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EFFECT OF ARTHROSCOPIC CARTILAGE DEFECT REPAIR WITH BONE MARROW DERIVED CELLS ON THE LUBRICANT PROPERTIES OF SYNOVIAL FLUID

A thesis submitted in partial satisfaction of the requirements for the degree Master of Science in Bioengineering

by

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2011
The thesis of Murray J. Grissom is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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Chair

University of California, San Diego

2011
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Selected Abstracts


Articular cartilage is a connective tissue covering the ends of long bones in synovial joints that facilitates low-friction joint articulation. In the case of high loads and low sliding velocities, lubrication of synovial joints occurs in a boundary, surface-to-surface contact mode, which is governed by interactions of biomolecules, such as hyaluronan (HA) and proteoglycan-4 (PRG4), with cartilage. Injuries increase the risk of osteoarthritis (OA) at a later age, but the mechanism by which this occurs is unclear. While the lubricating ability of synovial fluid in the boundary mode becomes abnormal following injury, the time-dependent changes in SF lubricating and biochemical properties following arthroscopic treatment of acute injury is unknown.
Thus, the aim of this study is to investigate the changes in SF lubricating ability and biochemical composition, as joints transition from normal to injury and repair states, and whether restoration of deficient lubricant molecules can restore normal lubricating ability.

Initially normal lubricating ability, indicated by a low steady-state friction coefficient, of SF became deficient shortly following injury, but returned to normal by a longer time post-surgery. These changes were correlated with a decrease in the concentration of HA and a shift in HA towards lower molecular weight (MW) forms. Furthermore, these changes were observed independent of the type of articular cartilage repair treatment applied. Supplementation of deficiently lubricating SF samples with high MW HA restored the boundary lubricating ability, indicating the importance of HA as a lubricant molecule and also suggesting its potential therapeutic ability.
CHAPTER 1

INTRODUCTION

1.1 General Introduction to the Thesis

Healthy synovial fluid (SF) facilitates joint movement by reducing friction and wear of articular cartilage surfaces [1, 2]. These properties work to maintain healthy, intact cartilage, which is important for bearing high loads and minimizing contact stresses in the joint [3]. In the case of high loads and low sliding velocities, the proportion of load supported by interstitial fluid pressurization of synovial fluid subsides, and lubrication occurs in the boundary mode, which is governed by interactions of biomolecules with cartilage [1, 4, 5]. Hyaluronan (HA) and proteoglycan-4 (PRG4) are putative lubricant molecules that have been shown to lower friction between articulating cartilage surfaces both individually and in combination [6]. Injuries to a joint may not only cause acute cartilage damage, but may also lead to long term degradation if the lubricating ability of SF becomes compromised. The relation between diminished lubrication and altered biochemical composition of SF remains to be well-established.

The overall motivation of this thesis work was to contribute to understanding of the changes in biochemical and lubricating properties of synovial fluid as joints recover from an injured state, as well as to expand on knowledge of counteracting
impaired lubrication. The objectives of this work were to thus characterize the lubricating ability and biochemical composition of normal, injury, and repair synovial and to utilize that information to investigate counteracting deficiently lubricating samples. Towards this goal, SF was analyzed for its boundary lubrication properties and for its concentration of protein, HA, and PRG4, as well as molecular weight (MW) distribution of HA. Deficiently lubricating samples were supplemented with high MW HA to investigate the effect of addition of exogenous, functional HA towards lubrication properties.

1.2 Structure, Composition, and Function of the Synovial Joint

Key features of synovial joints are: 1) articular cartilage, which supports high loads [3] and facilitates low-friction and low-wear joint articulation [7], 2) synovial fluid, a viscous fluid that contains the putative lubricant molecules HA and PRG4, and 3) encapsulation by the synovium, a thin lining of the joint that contains synoviocytes that synthesize and secrete HA [8, 9] and PRG4 [10, 11]. Muscles, ligaments, and tendons provide support and stability for motion.

Articular cartilage can withstand compressive forces several times one’s body weight [12, 13], but over a lifespan, cartilage-on-cartilage articulation becomes a biomechanical challenge [14]. Articular cartilage is a multiphasic tissue, with a fluid water phase (68-85% wet weight) and solid collagen (10-20% wet weight) and proteoglycan (5-10% wet weight) phases [15-20]. Proteoglycans have a high osmotic, or swelling, pressure due to their high density of negative charges, allowing hydration of cartilage under high loads [17, 18, 21, 22]. On the other hand, the collagen network of cartilage counteracts this swelling by withstanding high tensile loads to maintain the integrity of cartilage and allow for normal cartilage function [17, 18]. The high
fixed charged density of cartilage creates an osmotic pressure difference between SF and cartilage, due excess macromolecules and small ions [18]. The distribution of solutes between cartilage and external solution has been shown to be MW-dependent, where the distribution decreases with increasing MW such that the largest molecules able to penetrate cartilage are near the size of hemoglobin (MW~65kDa) [23].

With increasing age, the integrity of cartilage deteriorates, with increasing progression of tissue degeneration and osteoarthritis (OA), a degenerative joint disease that affects ~20 million Americans with an economic impact of $60 billion annually [24, 25]. OA cartilage is characterized by fissures or fibril structure [26], low glycosaminoglycan content [27], reduced collagen integrity [28], and softness in compression [29], resulting in reduced function. This painful and debilitating disease presents a major medical challenge and requires improved treatment and/or prevention.
1.3 Mechanisms of Synovial Joint Lubrication

Various theories have been developed on the mechanisms of synovial fluid lubrication (Figure 1.1). The factors that determine which mechanism may be dominant are the normal and tangential forces on articular surfaces and the history of loading and motion [30, 31]. General classifications for the different physicochemical modes of lubrication are fluid pressure/film or boundary [3, 32].

Types of fluid pressure/film lubrication modes include hydrostatic (Figure 1.1A), elastohydrodynamic (Figure 1.1B), squeeze film (Figure 1.1C), and boosted lubrication (Figure 1.1D). Hydrostatic pressure mode typically occurs at the onset of loading and for a prolonged duration, as the interstitial fluid within cartilage pressurizes due to its biphasic nature and also if fluid is forced into the asperities between articulating surfaces through a weeping mechanism [33]. This mode allows for significant bearing of normal load with resistance to shear [3]. Other lubrication modes that occur at the onset of motion/loading are elastohydrodynamic, where pressure in the viscous film between surfaces causes elastic deformation of articular surfaces, and squeeze film, where the viscous lubricant is driven from beneath the articulating surfaces under a normal force. In boosted lubrication, trapped lubricant in pressurized pools contribution to the separation of articulating surfaces [2, 3].

In the boundary lubrication mode (Figure 1.1E), articulating surfaces come into contact, and lubrication is mediated by the properties of surface lubricant molecules. This mode is thought to be where most the friction of articulating surface occurs, corresponding to ~10% of the total area of articulating surfaces [34]. As load time increases and hydrostatic pressure subsides, the lubricant-coated articular surfaces bear increasingly higher portions of load relative to the pressurized fluid, and the boundary mode becomes more dominant [33, 35]. Wear patterns of articular
cartilage suggest that boundary lubrication is critical towards protecting the integrity of the articular surface [36].

A number of factors influence which lubrication mode is dominant. When lubricant film may flow between sliding surfaces that may deform elastically, elastohydrodynamic lubrication occurs. Other important factors include pressure, surface roughness, and relative sliding velocity. When velocity decreases, lubricant films adhere to articulating surfaces and a mixed lubrication regime occurs; for low velocities, a very thin layer only a few molecules thick remains, and boundary lubrication is dominant.

Boundary mode lubricating ability may be quantified by a friction coefficient (\(\mu\)), which is a ratio of the frictional force between two articulating surfaces and the normal force. A protocol recently developed in our lab [6], based on previous work by Davis [37, 38] and Fung and Malcolm [39, 40], allows calculation of the boundary mode friction coefficient from an annulus-on-disk rotational test configuration. While many different in vitro mechanical test systems have been developed for analyzing boundary lubrication, each has its advantages and disadvantages. Both latex-on-glass [37, 41-43] and cartilage-on-glass [5, 44, 45] systems may be easily manipulated for reproducibility and may mimic some, but not likely all, of physiological molecular interactions of articulating surfaces. Whole-scale cartilage-on-cartilage tests retain realistic and complex geometry, but many modes of lubrication are likely operative [46, 47]. Cartilage-on-cartilage lubrication tests lack this complex geometry, but retain a physiological test surface that may be tested under conditions favoring the boundary lubrication mode. At relatively low effective sliding velocities in a rotational configuration, interstitial fluid pressure may dissipate and plowing friction losses may be minimized. Utilizing this cartilage-on-cartilage test, molecular interactions that
occur in physiological articulation may be observed, and the lubricating ability
normal, injury, and repair SF may be characterized.
Figure 1.1: Modes of lubrication of articular cartilage (modified from [32]).
1.4 Molecular Mechanisms of Articular Cartilage Boundary Lubrication by Synovial Fluid

Several molecules present in high concentrations in SF [48-51] that adsorb to the articular surface of cartilage include PRG4 (Figure 1.2A) [52], surface active phospholipids (Figure 1.2B) [53], and HA (Figure 1.2C) [54], all of which have been hypothesized to function as lubricant molecules. Whether these molecules contribute towards lubrication has been a subject of debate for decades.

Lubricin/SZP/PRG4 (which collectively will be referred to as PRG4 here) has contributed towards boundary lubrication for cartilage-on-glass [55-57], latex-on-glass [11, 58-62], and cartilage-on-cartilage interfaces [63]. O-linked β(1-3)Gal-GalNAc oligosaccharides within a large mucin like domain of 940 amino acids [64], encoded for by exon 6, were subsequently shown to mediate, in part, this boundary lubricating ability [60]. Mutations in the prg4 gene that encodes PRG4 causes camptodactyly-arthropathy-coxa vara-pericarditis (CACP) disease syndrome in humans, which results in camptodactyly, noninflammatory arthropathy, and hypertrophic synovitis, with coxa vara deformity, pericarditis, and pleural effusion [65]. PRG4-null mice exhibit cartilage deterioration and subsequent joint failure [66]. Thus, PRG4 is an essential component of healthy synovial joints.

SAPL are synthesized and secreted by type B synoviocytes [67, 68] and are present at the articular surface predominantly as phosphatidylcholines, dipalmitoyl phosphatidylcholine (DPPC) [51, 69, 70]. While a slight reduction in friction was observed when DPPC was tested at a cartilage-steel interface [71] and friction was considerably reduced when a high concentration of phosphatidylcholine was tested at a latex-glass interface [72], friction was not significantly lowered when a physiological level of DPPC was tested at a cartilage-on-cartilage interface [63].
However, the role in boundary lubrication of other phospholipid species present in SF, such as phosphatidylcholines, phosphatidylethanolamines, and sphingomyelin, remains to be determined.

HA is a multi-functional high MW polysaccharide built from repeating disaccharide units with the structure \([\text{D-glucuronic acid } (1-\beta-3) \text{ N-acetyl-D-glucosamine } (1-\beta-4)]^n [73]\). The molecular weight of HA is usually several hundred thousand to millions, reaching up to a molecular mass of \(10^6–10^7\) Da [74-76]. The contribution of HA towards boundary lubricating properties also remains variable. Trapped HA may contribute to a load bearing function [77, 78]. Whole joint apparatuses in which mixed modes of lubrication were present have reported HA to be both effective [79, 80] and ineffective [81-84] as a boundary lubricant. HA has also reduced friction between latex-glass [59] and cartilage-cartilage [63] interfaces.
**Figure 1.2:** Putative articular cartilage boundary lubricants present at the articular surface. Vertical section showing (A) PRG4 in a thin layer at the articular surface and in superficial zone chondrocytes, (B) oligolamellar structure (by TEM [85]) typical of SAPL, and (C) HA in a thin layer at the articular surface [86].
1.5 Altered Lubrication and Synovial Fluid Composition in Health, Injury, and Disease

Diminished lubricating ability of acute injury SF, relative to normal, has been observed in SF obtained from: 1) acutely injured horses, tested in cartilage-on-cartilage lubrication test [87], 2) rabbits following ACL transection (ACLT), tested under a cartilage-on-glass system [41], 3) patients with knee joint synovitis, tested under a latex-on-glass system [42], and 4) guinea pigs following ACLT, tested under a whole-joint pendulum system [47]. With injury and disease the concentration of HA and PRG4 may also be modulated (Table 1.1).

For humans, normal synovial fluid concentration of HA (C_HA) and PRG4 (C_PRG4) are in the range of 1.5-4.0 mg/ml [88-90] and 50-350 µg/ml [91, 92], respectively. In acutely injured patients’ synovial fluid (AI-SF), there are studies reporting C_HA ranges of 2.4-3.0 mg/ml [42, 93], not significant from normal. For AI-SF C_PRG4, there are both studies showing C_PRG4 to be lower than normal, approximately 50 µg/ml shortly after ACL injury [91], and also higher than normal, in ranges of 280-760 µg/ml for patients undergoing arthrocentesis [92]. In comparison, C_HA in normal equine synovial fluid (eSF) is in the range of 0.26-1.3 mg/ml [87, 94-97], and in acutely injured horses there are both studies indicating an increase [87] and no change [95] in C_HA relative to normal.

The molecular weight (MW) distribution of HA has been reported as not different between AI vs. NL equine SF (eSF), between 2-3 MDa, but the method of high-performance liquid chromatography in this study resulted in an upper detection limit of 3 MDa [96]. Studies on human SF (hSF) able to detect HA above 3 MDa reported normal ranges between 6-7 MDa [89, 93], but these studies did not make statistical comparisons to AI-hSF.
Only a few studies have simultaneously investigated the mechanical and biochemical properties of AI-SF and NL-SF, allowing for correlations between lubricating ability and biomolecules. Decreased $C_{HA}$ was correlated with increased friction in an equine study [87], while diminished lubricating ability was correlated with decreased $C_{PRG4}$ in a rabbit study [41]. However, neither study investigated both $C_{HA}$ and $C_{PRG4}$. A thorough investigation of the lubricating and biochemical properties of synovial fluid as the joint transitions from normal to injury or repair states would extend upon this knowledge and further understanding of changes in the lubricating ability with injury and repair progression.

Many surgical procedures may also acutely alter the joint environment by making changes to the subchondral bone. Currently the most widely used treatment for cartilage repair in the knee is microfracture (MF) [98], which involves drilling small holes into the subchondral bone to stimulate bone marrow cells to enter the defect and form repair tissue [99]. However, the regenerated cartilage has a fibrous nature, which has load-bearing and wear-resistant properties inferior to those of native hyaline articular cartilage [100]. The current study also involves an alternative treatment (termed BMC) in which bone marrow aspirate is concentrated through a simple, cost-effective, and autogenous centrifugue procedure and is implanted into the cartilage defects [101]. Bone marrow aspirate concentrate has been shown to provide a source for isolation of MSCs capable of regenerating cartilage [102-104], and previous studies on BMC treatment in horses have suggested cartilage repair superior to MF [101]. However, since both of these treatments require an enlargement of the subchondral bone defect, it is possible that the lubrication quality of SF may become abnormal.
Table 1.1: Synovial fluid composition in health and disease. mean±SD [41, 42, 87-89, 92-97, 105]

<table>
<thead>
<tr>
<th>Synovial Fluid Constituent</th>
<th>Species</th>
<th>Concentration [ mg/ml ]</th>
<th>Molecular Weight (kDa)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NL</td>
<td>AI</td>
</tr>
<tr>
<td>Hyaluronan (HA)</td>
<td>human</td>
<td>1.5 – 4.0</td>
<td>2.4 – 3.0</td>
</tr>
<tr>
<td></td>
<td>equine</td>
<td>0.26 – 1.3</td>
<td>0.21 – 0.42</td>
</tr>
<tr>
<td>Proteoglycan-4 (PRG4)</td>
<td>human</td>
<td>0.05 – 0.35</td>
<td>0.28 – 0.76</td>
</tr>
<tr>
<td></td>
<td>rabbit</td>
<td>0.28±0.03</td>
<td>0.02-0.76</td>
</tr>
</tbody>
</table>
1.6 References


CHAPTER 2

EFFECT OF ARTHROSCOPIC CARTILAGE DEFECT REPAIR WITH BONE MARROW DERIVED CELLS ON THE LUBRICANT PROPERTIES OF SYNOVIAL FLUID

2.1 Abstract

Objective. To compare equine synovial fluid (eSF) obtained from horses undergoing treatment of full-thickness chondral defects by (1) microfracture (MF), (2) bone marrow cell injection (BMC), or (3) both MF+BMC on changes, relative to a pre-injury state (t=0d), at 10 days (t=10d) and 3 months (t=3mo) following surgery in (A) lubrication properties, (B) composition of putative boundary lubricant molecules hyaluronan (HA) and proteoglycan-4 (PRG4), and (C) correlations between lubrication and biochemical properties. High molecular weight (MW) HA was investigated for its ability to restore lubricating ability in deficiently lubricating samples.

Methods. Cartilage-on-cartilage boundary mode lubrication tests were performed to determine static ($\mu_{\text{static}}$) and kinetic ($\mu_{\text{kinetic}}$) friction coefficients for portions of eSF samples. The concentration and MW form of HA ($C_{\text{HA}}$) and PRG4 ($C_{\text{PRG4}}$) were determined for other eSF sample portions and were tested for influencing lubrication
properties by linear regression. Experimental groups that were deficient in lubrication function were tested further for the ability of exogenous high-MW HA to restore lubrication function.

**Results.** Lubrication and biochemical data varied with time after surgery but generally not between treatment groups. Relative to t=0d, $\mu_{\text{kinetic}}$ was 68% higher at t=10d but returned to baseline levels at t=3mo. Correspondingly, $C_{HA}$ was 45% lower and $C_{PRG4}$ was 510% higher in t=10d samples relative to t=0d levels while in t=3mo samples $C_{HA}$ (for MF and BMC groups) was 110% higher than t=0d levels and $C_{PRG4}$ returned to baseline levels. Kinetic friction coefficients decreased with increasing $C_{HA}$ and increased with increasing $C_{PRG4}$. Addition of 4 MDa HA to bring t=10d pools to $C_{HA}$=0.6 or 2.0 mg/ml restored normal lubricating ability.

**Conclusion.** The finding that SF function and composition is modulated over time following surgical repair of a cartilage defect generally independent of the type of repair suggests a common local response that adversely affects joint lubrication post-operatively. The functional deficiency in SF lubrication can be counteracted in vitro by addition with high-MW HA.
2.2 Introduction

In healthy synovial joints, friction between articulating cartilage surfaces is diminished through various lubrication mechanisms, including boundary mode surface-to-surface contact \[1, 2\]. The lubricating ability in the boundary mode is governed by interactions of synovial fluid (SF) biomolecules with the cartilage and occurs in the case of high loads and low sliding velocities, as the proportion of load supported by interstitial fluid pressurization of SF subsides \[1, 3, 4\]. When this lubricating ability is diminished, there is evidence suggesting increased cartilage wear \[5, 6\] and shear strain \[7\], which in turn may regulate lubricant \[8, 9\] and matrix \[10\] metabolism. Such diminished lubricating ability has been observed in SF following acute injuries in equine \[11\], human \[12\], rabbit \[6\], and guinea pig \[13\] studies, indicating that articular cartilage may be susceptible to degradation following injury. Investigating pathways to reverse this lubrication deficiency following injury would thus require characterization of SF lubrication properties and the biomolecules that modulate them.

Previous investigations of cartilage-on-cartilage boundary lubrication have identified proteoglycan-4 (PRG4) and hyaluronan (HA) as putative lubricant molecules through observations of decreased friction between saline and cartilage following supplementation of saline with combinations of PRG4 and HA \[14\]. Given differences in experimental designs (i.e. type of species, joint, or injury) and biochemical methods between past studies on HA and PRG4 content in SF, different trends between normal (NL) vs. acute injury (AI) SF biochemical composition have
been reported. The concentration of HA (C_{HA}) in AI-SF has both been reported as lower [11, 15] and not different [16-18] from C_{HA} for NL-SF. The molecular weight (MW) distribution of HA has been reported as not different between AI vs. NL equine SF (eSF), between 2-3 MDa, but the method of high-performance liquid chromatography in this study resulted in an upper detection limit of 3 MDa [18]. Studies on human SF (hSF) able to detect HA above 3 MDa reported normal ranges between 6-7 MDa [19, 20], but these studies did not make statistical comparisons to AI-hSF. The concentration of PRG4 (C_{PRG4}) in AI-SF has both been reported as lower [6, 13, 21] and as higher [22] than in NL-SF.

Only a few studies have simultaneously examined the mechanical and biochemical properties of AI-SF and NL-SF, allowing for investigation of correlations between lubricating ability and biomolecules. Decreased C_{HA} was correlated with increased friction in an equine study [11], while diminished lubricating ability was correlated with decreased C_{PRG4} in a rabbit study [6]. However, neither study investigated both C_{HA} and C_{PRG4}. A thorough investigation of the lubricating and biochemical properties of synovial fluid as the joint transitions from normal to injury or repair states would extend upon this knowledge and further understanding of changes in SF lubricating ability with injury and repair progression.

Such alterations in lubricating ability may be expected following cartilage defect repair treatments, since many procedures may alter the joint environment by making changes to the subchondral bone. Currently the most widely used treatment for cartilage repair in the knee is microfracture (MF) [23], which involves drilling small holes into the subchondral bone to stimulate bone marrow cells to enter the
defect and form repair tissue [24]. However, the regenerated cartilage has a fibrous nature, which has load-bearing and wear-resistant properties inferior to those of native hyaline articular cartilage [25]. The current study also involves an alternative treatment (termed BMC) in which bone marrow aspirate is concentrated through a simple, cost-effective, and autogenous centrifuge procedure and is implanted into the cartilage defects following MF treatment [26]. Bone marrow aspirate concentrate has been shown to provide a source for isolation of MSCs capable of regenerating cartilage [27-29], and previous studies on BMC treatment in horses has suggested cartilage repair superior to MF [26]. However, since both of these treatments require an enlargement of the subchondral bone defect, it is possible that the lubrication quality of SF may become abnormal.

The objective of this study was to evaluate both time-dependent and treatment-dependent changes in lubricating and biochemical properties of synovial fluid aspirated from horses undergoing MF, BMC, or MF+BMC treatment of experimentally induced full-thickness cartilage defects. Our hypothesis was that initially normal lubricating ability would be diminished shortly following surgery and return to normal by a relatively longer time post-surgery and these changes in lubrication properties would be associated with changes in biochemical composition.
2.3 Materials and Methods

Materials. Lubrication testing materials are as described previously [2]. Additionally, hyaluronan (HA) was obtained as 4,000 kDa (Healon®, Advanced Medical Optics, Santa Ana, CA). The antibody to PRG4 was anti-Lubricin from AbCam (Cambridge, MA); non-specific rabbit IgG was from Pierce (Rockford, IL); mouse anti-rabbit IgG secondary antibody was from Jackson ImmunoResearch (West Grove, PA). *Streptomyces hyaluronidase* was from Seikagaku (Tokyo, Japan). SeaKem® gold agarose was from Lonza (Rockland, ME); 50X TAE (2M Tris, 0.5M EDTA) electrophoresis buffer was from Life Technologies (Carlsbad, CA); Hybond™-P polyvinylidene difluoride (PVDF) membrane for Western blotting was from GE Healthcare (Piscataway, NJ). Stains-All was from Sigma-Aldrich (St. Louis, MO).

Synovial Fluid Samples. Equine SF: With IACUC approval, one of the authors (LAF) created bilateral experimental cartilage defects (15 mm in diameter) extending down to, but not through, the subchondral bone in the mid-lateral trochlear ridge of adult horses (2-6 y.o., n=12). Bone marrow aspirate concentrate was autogenously isolated from the sternum of horses, as described elsewhere [26]. For each horse, one stifle joint was treated with MF (n=12) while the contralateral was treated with either BMC injection (n=8), as described elsewhere [26], or MF with BMC (MF+BMC) injection (n=4) (Figure 2.1). From each of these two joints, eSF was aspirated at different times, at day 0 (the pre-injury state, 0d), at 10 days (10d) and at 3 months (3mo) following surgery, and the total eSF volume aspirated was
noted. The eSF was clarified by centrifugation (3,000g, 30min, 4°C) and stored at −80°C before subsequent analysis.

**Experimental Design.** To determine functional and compositional differences between eSF samples as a result of treatment (BMC, MF, MF+BMC) or duration (t=0d, 10d, 3mo) post-surgery, biomechanical lubrication tests and biochemical analyses were performed. Lubrication tests assessed the friction-lowering properties of eSF sample portions in the boundary lubrication mode. Other portions were analyzed for their concentration of total protein, PRG4, HA, as well as MW distribution of HA. Univariate and multivariate linear regression analyses were performed to examine the influence of biochemical composition on lubrication function. Based on deficient lubricating properties and lower concentration of HA in t=10d samples, pools (n=8) of t=10d eSF were supplemented with high MW HA (Healon®) to 0.6 mg/ml (physiological) and 2.0 mg/ml (more typical of C_HA following therapeutic injection of HA [30]) to develop a dosage response. Pools were made from either MF (n=4) or “BMC” (n=4) individual samples, where “BMC” contained both BMC and MF+BMC samples. Given limited eSF volume and similarity between BMC and MF+BMC lubrication properties (and also between biochemical properties measured in this study), pooling of BMC with MF+BMC (“BMC” group) for the HA supplementation study was justified.

**Lubrication Test.** Portions of eSF were analyzed for start-up (static, \( \mu_{\text{static}} \)) and steady-state (kinetic, \( \mu_{\text{kinetic}} \)) coefficients of friction as measures of boundary lubrication function in a cartilage-on-cartilage articulation test [2]. Intact articular surface pairs used were osteochondral cores and annuli (n=65 pairs) paired from the
same adult bovine knee (n=22 harvested), stored in phosphate buffered saline (PBS) supplemented with protease inhibitors (PIs) (2 mM Na-EDTA, 1 mM PMSF, 5 mM Benz-HCL, and 10 mM NEM) at –80°C prior to testing. Osteochondral substrates were bathed in test lubricant for ~24h at 4°C prior to lubrication testing with 18% cartilage compression, an effective sliding velocity of 0.3 mm/second, and pre-spin pause time (T_{ps}) of 1.2, 12, and 120 seconds. Friction coefficients were calculated using the equilibrium axial load following 30-minute stress relaxation and peak torque (|τ|) measured either within the first 10° of the start of rotation, for μ_{static}, or from an averaged |τ| during steady-state sliding, for μ_{kinetic}. Consistent with previous work, μ_{kinetic} did not vary significantly with T_{ps}, thus μ_{kinetic} data is presented as the average for all T_{ps}. Substrates were screened with PBS+PIs as a negative control.

Biochemical Analyses of Lubricant Molecules. Other portions of eSF were assayed for the concentrations of protein, HA, and PRG4. Protein concentration (C_{Protein}) was determined by Bicinchoninic acid assay. PRG4 concentration (C_{PRG4}) was quantified by Western blot using anti-Lubricin antibody [31]. *Streptomyces hyaluronidase*-digested portions of eSF samples were run (0.5-2.0 µl/lane) on 2% agarose gel, transferred to a PVDF membrane, then the membrane was probed with anti-Lubricin antibody or non-specific rabbit IgG, followed by a mouse anti-rabbit light-chain specific secondary antibody conjugated to horseradish peroxidase, and then quantified by ECL-Plus detection and digital scanning with a STORM 840 Imaging System (Molecular Dynamics, Fairfield, CT). PRG4 concentrations were determined by analysis with ImageQuant software (Molecular Dynamics) and comparison to an equine PRG4 standard. HA concentration (C_{HA}) was quantified by
an ELISA-like assay using HA binding protein [32]. *HA MW distribution* was determined by horizontal agarose gel electrophoresis of proteinase K-digested portions of eSF samples (300 ng/lane) through a 1% agarose gel [33]. Gels were stained with Stains-all and imaged and processed to determine the distribution of HA in 7.0-2.5, 2.5-1.0, 1.0-0.5, 0.5-0.25, and 0.25-0.12 MDa ranges.

**Statistical Analysis.** Data are expressed as mean ± SEM. Left and right knee data were correlated by linear regression and slopes were compared to 1 by t-test. Similarity between left and right joints at t=0d− (pre-surgery state) demonstrated dependence of left and right knee measures at day 0, indicating t=0d− controls could at most have an n=12 (as opposed to n=24). Planned comparisons were to assess the effect of time post-surgery (0d−, 10d, 3mo) within each treatment group (MF, BMC, MF+BMC), and to assess the effect of treatment within each time post-surgery (10d, 3mo). Planned comparisons were made by unpaired t-tests because of decreased sample size resulting from missing cases when using a paired design. Correlation of friction data with C_{HA} and C_{PRG4} was determined using linear regression. The effect of HA supplementation to deficiently lubricating eSF was assessed by repeated-measures ANOVA, with pair-wise comparisons made by paired t-test. Bonferroni corrections were applied to multiple comparisons.
Figure 2.1: Adult horses (n=12) were treated for 15 mm diameter full-thickness chondral defects in the mid-lateral trochlear ridge with MF (n=12) in the right (R) or left (L) knee and either BMC (n=8) or MF+BMC (n=4) in the contralateral knee.
2.4 Results

**Left vs. right knee comparisons.** Left and right joints were similar for all assays at t=0d but in some cases became dissimilar by t=10d or t=3mo. As a representative example, correlation results for C_HA are shown in Figure 2.2. An initial similarity between left and right knee C_HA at t=0d (p=0.9) was lost by t=10d (p=0.02) but returned by t=3mo (p=0.08). Summarized results of the same analysis for all assays are given in Table 2.1. In general, biochemical assays showed a stronger correlation (higher R^2) than lubrication data, with the exception of C_HA between 1.0-0.12 MDa. The initial similarity between joints at t=0d indicated that in making time comparisons for each treatment, day 0 controls should at most have an n=12.

**Lubrication function of eSF.** Static (T_{ps}=120s) (Figure 2.3A) and kinetic (Figure 2.3B) friction coefficients of eSF did not show any treatment (Tx) effects within each time post-surgery (p=0.13-0.96). The kinetic friction coefficients at t=0d (before the repair procedures) averaged 0.029, and relative to that value, μ_kinetic for t=10d samples was +68% (each, p<0.01). μ_{static,T_{ps}=120s} tended to show a similar trend, but it was not significant (0.05<p<0.08). μ_{static,T_{ps}=120s} and μ_kinetic were +40% and +70% higher in t=10d samples, relative to t=3mo (each, p<0.05). Static and kinetic friction coefficients were similar to t=0d levels at t=3mo (p=0.20-0.87). Similar trends as those for μ_kinetic were observed for μ_{static} for T_{ps}=1.2s and 12s (data not shown).

**Biochemical analysis of eSF.** Aspirated eSF volume (Figure 2.4A) and concentration of total protein (Figure 2.4B), PRG4 (Figure 2.4C-D), and HA (Figure 2.4E) averaged 5.71 ml, 18.2 mg/ml, 9.82 μg/ml, 0.320 mg/ml at t=0d, respectively. Western blot for PRG4 demonstrated that C_{PRG4} varied with time post-surgery but
with no apparent description of structure (Figure 2.4C). Since $C_{PRG4}$ was similar between all Tx groups ($p=0.46-0.89$), the MF+BMC group is omitted in Figure 2.4C. Relative to normal ($t=0d$) levels, volume, $C_{Protein}$, and $C_{PRG4}$ levels were +69%, +93%, and +510% in $t=10d$ samples relative to $t=0d$ levels (each, $p<0.05$), respectively, and returned to normal levels by $t=3mo$ ($p=0.36-0.75$). Relative to $t=0d$ levels, $C_{HA}$ (Figure 2.4E) was -45% by $t=10d$ for all Tx groups and +110% by $t=3mo$ for groups MF and BMC (each, $p<0.05$), but was similar to MF+BMC at $t=3mo$ ($p=0.49$). With the exception of volume of MF+BMC group being +38% that for MF and BMC groups at $t=10d$ (each, $p<0.05$, Figure 2.4A) and $C_{HA}$ for MF being +84% that for MF+BMC at $t=3mo$ ($p<0.05$, Figure 2.4E), there were no other Tx effects observed within each duration post-surgery for volume, $C_{Protein}$, $C_{PRG4}$, and $C_{HA}$ ($p=0.25-0.93$, Figure 2.4).

HA MW analysis generally showed a shift, from normal, towards increasing levels of low MW $C_{HA}$ by $t=10d$ and increasing levels of high MW $C_{HA}$ by $t=3mo$ (Figure 2.5). Quantification of gel electrophoresis images of HA (Figure 2.5A) provided an HA MW distribution. Since HA MW percentage distribution (data not shown) was similar between all Tx groups within each time-point, MF+BMC group is omitted in Figure 2.5A. Relative to $t=0d$ levels, $C_{HA(2.5-7.0 \text{MDa})}$ (Figure 2.5B) was -73% by $t=10d$ for all Tx groups and +110% by $t=3mo$ for groups MF and BMC (each, $p<0.05$), but was similar to MF+BMC at $t=3mo$ (0.60). For $C_{HA(1.0-2.5 \text{MDa})}$ (Figure 2.5C), a time effect was only observed within the MF group, with $t=3mo$ levels being +85% and +180% than $t=0d$ and $10d$ levels, respectively (each, $p<0.05$); for all other time comparisons $p=0.10-0.33$. For $C_{HA(0.5-1.0 \text{MDa})}$ (Figure 2.5D), $t=10d$ levels were
+250% that for t=0d and for the MF group t=10d levels were also +170% that for t=3mo (each, p<0.05); for all other time comparisons p=0.11-0.86. For C_{HA(0.25-0.50 MDa)} (Figure 2.5E) and C_{HA(0.12-0.25 MDa)} (Figure 2.5F), t=10d levels were +400% and +21x that for t=0d, respectively, and for the MF and BMC groups t=10d levels were also +530% and +17x, respectively, that for t=3mo (each, p<0.05); for all other time comparisons p=0.10-0.69. For all MW ranges, within each time post-surgery no Tx effect was observed (p=0.12-0.95), except for C_{HA(2.5-7.0 MDa)} for MF at t=3mo being +95% that for MF+BMC at t=3mo (p<0.05, Figure 2.5B).

Univariate and multivariate linear regression analysis. Certain correlations between lubrication properties and eSF biochemical composition were supported by studying the effects on lubrication resulting from chemical augmentation of eSF with high MW HA (Figure 2.6-2.7). Univariate regression showed a decrease in $\mu_{\text{kinetic}}$ with decreasing C_{PRG4}, increasing C_{HA}, and increasing C_{HA(2.5-7.0 MDa)} (each, p<0.001, Figure 2.6A-C, respectively). Similar correlation trends were observed for $\mu_{\text{static}}$ (data not shown). Kinetic friction also decreased for decreasing C_{HA(0.25-0.50 MDa)} and C_{HA(0.12-0.25 MDa)} (each, p<0.001, data not shown), but there was not a significant correlation between $\mu_{\text{kinetic}}$ and C_{HA(1.0-2.5MDa)} or C_{HA(0.5-1.0 MDa)} (p=0.06 and 0.19, respectively). Multivariate regression of $\mu_{\text{static}}$ for T_{ps}=120s indicated static friction coefficients decreased with increasing C_{HA} and decreasing C_{PRG4} (each, p<0.005, Figure 2.6E). These multivariate regression trends were also observed for $\mu_{\text{static}}$ for T_{ps}=12s, but for $\mu_{\text{kinetic}}$ and $\mu_{\text{static}}$ for T_{ps}=1.2s friction coefficients were correlated with C_{PRG4} but not with C_{HA} (data not shown).
Pools (n=4 per MF and “BMC” [BMC & MF+BMC] group) of t=10d samples with kinetic friction coefficients initially +61% that for t=0d samples (p<0.001, Figure 2.7A) were supplemented with high MW HA to bring $C_{HA}$ to physiological ($C_{HA}=0.6$ mg/ml) and super-physiological ($C_{HA}=2.0$ mg/ml) levels, which reduced friction (p<0.05 and p<0.001, respectively) in a manner independent of treatment (p=0.22). Similarly, static ($T_{ps}=120s$) friction reduction at each supplemented $C_{HA}$ level was observed in “BMC” pools (each, p<0.05, Figure 2.7B) with $\mu_{static,T_{ps}=120s}$ initially +57% that for t=0d samples (p<0.01). However, $\mu_{static,T_{ps}=120s}$ for MF pools was not initially different from t=0d samples (p=0.91) and was also initially -37% that for BMC pools (p<0.01, Figure 2.7B). Thus, $\mu_{static,T_{ps}=120s}$ for MF groups was not affected by addition of high MW HA (p=0.16).
Figure 2.2: Linear regression of left knee eSF C$_{HA}$ (y-axis) vs. right knee C$_{HA}$ (x-axis). *slope vs. 1: p<0.05, †intercept vs. 0: p<0.05.

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Table 2.1: Summary of left knee vs. right knee data correlations for given assays at various time-points. Corresponding $R^2$ are given along with p-value for comparison of regression slope to 1. *p<0.05.

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<th>$H_{kinetic}$</th>
<th>C_HA</th>
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Figure 2.3: Effect of post-surgery duration on (A) static ($T_{ps}=120s$) and (B) kinetic friction coefficients for treatment groups (■) MF, (■) BMC, and (■) MF+BMC. Post-operative times are 0d, 10d, and 3mo. Data are mean ± SEM. n=4-12, *p<0.05.
Figure 2.4: Changes with time post-surgery in (A) eSF volume, (B) concentration of total protein, (C) representative PRG4 Western Blot images of eSF samples probed with an antibody to PRG4 in comparison to eSF standard, and concentration of (D) PRG4 and (E) HA for treatment groups (■) MF, (■) BMC, and (■) MF+BMC. Data are mean ± SEM. n=4-12, *p<0.05.
Figure 2.5: Changes with time post-surgery in (A) representative HA MW gel electrophoresis images of proteinase K digested eSF samples, and (B-F) concentration of HA for defined molecular weight bins for treatment groups (■) MF, (■) BMC, and (■) MF+BMC. Data are mean ± SEM. n=4-12, *p<0.05.
Figure 2.6: Univariate regression for steady-state friction coefficients versus (A) $C_{\text{PRG4}}$, (B) $C_{\text{HA}}$, and (C) $C_{\text{HA}(2.5–7.0 \text{ MDa})}$, and (D) multivariate regression for static ($T_{ps}=120s$) friction coefficients versus $C_{\text{HA}}$ and $C_{\text{PRG4}}$ for combined data ($n=64$) of treatment groups for all time-points ($t=0d–3mo$). Regression lines and regression plane are plotted for univariate and multivariate regression analyses, respectively.
Figure 2.7: Pair-wise comparison of (A) kinetic and (B) static (Tps=120s) friction coefficients for native t=10d eSF pools to: 1) the same pools supplemented with 4 MDa Healon to bring C_{HA} to final levels of 0.6 and 2.0 mg/ml and 2) μ_{kinetic} and μ_{static,Tps=120s} for t=0d individual samples, respectively. Data are mean ± SEM. n=4 for Native, 0.6 and 2.0 mg/ml groups, n=10-11 for t=0d groups. “BMC” pools contain both BMC and MF+BMC samples. †p<0.05, *p<0.01.
2.5 Discussion

This study indicates that synovial fluid lubrication function and biochemical composition are modulated over time following surgical repair of a cartilage defect, generally independent of the type of repair. To show this, it was important in the statistical design to consider the similarity between left and right joints in the normal, pre-injury state (Figure 2.2, Table 2.1). Boundary lubrication function was diminished shortly post-surgery (by t=10d) and returned to normal by a longer time post-surgery (by t=3mo), as indicated by increased steady state friction coefficients for t=10d samples, relative to t=0d and 3mo samples (Figure 2.3). This lubrication deficiency for t=10d samples is associated with an increase in volume (Figure 2.4A), concentration of protein (Figure 2.4B), and PRG4 (Figure 2.4C, 2.7) and decrease in concentration of HA and shift towards lower MW forms of HA (Figure 2.5, 2.7). High MW HA viscosupplementation of deficiently-lubricating t=10d samples to physiological and further elevated levels of C_{HA} resulted in full restoration of normal lubricating ability (Figure 2.7).

The experimental design allows for a well controlled study to investigate changes in the lubricating ability and composition of synovial fluid as it transitions from normal to injury and repair states, but is not without certain design limitations. Horses provide for a large animal-model and the stifle joint undergoes weight-bearing and active loads. Since comparisons between normal (t=0d), injury (t=10d), and repair (t=3mo) states are made within each animal, this study avoid issues of inter-animal variability inherent in a study making these comparisons across different populations of animals. The bilateral design ensured that similarities between left and right joints
in the initial pre-injury state would be considered in the statistical design so that sample size would not be incorrectly increased. However, throughout the duration of the study, there may be aging effects that were not investigated, but given the t=3mo duration of the study and the normal age of horses on the order of tens of years, this effect is likely to be insignificant. Additionally, without a sham surgery or sham experimental chondral defect, it is not clear whether duration post-surgery effects observed in this study were an effect of the defect, surgery, or arthroscopy. However, the focus of this study is to investigate the combined effect of defect and surgery, as would be relevant to clinical arthroscopic surgery. Lastly, the sample size of the MF+BMC group is limited (n=4) in comparison to the MF (n=12) and BMC (n=8) group. In particular, the low MF+BMC $C_{HA}$ value at t=3mo in turn affects the results of the $C_{HA}$ in defined MW bins, and this $C_{HA}$ value could possibly result from low sample size. On the other hand, the MF+BMC group results are generally consistent with the results from the MF and BMC groups for the other biochemical assays and lubrication tests and do not majorly alter the concluding statements of the study.

The finding of diminished lubrication shortly post-surgery is consistent with studies on AI-SF and the finding of naturally restored lubricating ability by t=3mo is a novel finding. The elevated t=10d coefficient of friction (COF) was consistent with observations of diminished lubricating ability, relative to a normal state, of synovial fluid obtained from: 1) acutely injured horses, tested under the same cartilage-on-cartilage lubrication test employed here [11], 2) rabbits following ACL transection (ACLT), tested under a cartilage-on-glass system [6], 3) patients with knee joint synovitis, tested under a latex-on-glass system [12], and 4) guinea pigs following
ACLT, tested under a whole-joint pendulum system [13]. The restoration of normal lubricating ability by a longer duration (t=3mo) post-surgery may be analogous to observations of normal lubricating ability of chronic injury eSF [11] and hSF from patients with various grades of OA [12, 34].

Differences in the friction coefficient values between those presented here and other work may be attributed to differences in lubrication testing system and protocol. The cartilage-on-cartilage test used here is advantageous in mimicking the molecular interactions occurring in a physiological articulation that may not occur in when artificial surfaces are used [2]. Thus, the trends observed in this study may expand upon previous studies on boundary lubrication of injury SF between non-cartilage interfaces. While equine cartilage was not used as a substrate for lubrication tests, the kinetic friction coefficient of normal (t=0d) eSF (µ kinetic=0.029) was similar to normal bovine synovial fluid (bSF) using bovine cartilage substrates (0.025) [14]. Use of PBS as a negative control for lubrication tests (data not shown) was consistent with previous values for kinetic friction coefficients for cartilage-on-cartilage tests (0.22 vs. 0.24) [14]. The t=0d kinetic friction coefficients were also comparable to previous results of steady-state friction coefficient for normal eSF using the same cartilage-on-cartilage lubrication test (0.024) [11] as well as to results from latex-on-glass lubrication tests for normal bSF (0.021) and human synovial fluid (hSF) from patients with degenerative joint disease (0.024), which were proposed to have normal lubricating ability [34].

More general joint characteristics of volume and concentration of total protein were also consistent with previous studies. The increased volume for t=10d eSF
samples was in agreement with observations of increased hSF volume, relative to normal, for patients with ACL tears combined or not combined with medial or lateral meniscal tears [35] and is also consistent with findings of largest increases in eSF volume occurring in cases of active synovitis [36], which may be expected shortly post-surgery. The concentration of protein in t=0d samples (C_{protein}=18.2 mg/ml) is comparable to documented values for normal horses (18.1 mg/ml) [37]. Increased C_{protein} for t=10d samples is consistent with increased protein concentrations, relative to normal, in eSF from horses with carpal chip fracture with articular cartilage injury [38]. Given the protein concentration of 35.7 mg/ml for t=10d samples, the accompanying finding of increased volume is consistent with the theory that abnormally high eSF protein concentrations between 25 – 40 mg/ml may be an indication of inflammation [36]. This theory may be supported by correlation of volume vs. C_{protein} for pooled t=0d-3mo data, which results in a significantly positive linear slope (p<0.001, R^2=0.22) (data not shown). Thus, characterization of protein concentration and volume is suggestive of joints becoming inflamed in response to the surgical treatment by t=10d.

Analysis of concentrations of putative lubricant molecules HA and PRG4 is variable with previous studies, but within the context of numerous differences between experimental design and methods. The HA concentration in t=0d samples (C_{HA}=0.32 mg/ml) was within the published range of 0.26-1.3 mg/ml for normal eSF [11, 17, 18, 39, 40]. The decrease in C_{HA} for t=10d samples was consistent with diminished HA concentrations for both eSF from acutely injured carpal or metacarpophalangeal joints [11] and hSF from patients with traumatic arthritis and
hydrarthrosis [15]. However, this trend was not in agreement with other eSF studies showing no change in $C_{HA}$, relative to normal, for horses: 1) undergoing fibrin treatment for an analogous experimental cartilage defect as the current study [16], 2) with acute traumatic synovitis [17], and 3) with acute traumatic arthritis [18]. The elevated PRG4 concentration in $t=10d$ samples, relative to $t=0d$ samples, is consistent with previous studies on hSF from patients undergoing arthrocentesis [22] and with observations of increased PRG4 synthesis for in vitro superficial zone cartilage when undergoing injurious compression [41]. However, an opposite trend was observed in studies on synovial fluid obtained from rabbits [6] and guinea pigs [13] following ACLT or from patients with ACL injury [21]. The differences in trends between normal vs. acute injury SF $C_{HA}$ and $C_{PRG4}$ may be explained by differences between experimental design, acute injury type, joint type, species, and method of quantifying HA content. Joint destabilization injuries, such as ACLT, may alter the mechano-environment [42] differently than the cartilage defect injuries here, which may result in different rates of secretion and joint efflux of HA and PRG4. This study expands upon previous work by examining the alterations in HA and PRG4 concentrations following cartilage defect repair surgery.

The findings of a shift towards low MW HA shortly post-surgery and increasing levels of high MW HA by a longer time post-surgery are novel results that extend upon previous work. Previous studies on eSF HA MW had an upper detection limit of 3 MDa by using high-performance liquid chromatography [18], and thus those results showed normal HA MW to be predominantly in the 2-3 MDa range [18], while other hSF studies reported normal ranges to be 3-7 MDa [19, 20]. Perhaps because of
the 3MDa upper limit, no significant change was found in HA MW distribution between normal and acute traumatic arthritis eSF [18]. The current findings expand upon this knowledge, because without a 3 MDa upper detection limit, a shift towards low MW HA is observed at t=10d, relative to normal. The finding of increased concentrations of high MW (2.5-7.0 MDa) HA in t=3mo samples, relative to normal, characterizes the relatively longer response of HA concentration and structure towards surgery.

The correlations between lubricating ability of eSF and HA and PRG4 content are variable with previous work, but also within the context of differences between experimental design and methods. Increased lubricating ability accompanied with increased $C_{HA}$ is consistent with an AI eSF study [11]. The trend of decreased lubricating ability with increasing $C_{PRG4}$ is not consistent with ACLT rabbit study also showing diminished lubricating ability but a decrease in $C_{PRG4}$ [6].

To further investigate these biochemistry-lubrication correlations, deficiently lubricating t=10d samples were supplemented with high MW HA rather than PRG4 because $C_{PRG4}$ was already 6.1x the normal (t=0d) value. Furthermore, HA is interesting to investigate from a clinical point of view, because of its wide use as a viscosupplement. The restoration of lubricating ability of eSF following high MW HA supplementation occurred for both $C_{HA}=0.6$ mg/ml (physiological) and 2.0 mg/ml (more typical of $C_{HA}$ following therapeutic injection of HA [30]). Given this finding, the results of the correlation do not imply that increased $C_{PRG4}$ is the cause of diminished lubrication ability. The diminished lubricating ability in t=10d samples may be due to either the diminished $C_{HA}$, as supported by the current HA
viscosupplementation study, or the elevated presence other unstudied components present in eSF post-surgery that may interfere with the lubricating mechanisms of PRG4. In either case, the current findings suggest that high MW HA viscosupplementation may be used in vitro to counteract diminished lubricating ability of SF resulting from arthroscopic cartilage repair surgery.

The current study provides evidence that cartilage repair surgeries alter the state of a joint over time post-surgery, causing changes in the composition and function of synovial fluid. Compositional and structural characterization of lubricant molecules, in relation to SF lubricating ability, allows investigation of potential mechanisms of diminished lubricating ability and is suggestive of potential therapeutic interventions involving intra-articular injection of high MW HA. Such an intervention would benefit from the current investigation of changes in SF mechanical and biochemical properties with duration post-surgery because HA has a short half-life in synovial joints (20.8 hours in sheep hock joints [43]). A recent compartmentalized model estimates that following typical therapeutic injection of 20 mg of HA, 63% of the injected HA would be cleared from the joint within 0.25-2.66 days [30]. Given the time post-surgery differences in lubrication properties, this study also suggests that therapeutic HA injection would have an optimal timeframe post-surgery for application.

2.6 Acknowledgments

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2.7 References


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