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A histological analysis of subchondral bone cysts in osteoarthritic hips

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Thesis

A HISTOLOGICAL ANALYSIS OF SUBCHONDRAL BONE CYSTS
IN OSTEOARTHRITIC HIPS

by

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A HISTOLOGICAL ANALYSIS OF SUBCHONDRAL BONE CYSTS IN OSTEOARTHRITIC HIPS

AMELIA WISE

ABSTRACT

Osteoarthritis (OA) is a debilitating disease that affects millions of people. It is characterized by the degeneration of articular cartilage, narrowing of the joint cavity, damage to the subchondral bone, loss of synovial fluid, and osteophyte formation. These symptoms can cause muscle weakness, decrease in range of motion at the joint, and pain. Pain is the symptom that is most frequently treated. However, pinpointing the exact origin and location that is causative to the pain can be difficult. Patients of OA commonly have areas of subchondral edema identified on an MRI as marrow bone lesions (BML). On a CT image, these BML are found to be areas of bone resorption within the subchondral bone of the affected joint called subchondral bone cysts (SBC). These cysts are hypothesized to be a source of pain in OA as well as progression of the disease. The synovial intrusion theory for cyst formation states that there is a physical connection made between the joint cavity and the SBC through which synovial fluid travels. The bony contusion theory describes micro cracks developing below the articular cartilage within the subchondral bone causing bone necrosis and SBC formation.

This study was undertaken to investigate the histological presentation of the contents of the SBC and the surrounding area.
Seven human femoral head samples, ranging from age 49-72 years old, from patients who received total hip arthroplasty due to OA were examined. Three areas were sectioned from each femoral head including an area containing a cyst, an area of primary compressive bone, and an area at the medial side of the femoral head. These sections were then stained for bone, cartilage, and nerves and examined histologically.

Sclerotic bone was shown to surround each cyst cavity, while cysts were composed of a mixed connective tissue infiltrated with multiple blood vessels and potential nerve fibers. With further investigation of these structures, the location of the nerve fibers within the SBC could be a possible source of pain in OA and a target for future treatments and therapies. Within the femoral head, cysts were found to be both shallow and deep to the articular cartilage. Shallow cysts may support the synovial intrusion theory for cyst formation while deep cysts could support the bony contusion theory. In relation to articular cartilage, cysts were not only found below a degraded articular cartilage surface as expected but also below intact, less degraded cartilage.

This in depth look at the cells and tissues present within and surrounding the cyst provides information to better understand the pathology of OA and possibly an alternative method for treating the disease.
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LIST OF ABBREVIATIONS

BML..............................................................bone marrow lesion
BU..............................................................Boston University
COX..............................................................cyclooxygenase
dMOADs............................................................disease modifying osteoarthritis drugs
ECM..............................................................extracellular matrix
HSC ..............................................................hematopoetic stem cells
MRI..............................................................magnetic resonance imaging
NSAIDs..........................................................nonsteroidal anti-inflammatory drugs
OA..............................................................osteoarthritis
PCG............................................................primary compressive group
PFA..............................................................Paraformaldehyde
PTH..............................................................parathyroid hormone
RANK............................................................receptor for nuclear factor-kβ
RANKL..........................................................receptor for nuclear factor-kβ ligand
SBC............................................................subchondral bone cyst
SMOADS.........................................................structure modifying osteoarthritis drugs
TRAP............................................................tartrate-resistant acid phosphatase
VITD............................................................1,25 dihydroxyvitamin D
INTRODUCTION

Bone Modeling and Remodeling

Normal bone contains both organic and inorganic components (Paulsen, 2010b). The organic components include osteoblasts, osteocytes, osteoclasts, type I collagen and non-collagenous proteins (Li et al., 2013). The inorganic compounds are mainly calcium and phosphate along with bicarbonate, citrate, magnesium and potassium (Paulsen, 2010b; Li et al., 2013). The osteoblasts function to lay down bone by forming a matrix of collagen fibers and an extracellular matrix (ECM). This matrix is mineralized by the deposition of hydroxyapatite (HA) crystals, the inorganic component of bone. Specific skeletal stem cells give rise to the osteogenic lineage first by committing into osteoprogenitor cells that reside within the endosteum and periosteum. These osteoprogenitor cells then fully differentiate into osteoblasts. Osteocytes are matured, differentiated osteoblasts that reside in osteons and are fully encapsulated in mineralized matrix. Although osteocytes do not proliferate via mitosis, they are still capable of turning over and affecting the composition and homeostasis of the matrix (Paulsen, 2010b).

Osteoclasts are multinucleated, monocytes and are the bone resorbing cells. Osteoclasts reside in Howship’s lacunae and secrete enzymes and acid that break down the bone matrix (Paulsen, 2010b). Osteoclasts must first be activated from osteoclast precursors, which is a point of osteoclast regulation. The receptor for nuclear factor-κβ (RANK) is expressed on macrophages, monocytes and osteoclast precursors. RANK
binds to receptor for nuclear factor-κβ ligand (RANKL) on either osteoblasts or stromal cells to activate the osteoclast precursor into an active osteoclast. Osteoprotegerin (OPG) is a RANKL antagonist and will bind to RANK and block RANKL from binding. This prevents osteoclasts from being activated by the osteoblasts or stromal cells shown in Figure 1 (Kemp, Burns & Brown, 2008).

Figure 1. Osteoclast activation. RANKL located on an osteoblast or a stromal cell bind to RANK on an osteoclast precursor and activate it into a functioning osteoclast. OPG is shown acting as a RANKL antagonist. (Shoback, Sellmeyer & Bikle, 2011).

Bone modeling occurs from the time of fetal development until around age 20 when the longitudinal growth of the long bones cease. Throughout the lifetime of an individual there is the continuous remodeling of the organic and inorganic components of
the bone to maintain hormonal homeostasis and stability of the architecture of the skeleton (Li et al., 2013).

The major hormones that regulate bone remodeling and maintain balance between the resorptive osteoclasts and bone forming osteoblasts are 1,25 dihydroxyvitamin D (VITD) and parathyroid hormone (PTH). PTH and VITD stimulate bone resorption by increasing the activity of the osteoclasts. VITD facilitates the intestinal absorption of calcium and phosphate to increase levels in the serum. The hormone calcitonin on the other hand inhibits the activity of osteoclasts, decreasing bone resorption (Parra-Torres et al., 2013).

Bone is classified as either dense (cortical) or spongy (trabecular) and is remodeled in distinct ways in response to changes in the mechanical loads placed on the bone or to alterations in hormone levels (Parra-Torres et al., 2013). Trabecular bone is surrounded by a mesenchymal cell canopy of bone lining cells. Blood vessels provide osteoclast precursors that differentiate into bone resorbing osteoclasts. Osteoblasts then lay down a new bone matrix. Macrophages ingest the degraded bone created by the osteoclasts (Li et al., 2013). Cortical bone is remodeled by osteoclasts creating a resorptive channel down the Haversian canal followed by osteoblasts laying down new bone down through the channel (Parra-Torres 2013).
Figure 2 shows the contrast between trabecular and cortical bone remodeling. Top: Trabecular bone is remodeled through a canopy of bone lining cells. Bottom: Cortical bone creates a resorptive canal. HSC: Hematopoietic stem cells. MSC: Mesenchymal stem cell (Sims & Martin, 2014).

Figure 2 shows the contrast between trabecular and cortical bone remodeling. Trabecular bone remodeling shown by the top image illustrates the canopy of bone lining cells with hematopoetic stem cells (HSC) being delivered by blood vessels to infiltrate...
the canopy. The HSC differentiate into osteoclasts and degrade the bone. Mesenchymal stem cells differentiate into osteoblasts which then lay down new bone.

*Joint*

Joints are defined by the joining of adjacent bones at articular cartilage surfaces. The hip, for example, is where the head of the femur connects into the acetabulum of the hip. These surfaces are extremely important for providing smooth, frictionless, limited motion and for the maintenance of the articular surfaces (Li et al., 2013). Joints are composed of complex structures that provide the support to maintain the articulating surfaces. These structures include the joint capsule, articular cartilage, ligaments, and muscles (Felson, 2015).

The joint capsule contains an outer fibrous layer and an inner synovial tissue layer made up of synoviocytes, which secrete synovial fluid (Felson, 2015). The synovial fluid, consisting of hyaluronic acid and lubricin, lubricates the joint to prevent friction between the two cartilage surfaces (Mescher, 2016; Felson, 2015). The articular cartilage and synovial fluid produced by the joint capsule are important for smooth movement of the bones at the joint and distributing the mechanical load across the surface of the joint (Kapoor, 2015).
Articular Cartilage

Articular cartilage surrounds the ends of connecting bones and contains two distinct layers: the superficial, non-calcified cartilage layer which is permeable to small molecules and the deep, calcified cartilage layer. The tidemark is the line that separates these two layers (Li et al., 2013). Hyaline articular cartilage is made of mainly chondrocytes and an ECM produced by the chondrocytes containing type II collagen, proteoglycans such as aggrecan, and other non-collagenous proteins. The combination of aggrecan, hyaluronic acid and collagen fibrils of the ECM provides a highly hydrated bio-polymer that provides the compressive stiffness and flexibility to the tissue (Felson, 2015).

Chondrocytes maintain homeostasis by regulating the levels of ECM degradation enzymes (Kapoor, 2015). Chondrocytes are senescent, meaning as they age, they have a very low mitotic replication rate and have not been found to contain a pool of progenitor cells capable of replenishing the aged or damaged chondrocytes (Loeser, 2010). Therefore, as these chondrocytes age, they are unable to maintain homeostasis. This will increase the risk for diseases such as osteoarthritis (OA) due to the inability of articular cartilage to rebuild itself. Injury to the articular cartilage over time causes tears in tissue that reside in the synovial cavity where the degraded cartilage is phagocytized by synoviocytes. This causes a release of numerous biochemical products such as pro-inflammatory substances, matrix metalloproteinases and other matrix degrading enzymes.
(Kapoor, 2013). Other factors that may contribute to chondrocyte death are telomere shortening and oxidative stress (Loeser, 2010).

Figure 3. Layers of articular cartilage and subchondral bone. Non-calcified cartilage and calcified cartilage are separated by the tidemark line. The subchondral bone plate and subchondral trabecular bone are separated by the cement line (Li et al., 2013).

The cement line is formed between the calcified cartilage and the subchondral bone below which form a functional group called the osteochondral junction. The osteochondral junction consists of the non-calcified cartilage, tidemark line, calcified cartilage, cement line and subchondral bone shown in Figure 3. These components of the osteochondral junction form an intimate interaction at the area of the joint. Blood vessels and nerves run across the concrete line that provide communication from the subchondral bone to the calcified cartilage for the maintenance of homeostasis in the joint cavity. The
number and extent of channels depend on many factors such as the age of the individual and the amount of loading forces that have been placed on the joint. Below the articular cartilage lies the subchondral bone (Li et al., 2013).

\textit{Subchondral Bone}

The subchondral bone provides support to the overlying cartilage and distributes the mechanical load across the joint (Li et al., 2013). There are two distinct layers of subchondral bone including the superficial subchondral bone plate and the deep subchondral trabecular bone, which is porous and is designed as a shock absorber (Kapoor, 2015). The subchondral bone adapts to load bearing mechanical forces on the joint by remodeling the bone in the area. Both the subchondral bone plate and subchondral trabecular bone contain blood vessels and nerves that travel into the overlying calcified cartilage to provide nutrients to the surrounding area (Li et al., 2013).

\textit{Development of Osteoarthritis}

OA is the most common degenerative joint disease involving both the articular cartilage and underlying subchondral bone (Desautels, Gikas & Guang, 2015). Characteristics of OA include degradation of articular cartilage, loss of synovial fluid, formation of osteophytes, remodeling of subchondral bone, development of sclerotic bone, muscle weakness due to uneven loading, and decrease in range of motion at the joint (Kapoor, 2015). There are currently no pharmaceutical drugs for the prevention or
cure for OA, but only treatment for the pain and swelling with non-steroidal anti-inflammatory drugs (NSAIDs) such as cyclooxygenase (COX-2) inhibitors. Alternative treatments such as physical therapy, acupuncture, and weight loss can be prescribed. Other pain medications include acetaminophen, Tramadol and opiates. New therapies are being developed called disease-modifying osteoarthritic drugs (DMOADs) and structure modifying osteoarthritic drugs (SMOADS) which are aimed at treatments for the underlying cause of OA progression rather than the resulting pain (Bingham, 2007).

Magnetic resonance imaging (MRI) is effective for diagnosing and monitoring the progression of OA. However, 50% of patients with characteristics of OA do not demonstrate symptoms, which can delay initial diagnosis (Hunter et al., 2013). The Kellgren-Lawrence grading is the most common system used to diagnose OA. This system grades OA from 0-4 with osteophyte formation being present at lower grades to joint narrowing, sclerosis, cyst formation and deformity of the joint exhibited at higher grades (Zhang & Jordan, 2010).

Pain and Inflammation

While pain is the most common symptom reported, pinpointing the exact cause and location can be difficult because of the location of the nociceptive nerve fibers, location of the OA (hand, hip, knee etc.) and patient’s individual sensitivity to pain (Lange-Brokaar et al., 2012). Pain receptors are found in the synovium, ligaments, muscles, joint capsule, subchondral bone and surrounding tissues (Salaffi, Ciapetti &
Carotti, 2014). While these tissues are vascular and neural, articular cartilage is not, meaning it cannot be the direct source of the pain. Thus, pain is secondary to the damage of the surrounding tissues, which are innervated by nerves and blood vessels (Hunter et al., 2013).

OA is characterized as a form of non-inflammatory arthritis, focused around the mechanical wearing of the joints (McErlain et al., 2012). Recent studies however have examined the release of cytokines (tumor necrosis factor, pro-inflammatory interleukins, chemokines, nerve growth factor, leukotrienes, prostaglandins) and metalloproteinases. Damage at the articular surface can cause inflammation in the synovial tissue. The release of these cytokines and metalloproteinases then cause the infiltration of macrophages, T cells and mast cells toward the area of inflammation (Lange-Brokaar et al., 2012). This immune response causes afferent nociceptive nerve fibers to become more sensitive and contribute to the cause of OA pain. Angiogenic factors are stimulated by endothelial cells and fibroblasts and further perpetuate the cycle of inflammation by the release of additional pro-inflammatory factors. Therefore, pain from OA could be the result of stimulation of afferent nociceptive fibers responding to tissue damage and inflammation at the joint (Salaffi, Ciapetti & Carotti, 2014).

**Phases of Osteoarthritis**

There are two phases of osteoarthritis: destructive and productive. In the destructive phase there is structural damage at the joint and surrounding structures. In the
productive phase, the subchondral bone remolds into osteophytes at the articular surface and remodeling of the subchondral bone below (Salaffi, Ciapetti & Carotti, 2014).

In the early stages of OA, subchondral bone is damaged causing a loss of the subchondral bone plate and deterioration of the subchondral trabeculae. This is representative of the destructive phase. The progression of OA in the destruction phase can be perpetuated by not only degradation of the articular cartilage but also by micro cracks in the subchondral bone, causing necrosis, and remodeling of the subchondral bone. Angiogenesis is followed by an expansion of the pores that connect the subchondral bone to the overlying calcified cartilage. These micro cracks can cause damage to the bone canaliculi (Li et al., 2013). The canaliculi provide the physical connection between osteocytes in lamellar bone (Paulsen, 2010). Without this communication, there is apoptosis of the osteocytes causes bone resorption of the necrotic bone (Li et al., 2013).

In the late stage of OA development, osteophytes form at the surface of the joint. Advanced OA ultimately will lead to a total joint replacement, which can be costly and have high morbidity and mortality rates (Bingham, 2007).

Subchondral Bone Cysts

Another aspect of OA progression involves the development of subchondral bone cysts (SBC). On an MRI, bright signals identify pockets of edema called bone marrow lesions (BML). At first, it was not understood why these areas were forming until
researchers discovered that they were due to extensive bone remodeling of the subchondral bone (Loeser, 2010). When examined on a CT image, certain bone marrow lesions are further characterized as (SBC), which are areas of consisting of resorbed bone and fibrous tissue, commonly present in patients with OA. Two theories attempt to explain how these cysts develop. The bony contusion theory, also called the microfracture or bone remodeling theory, suggests that the wear and tear on the cartilage causes degradation and uneven loading of weight onto the bone. This results in microfractures within this weakened area of subchondral bone causing osteonecrosis and initiation of bone remodeling. Osteoclasts resorb this old damaged bone and macrophages phagocytose the necrotic bone products. Osteoblasts then lay down dense bone surrounding this cavity leaving fibrous tissue inside the SBC (Desautels, Gikas & Guang, 2015). The synovial fluid leakage theory hypothesizes that synovial fluid leaks through the osteochondral junction through microcracks of the subchondral bone, creating and enlarging the SBC (Li et al., 2013).

There are two phases to SBC formation: destructive and productive. The destructive phase of cyst development occurs when the subchondral trabecular bone dies and the resorbed bone forms a fibrotic cyst. The productive phase occurs when sclerotic bone forms around the cavity of the cyst with increase in both density and volume. Although there is an increase in density, there is a reciprocal decrease in mineralization. The density in the area may increase in order to compensate for the stability that is compromised by the hypomineralization (Li et al., 2013).
Desautels, Gikas, & Guang (2015) CT imaged femoral heads of patients with OA who had received total hip arthroplasty and examined these SBC. They found 1) that these SBC were found directly underneath cartilage degradation areas; 2) they detected structural changes in the primary compressive bone of the trabeculae, and 3) they found dense sclerotic bone surrounding the cyst area. There was a positive correlation between the number of cysts the patients had and age. They also found that the deeper cysts were smaller while the more superficial cysts were larger. This would support the synovial fluid theory because there was a physical connection between the articular surface and the cyst, enlarging the cavity through synovial fluid intrusion. It is still unclear how exactly these cysts are forming, whether by the bony contusion theory or the synovial fluid intrusion theory, but it is evident that these cysts are evidence of bone subchondral interacting with a number of surrounding tissues in the development and progression of OA.

Chen et al. (2015) investigated patients with knee OA and examined bone remodeling occurring in relation to articular cartilage damage. Of the 97 patients enrolled with knee OA, 74.2% of them presented with SBC. Histological examination showed sclerotic bone surrounding the cysts with increase bone volume and stiffness of trabeculae. Cartilage was found at the perimeter and within the cyst which synthesized type I rather than type II collagen. Type I collagen forms fibrous cartilage and type II collagen forms hyaline cartilage which is found at the surfaces of joints. In contrast to the Desautels, Gika, & Guang (2015) study, Chen et al. (2015) study suggests that the articular damage that occurs in patients with OA leaves the subchondral bone more
susceptible to osteonecrosis and cyst formation, consistent with the bony contusion theory.

**Objectives**

OA is a multifactorial disease that is not completely understood. First, OA was characterized by the mechanical wear on the cartilage at the joint. Now, local tissues are being investigated for their role in the pathogenesis and progression of OA. Treatments are available to treat the downstream effects of pain and inflammation caused by the disease rather than drugs to prevent or mitigate the underlying causes. Investigation into these factors will be most pertinent into understanding what is causing and advancing OA and how to treat OA more effectively.

The objective of the present study is to analyze the histological presentation of subchondral bone cysts in patients who have received total hip arthroplasty due to OA. Specifically:

1. Examine the location of the SBC relative to the articular surface of the femur head.
2. Carry out a qualitative examination of the tissue contents of the SBC, specifically assessing the presence of blood vessels, nerve fibers, and cartilage.
3. Relate the extent of articular damage in primary compressive areas and medial sides of femur heads to cyst presentation.
METHODS

Experimental Design

A total of thirty femoral head samples were obtained from patients with OA at Boston Medical Center who underwent total hip arthroplasty. These patients ranged in age 43-77, both men and women. Once obtained, they were fixed in 4% paraformaldehyde (PFA). The samples were then subjected to μCT image analysis and using a coordinate, SBC were labeled (Desautels, Gikas, & Guang, 2015).

Image Correlation and Femur Sectioning

Seven femoral head samples were randomly selected from the total thirty samples. The methods for selection and sectioning of the areas for histological assessment are outlined in Figure 4. The μCT images were labeled for areas of small, medium and large cysts and used to determine the location of sectioning. First, the femoral head was positioned in a clamp to mirror the position in the left or right hip socket in the human body and matched with the μCT image, previously labeled for cysts. Three sections of each femoral head were marked for cutting: a section that contained cartilage on the medial side of the hip, a section including a cyst region, and a section including the primary compressive area of bone. Longitudinal sections from the articular cartilage to the neck were cut to include one or more of the above regions.
Figure 4. Method for Sectioning Femoral Head. Top left: µCT image of femoral head. Top right: Femoral head sample positioned to mirror µCT image. The green marks area of cyst within the femoral head. Middle left and right: µCT image of sectioned area with green depicting SBC. Bottom left: Sectioned sample. Bottom right: Stained section slide used for histological examination.
The medial side of the femoral head was determined using physical markers such as the ligament attachment of the femoral head to the acetabulum called the foveal ligament to orient the head to its natural position in the body.

This medial side contains the highest level of cartilage damage because of the continuous mechanical loading of this surface. The cyst area was determined by correlating the position of the femoral head in the clamp with the area of cyst marked on the μCT images.

Finally, the primary compressive group was located on the μCT image based on the architecture of the dense bone area and marked on the femoral head for locating during sectioning.

Figure 5. μCT Primary Compressive Group. μCT image of primary compressive group.
The primary compressive zone is characterized by dense trabecular bone where the majority of the load is placed on the joint and transmitted to the underlying cortical structure of the trochanteric neck. μCT was particularly useful for identifying this area. Since μCT detects the density of bone, the primary compressive zone, which is an area of dense bone, could be pinpointed. An example of a μCT image of a primary compressive group is shown in Figure 5.

For sectioning the heads, a low speed, rotating blade saw was utilized. As each section was cut, the sample was wrapped in phosphate-buffered saline (PBS) soaked gauze to prevent the samples from drying out. Each section of the femoral head was placed in a mesh bag with a small index card indicating the femoral head sample #, area of the head, and if an area was cut into several pieces, each piece was then labeled. The mesh bags were placed in 500 mL jars of 4% PFA for one week for further fixation. PFA was then drained and the samples were rinsed with distilled water.

Decalcifying

The mesh bags were placed in 2,000 mL jars containing 14% ethylenediaminetetraacetic acid (EDTA). The jars were placed on stir plates and agitated for one week. The samples were then removed from the mesh bags, x-rayed, and examined for inorganic mineral content. Samples which showed complete demineralization were removed from the EDTA solution and placed in 70% ethanol
(EtOH). Samples, which still showed mineralization, were placed in fresh EDTA and placed back on the stir plate for an additional week. This procedure was repeated until all the samples were completely demineralized. The samples were then processed, sliced on the microtome and placed onto slides. The samples were cut into fifty consecutive 5μm slices in order to make sure enough that slides would be available for staining and to account for any ripped or folded tissue slices.

**Staining and Imaging**

Slides in each area were prepared for histological investigation and were stained with Hemotoxylin and Eosin (HE), Safranin O/fast green, tartrate-resistant acid phosphatase (TRAP), and Cresyl Violet Nissl substance stain. The TRAP did not effectively stain the tissue samples. This could be a result of the over-fixation by PFA prior to sectioning. A series of examples illustrating specific aspects of this histological examination are presented with scoring procedure that was used on each sample.

Safranin O/fast green stain was used to stain cartilage. Red indicated the binding of basic Safranin to acidic proteoglycans of the ECM present in cartilage. Blue indicated binding of fast green to non-collagenous tissue. This contrast was used to distinguish between cartilage and bone in the samples (Mescher, 2016). The Nissl stain used Cresyl violet to stain the Nissl substance of nerve fibers a dark purple. The Eosin counterstain stained the cytoplasm of cells pink.
RESULTS

Areas examined histologically under the microscope include the cyst, primary compression group, and medial side of the femoral head. OA progression is thought to follow a destructive phase occurring at the articular cartilage surface and then a productive phase of osteophyte formation and remodeling of subchondral bone. SBC progression is thought to follow a destructive phase of osteonecrosis of the subchondral trabecular bone and then a productive phase of sclerotic cavity formation around the SBC. Each sample showed examples of theses phases of OA and SBC progression. The bony contusion theory and synovial intrusion theory were examined.

Cysts

Of the seven femoral heads sampled, six contained cysts. Sample #102 was expected to contain a cyst because the μCT image identified one in the area, but of the sectioned areas, none were found. This may have resulted from sectioning in an incorrect area where the μCT image indicated a cyst was present, but the femoral head could have been misaligned and therefore a cyst was not captured in the section. There is also the possibility that this femoral head sample did not contain a cyst at all. Based on the histology, the subchondral bone cyst is identified as an area containing fibrous tissue instead of trabecular bone within the femoral head encapsulated by sclerotic bone.
Characteristics of the SBC examined were the location of the cysts, encapsulating sclerotic bone, blood vessels, nerves and cartilage within cyst.

Figure 6. Cyst. Six of the seven samples collected contained a SBC. Arrows denote the cyst location within the subchondral bone. Each sample displayed above contained a cyst. Sample #102 did not contain one.
Figure 5 shows Safranin O/fast green stain of the six samples that contained cysts. The arrows point to the SBC and the fibrous tissue of the cyst is easily distinguished from the trabecular bone.

**Location**

Cysts appeared both directly below the articular surface as well as deep within the head. Figure 5 shows the location of each of the SBC. Samples #110 and #124 showed cysts deep to the articular cartilage surface. Samples #114, #121, #127, and #129 all contained superficial cysts to the articular cartilage surface.

With these cysts presenting deep to the articular surface within the head, the location does not support the synovial intrusion theory of cyst formation. According to this theory, there is a physical connection between the synovial capsule and the cyst where synovial fluid travels into the area of the cyst, creating and enlarging the area. Cysts that appear deep within the femoral head with no apparent connection to the joint cavity could lead to the conclusion that the cyst formation is due to the bony contusion theory. According to the bony contusion theory, mechanical damage at the joint causes micro fractures within the subchondral bone and subsequent osteonecrosis, osteoclast
invasion and cyst formation. This occurs deep below the articular surface with the need of a direct channel connection to the cartilage (Güven et al., 2013).

_Sclerotic Bone_

Dense, sclerotic bone was found surrounding every cyst, shown in Figure 5. This sclerosis of the SBC cavity shows a clear example of the productive phase of cyst formation. The bone necrosis and bone resorbing osteoclasts clean up the area leaving fibrous connective tissue within the cyst while osteoblasts lay down bone at the perimeter of the cavity to strengthen the overlaying surface and provide stability to the overall femoral head structure. This sclerotic bone would been most likely be seen in both a cyst formed by the bony contusion theory or synovial fluid intrusion theory.

_Figure 7. Sclerotic Bone._ Arrows denote SBC within femoral head samples with sclerotic bone surrounding the cavity.
Examples of dense sclerotic bone surrounding the cyst cavity is demonstrated in Figure 6. The images show a distinct color and structure between the dense sclerotic bone and spongy trabecular bone throughout the rest of the femur head.

Blood Vessels

Every identified cyst contained multiple blood vessels. This could be identified using the Safranin O/fast green stain shown. Red indicated binding of the basic Safranin to acidic proteoglycans of the ECM present in cartilage. A thin endothelial layer of cells surround the blood vessels. The vessels that still contain red blood cells stain as blue biconcave disks. Other vessels examined do not contain red blood cells appear empty. Figure 7 shows blood vessels in each of the six samples that contained SBC. Some blood vessels clearly contain red blood cells and others are devoid of any contents.
Figure 8. Blood vessels. Arrows denote blood vessels within SBC
Nerves

Nerves are typically found running through tissue in conjunction with blood vessels (Paulsen, 2010). As there were multiple blood vessels observed in the initial histological observation of the samples, it was hypothesized that nerves could be present close by. Nerves were first thought to be identified on the Safranin O/fast green stain distinguished from blood vessels by being encapsulated by a thin layer of cells but not containing RBC. They instead contained other non-specific contents that were not distinctly biconcave disks as red blood vessels are.

Figure 10. Nerves and Nissl substance. Arrows denote potential nerve fibers within the SBC.
Figure 10. Nerves. Arrows denote blood vessels within SBC. All six samples containing SBC were found to contain nerve like structures.
All samples that contained SBC also were found to have structures resembling nerves running through the area of cyst along with the blood vessels shown in Figure 8. These structures were attempted to be further identified using the Cresyl Violet Nissl stain with an Eosin counterstain. Cresyl Violet stains the Nissl substance located in the cell body and dendrites of neurons a dark purple shown in Figure 9, image B. This method is useful when distinguishing between blood vessels and neurons. Both nerves and blood vessels are encapsulated by a thin epithelium. However, with the Nissl stain and Eosin counterstain, the cytoplasm of the nerve will therefore stain pink with purple nuclei. Since red blood cells do not contain nuclei, blood vessel contents will either be unstained if red blood cells are absent or stain only the cytoplasm of the red blood cells pink from the Eosin without purple nuclei. This comparison is suggested in Figure 9, image A.

Nissl bodies are rough endoplasmic reticulum with ribosomes present in neural tissue. Nissl bodies are found in the soma and the dendrites of neurons but not in the axon (Paulsen, 2010c). When examining the slides stained with Cresyl Violet and counterstained with Eosin, the nerve fiber will show dark purple spots within the cytoplasm. These dark purple spots are the Nissl bodies staining with the Cresyl Violet. Panel B shows a 40x magnification of a potential nerve and two blood vessels.

It remains unclear if these structures are actually nerves. Further investigation to specifically identify them as nerves is needed.
**Cartilage Within Cyst**

Of the femoral heads sampled that contained a cyst, 50% were also found to contain areas cartilage within the cyst. This was identified with the Safranin O/fast green stain which binds to proteoglycans present in cartilage.

*Figure 11. Cartilage within Cyst.* Arrows denote cartilage within the SBC.
The left vertical row shows the overview image of the cysts that contained subchondral cyst cartilage at 10x. Panel B zooms into the SBC at 20x to see the cartilage appearing. The cartilage stains red and the surrounding tissue blue. Chondrocytes are found sitting in lacunae throughout the area of cartilage. Figure 11 shows a 20x magnification of the chondrocytes residing in the cartilage matrix.

**Figure 12. Chondrocytes.** 20x magnification of an area of cartilage within the SBC. Arrows denote chondrocytes within lacunae.
Table 1 provides a summary of the characteristics of the sections containing SBC.

Of the six samples that contained a cyst, all cysts contained blood vessels, sclerotic bone surrounding the cavity, and nerves. Half of the cysts contained cartilage residing inside the cavity.

<table>
<thead>
<tr>
<th>Sample #</th>
<th>102</th>
<th>110</th>
<th>114</th>
<th>121</th>
<th>124</th>
<th>127</th>
<th>129</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyst</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sclerotic Bone</td>
<td>N/A</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cartilage</td>
<td>N/A</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Blood Vessels</td>
<td>N/A</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Nerves</td>
<td>N/A</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 1: Histological Presentation of Cysts
Primary Compressive Group

Areas examined on the primary compressive group include SBC presentation, SBC location and cartilage fibrillation. Based on the progression of OA and cyst formation, the degraded cartilage surface is followed by the cyst formation due to osteonecrosis and the subsequent sclerotic bone formation. All of the primary compressive area sections showed intact, thick cartilage. Figure 13 shows images of Safranin O stained primary compressive group sections. The Safranin stained the proteoglycans red and intact cartilage can be seen. Since these sections were hypothesized to not have progressed past the destructive phase of OA progression, cysts were not expected below the surface of intact cartilage. However, cysts were found below intact cartilage in two samples.

Two of the five samples sectioned for the primary compressive group contained cysts. Arrows in Figure 13 in samples #114 and #129 point out the cysts. Both the cysts found were deep below the articular surface.
Figure 13. Primary Compressive Group. Arrows denote SBC within sectioned sections from primary compressive group.
According to Desautels, Gikas, & Guang, (2015), cysts were found via μCT overwhelmingly below areas of greatest articular cartilage damage. Therefore, cysts were expected to be identified histologically below articular cartilage damage. However, cysts were histologically found in various areas of the femoral head such as below intact cartilage. The primary compressive zone of the femoral head samples all showed thick intact articular cartilage and dense trabecular bone. However, some samples such as sample #114 and #129 contained a cyst within the section with intact articular cartilage. A summary of the characteristics found in primary compressive group sections is show in Table 2.

<table>
<thead>
<tr>
<th>Sample #</th>
<th>102</th>
<th>110</th>
<th>114</th>
<th>121</th>
<th>124</th>
<th>127</th>
<th>129</th>
</tr>
</thead>
<tbody>
<tr>
<td>Association with Cyst</td>
<td>-</td>
<td>N/A</td>
<td>+</td>
<td>N/A</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Location of Cyst (S=superficial, D=deep)</td>
<td>N/A</td>
<td>N/A</td>
<td>D</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>D</td>
</tr>
<tr>
<td>Fibrillation</td>
<td>-</td>
<td>N/A</td>
<td>-</td>
<td>N/A</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Medial Cartilage Surface

The medial side sections showed degraded cartilage and thinned subchondral trabecular bone. This is representative of the destructive phase of OA progression. Areas examined the section’s association with a cyst, the location of a cyst, subchondral bone sclerosis, and articular cartilage fibrillation.

Figure 14. Medial Cartilage Surface. Arrows denote SBC within sectioned sections from primary compressive group.
All of the medial cartilage sections contained a subchondral bone cyst with sclerotic bone encapsulating the cyst, shown in Figure 14. These cysts all appeared to be deep to the articular surface. The articular surface also showed degraded cartilage shown by the Safranin O not staining the proteoglycans that would normally be present at the cartilage surface.

The early destructive phase of OA is identified by degraded articular cartilage and damage to subchondral bone. The later productive phase is osteophyte formation and subchondral bone remodeling. The destructive phase of cyst formation is the necrosis of the trabecular bone and the productive phase is sclerotic bone cavity formation. Sample #127, for example, showed an early destruction phase of degraded cartilage and thinned subchondral trabecular bone as well as sclerosis and cyst formation. This is what was hypothesized to be seen in these medial cartilage surface samples.

A summary of the characteristics found within medial cartilage surface sections is seen in Table 3.

<table>
<thead>
<tr>
<th>Sample #</th>
<th>102</th>
<th>110</th>
<th>114</th>
<th>124</th>
<th>127</th>
<th>129</th>
</tr>
</thead>
<tbody>
<tr>
<td>Association with Cyst</td>
<td>N/A</td>
<td>+</td>
<td>N/A</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Location of Cyst (S=superficial, D=deep)</td>
<td>N/A</td>
<td>D</td>
<td>N/A</td>
<td>D</td>
<td>D</td>
<td>D</td>
</tr>
<tr>
<td>Subchondral bone sclerosis</td>
<td>N/A</td>
<td>+</td>
<td>N/A</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fibrillation</td>
<td>N/A</td>
<td>+</td>
<td>N/A</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
DISCUSSION

Three areas of the femoral heads were examined: an area containing a cyst, the medial side containing degraded cartilage and the primary compressive area. SBC are identified as areas of bone resorption below the articular cartilage within subchondral bone that are commonly found in patients with OA. These cysts were first identified as bone marrow lesions on MRI by a hyperintensive signal marker (Li et al., 2013). To further characterize these bone marrow regions as SBC, CT imaging is utilized. A CT image has the advantage of being able to detect density of the mineralized tissue, specifically in the subchondral bone to detect both SBC as well as sclerotic bone surrounding the area and any adjacent structural remodeling throughout the subchondral bone (Bousson, 2012). Of the seven samples sectioned, six contained subchondral bone cysts. Every cyst was surrounded by sclerotic bone and contained multiple blood vessels, potential nerve fibers and fibrous tissue. SBC development in relation to OA progression and SBC in relation to theories of formation are discussed.

SBC Nerves

All of the samples that contained SBC also displayed structures that resembled nerve fibers throughout the cyst. Further investigation of these structures is needed to classify them as nerves. The finding of nerves within the cyst cavity could be an explanation for the source of pain in osteoarthritis. Previously, it was thought that the pain in OA was caused by damage to the surrounding structures such as the synovial
cavity or the subchondral bone below the degraded articular cartilage since cartilage is aneural and avascular. However, this study showed structures resembling afferent nerve fibers residing within the cyst itself running next to the blood vessels. This could be an additional cause for pain with the progression of OA.

**SBC Cartilage**

Of the six samples that contained cysts, three of them contained cartilage within the cyst themselves. This suggests that mesenchymal stem cells have differentiated into mature chondrocytes to lay down the cartilage. Chen et al. (2015) had identified cartilage within and surrounding the cysts of their samples as type I collagen rather than type II collagen that composes articular cartilage. This suggests that this cartilage is not articular cartilage but rather originated from other sources such as mesenchymal stem cells.

**SBC Blood Vessels**

All of the cysts examined contained both fibrous tissue rather than trabecular bone along with many blood vessels. This finding is consistent with the Zanetti et al. (2000) study. Zanetti et al. (2000) examined 16 patients who had received a total knee replacement due to OA. They found that 2% of the bone marrow region examined through MRI consisted of blood vessels. It is well known that vascularization is pertinent to bone remodeling in order to couple bone resorption and formation (Findlay, 2007).
Therefore, a finding of multiple blood vessels in the SBC where bone has been remodeled substantially was expected.

**SBC Sclerotic Bone Cavity**

This study found that the cysts were all surrounded by a sclerotic bone cavity through histological examination. This was consistent with the literature about SBC. The reason this sclerotic bone forms is thought to be a remodeling process to protect the stability of the femoral head (Li et al., 2013). Chen et al. (2015) also identified sclerotic bone surrounding the bone cysts they identified. Although the bone surrounding the cyst cavity had a higher density, it was shown to be hypomineralized.

**OA Progression and SBC Development Based on Cyst Location in Relation to Articular Surface**

Early destructive phase of articular cartilage and subsequent subchondral bone damage followed by the productive phase of sclerotic cyst capsule of OA does not prove to be as straightforward as occurring consecutively. Li et al. (2013) describes an early destructive phase and then a productive phase of OA progression. The cyst presentation did not always directly correlate with articular cartilage damage suspected to be present in OA patients. For example, primary compressive area sections with thick articular cartilage showed cysts beneath the articular surface. The medial sections, which all
contained degraded cartilage, did all contain cysts, which was expected based on literature describing progression of cyst formation.

**SBC and Theories of Formation Based on Cyst Location within Subchondral Bone**

In regards to supporting or refuting the theories of cyst formation, it remains unclear. The bony contusion theory explains mechanical damage to the joint cavity causing micro cracks and necrosis to subchondral bone. The synovial fluid theory suggests that there is a physical connection between the synovial fluid bathing the joint cavity and the cyst beneath. With the synovial fluid theory, the cysts are expected to reside close to the articular surface as physical channels are formed. Most cysts in this study however were identified deep within the femoral head, which would support the bony contusion theory, specifically when cysts they are deep and below intact cartilage. The cartilage in this situation may or may have not yet been damaged but micro cracks develop, resulting in osteonecrosis and formation of these cysts.
CONCLUSION

This is the first study to specifically examine the histology of subchondral bone cysts present in osteoarthritic femoral heads. There is little research done on the histological characterization of the cells and tissue inside the subchondral bone cysts. Still, there is controversy about the exact mechanism of these cysts forming. Histologically examining the cysts as well as surrounding tissues provides a better understanding of the composition of the SBC. This may be important for future research on the pathology and progression of OA beyond the articular cartilage damage previously characterizing the disease.
REFERENCES


VITA

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amelia@bu.edu, 207.319.8273
Year of Birth: 1990

EDUCATION

Boston University School of Medicine 2014-Expected May 2016
University of Connecticut May 2012
Bachelor of Science in Biological Science

University of Connecticut Neuroscience May 2010- July 2010
Educational Abroad Program

CLINICAL/HEALTHCARE EXPERIENCE

Brigham and Women’s Hospital
Emergency Services Assistant (ESA)
November 2013-March 2014, June 2015-Present
- Perform 12 lead EKGs, obtain vital signs, collect clean catch urine specimens, assist in trauma settings; move patients from backboards and undress patients, retrieve equipment for providers, assist in procedures, perform CPR, apply the automatic CPR machine if needed, perform visual acuity examinations, transport patients to CT, radiology, ultrasound and specialty units of the hospital

Mercy Hospital
Emergency Department Technician (ED Tech)
August 2012-August 2014
- Performed phlebotomy, perform 12 lead EKGs and placed patients on cardiac monitors, obtained vital signs, utilize glucometers to test blood sugar levels, cleaned and dressed wounds; applied ace wraps, knee immobilizers, wrist splints, postop boots, educated patients on using crutches and canes, set up suture trays and assisted in procedures, bathed and toileted patients, sat with behavioral patients on one-to-one watches
Lisbon Emergency
Emergency Medical Technician
May 2011-July 2013
- Responded to medical and trauma calls in the community to transport patients to appropriate medical facilities, completed medical and trauma assessments to determine appropriate treatment; obtained patient’s past medical histories, demographics, blood glucose levels, and vital signs; performed CPR, spinal immobilization and administered oxygen, epinephrine, and oral glucose

RELEVANT EXPERIENCE
Department of Orthopedic Surgery, Boston University
Graduate Researcher
June 2015-Present
- Analyze the histology of subchondral bone cysts associated with patients who have received total hip replacement due to osteoarthritis

EDUCAN!
Mentor
February 2016-April 2016
- Mentor one-on-one with 5th grade students at an elementary after-school program, emphasizing a growth mindset to succeed in both in school and in their personal lives

Rosie’s Place Volunteer
Activities, Food Pantry, Server
February 2016-Present
- Rosie’s Place is a women’s shelter based in South Boston which provides support for homeless and underserved women by offering beds, a food pantry, activities and advocacy services. Organize and carry out with weekly activities such as bingo, prepare and serve meals in the kitchen, and assist distributing food in the food pantry

Haley House Volunteer
Take Back the Kitchen (TBK)
July 2015- August 2015
- Assisted chef in setting up kitchen and preparing food, guided participants through a recipe, and cleaned work area after session. TBK empowers
underserved communities in the Boston area by leading cooking classes for children, adults and families
  ● These groups are taught why eating healthy is important, where foods come from, and gives them the technical skills to be able to not only cook for themselves but to bring back the skills they have learned to teach others.

DeBlas Lab, University of Connecticut
Research Assistant
September 2010-May 2011
  ● Assisted graduate students with aliquoting chemicals into tubes using micropipettes and researching scientific articles on the internet to be used in experiments, acquired price quotes over the phone for laboratory items and compiled necessary information to prepare order forms for purchase, processed incoming orders and filed receipts

ADDITIONAL EXPERIENCE
Biology Club, University of Connecticut
Vice President, September 2011-May 2012
Secretary, September 2010-May 2011
Member, September 2009- May 2010
  ● Arranged for professors in the fields of Molecular and Cell, Physiology and Neurobiology, and Ecology and Evolutionary Biology to speak at weekly meetings about their research and organized field trips

University of Connecticut Global Health, Education and Economic Development (H.E.E.D.)
President, September 2011-May 2012
Campus Relations Director, September 2010-May 2011
  ● Global HEED is a student-led, campus group focusing on bringing awareness of the needs of underserved communities globally. Assisted in organizing a Hunger Banquet, facilitated debates at weekly and organized local community projects

Patient and Family Care Centered Committee
December 2015-present
  ● The Patient and Family Care Centered Committee consists of clinical representatives from the ER including nurses, physicians and technicians.
  ● Meet once a month with the Patient and Family Advisory council to discuss quality improvement for the community we serve. I have learned about the
current issues our emergency room as well as surrounding communities face and how these issues can be resolved.

CERTIFICATIONS/SKILLS
- Emergency Medical Technician
- American Red Cross Certified in CPR, First Aid, AED