique geometric features together attempt to permanently *fix* the implant into the patient's bone. Despite continuous TKA geometry advancements, aseptic tibial loosening continues to drive the development of new and unique fixation features.

Orthopedic medical device companies all sell tibial components with unique fixation features. The most popular geometries often include: cones, keels, the combination of the two, or cruciform-like structures. In addition, systems that require the use of cement also exhibit a recess, ridges, pockets, or hole-like features to maximize cement contact surface area for adhesion. Trays that are not singularly purposed for as a *primary* knee replacement may also have uncommon features such as a modular cone, screw holes, or tapered cone geometries for tibial augmentations, stem elongation, or insertion of peg features, which may also contribute to cement adhesion.

Undercut cement locking features are also present in some trays, but is not widely common. The use of undercut cement pockets or peripheral undercut regions provides an added mechanical locking feature beyond the usual cement to implant adhesion interface. Coupling that with the metal to cement adhesion can significantly increase the bond strength between the two. In addition, some trays exhibit grit blasted surfaces to achieve a desired roughness. Although variations in surface roughness certainly exist between implant manufacturers and could influence cement bonding, roughness values were not extensively explored for the knee systems evaluated in this thesis.

Due to the complexity of such fixation geometries, manufacturing a complete TKA system is likely non-trivial. The components are routinely made of non-ferrous metals, typically consisting of Titanium (Ti), Zirconium (Zr), Cobalt Chrome (CoCr), and Cobalt Chrome Molybdenum (CoCrMo) alloys. The manufacturing process often includes a combination of both casting and CNC machining to complete polyethylene insert locking mechanisms and revision features. For the production of the metal alloys, ASTM standards such as F-75 and F-136 are strictly followed in addition to the strict sanitation processes. Polyethylene cemented tibial component variants also exist as a cost effective alternatives to the metal backed trays, but was not explored due to the focus on metal backed trays in this paper. Unlike the femoral component, the tibial component is most commonly symmetric, though asymmetric trays do exist. Clary et al explored the effect of tibial component coverage on the tibia and found that maximizing coverage without overhang of the implant is vital when understanding implant kinematics (Clary, 2014).

The nine implants commercially available contemporary implants explored in this study are metal backed, posterior stabilized (PS) TKA knee systems. They include the Depuy Synthes Attune, Attune S+, Attune Revision, and SIGMA (Warsaw, IN); ZimmerBiomet NEXGEN, Persona, and Vanguard (Warsaw, IN); Smith and Nephew Genesis II (Memphis, TN); and Stryker Triathlon (Mawah, NJ) trays. Such group of modern implants holistically captures the most common fixation features, in addition to the latest and most advanced lightweight metal alloys.

Implant underside geometries vary among competitive systems. They are necessary to provide the interlock between the implant and the patient's bone, with the addition of a prosthetic locking cement. Cone shapes, keel structures, and the combination of the two are among the most popular. Some implants systems incorporate the use of cement-locking features such as undercut regions or cement pockets to increase fixation area while also providing a mechanical locking interface. Underside tray roughness coatings significantly vary among designs. More rough surface finishes can ideally provide greater adhesion of the bone cement. Surface coatings and geometric features that increase surface area, such as ridges and bumps, also contribute to maximizing the implant-cement bond.

In addition to variations in TKA geometries, some systems utilize a cement mantle around the cone or keel structure of the tibial component. The cement mantle is created via the use of tibial bone preparation instruments which remove a greater volume of bone than the implant itself can fill (Figure 13). Therefore, a cement barrier is needed to fill the space in between the cone or keel structure and the intramedullary cancellous

bone. Implant systems which do not utilize a cement mantle are often referred to as a *Line-Line Fit*, whereas those who do are often referred to as *Standard Fit*. The depth of the cement mantle is vital to the implant's initial stability. Cement mantles of greater depth require more cement and could result in lower initial stability of the implant due to fewer supporting bone structures and a greater volume of cement surrounding the implant.

Geometry Underside Overview:

Depuy Synthes Attune Standard and Line-Line

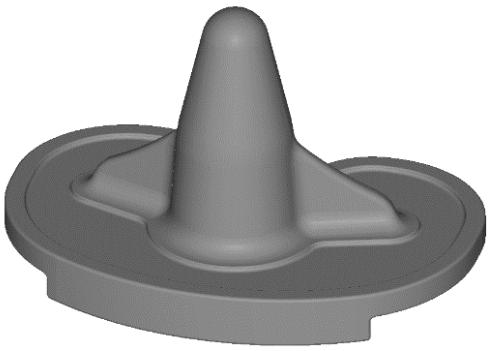

Figure 4: Depuy Synthes, Attune tibial tray geometry.

- Combination of tapered cone and keel structure
- Standard and Line-Line cone preparations

Depuy Synthes Attune S+ Standard and Line-Line

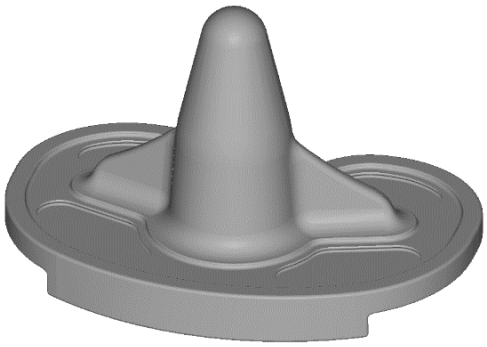

Figure 5: Depuy Synthes Attune S+ tibial tray geometry.

- Combination of tapered cone and keel structure
- 4 undercut cement pockets in plateau region
- Standard and Line-Line cone preparations

Depuy Synthes Attune Stemable

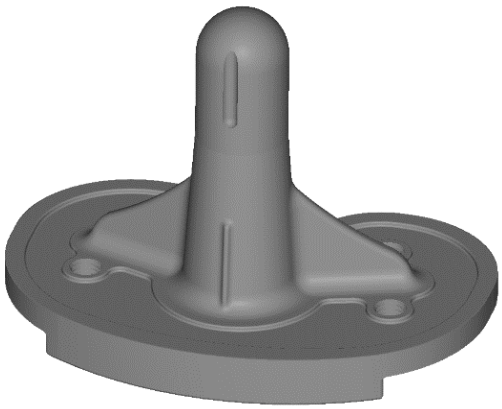

Figure 6: Depuy Synthes Attune Stemmable tibial tray geometry.

- Central cone and keel structures
- No undercuts, although augmentation holes also serve as cement pockets to increase surface area

Depuy Synthes Sigma

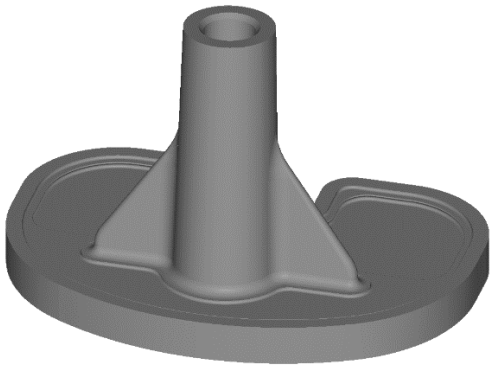

Figure 7: Depuy Synthes Sigma tibial tray geometry.

- Peripheral undercut cement locking feature around plateau underside
- Central cone with medium width keels

Zimmer Biomet Vanguard

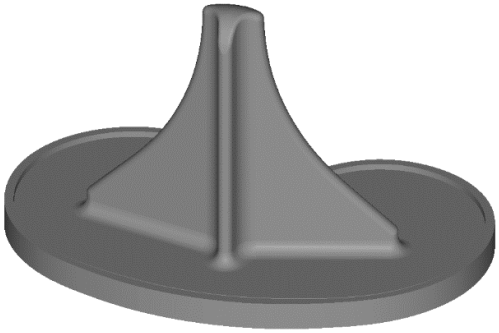

Figure 8: Zimmer Biomet Vanguard tibial tray geometry.

- Keel and cruciform-like structure
- No undercuts or cement pocket

Zimmer Biomet NexGen

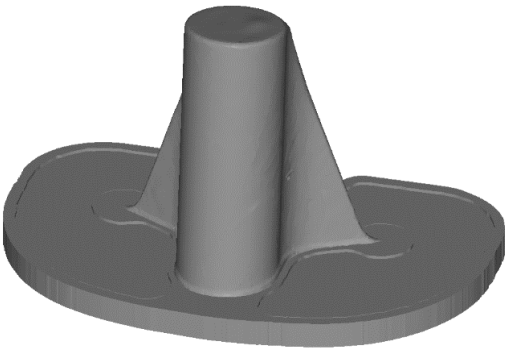

Figure 9: Zimmer Biomet NexGen tibial tray geometry.

- Anteriorly centered cone, with tapered keel structure
- No undercuts or pockets

Zimmer Biomet Persona

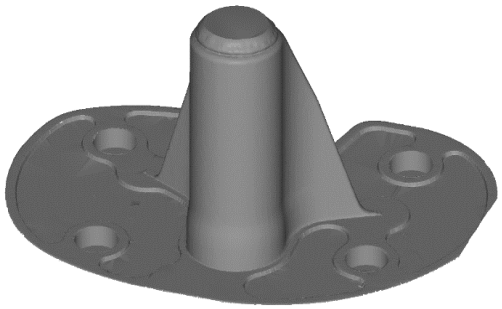

Figure 10: Zimmer Biomet Persona tibial tray geometry.

- Assymetrical cone with tapered keel flanges
- Cement pockets
- No undercut cement locking
- Shallow ridges that increase surface area

Stryker Triathlon

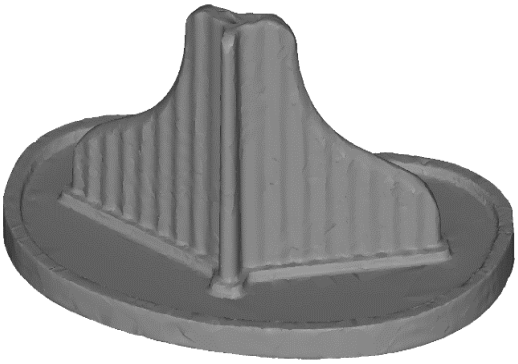

Figure 11: Stryker Triathlon tibial tray geometry.

- Very wide tapered keel and cruciform structure
- Ripple-like surface increase keel surface area
- Cement pocket ridge around periphery of plateau

Smith & Nephew Genesis II

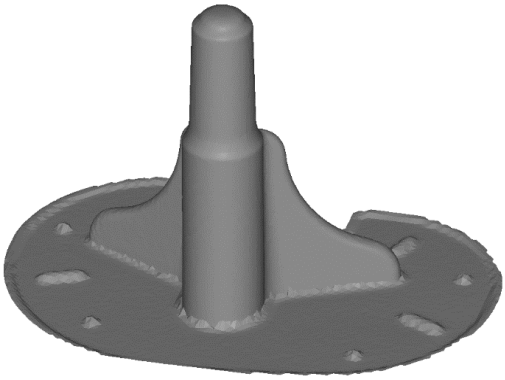

Figure 12: Smith & Nephew Genesis II tibial tray geometry.

- Central cone structure with tapered distal cone, stem compatible
- Very wide keel structure with distal taper
- Ribs in plateau to increase surface area
- Non-center cone and asymmetric plateau

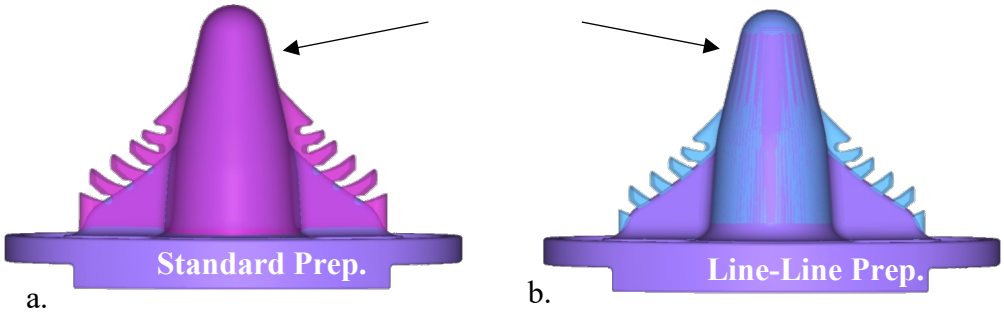

Figure 13: Attune (DePuy Synthes) and its corresponding intramedullary tibial bone resection surgical instrument. a) Standard Prep. illustrating greater resection of the cone region to create the cement mantle. b) Line-Line Prep. illustrating a zero-mm gap around the cone region and therefore no cement mantle being formed.

Table 1: Implant geometry feature overview

TKA System	Cone	Keel	Cruciform	Cement Pockets	Undercut Features	Material
Attune	X	X				CoCr Alloy
Attune S+	X	X			X	CoCr Alloy
Attune Revision	X	X		X		CoCr Alloy
Sigma	X	X			X	CoCr Alloy
Vanguard			X			CoCrMo/Ti Alloy
NEXGEN	X	X				Ti Alloy
Persona	X	X		X		Ti Alloy
GenesisII	X	X				Zr Alloy
Triathlon			X			CoCr Alloy

Chapter 3: Experimental Study

3.1 Introduction

Total knee arthroplasty (TKA) is a novel procedure used to treat end-stage osteoarthritic and osteolytic degenerative diseases (Ito, 2003). The American National American Joint Registry has estimated that over 500,000 TKA procedures are performed in the United States alone and is expected to increase by 600% between the years of 2016 and 2030 (AJJR, 2018). Despite recent advancements in implant design and cement techniques, revision surgeries are still common. Prior studies have reported that approximately 3% of all TKA procedures result in a revision surgery, and 60% of all revisions occur within the first five years post-operation (Khan, 2016).

Implant loosening follows joint infection as the primary indications for revision surgery. After two years post-op, aseptic loosening of the tibial component remains the primary mode of TKA failure and indication for revision surgery. Aseptic loosening is characterized by the debonding of the tibial component from the polymethyl methacrylate (bone cement) interface (McTighe, 2009). Contemporary TKA systems today utilize cement bonding features such as metallic surface coatings and backside cement pockets, but are not singularly responsible for weak fixation or tibial component loosening (Khan, 2018, Billi, 2014). Endogenous contaminants such as blood, bone debris, and bone

marrow lipids may present complications for adequate TKA component-PMMA-bone fixation strength. While the mechanisms of tibial loosening are multifactorial, previous research has shown that third-generation cement techniques, including the use of jet lavage and cement pressurization, can reduce contamination of the implant surface by lipids endogenous to the surrounding cancellous bone and thereby enhance fixation strength between the implant and bone (Rossi, 2010).

Variance in cementation technique and lower extremity manipulations are few among many potential mechanisms of introducing contaminating agents into arthroplasty procedures. Although cement technique is independent of tray design, a surgeon's cementation method is often learned during residency and does not consider manufacturer recommendations. Despite this, manufacturers provide recommendations on working temperature, mixing time, and application methods to ensure the best performance of their product. Cawley et al. reported gun cementation provides consistent cement penetration with minimal tibial component micromovement, whereas his colleagues indicate factors such as spatula or hand-packing, pulsatile lavage, high or medium viscosity PMMA, and plateau or stem geometries contribute to adequate penetration of PMMA into the cancellous tibial bone contributing to tibial tray fixation.

Lipid Marrow Infiltration (LMI) is a phenomenon in which lipids within bone morrow or surrounding cancellous bone pockets contaminate the tibial component surface following implantation. Surface contamination is believed to be a mechanism for aseptic loosening (Sugita, 1999). To mitigate contamination, surgical procedure calls for pulsatile jet-lavage methods to remove and clear debris from the prepared bone. Helwig

et al. have indicated pulsatile jet-lavage led to significantly better cement penetration and cement-to-bone contact as compared to syringe irrigation or brush cleaning techniques, but inconsistencies in such methods could lead to a greater prevalence of arthroplasty failure. Some argue that precautions to lipid exposure should be taken due to its ability to inhibit PMMA component binding. Despite recent focus on improving cement techniques, it is common for surgeons to evaluate knee balance by applying varus-valgus traction and flexing the knee through the range of Motion before the bone cement is fully cured.

Intra-operative manual manipulations are routinely performed to assess the balance of the knee (Phillips, 1996). Such assessments are used to determine if ligament release or polyethylene insert thickness adjustments need to be made. Often, the manipulation consists of passively ranging the knee from full extension to deep flexion, with varus and valgus joint laxity examinations done in between each range of Motion (ROM) cycle. Manipulations performed by the surgeon before the curing time specified in the cement manufacturer's documentation may displace the tibial component from its original position. Motion of the tray during cementation is hypothesized to open up the tray-cement interface, reducing implant-cement binding, and possibly providing cavities for lipids to infiltrate. It is believed that lipids from the surrounding marrow within the cancellous bone travel through the PMMA and onto the implant surface, but the mechanism is still not fully understood. One hypothesized mechanism in which marrow lipids travel is through the introduction of lower extremity manipulations executed prior

to complete cement polymerization. In addition, the timing and degree to which the examinations are performed are not consistent among surgeons.

Understanding where the lipids originate and the path in which they migrate to the surface of the implant may prove useful when considering changes in cement technique. Previous studies replicate *in vivo* conditions through the use of artificial contaminant applications, confirming the need for a similar cadaveric study. The benefit of using cadaveric specimens is the degree of similarity to true live patients as compared to experiments done in artificial environments (Billi, 2014). We hypothesized three mechanisms in which lipids travel to the surface of the implant: 1) lipid pools at the bottom of the intramedullary canal resection and travels upward, 2) lipids exist around the rim of the tibial plateau due to the surrounding fatty tissue, 3) marrow lipids exist in the area surrounding all bone cuts, upon cement pressurization, lipids travel upward and collect on the tibial plateau surface.

The purpose of this study is to explore the effect of surgeon behavior during the cement process and variations in TKA systems on tibial tray pull-off force, and to what degree LMI occurs during implantation. To our knowledge, very little biomechanical literature regarding LMI exists, yet it is commonly understood as a source of TKA cementation contamination. We hypothesize that ROM manipulation at the knee prior to adequate PMMA polymerization provides an avenue for LMI to contaminate proper tibial cement interface. We believe understanding the effects of cementation technique and intra-operation manipulations of the lower extremity will provide useful information to the members of the orthopedic community.

In this study, the effects of cementation technique, intra-operative knee balance assessment, and tibial preparation instrumentation variation on tibial tray retention strength were explored. Nine different TKA systems were implanted into cadaveric specimens and subjected to a knee manipulation during the cement curing process. Tray pull-off tests were performed on the specimens and compared with control specimens which did not have the knee manipulation. The underside of each tibial component was analyzed to understand where lipid infiltration occurred and the mechanism by which they traveled to the tray. Our preliminary studies have allowed us to develop two hypotheses:

1) Cementation technique does have an effect on tray retention force. Cement with greater cancellous bone penetration could prove to act as a barrier against contaminating lipids.
2) Knee manipulation during cement-curing increases lipid contamination of the cement-implant interface that compromises tibial tray fixation.
3) Tibial prep instrumentation that provides a tighter fit between the implant and bone (i.e. “No Clearance”) may mitigate the risk of poor initial fixation.

3.2 Experimental Cohorts

Study 1: Impact of implant "fit" and cementation technique mitigating risks of poor fixation

Specimens assigned to Study 1 were incorporated into a 2-factor design-of-experiments focused on factors which may reduce the deleterious impact of tray motion during cementation when using the Attune TKA system (DePuy, Warsaw, IN). The first factor was whether cement was applied only to the tibial bone or was applied to both the tibial bone and the underside of the tibial implant. The second factor was the fit between the intramedullary cone of the tibial implant and the cavity that was prepared in the tibial bone. Two alternative sets of tibial instruments were used for tibial bone preparation. The first set of tibial instruments created a "standard" fit with the implant which included a 1-mm space around the keel structures of the intramedullary cone to allow for a cement mantle. The second set of instruments created a created a "line-to-line" fit between the tibial implant and the bone whereby the prepared bone had the exact shape of the tibial implant.

Table 2: Study 1 experimental Protocol. All knees received the Attune TKA system with motion protocol. Cementation technique and tibial preparation instrumentation were varied. Designation between each specimen's control and experimental knee were randomized.

Control (Contralateral Knee)	**Experimental Group 1**
• Standard Prep. • Cement on Bone Only (BO)	• Standard Prep. (Clearance) • Cement on Bone and Implant (BH)
Experimental Group 2	**Experimental Group 3**
• Line-Line (No-Clearance) Prep. • Cement on Bone and Implant	• Line-Line (No-Clearance Prep.) • Cement on Bone Only

Study 2: Impact of knee Motion during tibial cementation on fixation strength.

Study 2 cadaveric specimens were assigned to one of nine contemporary TKA systems to understand the general effect of the Motion protocol during cementation, where one knee was received the experimental protocol. Each TKA is commercially available globally. The first knee (randomly chosen) first knee received the No-Motion cohort, where the knee balance manipulation protocol was not prescribed during the cement curing phase, and the knee was left un-agitated for the duration of the cement polymerization. The specimen's contralateral knee which received the knee balance manipulation between seven to nine minutes after initial cement mixing began. The peak pull-out strength and lipid contamination metrics were compared against both groups by their implanted TKA system, the instrumentation intramedullary canal preparation type associated with the system, and lastly all systems grouped together.

3.3 Methods

Cadaveric Specimens

TKAs were performed on a total of 48 lower extremity cadaveric specimens (96 knees, age = 68 ±11 years, height = 66 ±3inch, BMI = 24 ±7, 39 female / 57 male, ScienceCare) and evaluated for the tibial component's retention strength. All specimens were fresh-frozen and were allowed to fully thaw to room temperature (65° F) prior to surgery. Specimens with prior lower extremity injury, surgery, or compromised knee ligaments were excluded from the experiment. Specifically, all specimens considered for testing were subject to the following inclusion criteria: cadavers where both extremities were without prior surgery or trauma no retained hardware, intact knee ligaments and, radiographs without evidence of severe osteoporosis or advanced arthritis. Specimens that violated any of the followed exclusion criteria, were not included: cadavers with prior surgery or retained hardware, incompetent knee ligament structures, severe osteoporosis, or anatomical variance between sides that would influence ability to perform same sized implants.

TKA Systems

Specimens assigned to Study 1 all received the Attune (DePuy Synthes, Warsaw, IN, n=30) TKA system with either Line-Line or Standard tibia bone prep., depending on its experimental condition. Specimens assigned to Study 2 were randomly assigned to one of nine posterior-stabilized fixed bearing TKA systems (n=66). Study 2 TKA systems included Sigma, Attune, Attune Revision, Attune S+ (DePuy Synthes, Warsaw, IN), Triathlon (Stryker, Mahwah, NJ), NexGen, Persona, and Vanguard (ZimmerBiomet, Warsaw, IN), and Genesis II (Smith and Nephew, Memphis, TN). The Attune and Attune S+ knee system instrumentation provided two cone preparation methods: line-line (LTL) and standard (STD). Standard instrumentation creates a one millimeter circumferential gap between the cone and bone ("clearance fit"), whereas line-line created a tighter (sub-millimeter) fit ("no clearance fit"). All other knee systems were laser scanned (3D Scanner HD and Scan Studio, NextEngine Inc, CA) to understand if variation in the fit between prepared tibial bone and the tibial implant existed along the intramedullary cone, versus preparation of a gap around the keel structures to accommodate a cement mantle (Figure 13). Instrumentation to implant cone geometry differences of less than one-half millimeter were classified into a *no clearance fit* group whereas those of greater than one millimeter were considered as *clearance fit*. The *no clearance fit* group consisted of knee systems Triathlon, NexGen, Persona, Vanguard, GenesisII, Attune (LTL), and Attune S+ (LTL). The *clearance fit* group consisted of knee systems Attune (STD), Attune (STD), Attune Revision, and Sigma.

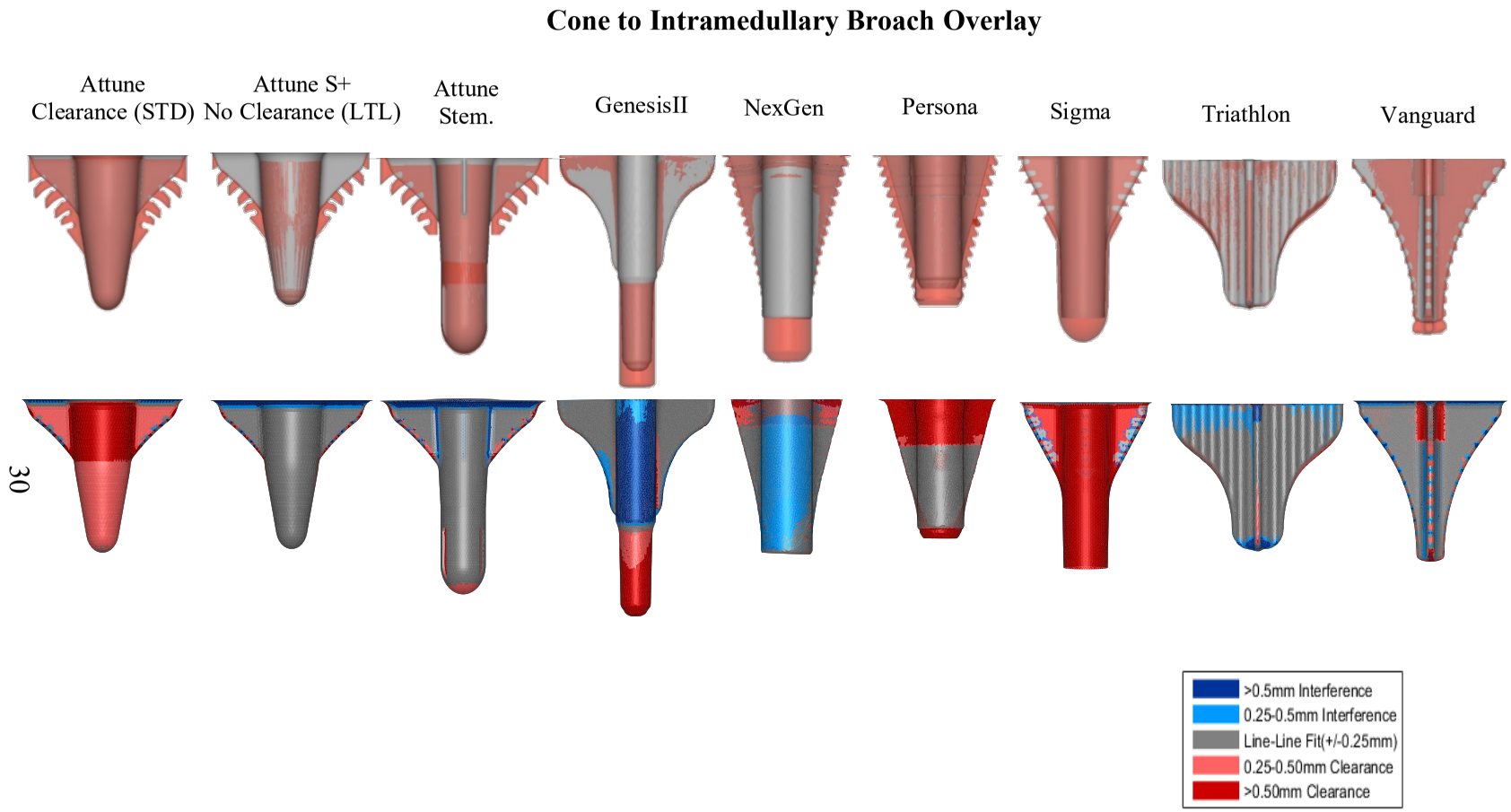

Figure 14: Tibial component cone and broach instrumentation morphology comparisons. Implant (grey), Instrumentation (red); Top row: implant cone region overlay with bone preparation instrumentation. Bottom row: level of clearance or no-clearance interference or clearance of the implant via tibial bone resection. Interference (blue), Clearance (red); Attune and Attune S+ only vary in their plateau geometries, cone geometries are identical. Therefore, only once preparation configuration (line-line or standard) is presented for each implant in this figure.

Surgery

All surgeries were performed by a single surgeon using the specified instrumentation per each manufacturer's recommendation. Each specimen in Study 1 was randomly assigned into one of three experimental categories: 1) line-to-line tibial preparation with cement applied only to the bone, 2) line-to-line tibial preparation with cement applied to both the bone and implant, or 3) standard tibial preparation with cement applied to both the bone and implant (Table 1). The first knee of each specimen was considered the experimental knee and TKA surgery was performed as per the guidelines of the assigned experimental group using the Attune primary knee system. The contralateral knee received TKA as per the control group guidelines: Standard tibial preparation with cement applied only to the bone. After cementation, both knees in each specimen underwent the previously described manual knee balance assessment intraoperatively.

In Study 2, the first knee of each specimen was implanted with an "ideal" cement technique, cement was digitally pressurized into the bone, the implant was fully impacted into the cement mantle, the excess cement was removed, and the cement fully cured under digitally-applied pressure. The contralateral knee was cemented in the same fashion, with the addition of the standardized knee balance assessment protocol performed seven minutes after initial mixing of the bone cement. High-viscosity SmartSet^{TM} poly methyl methacrylate (PMMA) bone cement (Depuy Synthes Warsaw, IN) was used for all specimens following thorough pulsatile lavage (MicroAire, Charlottesville, VA) cleaning to clear debris from the cancellous bone.

Knee Balance Assessment

The knee balance assessment consisted of a manually-applied varus and valgus traction of the knee in full extension, followed by flexing and extending the knee through the full range of Motion, then repeating the sequence again for a total of three times (Figure 15). Following the balance assessment, the knee was left Motionless in full extension for twenty minutes until complete polymerization of the cement, per the manufacturer's recommendation.

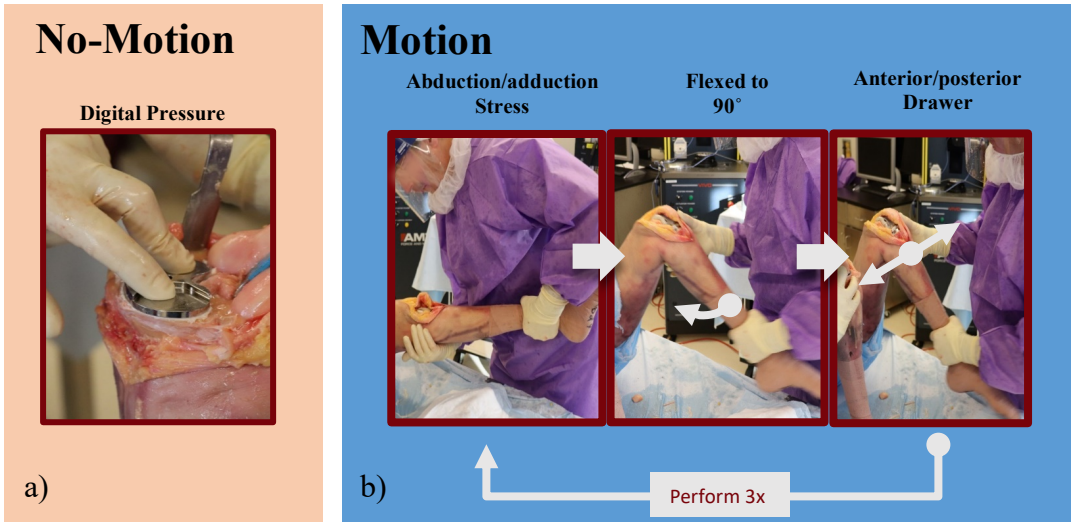

Figure 15: Intraoperative experimental protocol for "Motion" and "No Motion" cohorts. a) "No Motion" cohorts which only received digital pressure for the duration of cement polymerization. b) Knee balance manipulation performed by the operating surgeon between 7-9 minutes following cement mixing begins with an abduction/adduction stress, knee flexed to 90˚ followed by an anterior/posterior drawer test, performed a total of three times.

Mechanical Testing of Tibial Component-Bone Interface

Two hours following surgery, the implanted tibiae were extracted from the specimen, skeletonized, and sectioned 150-mm below the joint line. The distal aspect of the tibial specimens were secured in tubular fixtures with a PMMA based resin and mounted onto the base of an Instron 5982 uniaxial test-frame. Tibial trays were attached to the actuator via a universal joint and fixturing custom to each knee system's tibial tray (Figure 16a). Each tray was pulled proximally from the bone under displacement control at a rate of 5 mm/minute until failure of the cemented-implant (Type I) or cement-bone (Type II) interface, where peak retention force prior to failure was recorded (Figure 16b).

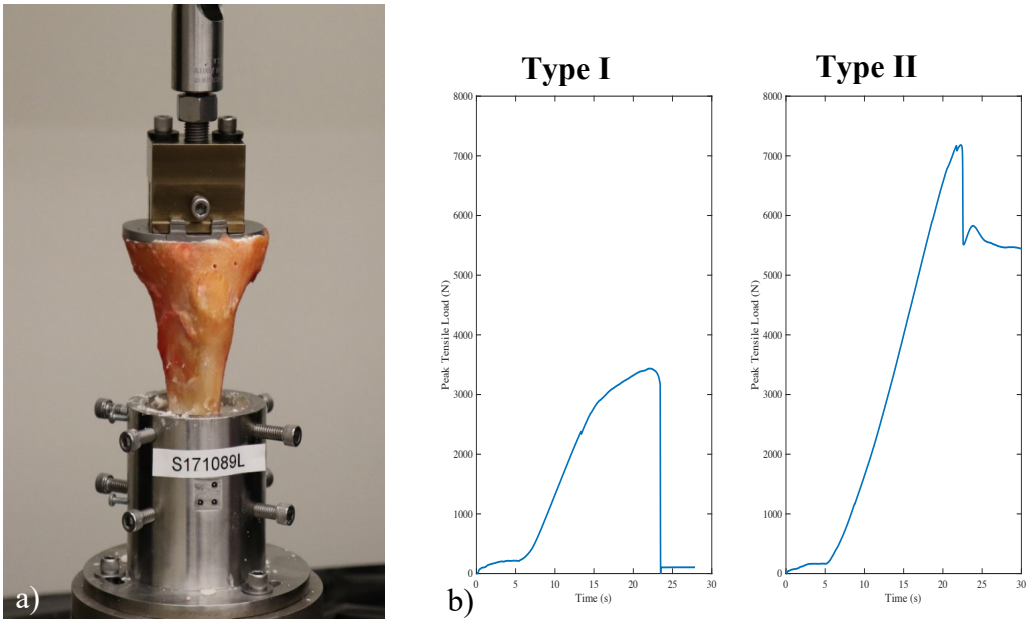

Figure 16: a) Experimental setup with skeletonized tibia potted into a fixture loaded into Instron 5982 Uniaxial Test Frame for mechanical pull-off testing. Testing stopped upon a 2000N drop from peak pull-off value; b) Specimen failure case of implant-cement interface (Left, Type I) or cement-bone interface (Right, Type II).

Lipid Contamination Analysis

Upon the completion of the mechanical testing, the specimen's failure mechanism was characterized as 1) failure at the implant-cement interface, 2) failure at the cement-bone interface, or 3) mixed failure between the implant-cement-bone interfaces. Tibial bases that failed at the implant-cement interface were photographed to characterize the amount of lipid contamination on the implant surface. Two-dimensional triangular surface meshes of the tibial tray were overlaid and manually aligned to the images using Hypermesh (Altair), where elements corresponding to contaminated areas of the implant surface were identified. A custom Matlab script was used to quantify the contaminated areas of the cone and plateau regions ("wet" areas of the tibial base where lipids were present) normalized by the total surface area of the base (Figure 17). Quadrants of the plateau were established by defining the midpoint along the anterior-posterior direction and medial-lateral axes of the implant aligned in a Grood and Suntay coordinate system. Proximal, middle, and distal regions of the cone or keel structures were defined by dividing the surface area of the most distal point to the most proximal point of the cone or keel into thirds of equal surface areas (Figure 18).

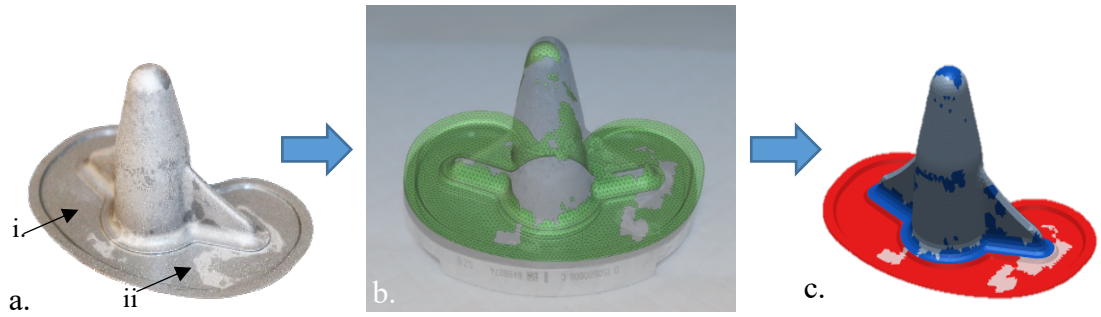

Figure 17: Process roadmap of quantifying surface lipid contamination of implants that failed cleanly at the implant-cement interface; a. raw real image of an implant with lipid contaminated (i.) and uncontaminated (ii.) regions); b. 2D triangular mesh (Altair Hypermesh) superimposed over real image of lipid-contaminated tibial tray; c. 3D mesh (Altair Hypermesh) where dark blue and dark red regions represent lipid contamination of the implant plateau and cone regions.

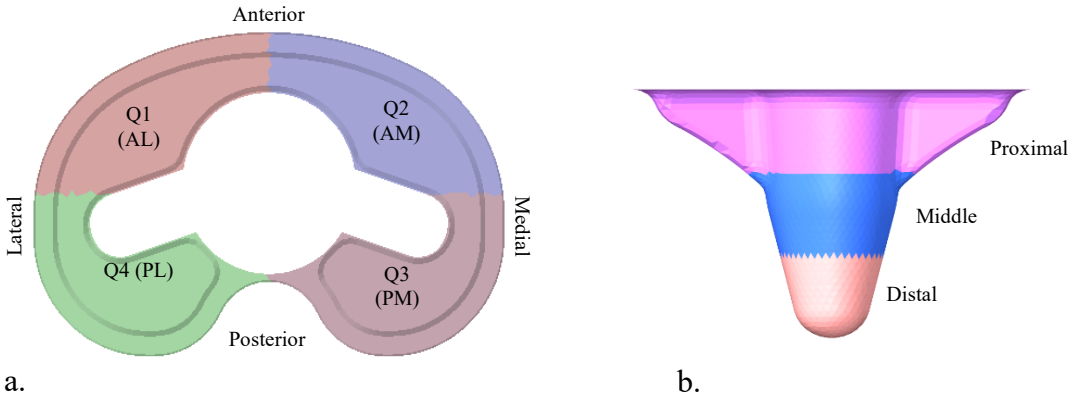

Figure 18: A visual representation of the regions in which each lipid cell's location was allocated into; a. the plateau is divided into quadrants where the surface area of each quadrant bisects the medial/lateral and anterior/posterior axes; b. the cone is divided into thirds where the surface area is equal among each region.

Intra and Inter-observer Reliability

To validate the contaminated surface assessment methods, a repeatability study was performed to quantify both intra- and inter-observer variability. An Attune (DePuy Synthes, Warsaw, IN) size 5 tibial base with randomly chosen, moderately wet, area was

analyzed by five independent observers, five times each. Intra- and inter-observer measurements yielded mean percent wet area ± 1 SD of 67±3% and 69±3%, respectively.

Statistical Analysis

A 2-factor Analyses of Variance (ANOVAs) with Tukey-Kramer post hoc tests were performed on data from Study 1 to understand the effect of cementation application and bone preparation on implant retention strength and ultimately establish surgical methods for Study 2. In addition, Analyses of Variance (ANOVAs) with Tukey-Kramer post hoc tests were repeated for Study 2 to identify whether implant type or the balance assessment significantly affected retention strength and the contaminated surface areas across implant designs. Paired T-tests were executed to understand if significant differences existed between experimental groups. Study 2 Motion and No-Motion cohorts for each implant type were combined and correlation coefficients were calculated between the tibial base uncontaminated surface areas and retention strengths for each design. Statistical analyses were repeated after grouping all implant designs together based on their assigned surgical protocol. For all statistical analyses, a confidence level of 5% was assumed to be a sufficient acceptance criterion.

3.4 Results

Study 1: Cement Technique and Tibial Bone Preparation

Surgical implantation technique was explored by varying both cement application and tibial bone preparation. Mean retention force was greatest in specimens which received the Attune Line-Line bone preparation and cement on both surgical protocol (3021N ± 737N). This experimental group did present high lipid contamination and the lowest contamination difference between its experimental and control knees (83% mean contamination, 0.4% difference). Specimens which received the Attune Standard prep. with cement on both yielded the lowest mean peak retention strength (1686N ± 790N). Paired t-tests to between experimental and control protocol knees presented no statistical significance across all groups in both mean peak retention forces and lipid contamination percent area. (Figure 19, 20). Furthermore, Two-way ANOVA analysis presented no significant difference in both mean peak retention forces and mean lipid contamination percent area among all experimental groups against their control.

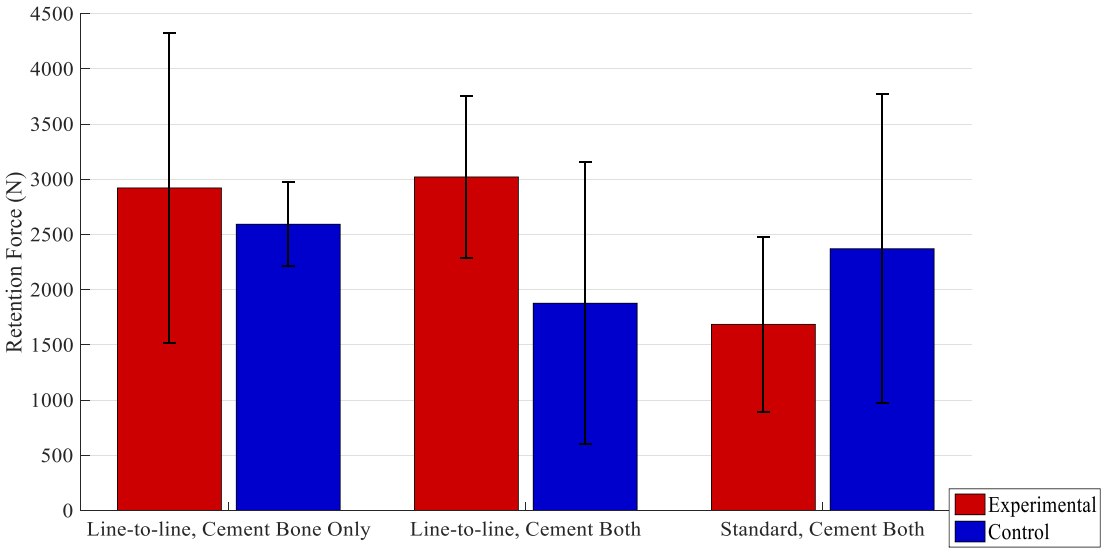

Figure 19: Peak retention force (N) between experimental cementation techniques compared to its contralateral knee control. Control comparison was the contralateral knee which received the Motion protocol, standard bone preparation instrumentation, and cement was applied to the bone only. Paired t-test presented no statistical significance between experimental and control groups. Two-way ANOVA also presented no significant mean differences among cementation and bone preparation groups.

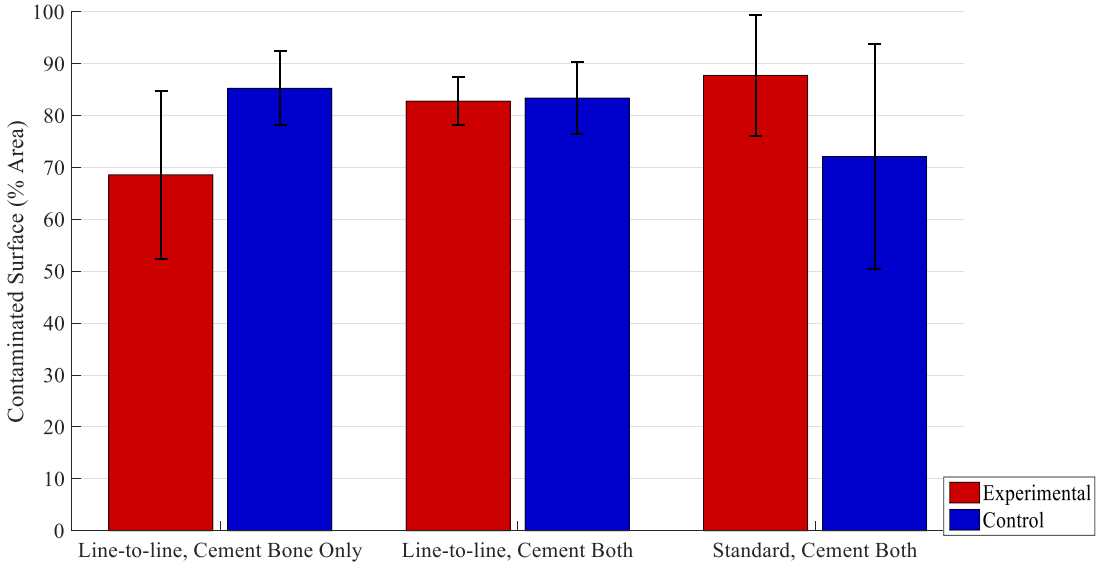

Figure 20: Lipid contamination (%) between experimental cementation techniques compared to its contralateral knee control. Control comparison was the contralateral knee which received the Motion protocol, standard bone preparation instrumentation, and cement was applied to the bone only. Paired t-test presented no statistical significance between experimental and control groups. Two-way ANOVA also presented no significant mean differences lipid contamination area among cementation and bone preparation groups.

Study 2: No Motion vs. Motion

Retention forces and lipid contaminated surfaces of cemented fixed-bearing TKA systems were analyzed in cadaveric specimens. Knees were subject to either intra-operative knee balance assessment (experimental) or no manipulation at all (control) for the duration of allotted bone cement curing process. Specimens which failed at the cement-bone interface (Type II, n=14) fractured with an average peak of 5176 ± 1557N (Figure 21). All other specimens failed at the cement-implant interface (Type I, n=82), which was considered as the ideal failure.

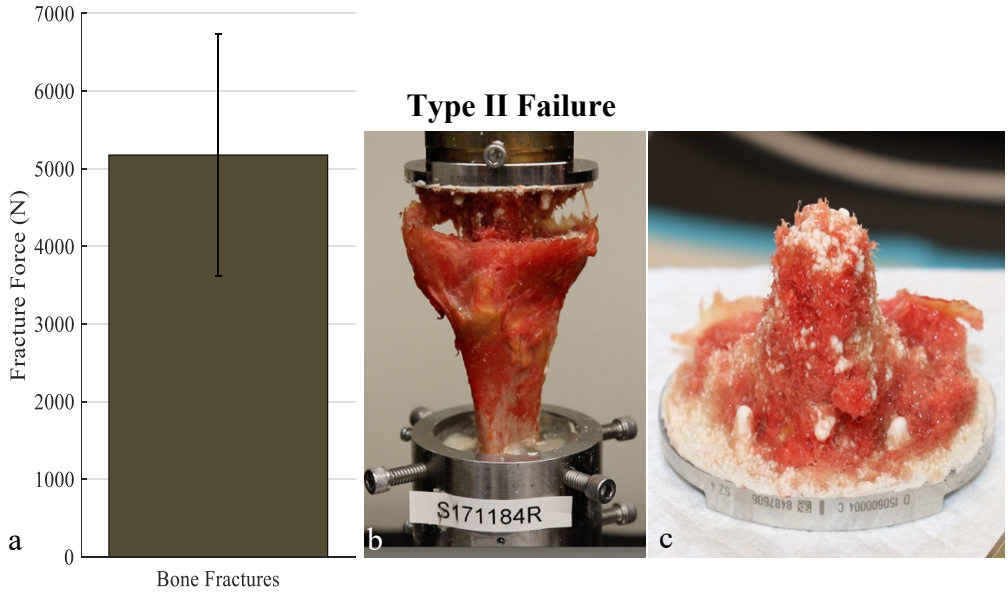

Figure 21: a. Mean peak tensile loading of specimens upon bone failure at cement-bone interface (Type II failure); b. Specimen failure case of specimen loaded into uniaxial test frame (Instron 8872); c. tibial implant underside of bone failure case.

All TKR systems experienced a decrease in retention force after intraoperative knee balance assessment manipulation (Figure 22). Holistically, a significant difference was observed between all No-Motion and Motion experimental groups when TKA systems were grouped together ($p=7.10e-5$, Figure 23). Attune (Standard Prep.), Attune S+ (Standard Prep.), and Attune Revision TKA systems all experienced significant reductions in tibial tray retention force ($p \leq 0.05$). Attune S+ system yielded the highest mean peak retention force of 6353N ± 1411 N, whereas Attune yielded the lowest mean peak 1045N ± 616N among both Motion and No-Motion groups. Stryker's Triathlon TKA system was mostly unaffected to any deleterious effects of manipulation with only a mean difference of 153N between Motion and No-Motion cohorts. NexGen (Zimmer Biomet), Triathlon (Stryker), and Vanguard (Zimmer Biomet) systems also exhibited decreases in retention forces after knee balance assessment with mean differences of 736N, 1313N, 153N, and 1289N, respectively. Although no significant differences in retention forces were found in Attune (LTL Prep.), Attune S+ (LTL Prep), GenesisII, NexGen, Persona, Sigma, Triathlon, and Vanguard systems, a clear negative trend ($R^2=0.304$) in all systems were observed when correlating retention forces to contaminated surface area (Figure 24).

Significant differences in total lipid contamination area were observed among designs grouped together between No-Motion and Motion cohorts (Paired T-test with Tukey Post-hoc analysis, Figure 25). However, when isolating TKA systems, only Attune Primary (Standard Prep.) and Attune Primary S+ (Standard Prep.) exhibited significant differences in total lipid contaminated area between Motion and No-Motion groups

(Figure 16, p=0.0002, p=0.038), while no other implant system exhibited statistically significant total lipid contamination.

In addition to significantly lower mean peak retention strengths, paired T-test analysis also highlighted a statistically significant increase in lipid contamination when all TKA systems were grouped together based on their prescribed motion protocol. Total and sub region percent areas of the keel or cone and plateau regions all presented significance (Table 2).

Two-way analysis of variance presented statistical significance in mean peak retention force across No-Motion or Motion experimental cohorts in five of the eleven TKA system configurations. The mean peak retention force of Attune Std. was significantly different compared to Attune S+ LTL (p=0.00473) and Persona (p=0.0044). Mean peak retention force of Attune S+ LTL was also significantly different than Sigma (p=0.0165) and Vanguard (p=0.0029) knee systems. Lastly, Persona's mean peak retention force was statistically significant than Sigma (p=0.0153) and Vanguard (p=0.0027) as well. No significance was observed across all percent lipid contamination area across No-Motion and Motion cohorts.

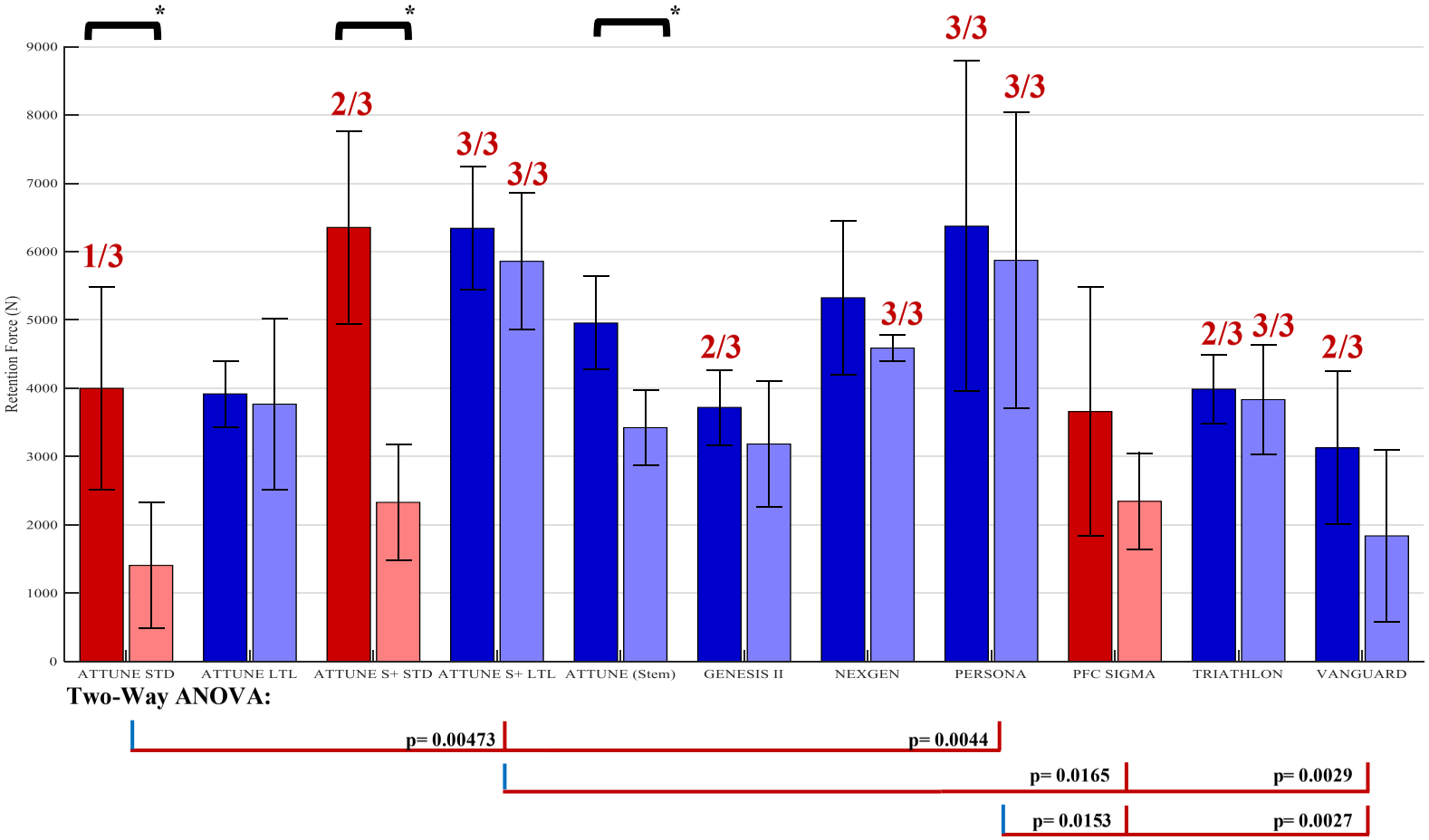

Figure 22: Peak retention force (N) across No-Motion (left bar) and Motion (right bar) cohorts for each TKA system (figure top). Red numbers indicate number of specimens in cohort that failed at the cement-bone interface (Type II). Statistical significance was observed between No-Motion (Cement Mantle) and Motion (Cement Mantle) of Attune Std., Attune S+ Std., and Attune Stem. systems, with P-values of 0.005, 0.013, and 0.039, respectively. Two-way analysis of variance (ANOVA, figure bellow) illustrates differences in mean variance between TKA systems. Red tick marks illustrate mean statistical significance compared to highlighted systems of blue tick marks.

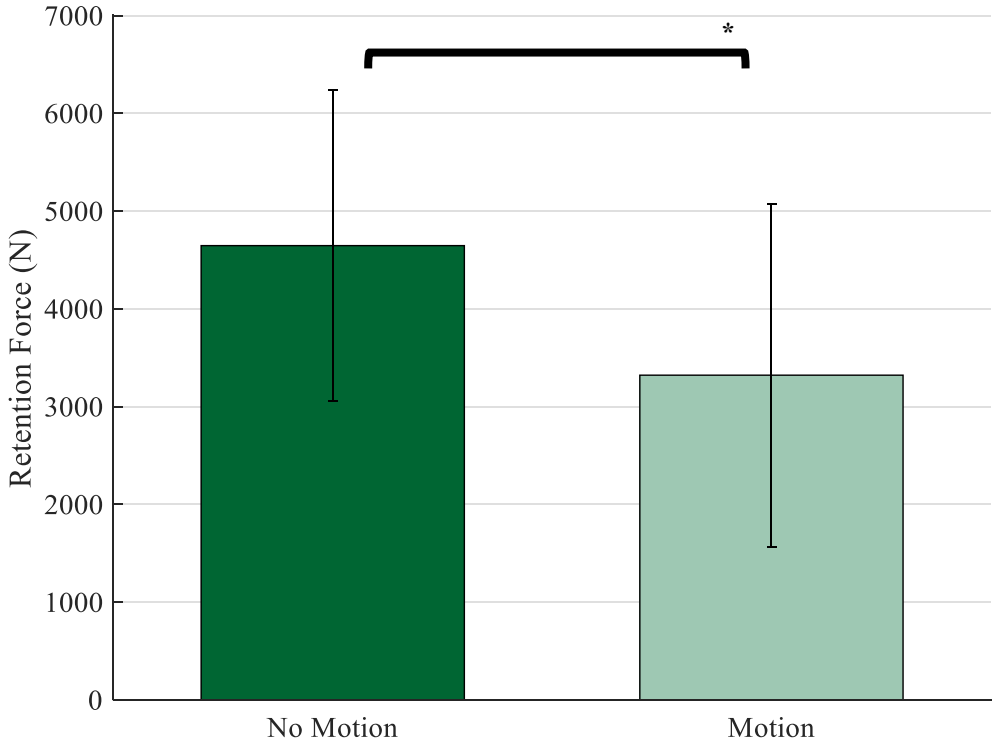

Figure 23: Peak retention forces (N) for all specimens and designs grouped together across No-Motion and Motion cohorts. Statistically significant difference was observed (p=7.10e-5).

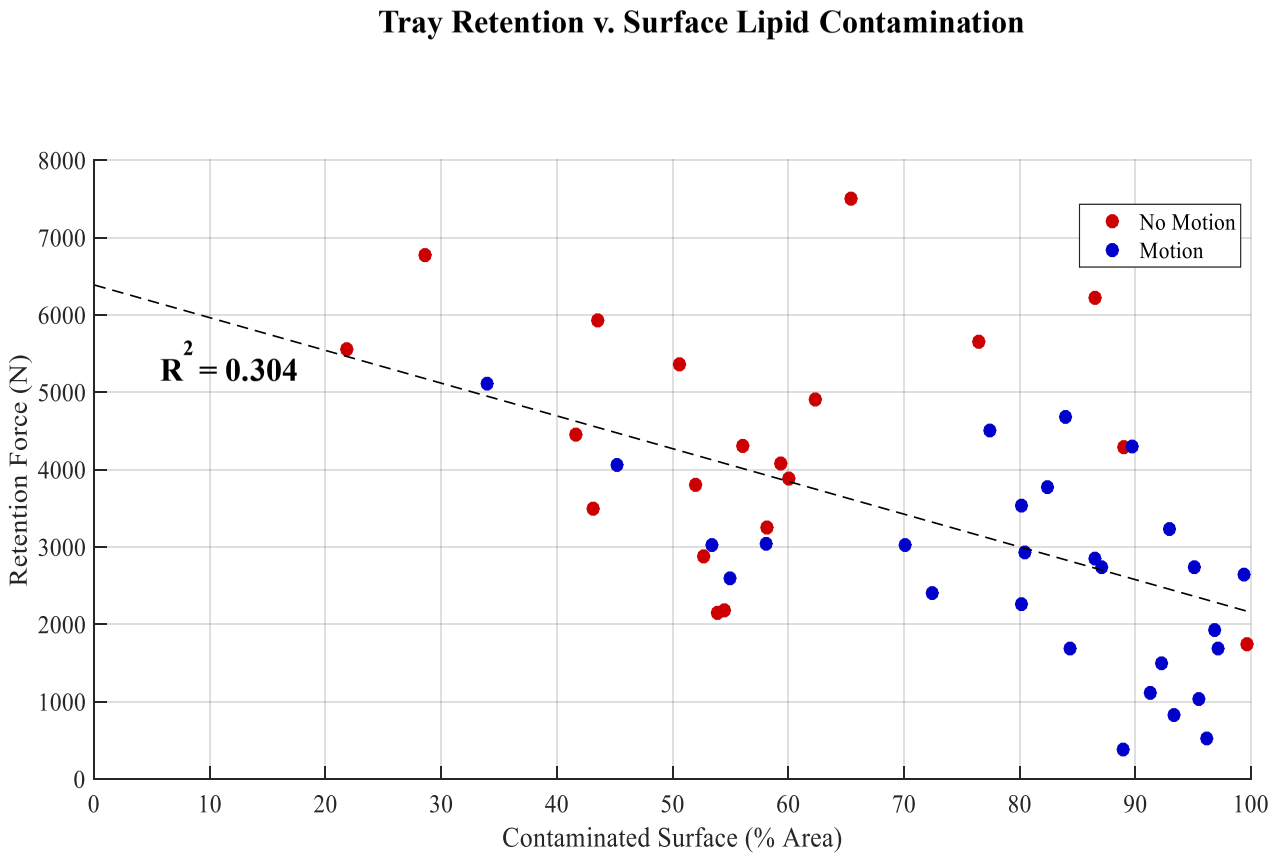

Figure 24: Tray retention metrics for all Study 2 specimens, TKA systems and experimental groups were plotted against their percent total contaminated surface area. A linear regression line was fit to the data (R^2=0.304).

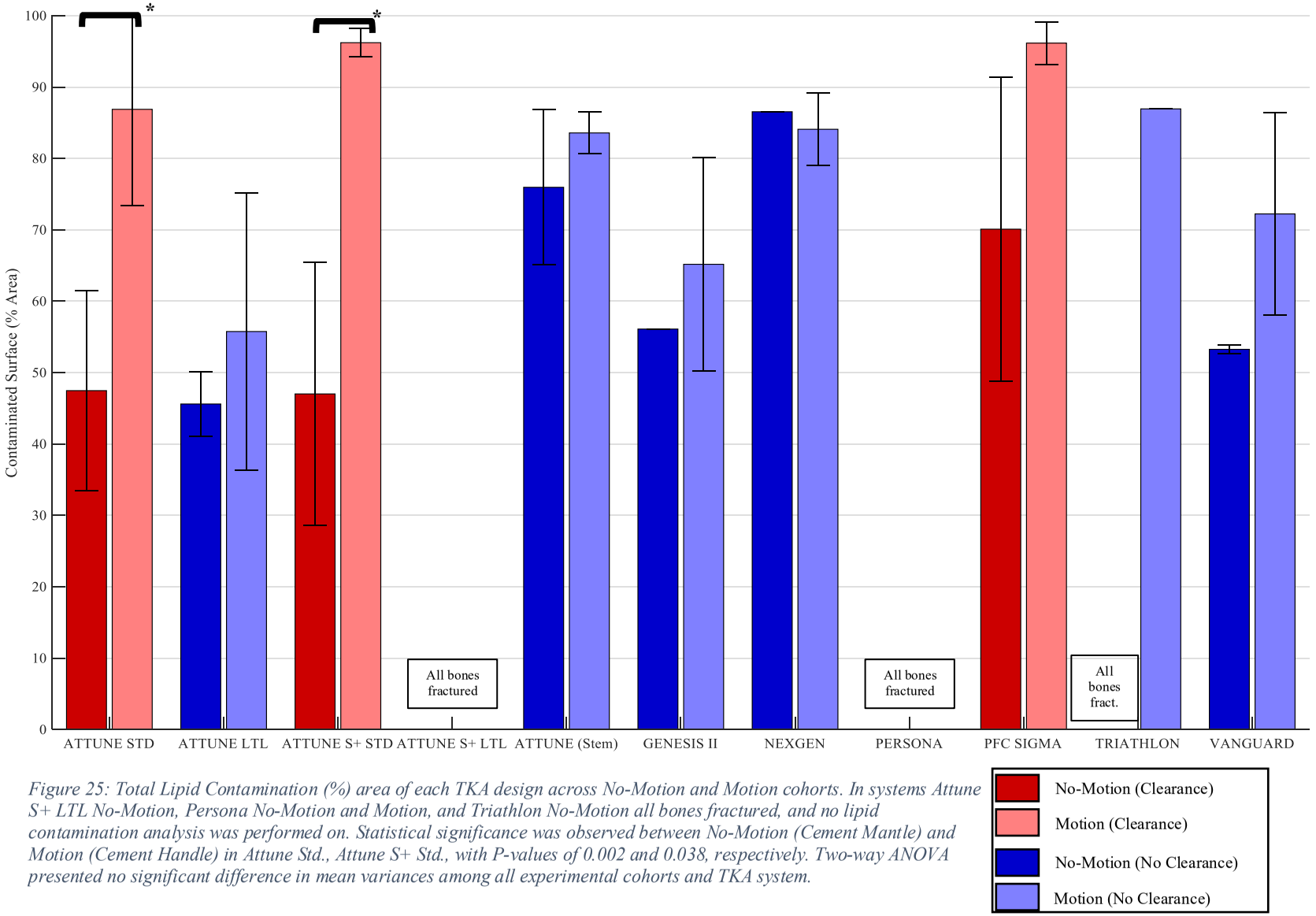

Figure 25: Total Lipid Contamination (%) area of each TKA design across No-Motion and Motion cohorts. In systems Attune S+ LTL No-Motion, Persona No-Motion and Motion, and Triathlon No-Motion all bones fractured, and no lipid contamination analysis was performed on. Statistical significance was observed between No-Motion (Cement Mantle) and Motion (Cement Handle) in Attune Std., Attune S+ Std., with P-values of 0.002 and 0.038, respectively. Two-way ANOVA presented no significant difference in mean variances among all experimental cohorts and TKA system.

Clearance vs. No Clearance

All Study 2 TKA systems and their corresponding specimens were evaluated for their tibial bone preparation instrumentation and assigned to either a Clearance or No Clearance group for statistical analysis. Within the *clearance* group, specimens which received the Motion protocol experienced a significantly lower peak fixation strength and significantly greater total and mean cone and plateau. A significant difference was observed between No-Motion and Motion cohorts in both clearance and No Clearance groups, with mean pull out strengths of 4503N ± 1800 vs. 1873N ± 917 and 4719N ± 1509 vs. 4046N ± 1621, respectively. In addition, a strong negative correlation was also observed (R^2=0.061) when comparing peak pull off strength and lipid contamination among the Motion and No-Motion clearance cohort (Figure 26). Furthermore, the clearance group exhibited significant lipid contamination across all regions of the plateau and cone except for quadrant 3, whereas the No-Clearance group only showed significant increase in contamination on the cone or keel region as a whole (p=0.0319). Only the proximal third of the cone exhibited a significant increase in lipid contamination (p=0.0468) in No Clearance Line-Line Prep. experimental cohort.

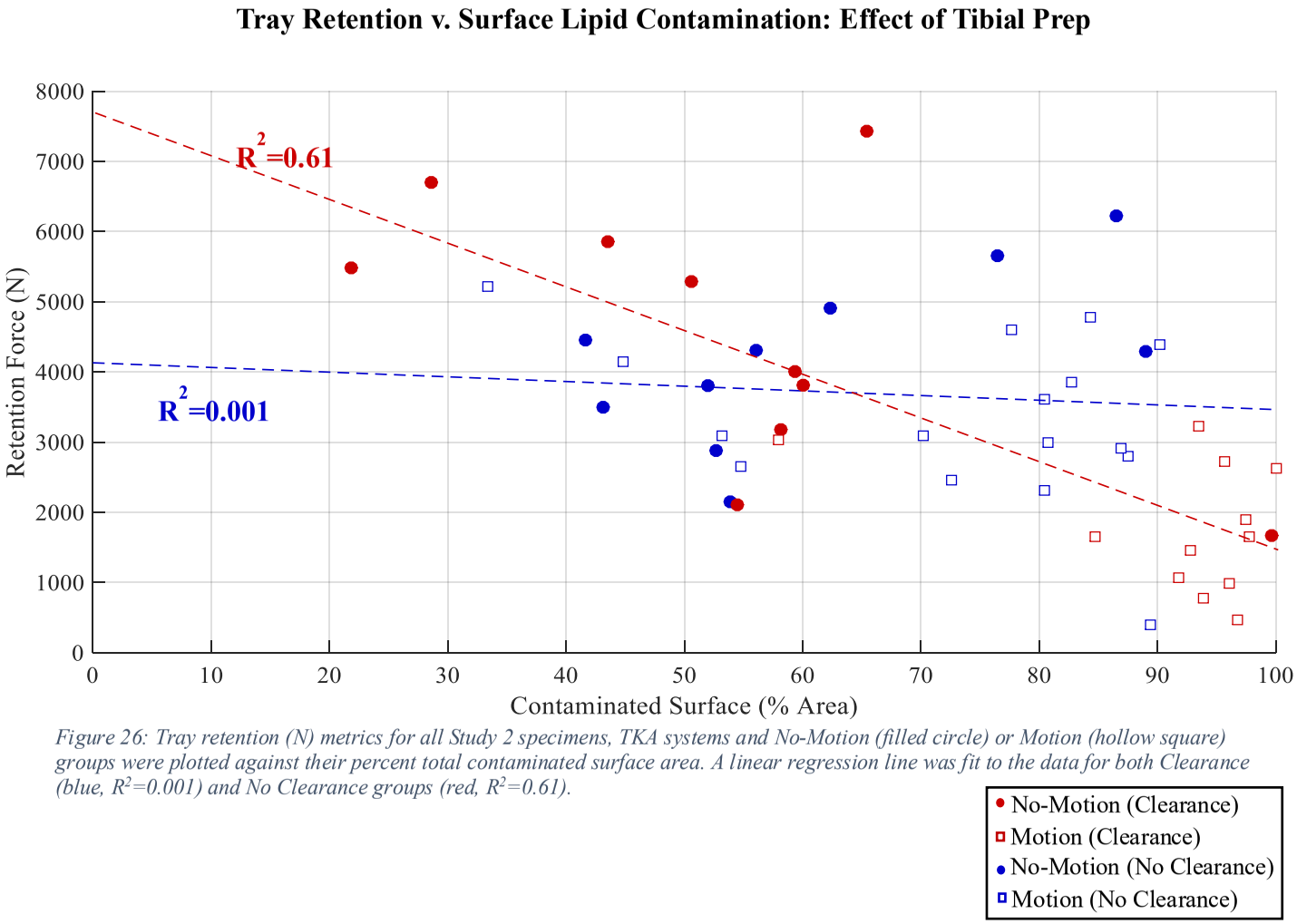

Figure 26: Tray retention (N) metrics for all Study 2 specimens, TKA systems and No-Motion (filled circle) or Motion (hollow square) groups were plotted against their percent total contaminated surface area. A linear regression line was fit to the data for both Clearance (blue, R^2=0.001) and No Clearance groups (red, R^2=0.61).

3.5 Discussion

TKA success can be heavily influenced by surgeon behavior. This study reports the effect of intraoperative knee balance assessment on tibial baseplate fixation strength of nine contemporary and commercially available TKA systems. In addition, it also explores the robustness of surgical technique and bone preparation procedures to the deleterious effects endogenous contaminants on implant fixation. To our knowledge no published literature exists regarding endogenous contaminants and their contributing failure mechanism pathways. This study is the first to isolate the effect of these contaminating agents to further understand the failure mechanism of aseptic tibial loosening conditions.

Through our own preliminary studies, it was evident that an endogenous contaminating agent are present on the underside of tibial implants (Figure 2) which fail at an uncharacteristically low tensile load, and may contribute to aseptic tibial loosening. In addition, when surgeons use a single batch of cement for multi-component cementation, their knee balance assessments are often performed while the cement in still in its curing (polymerization) phase as noted in the manufacturer's instruction manual, which we hypothesize contributes to aseptic loosening.

Our study explored the effect of cementation technique by varying cement application: bone only, implant and bone, as well as tibial resection preparation: Line-Line and Standard. Their fixation strength was tested by first defining it as the peak axial tensile yield strength of the tray, whereas failure was defined as a drop of 2000N with increasing displacement, then loading it axially. Of the specimens that failed at the

cement-implant interface (Type I) as opposed to the cement-bone interface (Type II), lipid contamination analysis was performed to further understand the pathway in which lipids migrate to and contaminate the implant cement-interface. Correlations between fixation strength and lipid contamination were assessed and compared across implant types.

In study 1, there was no significant difference between control and experimental knees when analyzing both retention strength and lipid contamination. The standard prep. was used as the control because of its popularity and abundance with the Attune knee system, while the Line-Line was a next generation of the surgical instrumentation. Therefore, it was assumed that the Line-Line prep. would rescue fixation force in an un-deal implantation technique, yet it was not evident. In addition, the use of cement on both the bone and implant was hypothesized to increase bone cement interdigitation, and therefore pushing the lipids further away from the intramedullary canal and tibial plateau. Both experimental techniques and their variations did not rescue the effects of knee motion during cement curing. No significant difference was observed between control and experimental knees, and no rescuing abilities were present. We believe the variation in specimen bone quality may have been a contributing factor to our observations. It is likely that the variation in bone densities and qualities may have been more significant than the possible benefits of using one cement or surgical technique over the other.

In study 2, when compared to their No-Motion contralateral knee, all eleven knee systems individually studied showed a statistically significant reduction in fixation strength after the knee balance manipulation was applied during the PMMA cement

curing phase. Variations in cement placement (cement on implant only or bone and implant) procedure presented no significant ability to mitigate the effect of intraoperative knee balance assessment on fixation strength nor lipid contamination. Furthermore, all cohorts that failed at the cement-implant interface (Type I) exhibited a statistically significant increase in lipid marrow contamination between Motion and No-Motion cohorts. When studied together, a strong negative correlation can be seen between fixation strength and lipid contamination.

All TKA systems classified as clearance exhibited statistically significant reductions in fixation strength and increases in lipid contamination, suggesting that a cement-mantle surrounding the implant may prove to be detrimental to the success of tibial base plate implantation. More specifically, the No-Clearance group exhibited an increase in lipid contamination around the proximal cone region and could be correlated with the drop in retention force after a knee balance assessment manipulation.

Significant differences compared to Attune and Attune S+ with Standard prep. was seen in the study 2 specimens implanted with these systems. This was contrary to what we observed in study 1, and may likely be due to the use of specimens with greater bone quality and higher density. Therefore, we can conclude that the tighter fit of the bone in Line-Line or a No-Clearance knee systems may be more robust to intraoperative knee balance manipulations than there Clearance knee system competitors.

Most undercut cement pockets and cement locking features observed existed solely within the plateau region of the tray. Although pooling was observed, no significant difference was observed in plateau contamination between No-Motion and

Motion cohorts. However, results from this study indicate that lipid pooling in the proximal region of the cone significantly reduces fixation strength and may contribute to aseptic tibial component loosening. Therefore, future design efforts should be focused around maximizing the cone or keel structures fixation ability in addition to that of the plateau.

Our study confirms that when the final knee balance assessment is performed during the cement's curing phase, the intraoperative manipulation can increase surface contaminants at the cement-implant interface, compromising initial tray fixation.

It is important to note that five out of six tested specimens implanted with Stryker Triathlon fractured during mechanical testing, in addition to all specimens of Attune S+ (Line-Line) and Persona systems, therefore an adequate sample size was unachievable to understand lipid contamination of the No-Motion Triathlon-implanted specimens (Appendix D).

In a clinical setting, this study has illustrated that an implant system which does not prepare a cement-mantle (Clearance) in which the surgeon does not move or perform a final knee balance manipulation throughout the entirety of the cement curing phase will reduce lipid contamination and ensure a strong bond between the implant and bone cement and potentially lower the likelihood for revision surgery. Although this will increase the operative time and may limit the number of procedures a surgeon could typically perform in a day, it may enhance the outcome for the patient and the success rate for the surgery.

Maximizing tray retention strength may minimize the likeliness of contaminating agents sacrificing initial tray fixation, ultimately leading to a revision surgery. However, a balance in retention strength vs. revisability must be met when designing a TKA system with the goal of maximizing retention strength because tibial base plate loosening is not the only indication for a revision surgery. Typically, a revision surgery requires the removal of far more bone tissue than a primary surgery. Therefore, a tibial baseplate with a retention strength beyond the modulus of cancellous bone, may complicate its removal and could require the surgeon to remove more bone to remove the implant as compared to another implant with a lower peak retention strength. Patients who suffer from degenerative osteolytic conditions are likely not capable of recovering from excessive bone loss and may encounter a myriad of other issues as a result.

Changes in surgical procedure may prove to reduce the risk of arthroplasty failure and likeliness of revision surgery. With our findings, we can conclude that LMI does occur when a final knee balance manipulation is performed prior to cement polymerization. It is likely that the mechanism of the manipulation pressurizes cancellous bone and drives the movement of lipids through the cancellous cavities up on to the surface of the tibial component. The link between retention strength and survivorship is unclear, but we have shown aseptic loosening is multifactorial, and knee Motion and lipid contamination are likely contributors.

3.6 Limitations and Future Directions

This study has several limitations. The first being that no chemical assay was performed on the presumed lipids present on the underside of the tibial tray after fixation strength testing. Although we speculate these contaminates are lipids originating from the specimen's bone marrow, these fluids are contaminating agents, and have been present at the implant-cement interface failures of every tested specimen. In addition, all surgeries and mechanical testing was performed on cadaveric specimens that were once frozen and then thawed before operation. The freezing and thawing actions coupled with post-mortem changes in lipid marrow viscosities may not truly represent *in vivo* conditions and should be noted.

Variations in cancellous bone densities between specimens may confound the retention strength of an implant among the specimens where the bone has fractured. Factors such as age, sex, BMI, and cause of death, coupled with the amount of time between tissue dissection and mechanical testing may influence the quality of the cancellous bone. Significant differences between cancellous bone densities could result in inconsistent PMMA bone cement penetration and pulse lavage decontamination of the tibial canal resection, therefore confounding retention force and lipid contamination metrics.

Although intra-observer and inter-observer differences were statistically insignificant, the mapping between two-dimensional images to three-dimensional representations of the contaminated lipid area is likely to over or under-represent the contaminated area accurately. In addition, no automated algorithm was used in the

alignment of the stereo images of the contaminated areas base trays to the three-dimensional implant mesh. Observer variability must be considered when interpreting the data.

Specimens that failed at the cement-bone interface (Type II failure) with low peak retention forces often presented with low bone quality, as reported by the surgeon. Despite screening cadaveric specimens against osteoarthritis, knee trauma and chronic smoking, it is believed that some chemo-based cancer treatments contribute to a decline in the patient's bone quality (Sugita, 1999). Although certain specimens were not excluded, we observed specimens reported with cancer treatments prior to death often exhibited inferior bone quality and could have contributed to failures at the cement-bone interface due to their uncharacteristically low peak retention force.

In the future advancements of this study, a few changes should be made to further understand the effects of lipid contamination and the best possible method to mitigate them. First, more commercially available knee systems should be studied. Analyzing more tibial implant underside features could allow us to enhance our understanding on which features work best in maximizing implant fixation and protection against contaminants. In addition, a greater number of commercially available High-Viscosity PMMA bone cements should be examined as well because many variations exist and are currently used by many surgeons around the world. Different pulse lavage systems should be implemented in the surgical method of this study, in combination with the use of an intramedullary plug, what is commonly used in the operating room today.

On the analysis end, an automated algorithm image-to-mesh alignment pipeline and implant lipid contamination quantification could reduce the inter and intra-observer error. Modern day neural networks and machine learning algorithms are capable of object detection and could be optimized to identify the dry versus wet areas of the tibial component's underside surface. And lastly, a chemical assay should be performed on the lipids present on the tray after a cement-implant failure to understand the molecular make-up of the contaminants, where an impermeable PMMA bonce cement could be developed in the future.

Chapter 4: Concluding Remarks and Recommendations

This study was the first to underline the deleterious effects of contaminating agents drawn from an intraoperative knee balance assessment in a cadaveric setting. It began by exploring various cementation and tibial bone resection methods to understand their robustness to Lipid Marrow Infiltration, which lead to the formation of an ideal TKA surgical method: using a TKA system with a keel-dominant tibial cruciform geometry (Triathlon-like geometry), Line-Line preparation with bone cement applied to the bone only, and no knee balance assessment performance for the duration of the cement's curing phase. This study was also the first to challenge the most successful commercially available TKA systems on the market in their ability to mitigate endogenous contaminants. As a result, we were able to conclude that modern day tibial TKA components must be able to withstand no less than 4500N of peak retention force during an axial loading test.

In addition to retention strength, this study also successfully mapped the mechanism in which endogenous contaminating agents were compromising initial tibial tray fixation. The deleterious effects of a knee balance assessment manipulation during the cement's curing phase mobilized lipids to the implant surface and inhibited proper adherence of the bone cement. It also concluded that contamination of the cone region proved to be significantly more deleterious than contamination of the plateau region, which would indicate future design iterations should focus their efforts on new fixation features that

would maximize cone retention into the cancellous bone and cement as well as the plateau.

In the surgical setting, completely removing endogenous contaminants may not be possible, but preventing them from reaching the surface of the implant could mitigate the deleterious effects on tray fixation. Previous literature has already highlighted the benefits of using pulsatile lavage to irrigate and debris the intramedullary canal from contaminating agents, but it can be improved through the use of special nozzle fittings, specific to the TKA system's cone or keel geometry, to reach debris deeper in the canal in hopes of protecting the cone or keel from contaminants. A cone fitting for the lavage system coupled with a stronger vacuum could significantly reduce the amount of contaminating agents local to the distal cone feature. Also, suction applied to bine pin holes may also help to reduced pressure waves generated during impaction of the implant.

In addition, the use of a PMMA bone cement specifically designed to be impermeable to lipids found in the cancellous bone could provide the ultimate barrier needed to block the contaminants. It may also allow the surgeon to perform their knee balance assessments while the cement is in its curing phase, acting as a failsafe mechanism in case the surgeon does not wait for complete polymerization of the cement. A chemical assay of the contaminants could prove useful to both confirm the contaminants are lipids originating from the specimen's marrow, but also to provide the molecular makeup and the blueprint to a cement impermeable to such molecules found naturally in human bone.

In future directions of this study, greater effort should be made to avoid specimens with reported history of cancer and chemo-based cancer treatments to avoid any confounding results due to poor bone quality or medications whose side-effects lead to a decline in bone mineral density and bone quality.

To make the biggest impact and to ensure the best possible outcome for their patients today, surgeons implanting a cemented TKA system should not perform a knee balance assessment during the operation while the bone cement is curing, regardless of system being used.

Bibliography

1. Jun Ito, Tomihisa Koshino, Renzo Okamoto, Tomoyuki Saito, 15-year follow-up study of total knee arthroplasty in patients with rheumatoid arthritis, The Journal of Arthroplasty, Volume 18, Issue 8, 2003, Pages 984-992, ISSN 0883-5403, http://dx.doi.org/10.1016/S0883-5403(03)00262-6.
2. Phillips, A. M., Goddard, N. J., & Tomlinson, J. E. (1996). Current techniques in total knee replacement: results of a national survey. Annals of The Royal College of Surgeons of England, 78(6), 515–520.
3. Bäthis, H., Perlick, L., Tingart, M., Lüring, C., Zurakowski, D., & Grifka, J. (2004). Alignment in total knee arthroplasty. Bone & Joint Journal, 86-B(5), 682-687. Accessed August 11, 2017.https://doi.org/10.1302/0301-620X.86B5.14927.
4. Dongquan Shi, Xingquan Xu, Anyun Guo, et al., "Bone Cement Solidifiliation Influence the Limb Alignment and Gap Balance during TKA," BioMed Research International, vol. 2015, Article ID 109402, 3 pages, 2015. doi:10.1155/2015/109402
5. Cawley, D. T., Kelly, N., McGarry, J. P., & Shannon, F. J.(2013). Cementing techniques for the tibial component in primary total knee replacement. Bone Joint J, 95-B(3), 295-300. Accessed August 11, 2017. https://doi.org/10.1302/0301-620X.95B3.29586.
6. Jan Vanlommel, Jean Philippe Luyckx, Luc Labey, Bernardo Innocenti, Ronny De Corte, Johan Bellemans, Cementing the Tibial Component in Total Knee

Arthroplasty, The Journal of Arthroplasty, Volume 26, Issue 3, 2011, Pages 492-496, ISSN 0883-5403, http://dx.doi.org/10.1016/j.arth.2010.01.107.

7. Saari, T., Li, M.G., Wood, D. et al. Comparison of cementing techniques of the tibial component in total knee replacement. International Orthopaedics (SICOT) (2009) 33: 1239. https://doi.org/10.1007/s00264-008-0632-x
8. Rousseau, MA., Lazennec, JY. & Catonné, Y. Early mechanical failure in total knee arthroplasty. International Orthopaedics (SICO (2008) 32: 53. https://doi.org/10.1007/s00264-006-0276-7
9. Khan, M., Osman, K., Green, G., & Haddad, F. S. (2016). The epidemiology of failure in total knee arthroplasty. Bone Joint J, 98-B(1 Supple A), 105-112. Accessed August 11, 2017.https://doi.org/10.1302/0301-620X.98B1.36293.
10. Bozic, K.J., Kurtz, S.M., Lau, E. et al. The Epidemiology of Revision Total Knee Arthroplasty in the United States. Clin Orthop Relat Res (2010) 468: 45. https://doi.org/10.1007/s11999-009-0945-0
11. Katrin Nagel, Nicholas E. Bishop, Ulf J. Schlegel, Klaus Püschel, Michael M. Morlock, The Influence of Cement Morphology Parameters on the Strength of the Cement-Bone Interface in Tibial Tray Fixation, The Journal of Arthroplasty, Volume 32, Issue 2, 2017, Pages 563-569.e1, ISSN 0883-5403, http://dx.doi.org/10.1016/j.arth.2016.08.013.
12. Sugita T, Umehara J, Sato K, Inoue H. Influence of Tibial Bone Quality on Loosening of the Tibial Component in Total Knee Arthroplasty for Rheumatoid

Arthritis: Long-Term Results. ORTHOPEDICS. 1999; 22: 213-215. doi: 10.3928/0147-7447-19990201-10

13. Anne Gebert de Uhlenbrock, Vesna Püschel, Klaus Püschel, Michael M. Morlock, Nicholas E. Bishop, Influence of time in-situ and implant type on fixation strength of cemented tibial trays — A post mortem retrieval analysis, Clinical Biomechanics, Volume 27, Issue 9, 2012, Pages 929-935, ISSN 0268-0033, http://dx.doi.org/10.1016/j.clinbiomech.2012.06.008.
14. Rossi, R., Bruzzone, M., Bonasia, D.E. et al. No early toboa; tray loosening after surface cementing technique in mobile-bearing TKA. Knee Surg Sports Traumatol Arthrosc (2010) 18: 1360. https://doi.org/10.1007/s00167-010-1177-2
15. Billi et al. Factors Influencing the Strength of the Tibial Tray-PMMA Cement Bond. Orthopaedic Research Society. 2014, poster 1854
16. Helwig P, Konstantinidis L, Hirschmüller A, Miltenberger V, Kuminack K, Südkamp NP, Hauschild O. Tibial cleaning method for cemented total knee arthroplasty: An experimental study. Indian J Orthop [serial online] 2013;47:18-22. http://www.ijoonline.com/text.asp?2013/47/1/18/106887
17. Rand J, Trousdale R, Ilstrup D, Harmsen S. Factors Affecting the Durability of Primary Total Knee Prostheses. The Journal of Bone and Joint Surgery. Volume 85-A, Number 2, 2003. Pages 259-25.
18. McTighe T, Clarke I. Failure Mechanism on Total Knee Arthroplasty. Joint Implant Surgery and Research Foundation. Chagrin Falls, Ohio, USA. July, 2009.

19. Thiele K, Perka C, Matziolis G, Otto Mayr H, Sostheim M, Hube R. Current Failure Mechanisms After Knee Arthroplasty have Changed: Polyethylene Wear is Less Common in Revision Surgery. The Journal of Bone and Joint Surgery. Volume 97-A, Number 9, 2015. Pages 715-720.
20. Scuderi G, Insall J, Windsor R, Moran M. Survivorship of Cemented Knee Replacements. The Journal of Bone and Joint Surgery. Volume 71-B, Number 5, 1989. Pages 798-803.
21. Peters C, Mohr R, Craig A, Bachus K. Tibal Component Fixation with Cement: Full versus Surface Cementation Techniques. Orthopaedic Research Society. Session 36. February 2001.
22. Schlegel et al.: Effect of tibial tray design on cement morphology in total knee arthroplasty. Journal of Orthopaedic Surgery and Research 2014 9:123.
23. Lavernia C, Contreras J. Knee arthroplasty: growing trends and future problems. International Journal of Rheumatology. 2010. Volume 5. Number 5. Pages 565-579. https://doi.org/10.2217/IJR.10.49
24. A. Wood, A. Keenan, C. Arthur, S. Aitken, P. Walmsley and I. Brenkel, "The Functional Outcome of Total Knee Replacement in Young Patients: A 10-Year Matched Case Control Study," *Open Journal of Orthopedics*, Vol. 3 No. 2, 2013, pp. 128-132. doi: 10.4236/ojo.2013.32024.
25. Williams D, Garbuz D, Masri B. Total knee arthroplasty: Techniques and results. British Columbia Medical Journal. Volume 52. Number 9. November, 2010. Pages 447-454.

26. Sharkey PF1, Lichstein PM2, Shen C2, Tokarski AT2, Parvizi J2. 2014. Why are total knee arthroplasties failing today--has anything changed after 10 years? J Arthroplasty. 2014 Sep;29(9):1774-8. doi: 10.1016/j.arth.2013.07.024. Epub 2014 Jul 5.

27. Canalle T: *Campbell's Operative Orthopaedics (10th Edition).* Mosby, St Louis, USA, 246 (2003).

28. Kurtz S, Ong K, Lau E, Mowat F, Halpern M. Projections of primary and revision hip and knee arthroplasty in the United States from 2005 to 2030. J Bone Joint Surg [Am] 2007;89-A:780–785.

29. Price AJ, Longino D, Rees J, et al. Are pain and function better measures of outcome than revision rates after TKR in the younger patient? Knee 2010;17:196–199.

30. Canalle T: *Campbell's Operative Orthopaedics (10th Edition).* Mosby, St Louis, USA, 246 (2003).

31. Dalury, D. F., Pomeroy, D. L., Gorab, R. S., & Adams, M. J. (2013). Why are total knee arthroplasties being revised? *Journal of Arthroplasty*, *28*(8 SUPPL), 120–121. https://doi.org/10.1016/j.arth.2013.04.051

32. Ranawat, A. S., & Ranawat, C. S. (2012). The history of total knee arthroplasty BT. The Knee Joint: Surgical Techniques and Strategies, (26), 699–707. https://doi.org/10.1007/978-2-287-99353-4_63

33. DePuy Synthes. (2015). Guidance for Cementing Primary Total Knee Replacements Cementing Primary Total Knee Replacements. (n. d.).

34. American Joint Replacement Registry. (2018). Fifth AJRR Annual Report on Hip and Knee Arthroplasty Data 2018. *American Joint Replacement Registry*, 319. https://doi.org/1.1

35. Clary, C., Aram, L., Deffenbaugh, D., Heldreth, M. (2014). Tibial base design and patient morphology affecting tibial coverage and rotational alignment after total knee arthroplasty. *Knee Surgery, Sports Traumatology, Arthroscopy*, *22*(12), 3012–3018. https://doi.org/10.1007/s00167-014-3402-x

Appendix A: Peak pull-off force (PO N) mean and standard deviation comparisons among cementation technique cohorts. Control specimens consisted of the contralateral knee with Attune standard prep. system, Motion applied, and cement applied to the bone only.

Study 1: Surgical Technique

	STD + BH	LTL + BO	LTL + BH
Exp. PO (N)	1686 ± 790	2921 ± 1403	3021 ± 737
Control PO (N)	2371 ± 1401	2593 ± 379	1877 ± 1279
T-test (P<0.05)	0.4271	0.6668	0.1721
Exp C (%)	87.8 ± 11.6	68.6 ± 16.2	82.8 ± 4.6
Control PO (%)	72.2 ± 21.8	85.3 ± 7.1	83.4 ± 6.9
T-test (P<0.05)	0.3833	0.1543	0.9177

BO: Cement on bone Only

BH: Cement on both bone and implant

STD: Standard Prep. (Clearance)

LTL: Line-Line (No Clearance)

Control: Contralateral Knee- Attune, Motion, STD, BO

Appendix B: Implant specific peak pull out forces (PO N) and lipid contamination (C %) metrics between No-Motion (NM) and Motion (M) cohorts. Green cells indicate significant difference between No-Motion and Motion cohorts (p-value<0.05). Red cells indicate bone fractures and sample size of n=1, green cells indicate statistical significance. All specimens with the Attune S+ with the line-line instrumentation, Persona, and Triathlon No-Motion implants failed at the cement-bone interface and therefore no lipid metrics were calculated. Two out of three specimens which received the GenesiII No-Motion protocol, NexGEN No-Motion protocol, and Triathlon Motion protocol failed at the cement-bone interface and therefore no standard deviation is reported.

Study 2: Implant Specific Metrics

	Attune STD	Attune LTL	S+ STD	S+ LTL	Stem	GenesisII	NexGen	Persona	Sigma	Triathlon	Vanguard
NM PO (N)	4000 ± 1490	3918 ± 484	6355 ± 1411	6342 ± 905	4955 ± 682	3720 ± 549	5325 ± 1129	6373 ± 24164	3659 ± 1818	3987 ± 509	3129 ± 1122
M PO (N)	1407 ± 918	3768 ± 1255	2330 ± 847	5859 ± 1003	3424 ± 551	3184 ± 919	4589 ± 191	5873 ± 2168	2346 ± 706	3834 ± 800	1840 ± 1253
Stat (P)	0.0046	0.8562	0.0133	0.569	0.0389	0.435	0.3276	0.8026	0.3082	0.7932	0.2551
NM C (%)	47.5 ± 15.7	45.6 ± 5.6	47.0 ± 26.1	N/A	76.0 ± 13.3	56.1	86.54	N/A	70.1 ± 26.1	N/A	53.3 ± 0.8
M C (%)	86.9 ± 14.8	55.8 ± 23.8	96.2 ± 2.4	N/A	83.57 ± 3.6	65.2 ± 18.3	84.1 ± 6.2	N/A	96.15 ± 3.6	86.94	72.2 ± 17.3
Stat (P)	0.0002	0.5112	0.038	N/A	0.3934	0.7101	0.7655	N/A	0.1613	N/A	0.2382

Statistical Significance (P<0.05)

n=1

Appendix C: Peak pull-off force (PO N) and lipid contamination (C %) metrics of all specimens grouped together across all experimental cohorts. Green cells indicate significance between Motion and No-Motion cohorts (p-value<0.05).

Study 2: All Specimens

	Total	Cone	Plateau	Q1	Q2	Q3	Q4	Prox	Mid	Dist
NM C (%)	57.78 ± 19.2	25.2 ± 13.9	32.5 ± 10.8	65.4 ± 32.3	66.7 ± 29.8	72.1 ± 30.2	73.8 ± 25.3	47.7 ± 26.9	40.7 ± 34.4	58.1 ± 34.2
MO C (%)	80.99 ± 17.4	41.7 ± 11.5	39.3 ± 9.4	81.2 ± 22.4	86.9 ± 20.7	78.2 ± 28.2	89.8 ± 17.3	75.4 ± 24.0	76.8 ± 24.9	89.2 ± 24.4
T-test (P<0.05)	7.10E-05	4.90E-05	0.025	0.051	0.0079	0.4733	0.012	4.99E-04	1.12E-04	6.23E-04
NM PO (N)	4647 ± 1589									
MO (N)	3322 ± 1753									
T-test (P<0.05)	0.0013									

Appendix D: Peak pull-off force (PO N) and lipid contamination (C %) metrics of all specimens grouped via broach clearance definition via laser scanning metrology (Clearance vs. No-clearance). Green cells indicate significance between Motion and No-Motion

Study 2.5: Clearance vs. Interference

Clearance (Standard Prep)

	PO		Total	Cone	Plateau	Q1	Q2	Q3	Q4	Prox	Mid	Dist
NM PO (N)	4503 ± 1800	**NM C (%)**	54.2 ± 20.3	24.8 ± 13.5	29.3 ± 11.4	55.9 ± 30.7	53.8 ± 27.6	69.3 ± 26.9	67.1 ± 26.8	50.1 ± 29.3	36.9 ± 32.2	58.4 ± 33.4
MO PO (N)	1873 ± 917	**MO C (%)**	91.5 ± 10.8	46.6 ± 6.8	44.9 ± 5.8	94.7 ± 8.6	94.5 ± 12.3	90.8 ± 19.8	98.4 ± 3.9	87.7 ± 15.4	85.3 ± 14.6	97.1 ± 6.2
T-test (P<0.05)	1.73E-04	**T-test (P<0.05)**	3.773-5	1.55E-04	7.96E-04	7.20E-04	2.97E-04	0.0529	0.0011	0.0015	2.44E-04	0.0013

No Clearance (Interference)

(Line-Line Prep)

	PO		Total	Cone	Plateau	Q1	Q2	Q3	Q4	Prox	Mid	Dist
NM PO (N)	4719 ± 1509	**NM C (%)**	61.4 ± 16.1	25.7 ± 13.5	35.7 ± 8.4	61.4 ± 16.11	74.9 ± 29.2	79.5 ± 24.5	74.8 ± 31.6	45.3 ± 22.5	44.5 ± 34.3	57.9 ± 33.3
MO PO (N)	4046 ± 1621	**MO C (%)**	73.1 ± 16.8	38.0 ± 12.5	35.1 ± 9.2	73.1 ± 23.5	81.3 ± 23.2	68.7 ± 28.9	83.3 ± 19.8	66.2 ± 24.5	70.4 ± 28.1	83.2 ± 29.8
T-test (P<0.05)	0.1438	**T-test (P<0.05)**	0.1039	0.0319	0.8718	0.7268	0.8611	0.633	0.7341	0.0468	0.0557	0.065

Statistical Significance (P<0.05)