Validation of the Radiographic Union Score for Tibial Fractures (RUST) using Medical Imaging and Biomechanical Testing in an In-Vivo Rat Model

by

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A thesis submitted in conformity with the requirements for the degree of Master of Health Science
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University of Toronto

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Abstract

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Introduction. The lack of objective parameter to define bone fracture union makes it difficult for orthopedists to properly treat fractures. The Radiographic Union Score for Tibial fractures (RUST) is becoming widespread as an objective means of assessing fracture repair however its accuracy has yet to be validated.

Methods. A group of 24 rats underwent standardized femoral osteotomies. At their assigned endpoint, the healing femur was radiographed, scored with RUST and modified RUST, scanned with µ-CT, and tested in torsion.

Results. The RUST and modified RUST scores correlate highly with the mineralized callus volume, total callus volume, mineralized to total callus volume ratio, ultimate torque, stiffness, maximum shear strain and torsional strength. RUST showed slightly higher interobserver agreement among reviewers than modified RUST.

Conclusion. RUST and modified RUST have strong relationships many with imaging and biomechanical parameters providing evidence of the accuracy of the scores as assessment tools for fracture healing.
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Chapter 1

Background and Previous Literature

1.1 Motivating Problem

There are an estimated 50 million musculoskeletal injuries each year in the United States alone, of which long bone fractures are the most frequent [1, 2]. The tibia is the most commonly fractured long bone in the body with an estimated 500,000 tibial shaft fractures occurring every year in the US [3, 4]. In addition to their high frequency, tibial fractures are highly prone to complications including nonunion and delayed union of the bone fragments due to the sparse soft tissue envelope of the tibia. The frequency of nonunion has been reported to be as high as 9%-12% for cases treated with intramedullary nail fixation [5]. While tibial fractures account for the majority of bone healing complications, the overall rate of nonunion of the skeleton has been estimated at 1-5% of all fractures [6]. Determining the occurrence of such healing complications has proven to be a complication in and of itself as currently there is no consensus on an objective parameter to define bone fracture union [7, 8, 9]. This has serious implications for the following two main reasons.

First, determination of a healed bone fracture is paramount for orthopaedic clinical care. Patient, fracture and treatment are often unique in each clinical case which creates high variability in the time to heal and chances of successful repair. Such variability makes clinical decision-making very difficult [10]. In fact, a 2002 survey of 444 orthopaedic surgeons showed a lack of consensus in the assessment of tibial shaft fracture healing [11]. The definition of delayed union ranged from 1 to 8 months and acceptable fracture translation ranged from 5 mm to 15 mm. Such varying definitions of fracture union can interfere with clinical decisions with respect to weight-bearing status, hardware removal timing, and prediction and treatment of malunion or nonunion. Additionally, a study by Antonova et al. showed that the effective diagnosis of malunion and nonunion of
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Tibial shaft fractures will decrease morbidity and healthcare resource use and cost [12]. A better assessment of fracture repair will aid clinicians predict and manage complications resulting in superior patient outcome [10].

Second, the lack of an objective definition of fracture union presents an obstacle in clinical trials. While a single study will use a given parameter to deem a bone healed, it is difficult to compare the efficacy of various treatments if the parameters used in each study differ [13]. This lack of a standardized endpoint for fracture healing can also lead to misleading results, thus exposing patients to avoidable risk if these treatments are incorrectly legitimized.

Ultimately, an evidence-based assessment tool is required to assess the progression of bone healing to aid in clinical decision-making and clinical studies.

1.2 Physiology of Fracture Repair

1.2.1 Bone Physiology

The human skeleton serves many functions including providing structural support, permitting locomotion and protecting vital internal organs [14]. There are two types of bone: cortical and trabecular (also called cancellous or spongy). Both types of bone are made of a composition of inorganic material (e.g. hydroxyapatite), organic materials (e.g. collagen fibers, proteoglycans and other proteins) and bone cells. The fundamental functional unit of bone is the osteon. Osteons are lamellar structures comprised of collagen fibrils laid down in alternating orientations, mineral matrix, and bone cells [15]. In cortical bone, osteons are densely packed and called Haversian systems. Haversian systems are cylindrical in shape and surround a central canal containing blood vessels, nerves and other tissues. Its high density and calcification provide cortical bone with a high resistance to bending and torsion. In trabecular bone, osteons are semilunar in shape, are known as packets, and are sparsely organized into plates and rods. In contrast to cortical bone, trabecular bone’s framework and high turnover rate allow for high elasticity and metabolic function [16].

At the microscopic level, both mature cortical and trabecular bone are comprised of a lamellar structure (the osteon). However, immature or pathological bone is characterized by irregular and randomly oriented collagen fibrils and minerals. This is known as woven bone. It is weaker and more flexible than lamellar bone [14].

Adaptation to mechanical function has created a wide variety of shapes and bone compositions in the skeleton [17]. The bones in the human body are categorized as long
bones, short bones, flat bones and irregular bones. Figure 1.1 shows the basic anatomy of a long bone, the femur. Other long bones include the tibia, the humerus and the radius. Long bones contain three main sections: the diaphysis, metaphysis and epiphysis. The diaphysis is a long hollow shaft located in the middle of the length of a long bone and is composed primarily of cortical bone. On either end of the diaphysis are the metaphyses and epiphyses, separated by the growth plate in growing bone. The metaphysis and epiphysis are for the most part composed of trabecular bone [14].

Figure 1.1: Illustration of a long bone (human femur) with labelled diaphysis, metaphysis and epiphysis [18].

Throughout its existence, bone undergoes continuous remodeling in response to mechanical and metabolic stimuli. The rate and location at which bone is simultaneously degraded, resorbed and re-formed dictates a bone’s strength and composition [16]. Under high strain, bone’s mechanotransduction will induce bone formation whereas low strain conditions induce bone resorption. This phenomenon is known as Wolff’s Law [19]. In homeostasis, there is an equal balance of bone formation and resorption. The remodeling process is managed by the three main bone cell types: osteoclasts, osteoblasts and osteocytes. Osteoclasts are responsible for bone resorption as well as signaling the mesenchymal precursor cells to differentiate into osteoblasts. Correspondingly, osteoblasts form bone by secreting collagen which aggregates into a mineralized collagen matrix outside of the cell [16]. Once surrounded by the bone that it has created, osteoblasts
transform into osteocytes. Osteocytes play a crucial role in mechanotransduction by modulating signals and directing bone resorption and deposition [20].

In addition to the remodeling process, bone will form spontaneously without the need for preexisting bone, which occurs during growth or fracture repair. Bone formation occurs via two main mechanisms: intramembranous ossification and endochondral ossification. Both mechanisms comprise the transformation of mesenchymal tissue which is embryonic connective tissue [21]. Endochondral ossification involves the synthesis of cartilage tissue from mesenchymal stem cells and the subsequent replacement of the cartilage scaffold by bone. On the other hand, during intramembranous ossification, mesenchymal stem cells are directly converted into bone tissue without the mediation of a cartilage phase [19]. While there is a distinction between these two methods of bone formation, it refers exclusively to the process. The end result of both intramembranous and endochondral ossification is the same.

Due to bone’s varied architecture and material composition, its mechanical properties are complex. First, bone is a viscoelastic material meaning that its elastic properties and strength are dependent on loading rate and duration [22]. Second, bone is an anisotropic material due to its changing elastic properties and strength depending on the direction of loading. In particular, bone has been shown to be transversely isotropic: it is stronger and stiffer in the longitudinal direction than in the transverse direction [22]. A study by Reilly and Burstein found that in the longitudinal direction, cortical bone exhibits the highest ultimate strength in compression, followed by tension and is weakest in shear (torsion) [23]. Such complex mechanical characteristics of bone illustrate the necessity for controlled biomechanical testing in order to properly characterize bone strength.

1.2.2 Bone Repair Physiology

Bone has the unique ability to fully repair itself to a normal pre-injury state [19]. It is generally recognized that there are two mechanisms of bone healing: primary (direct) healing and secondary (indirect) healing.

Primary Healing

Primary healing is uncommon in the natural bone healing process. It requires surgical stabilization with perfect reduction of the fracture fragments and a rigid construct. Such measures are necessary in order to create interfragmentary compression with absolute stability (i.e. substantially decreased interfragmentary strain) [24]. Primary healing is characterized by the absence of callus at the fracture site. Rather, osteons bridge
the reduced fracture gap. Contact healing, where the two fracture ends are directly in contact, is a result of simultaneous bony union, lamellar remodeling and re-establishment of Haversian canals [25]. Gap healing, which occurs with a small gap less than 800 microns between the fracture ends, is a result of woven bone bridging the gap followed by remodeling to lamellar bone [19]. Larger gaps will heal via secondary healing.

Secondary Healing

Secondary healing is the most common method of bone healing. In contrast to primary healing, secondary healing does not require fracture fragment reduction and rigid stabilization; in fact, it is enhanced by micro-motion and weight-bearing [25]. Secondary healing is comprised of both endochondral and intramembranous bone formation and is characterized by the presence of callus at the fracture site. The fracture callus contains a variety of tissue types whose quantities change throughout the healing process. Tissue types include fibrocartilage, cartilage, granulation tissue, intramembranous bone and calcifying cartilage [26]. Secondary healing is widely described as occurring in four stages as illustrated in figure 1.2.

Figure 1.2: Illustration of the four stages of secondary healing of a fractured femoral diaphysis [27]. a) Inflammatory Stage b) Soft Callus Stage c) Hard Callus Stage d) Remodelled Stage.

The first stage of bone healing is the inflammatory stage. Bone marrow cells and cells from the peripheral and intramedullary blood create a hematoma in between and around the fracture ends [25]. Degranulating platelets, macrophages and inflammatory cells secrete a number of signaling molecules while growth factors are secreted from the hematoma to promote bone healing and vascular proliferation [28]. Mesenchymal stem cells are recruited to the fracture location. At the well vascularized outer cortex and periosteal surface, mesenchymal cells preferentially differentiate to osteoblasts leading
to intramembranous bone formation. The conditions at the rest of the fracture site lead to preferred differentiation of mesenchymal cells to chondroblasts for endochondral bone repair [19, 25]. This stage is not radiographically visible as the hematoma is not sufficiently dense [29].

The second stage of bone healing is the soft callus (fibrocartilage) formation. In this stage, fibroblasts and chondrocytes create a semi-rigid callus that bridges the fracture pieces and provides a mechanically stable structure [25]. This is accomplished by the replacement of the fibrinous tissue and hematoma into cartilagenous matrix by chondrocytes and generalized fibrous tissue by fibroblasts [28]. The soft callus is principally avascular. It is near the end of the second stage that the chondrocytes mineralize the cartilagenous matrix [28]. The second stage is the earliest that the evidence of callus formation will be seen on a radiograph [29].

The third stage of bone healing is the hard callus formation. Vasculature ingrowth in the soft callus increases the oxygen tension which promotes osteoblast differentiation and the formation of bone [10]. The soft callus is removed and replaced with irregular and under-remodeled woven bone creating a rigid construct. This stage is highly evident on radiographs with copious visible callus [29].

The final stage in fracture repair is the remodeling of the woven bone hard callus into its original lamellar configuration, be it cortical or trabecular. This process is similar to the remodeling of bone as described in section 1.2.1 and is mediated by osteoclasts and osteoblasts [28]. A large portion of the biomechanical strength of the bone is restored in the third stage but remodeling is required to regain full strength. This final stage occurs over a prolonged period of time and often, permanent evidence of fracture will remain on X-ray [29].

1.2.3 Complications in Bone Healing

While bones are able to return to their pre-injury state, they do not always do so without complication. Among the more common complications are delayed union and nonunion (also known as pseudarthrosis). Delayed union, as indicated by the name, is a fracture that is not united within the usual timeframe. Such fractures require special care moving forward to ensure that they do not proceed to pseudarthrosis. Pseudarthrosis can be caused by infection at the fracture site or infection of the bone. Pseudarthrosis can also occur with no infection and is often classified as hypervascular or avascular. Hypervascular, also known as hypertrophic, nonunions are rich in callus and are caused by insufficient immobilization of the fracture ends causing them to be unable to bridge the fracture with
hard callus. Intervention involving increased fracture stabilization is required. Avascular, also known as atropic, nonunions are characterized by the presence of tissue with no osteogenic potential or a lack of blood supply at the fracture site. Intervention for avascular nonunion involves debridging of the fracture site and biological enhancement in addition to increasing stability [30, 31]. Nonunions are associated with high economic cost and health burden to the patient [12]. It is therefore crucial to develop tools to accurately monitor fracture healing to predict healing outcomes.

1.3 Conventional Fracture Healing Assessment

While there is no objective definition to fracture union, there are currently several methods used by clinicians and researchers to assess a healing bone fracture.

1.3.1 Physical Examination

Traditional bone healing assessment by clinicians is done using patient-reported outcomes and the findings of physical examinations such as pain during weight bearing or the presence of tenderness at the fracture site based on palpation [9, 11, 32, 33]. Such measures are, however, controversial. A survey of orthopedic surgeons showed that 37.2% of surgeons always used pain at fracture site to assess healing, while 15.9% never do [11]. In addition to the lack of consensus over the use of palpation for fracture healing assessment, the subjectivity of the method has been shown to lead to incorrect conclusions and irrational treatment decisions [8]. Accordingly, a study on the ability of orthopedic surgeons to judge fracture stiffness concluded that clinical assessment alone may put 83% of patients at risk of refracture or malunion [34].

1.3.2 Radiographic

Radiographic methods remain the most common fracture assessment tool by orthopaedists [9]. The results of a large-scale survey demonstrate that 39.7 % to 45.4% of orthopedic surgeons used radiographic variables to assess fracture healing [11]. Its low cost, wide availability and relative low radiation exposure are the reasons for the overwhelming popularity of plain radiography among orthopedists when compared to other imaging modalities such as magnetic resonance imaging or computed-tomography [31]. Although plain radiography is common, its effectiveness has been put to question. Due to the regenerative nature of bone healing, it can be difficult to identify a healing bone versus a healed bone from a radiograph [8]. In a study published in 2007 by McClelland et
al., orthopedists and engineers estimated fracture stiffness based on tibial fracture radiographs. The study found that the assessment of fracture healing from radiographs alone was inaccurate and that caution should be used when interpreting results based solely on radiographic assessment [35].

In response to such findings, studies have been conducted to propose standardized radiographic tools. One such radiographic union score was proposed in 1985 entitled the Hammer scale [36]. It was based on; callus formation being homogeneous, massive, apparent or trace; stage of union being achieved, uncertain or not achieved; and fracture line being obliterated, barely discernible, discernible or distinct. The scale proved, however, to have very little correlation with the mechanical stage of union and had poor interobserver agreement likely due to the complicated criteria definitions (e.g. "barely discernible" versus "discernible") [36, 37]. One of the more successful radiographic parameters has been cortical bridging. A study on the correlation of radiographic criteria with biomechanical strength of fractured rabbit tibiae determined that cortical continuity was the best predictor of strength of a healing fracture [38]. Accordingly, a study by Lack et al. found that any cortical bridging within the first four months of fracture fixation was an accurate predictor of final healing [39]. These findings were utilized to create a radiographic standard with promising results as reviewed in section 1.4.

### 1.3.3 Biomechanics

A bone is often considered healed upon return of mechanical stability and load carrying capability [31]. One of the most prominent papers with regard to the biomechanics of fracture healing is the 1977 study by White et al. [40]. In this study, the researchers examined mechanical properties of rabbit tibiae at various time points in the healing process using destructive torsion testing. The location of fracture and maximum torsional moment sustained were used to define four distinct stages of biomechanical healing as summarized in table 1.1.

<table>
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<th>Stage</th>
<th>Stiffness</th>
<th>Location of Fracture</th>
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<td>1</td>
<td>Low</td>
<td>Failure through original fracture site</td>
</tr>
<tr>
<td>2</td>
<td>High</td>
<td>Failure through original fracture site</td>
</tr>
<tr>
<td>3</td>
<td>High</td>
<td>Failure partially through original fracture site and partially through intact bone</td>
</tr>
<tr>
<td>4</td>
<td>High</td>
<td>Failure entirely through intact bone</td>
</tr>
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The four stages outlined in table 1.1 are indicative of the progressive increase in biomechanical strength throughout the healing process. These stages can be correlated to the histological stages of fracture repair. Of note, the transition from low stiffness to high stiffness (stage 1 to stage 2) is indicative of the remodeling of the soft fracture callus to a hard fracture callus. Also, the failure through intact bone (stage 4) indicates the complete restoration of the fracture to a pre-injury state. This is analogous to the remodeling of the bone to organized lamellar structure. The identification of these four stages of healing requires re-fracturing the bone and is therefore of use in ex vivo animal-model research and not clinically.

In clinic, there exist non-invasive alternatives to measure biomechanical properties. One such alternative is the Shift Comparator as suggested by Edholm et al [42]. This device measured the relative deflection of the two bone fragments under an applied bending moment which was assumed to be inversely proportional to the stability of the fracture union. This method was further explored by Marsh who attempted to provide a rational definition of union, delayed union and nonunion using stiffness recovery patterns [43]. A disadvantage of the Shift Comparator is that it cannot be used with any form of fixation or casting; therefore, it has little clinical use. In addition to the Shift Comparator, another system was proposed that involved strain gauges attached to external fixators [44, 45, 46]. The results from such methods are questionable as they are highly dependent on the fixator stiffness itself: one loose screw can cause an increase in stiffness by up to 50% [47]. Much of the research into in vivo biomechanical measurements of bone healing was done in the late 20th century. It is believed such research has been hindered by the poorly understood relationship between bone geometry, measurement conditions, fracture length and fracture location [48]. Further, a recent study by Chen et al. used finite element modelling to conclude that in vivo biomechanical stiffness measurements are of limited reliability to assess healing quality particularly at late stages of healing [49].

In research studies using animal models where bone dissection is possible, biomechanical testing is the gold standard used to assess the quality of fracture healing. Biomechanics indicate the bone’s progress in regaining its mechanical functional capability. The quality of the newly formed bone or callus is often measured by load-at-failure (such as that in the aforementioned study by White et al.) which requires sample destruction or by evaluating the callus stiffness non-destructively.

Traditionally, there are four types of tests that can be performed to evaluate the mechanical properties of bone as illustrated in figure 1.3: axial tensile testing, axial compression testing, bending testing and torsion testing [50, 51, 52, 53]. Tensile testing
involves applying an axial tensile force to the specimen. It is simple to setup and requires no complex test fixtures however it is not physiologically relevant since bones are seldom loaded in tension in vivo. Similarly, compression testing applies an axial compressive force to the specimen. In contrast to tensile testing, compression testing cannot achieve a high level of accuracy due to end effects imposed on the specimen during testing [50]. End effects are caused by the restriction of lateral expansion where the force is being applied as opposed to the center of the specimen [54]. Further, Steiner et al. do not recommend the use of axial compression tests for the determination of fracture callus stiffness due to the strong influence and resulting error of experimental variations in embedding and fixation of the specimen [53]. Bending tests can take the form of three-point bending or four-point bending. The opposing forces along the axis of the bone cause tension on one side of the bone and compression on the other. In the case of the applied forces in figure 1.3, the top of the bone is in compression and the bottom of the bone is in tension. Failure will most often occur on the tensile side due to bone being weaker in tension than compression [55]. The advantage of bending tests is that the maximum load can be applied to a particular section of the bone, such as the fracture callus. Additionally, bending tests, in particular three-point bending tests, are most similar to the internal physiological loading in vivo. On the other hand, bending tests have high directional dependency which can lead to large errors when testing biological specimens which do not have a consistent geometry [53]. Finally, torsion testing applies a twisting force to the ends of the specimen. One advantage of torsion testing is that torsion places the length of the bone under uniform loading conditions. This culminates in fracture at the weakest point in contrast to bending tests which generally cause failure at the loading point. Moreover, torsional stiffness is unaffected by orientation of asymmetrical calluses [50, 53]. Ideally, torsion testing should be performed unconstrained in the axial direction as it has shown the least error by Steiner et al. however such experimental setup is highly complex [53]. Axially constrained torsional testing can hinder similar accuracy when careful to align the axis of the sample to the axis of rotation during testing to reduce linear movement and the resulting axial stress on the sample. Torsional testing is widely adopted in the literature as a means of evaluating bone stiffness and strength [52, 56, 57, 58, 59, 60].
During destructive torsion testing, increasing torque is applied to the bone and the resulting twist angle of the bone is measured until fracture occurs. This results in a graph such as that in figure 1.4. From this graph, a series of parameters can be calculated including stiffness, maximum yield torque, ultimate torque and angle at failure. In mechanical testing scenarios, the geometry of bone is often simplified to a hollow circular shaft. This allows for easy calculation of the polar moment of inertia ($J$) using the known relationship outlined in equation (1.1) where $r_o$ is the outer radius of the shaft and $r_i$ is the inner radius of the shaft. With the polar moment of inertia known, material properties of the bone can be computed such as the torsional strength ($\tau_{\text{max}}$) and shear modulus of elasticity ($G$). Torsional strength (or maximum shear stress) is the ability of a material to withstand a twisting load. It is computed using equation (1.2) where $T_{\text{max}}$ is the torque at failure, $r_o$ is the outer radius of the shaft (where the shear stress is a maximum) and $J$ is the polar moment of inertia. The shear modulus describes a material’s response to shear stress such as that under torsion and is calculated with equation (1.3) for elastic materials where $\tau$ is the stress and $\gamma$ is the strain at a given point.
Figure 1.4: Sample graph illustrated the relationship between torque applied and twist angle with identification of stiffness, maximum yield torque, ultimate torque and angle at failure.

\[ J = \frac{\pi}{2} (r_o^4 - r_i^4) \]  

\[ \tau_{max} = \frac{T_{max} r_o}{J} \]  

\[ G = \frac{\tau}{\gamma} \]

1.3.4 CT Imaging

Computed Tomography, or CT, uses multiple radiographs of an object to recreate and visualize internal three dimensional structures. \( \mu \)-CT is computed tomography that uses higher image capture resolution in order to visualize small structures such as the trabeculae in small animal bones. Computed tomography has been shown to be a superior imaging modality to plain radiography (X-ray) in the presence of a large callus as is often the case during fracture healing [61]. A study by Grigoryan et al. has shown that CT scans can detect certain signs of bone healing earlier than X-ray imaging, notably blurring of fracture margins and the formation of callus [62]. In addition to the detection
of early callus formation, CT has also been shown to quantify the fracture callus and use this information to determine the stability of tibial shaft fractures [63]. Similarly, a study by Lind et al. has shown a correlation between parameters measured with CT (e.g. cross-sectional area and polar strength strain index) and torsional testing results in rat long bones [57]. Finally, a study that simulated fixed gap spiral tibial shaft fractures in cadaveric specimens showed excellent correlation between measurements of the gap on specimens and on the computed tomography scans whereas plain radiography underestimated the true gap size in 30 of 33 cases [64].

As a result of the aforementioned advantages of computed tomography over plain radiography, it has been shown that the use of CT by orthopedic surgeons improved agreement of proposed treatments plans and influenced the change of treatment plans [65].

While the complete and detailed visualization of healing provided by computed tomography seemingly makes it the gold standard of healing assessment, it is accompanied by certain complications that limit its clinical use. Of note, image degradation is common as a result of the presence of metal implants frequently used for bone fixation [8, 31]. Additionally, its high cost and high radiation dose limit the use of CT by clinicians [31].

1.3.5 Others

There are a number of less common alternatives for fracture healing assessment which will not be further explored here in the interest of brevity. Such alternatives include ultrasound imaging, positron emission tomography, vibrational analysis and serologic markers [31].

1.4 RUST: Radiographic Union Score for Tibial Fractures

The Radiographic Union Score for Tibial fractures (RUST) was developed by a team of researchers from the University of Toronto and McMaster University in response to the lack of successful and widely adopted bone healing assessment scales [66]. Previous research in successful radiographic criteria (see section 1.3.2) determined that that the RUST score should be based on the following: callus formation and fracture line visibility.

RUST uses X-ray images to assign a numerical score to fractured tibiae. A maximum score of 12 is achieved when the bone is fully healed and a minimum score of 4 is given for no healing. Scores are awarded based on callus formation and fracture line visibility.
at each of the four cortices of two orthogonal x-ray images: anterior, posterior, medial and lateral [67]. All four cortex scores are subsequently summed. A breakdown of RUST and an example of a radiograph with given RUST scores are presented in table 1.2 and figure 1.5 respectively.

Table 1.2: RUST scoring system breakdown [67].

<table>
<thead>
<tr>
<th>Score per Cortex</th>
<th>Radiographic Criteria</th>
<th>Score per Cortex</th>
<th>Radiographic Criteria</th>
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<tbody>
<tr>
<td>1</td>
<td>Absent</td>
<td>2</td>
<td>Present</td>
</tr>
<tr>
<td>2</td>
<td>Visible</td>
<td>3</td>
<td>Invisible</td>
</tr>
</tbody>
</table>

A score is given to each cortex (anterior, posterior, medial and lateral) and the RUST score is the sum of all cortex scores.

Figure 1.5: Typical orthogonal X-rays of a tibial shaft fracture showing 4 cortices and their RUST scores. *modified from* [67].

Several independent studies have been published which measure and validate the observer reliability of RUST; agreement within a scoring system must be determined before clinical application to ensure repeatability and objectivity of the score. Of note, Whelan et al. used a group of seven reviewers to evaluate 45 human diaphyseal tibia fracture radiographs resulting in substantial intraobserver reliability (intraclass correlation coefficient (ICC) 0.88) and interobserver reliability (ICC 0.86) [66]. Accordingly, Ali et al. found that RUST had an interobserver ICC between 0.87-0.98 and intraobserver ICC
between 0.87-0.96 using two observers and 345 human diaphyseal tibia fracture radiographs at multiple time points [68]. Finally, Tawonsawatruk et al. used thirty sets of radiographs of rat tibial shaft fractures with six reviewers which resulted in interobserver and intraobserver agreement of 0.81 and 0.86 respectively [69].

RUST was originally intended to be used for diaphyseal tibial fractures fixated with an intramedullary nail. It can be hypothesized that the scoring system was designed this way for three reasons. First, intramedullary nails have been proven to have a better outcome over other surgical interventions such as a bone plating or external fixation [70]. Second, there is a high frequency of complications reported with tibial fractures and finally, intramedullary nails allow for the visibility of four cortices in orthogonal radiographs[71]. Accordingly, the three aforementioned studies on RUST’s observer reliability use the scoring system as it was originally intended: for diaphyseal tibial fractures fixated with an intramedullary nail. However, the RUST scoring system can be used toward assessing fractures of any long bone with any fixation type on the proviso that four cortices remain visible in two orthogonal radiographs. Correspondingly, Litrenta et al. have examined the use of RUST for metaphyseal femur and tibia fractures fixated with either intramedullary nail or plate [33]. Nails demonstrated substantial agreement (ICC 0.74) whereas plates had moderate agreement (ICC 0.67). In addition to being translated for use with metaphyseal femur fractures, RUST has also been examined for fractures of the tibia, femur, humerus and radius fixated with intramedullary nail and plate in a study by Perlepe et al. [72]. In said study, the authors used a derivative of RUST entitled RUS based on the same criteria as RUST (callus formation and fracture line visibility) and found that the scoring system had sufficient reproducibility to be tested in clinical practice [72]. Another derivative of the RUST score is RUSH or the Radiographic Union Score in Hip Fractures which used a similar scoring process for femoral neck and intertrochanteric fractures [73]. RUSH resulted in higher agreement for fracture healing compared with physician impression of healing when 6 reviewers (orthopedic surgeons and radiologists) independently assessed radiographs from 100 patients [74].

In addition to being adapted for multiple purposes, RUST has already been adopted in many recent studies as a comparative endpoint for: the characterization of success rates of secondary interventions in tibial nonunions, the evaluation of the relationship between nutritional status with tibial fracture outcomes, and the investigation of the efficacy of elastic intramedullary nails, among many others [41, 75, 76, 77, 78]. The RUST score is also being applied clinically. Van Houten et al. determined that their version of the RUST score (which omitted the anterior cortex due to lack of visibility caused by hardware) assigned at 6 weeks and 3 months after surgery is a strong predictive
factor for the development of delayed union and nonunion after open wedge high tibial osteotomy [79]. They found that for each 1-point increase in the RUST score at 6 weeks, there was a decrease in the odds for nonunion of 0.19.

1.4.1 Modified RUST

In recent studies, a modified RUST score has emerged and has begun to gain momentum as an improvement to the standard RUST score as described in section 1.4. The modified score allocates a value of 1 to 4 to each cortex according to table 1.3. In this modification, the cortical assessment of callus presence is further subdivided into simply “present” or “bridging” since the loss of visible fracture line occurs late in the healing process [33]. As callus formation is a crucial stage of healing, the scoring subdivision is intended to more precisely define union.

Table 1.3: Modified RUST scoring system breakdown [56].

<table>
<thead>
<tr>
<th>Score per Cortex</th>
<th>Radiographic Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Absent</td>
</tr>
<tr>
<td>2</td>
<td>Present</td>
</tr>
<tr>
<td>3</td>
<td>Bridging</td>
</tr>
<tr>
<td>4</td>
<td>Remodeled</td>
</tr>
</tbody>
</table>

A score is given to each cortex (anterior, posterior, medial and lateral) and the RUST score is the sum of all cortex scores.

A study by Tornetta III et al. using a sheep model has shown that the ICC is better for the modified RUST than the standard RUST indicating that the modified score may be a better tool for assessing the progress of union over time [56]. Additionally, Litrenta et al. found that the modified RUST score demonstrated slightly higher ICCs than the standard RUST when scoring metaphyseal fractures of the tibia and femur [33]. Similar to the original RUST score, further research is needed to relate the use of the modified RUST score to clinical outcome.

1.5 Small Animal Bone Healing Model

Many animal models have been used in the past to study bone healing mechanisms including dog, rabbit and sheep. In the second half of the 20th century, rats and mice (or small animals) have been used increasingly in the study of fracture healing. The major
advantages of rodents are the logistics: they are relatively inexpensive to buy, easy and inexpensive to house and their short breeding cycle makes them readily available [80]. It is sometimes thought that rats and mice are less suitable for fracture healing studies because their bone structure is more primitive than that in humans and large animals. Mice and rats do not have Haversian systems, rather they use resorption cavities for bone remodeling. However, this has been shown to be similar to full Haversian remodeling and rodents have been deemed a suitable model when microscopic bone structure is of minor importance [80, 81]. Further, the similarity of the stages and morphology of fracture repair between humans and rodents make rodents a suitable fracture model [82].

1.6 Research Method

While RUST is becoming increasingly widespread as an objective means of assessing long bone fracture repair, it has yet to be validated as an assessment tool for the determination of bone healing [31]. In order to do so, the RUST scale must be compared with available outcome measures of healing such as other imaging modalities and biomechanical properties. In particular, μ-CT is an excellent indicator of fracture healing and does not always correlate with X-ray imaging. Is it therefore of interest to evaluate the evolution of μ-CT parameters throughout the RUST spectrum. Additionally, it is of interest to evaluate the relationship between the biomechanical healing of a fracture with the bone’s RUST score.

Ultimately, bone specimens at various healing points after a standardized fracture must be assigned a RUST score by a group of clinicians and must be dissected in order to image the callus with μ-CT and perform biomechanical analysis. This will allow the correlation of healing outcomes with the RUST scale.
Chapter 2

Rationale, Objectives and Hypothesis

2.1 Study Rationale

The only study that has attempted to determine healing based on the RUST scoring system was performed by Litrenta et al [33]. In this study, the participating orthopedists defined whether they thought the fracture to be healed subsequent to which they assigned a RUST score to a series of radiographs. Expectantly, the agreement between reviewers was low for whether the fracture was healed. Nonetheless, the average minimum threshold score for union was a 9 for RUST and 11 for modified RUST. This study compared the RUST scoring system to clinical opinion. While this is an important step in the validation process, the RUST score must be compared to alternative healing outcomes to ensure that it does effectively assess the progress of a healing bone.

2.2 Study Objective

This study aims to:

1. Validate the Radiographic Union Score for Tibial Fractures (RUST) using $\mu$-CT imaging and \textit{ex vivo} biomechanics in a rat model.

2. Compare the strength of relationship with healing parameters of the RUST score versus it’s modified counterpart.
2.3 Study Hypothesis

It is hypothesized that the biomechanical strength of a bone as well as the mineral density of a fracture callus will increase with increasing RUST and modified RUST indicating that the score correlates with the progression of healing. Further, it is hypothesized that strength of the fracture callus will increase rapidly at lower RUST scores when the bone is early in healing. The increases will then taper off at higher RUST scores. A similar phenomenon has been reported in a study that correlated mechanical properties to fracture callus properties [48].

2.4 Specific Aims

In this study, the researcher will:

1. Compare the interobserver agreement among radiographic reviewers of RUST and modified RUST to that reported in the literature.
2. Determine whether the RUST and modified RUST correlate with imaging parameters such as callus volume and mineral density.
3. Determine whether the RUST and modified RUST correlate with biomechanical properties such as ultimate torque and shear modulus of elasticity.
4. Compare the interobserver agreement and strength of relationship with healing parameters between the RUST and modified RUST scores.
5. Determine whether a single score can be identified as the point when a fracture can be clinically considered healed.
Chapter 3

Pilot Work: Establishing a Fracture Model

3.1 Closed Fracture versus Osteotomy

Studies that address fracture healing often require inducing a fracture in a small animal model so that the healing progression can be monitored. There are generally two options that researchers use to create a fracture: closed fracture or open osteotomy [82]. Both methods present advantages and disadvantages. Closed fractures require the insertion of an intramedullary nail into the intact bone using a small incision at one end of the bone prior to external blunt trauma to the leg. Often a guillotine-like device is used or else manual fracture is done by the researchers. The major advantage of closed fractures is that they more closely mimic reality as clinical fractures in humans are caused by similar trauma. Additionally, they do not require major soft tissue damage by exposure of the bone: soft tissue damage is associated with delays in healing [83]. However, arguments are made against its semblance to reality considering the fracture occurs after the bone has been stabilized with the intramedullary pin. Efforts have been made to introduce the intramedullary nail post fracture without much adoption [82]. The major disadvantage of closed fracture models is the lack of control over the resulting fracture pattern [84]. In fact, a study by Aurégan et al. recreated the widely used fracture model device established by Bonnarens in 1984 and found that 68% of the fractures created were comminuted to varying degree [85, 86]. On the other hand, open osteotomy requires open surgical exposure of the bone and subsequent severing of the bone using a saw. Bone exposure requires lifting soft tissues from the bone and severing the periosteum which may affect healing. However, the control of using a bone saw means that a consistent
standardized fracture can reliably be created across specimens.

The objective of this study is to correlate RUST scores to biomechanical strength and medical imaging. This requires a group of bone specimens that differ only in RUST score in order to compare one score to another. Seeing as some fracture patterns may heal differently than others, there is a need for standardization across specimens. This calls for an osteotomy model.

3.2 Pilot Study 1: Tibial Osteotomy and Intramedullary Nailing

3.2.1 Background

The RUST score was originally intended to be used for diaphyseal tibial fractures fixated with an intramedullary nail given the frequency of complications that occurs with tibial fractures and the success of the intramedullary nail in healing tibiae. It was therefore desired to recreate said scenario while evaluating the RUST score.

The use of a stainless steel Kirshner wire (K-wire) as a proxy for an unlocked intramedullary nail in small animal models is widely used in the research community. This method is often used with a closed fracture as the insertion of a pin can be minimally invasive further limiting the soft tissue damage during closed fracture. Insertion of the K-wire into the medullary canal only requires a small incision at either the distal or proximal end of the bone [82, 87]. Its uncomplicated surgical procedure and lack of cortical occlusion under radiograph make intramedullary nailing an appealing fixation method. Researchers will therefore utilize intramedullary pins to fix osteotomies as well. Of note, a standardized tibial osteotomy technique was published by Miles et al. in 2010 that promised normal healing with reduced fracture fragments [88]. The procedure involved exposure of the right tibia via a 2 cm incision and blunt dissection of the soft tissues surrounding the bone. A rotary saw was used to osteotomize the tibia at the level of the hamstring insertion point (at the junction between the proximal and middle thirds of the tibia). A 0.89 mm diameter stainless steel wire is inserted retrograde into the proximal fragment and subsequently anterograde into the distal fragment. Pursuing this further, Shefelbine et al. further demonstrated that osteotomized rat tibiae stabilized with an unlocked intramedullary pin healed better with an intact fibula due to the provided rotational stability [89]. However, even the rats with fractured fibulae in the study showed healing to some degree.

Intramedullary nailing of a tibial osteotomy has even shown superior mechanical
properties when compared to external fixation in one study by Sigurdsen et al. [90]. Finally, while there are slight variations in surgical technique, similar methods of tibial osteotomy and intramedullary pin fixation are used in a number of papers evaluating various aspects of healing such as the effect of lasers on healing, intramedullary pin stiffness, pharmaceuticals and bacterial infection [91, 92, 93, 94, 95, 96, 97].

3.2.2 Objective

This pilot study intends to utilize the methodologies published in the literature regarding tibial osteotomies and intramedullary nailing in order to create a highly reproducible fracture model. In turn, this fracture model will be applied to the biomechanical and medical imaging validation of the RUST score.

3.2.3 Methodology

A group of 10 male Sprague-Dawley rats weighing between 250-300 g underwent tibial osteotomy based upon the aforementioned published methodologies and in accordance with the guidelines provided by the Animal Care Committee at the Keenan Research Centre in St. Michael’s Hospital. All surgical procedures were conducted under anesthesia with isoflurane inhalation (5% for induction and 2% for maintenance). The right lower leg was shaved and disinfected with providone-iodine prior to an anterolateral longitudinal incision being made thus exposing the proximal two thirds of the tibia, taking care to minimally damage soft tissues.

The literature reports the use of K-wires in a range of diameters, generally falling between 0.8 mm and 1.4 mm [88, 90, 91, 92, 93, 94, 95, 96, 97]. Cadaveric tests were done prior to the surgery and demonstrated that both 0.9 mm and 1.1 mm diameter K-wires fit in the medullary cavity with varying depths of insertion into the distal end of the tibia. As the literature reports the use of varied K-wire diameters, five of the animals were fixated with a 0.9 mm K-wire and the remaining five were fixated with a 1.1 mm K-wire.

A drill was used to create a small hole in the cortex in between the tibial plateau and the anterior cortex in the distal direction. The K-wire was then inserted into the medullary canal in the distal direction without reaming. The pin was subsequently withdrawn from the bone and an oscillating saw with sterile saline irrigation was used to create a complete transverse osteotomy. In three animals with 1.1 mm diameter K-wires, the osteotomies were made at the tibial apex, approximately at the proximal third of the bone, as reported by Miles et al. and Zhao et al. [88, 93]. After following the first
three animals post-surgery as is shown in section 3.2.4, it was noted that the osteotomy location was not hindering a good outcome. The next seven animals were osteotomized at midshaft as was reported by Shefelbine et al., David et al. and Alt et al. [89, 91, 94]. The fibula was left intact in all animals.

After the osteotomy, the K-wire was reinserted into the medullary cavity and the fracture was reduced. The K-wire was lightly hammered into the distal medullary canal where the cavity is the smallest. The proximal end of the K-wire that remained outside of the bone was trimmed flush with the surface of the bone at the tibial plateau and the soft tissue layers and skin were sutured. Immediately post-surgery, full weight bearing and unrestricted movement were allowed.

The animals were to be followed and radiographed weekly until the bones reached a RUST score of 12 in order to gain a temporal perspective on the fracture model.

### 3.2.4 Results

Figure 3.1 shows lateral radiographs of the osteotomized leg of each of the 10 rats prior to sacrificing in order of fracture fixation success. The animals in this pilot study showed a high variability in the level of success of fracture fixation. Only three of the 10 rats did not require euthanasia due to animal welfare. The remaining seven were sacrificed at either 1 week or 2 weeks due to unacceptable fracture conditions. All animals but one (d in figure 3.1) showed evidence of fibula fracture in the week after osteotomy surgery.

Of the three rats with best outcome (a, b and c in figure 3.1), two were stabilized with a 1.1 mm K-wire and one with 0.9 mm K-wire. The fracture site showed evidence of callus formation with slight bridging callus in two of the animals. The three animals in question were sacrificed at four weeks due to the discontinuation of this fracture model however they were deemed suitable to be followed for an extended period of time.

The three animals which were osteotomized at the tibial apex (d, e and f in figure 3.1) showed misalignment of the fracture fragments. This resulted in a larger fracture gap on the anterior cortex in contrast to compression between fracture fragments on the posterior cortex. One of the three animals exhibited nail retraction in addition to misalignment. The misalignment was decided to be unacceptable for the current study and these animals were sacrificed at one week post-operation.

The four remaining animals (g, h, i and j in figure 3.1) exhibited dire nail dislocation and required sacrificing at 1 or 2 weeks post-operation due to animal discomfort and fracture instability.
Figure 3.1: Lateral radiographs of 10 osteotomized tibias fixed with 0.9mm or 1.1 mm K-wire including description of the location of osteotomy and wire used in each animal.
3.2.5 Discussion

This pilot study resulted in the dismissal of the tibial intramedullary pin as a viable fracture model for this study. In particular, the results of the 10 animals that underwent osteotomy clearly demonstrated the lack of reproducibility of the model. The location of the osteotomy nor the diameter of the K-wire had an effect on the outcome of the fracture fixation.

There were two major issues that presented themselves during this pilot study. The first issue was misalignment of the fracture ends. This occurred more prominently in the animals whose osteotomy was at the proximal end of the bone. Misalignment of the tibial fracture fragments results in the fracture fragments of one cortex to be under compression with small fracture gap and the other cortex to be under tension with a large fracture gap. Such a disparity is highly undesirable, in this study in particular, as it favours the healing of one cortex over the other which would result in faster healing and higher RUST scores on one or two cortices compared to the others. This has the potential to skew the overall RUST scores to cortex combinations with high differentials (e.g. one cortex gets RUST = 3 and the others have RUST = 1) whereas it is desired to have a variation in cortex combinations. Additionally, if these fractures did heal, they would likely result in malunion (healing in anatomically incorrect position) which is unrepresentative of clinical success. The second issue that presented itself in this pilot study was proximal intramedullary pin dislocation. Six of the animals showed evidence of pin retraction occurring with both the 0.9 mm and 1.1 mm diameter K-wire but more prominently with the 0.9 mm K-wire. The retracted pin caused discomfort to the animal and soft tissue damage to the surrounding area. For the more extreme cases, the continued retraction of the pin ran the risk of loss of stabilization at the fracture location.

Both of the aforementioned issues can be explained by the challenges presented by the anatomy of the rat tibia. Similar to mice, rat tibiae have a triangular declining caliber and curved longitudinal axis [98]. The small diameter of the distal end of the medullary cavity limits both the diameter of K-wire that can be used as well as the depth to which the K-wire can be inserted into the tibia. While the K-wire will be tight at the distal end, the proximal medullary cavity’s larger diameter allows the proximal fracture fragment to angulate about the K-wire causing misalignment. Further, the curved longitudinal axis necessitates the insertion of the K-wire through the anterior cortex with lack of axial fixation. This, in combination with the reduced diameter of the distal tibia, provokes the K-wire to recede out of the medullary canal as the rat puts weight on the fractured leg.

Findings of difficulty with tibial osteotomies are not unique to this pilot study. Kratzel et al. found that intramedullary fixation with a tibial osteotomy was a suitable nonunion
model as the rats in their study did not show radiographic cortical bridging at 84 days post-operation [99]. The hypothesized causation being a prolonged initial inflammatory phase. Elsewhere, Molster et al. found that in 13 out of 48 specimens the nails had dislocated proximally in a similar fashion to that observed in this pilot study [92]. This phenomenon is explained by the author by the lack of shielding against axial stress provided by an intramedullary pin during walking. Also, similar to Kratzel et al., Molster et al. found that 9 of the remaining 35 specimens resulted in nonunion. The problem of nail retraction does not seem to be a problem of open osteotomy alone. After closed fracture of the tibia Schmidmaier et al. reported a similar issue in three of their 63 samples [82].

The complex anatomy of the tibia does not only pose challenges with fracture fixation but also with biomechanical testing. Cheung et al. explains that the curvature of the tibia means that its mechanical properties will vary along its length [100]. Any variation in fracture location or specimen placement will have a significant impact on the results of \textit{ex vivo} mechanical testing. Its triangular cross-section is an additional complication for mechanical testing.

Even with the aforementioned complications of the tibia, many studies still use tibial osteotomies to publish uneventful consistent healing [90, 91, 94]. Ultimately, this model did not provide safe fracture fixation in a reproducible fashion for the 10 animals tested in this pilot study. For this reason, it was decided to not move forward with the tibia.

### 3.3 Pilot Study 2: Femoral Osteotomy and Intramedullary Nailing

#### 3.3.1 Background

Where the tibia offers complexity, the femur offers simplicity. The rat femur is longitudinally straight with a tubular shape and constant diameter. This will greatly reduce error and variation in biomechanical testing and simplify the alignment of fracture fragments. Fundamentally the femur is widely regarded as the best bone in the small animal skeleton to study fracture properties [101]. One disadvantage of the femur over the tibia is the bulky soft tissue cover of the femur. Such tissue can shield the bone and make closed fractures more difficult to accomplish [87, 100]. Given that an osteotomy is to be performed in this study, the soft tissue covering is not an issue for bone fracture. However, the femur’s soft tissue covering does not replicate the human tibial situation with sparse soft tissue covering. This brings forward the RUST score itself and how it was
intended to be used. As discussed in section 1.4, RUST was originally intended for tibial fractures as they are the most prone to complication. However, RUST is being used with increasing frequency for any bone for which two cortices are visible on two orthogonal radiographs such as the femur, humerus and radius. The adoption of the RUST score to all long bones justifies the use of the rat femur in this study.

Femoral intramedullary pin fixation in rats is widely used in accordance to the method popularized by Bonnarens and Einhorn in a pivotal 1984 paper that describes a standard closed fracture guillotine device [86]. This method is reported to offer high levels of success but also questionable levels of fracture reproducibility [85]. Osteotomies are also an option for intramedullary pin fixation in rat femurs. Many studies have used such models in the literature. Notably, Sha et al. performed femoral osteotomies and intramedullary nailing which resulted in complete healing at 12 weeks post-surgery in all animals but one which exhibited nail migration [102]. Using a similar methodology, Ryhanen et al. tested intramedullary pin material using a femoral osteotomy model and found normal healing in the majority of the rats with the exception of nail retraction in select specimens [103]. Further, in one study evaluating specific hormonal influence on fracture healing, a complete transverse osteotomy with pin fixation found all healing bones in the untreated control group were in good alignment [104]. Reikeraas found that rat femurs fixed with large diameter intramedullary nails and rat femurs fixed with small diameter intramedullary nails healed with no significant differences after complete transverse osteotomy [105]. Such evidence in the literature motivates the hypothesis that this fracture model will have a successful outcome.

3.3.2 Objective

This pilot study intends to utilize the methodologies published in the literature regarding femoral osteotomies and intramedullary nailing in order to create a highly reproducible fracture model. In turn, this fracture model will be applied to the biomechanical and medical imaging validation of the RUST score.

3.3.3 Methodology

An 18 gauge injection needle was used for the intramedullary pin. The diameter of 1.27 mm was deemed to be suitable for the canal of the rat femur and the needle's beveled edge proved to serve two functions. The first function was to perforate through the femoral condyles when inserting the nail into the canal and the second function was to provide a small amount of fixation at the proximal end by penetration into the greater
trochanter [98].

A group of two male Sprague-Dawley rats weighing between 250-300 g underwent femoral osteotomy based upon the aforementioned published methodologies and in accordance with the guidelines provided by the Animal Care Committee at the Keenan Research Centre in St. Michael’s Hospital. All surgical procedures were conducted under anesthetization with isoflurane inhalation (5% for induction and 2% for maintenance). The right leg was shaved and disinfected with providone-iodine prior to making a small medial parapatellar incision. The patellofemoral joint was exposed and the patella was dislocated laterally exposing the femoral condyles. A small hole was drilled with a 1.1 mm drill bit under saline irrigation through the condyles and in line with the axis of the bone. The 18 gauge needle was inserted through the hole into the medullary canal as far as it would go without additional force. The needle was subsequently removed until approximately one third was left inserted into the bone. A lateral longitudinal incision was made and soft tissues separated and pushed aside to expose the midshaft of the femur while taking care to minimally damage soft tissues. An oscillating saw with sterile saline irrigation was used to create a complete transverse osteotomy at the femoral midshaft. The 18 gauge needle was then pushed back into the canal and the fracture fragments were reduced. The needle was inserted as far as possible by hand into the proximal canal and greater trochanter before the remaining length of needle was cut flush to the bone. The wound was closed in layers with resorbable sutures. Immediately post-surgery, full weight bearing and unrestricted movement was allowed.

The animals were to be followed and radiographed weekly until the bones reached a RUST score of 12 in order to gain a temporal perspective on the fracture model.

3.3.4 Results

The two rats in this study were followed until nine weeks after fracture at which point the fracture model was deemed unsuitable for this study. The x-ray images taken prior to sacrificing are illustrated in figure 3.2. In all of the nine weeks that followed the osteotomy, the animals did not return to normal walking patterns: exhibiting limping and severe distal limb rotation upon weight bearing. Figure 3.3 shows a picture of the extreme distal limb rotation at one week post fracture.

Both rats showed signs of callus formation at the site of fracture. Rat “b” in figure 3.2 exhibited slight callus bridging on the lateral and medial cortices. However, this rat had increasing distal nail migration and pronounced misalignment in the sagittal plane. Rat “a” in figure 3.2 had callus formation however no signs of bridging in any cortex.
Figure 3.2: Lateral and anteroposterior radiographs of 2 osteotomized femurs fixed with 18 gauge needle at nine weeks post-operation.

Figure 3.3: Image of rat in pilot study 2 with extreme rotation of distal limb at one week post-operation.

3.3.5 Discussion

This pilot study concluded that the fixation of a femoral osteotomy with a simple unlocked intramedullary pin is not suitable for this study and should be discouraged in future studies. Neither of the animals in this study exhibited uneventful normal healing. Admittedly, the sample size used in this pilot was small: the use of live animals was limited given the poor results of pilot study 1. However, the results of the two animals tested in this study as well as evidence in the literature have indicated that this fracture model should be discontinued.

Both of the rats that underwent the proposed fracture model did not heal in a standard fashion. While Rat “b” in figure 3.2 showed early potential for fracture callus bridging,
the increasing nail dislocation would not have allowed the rat to reach a healed point. Even if the animal had reached healing, the misalignment in the sagittal plane would have resulted in malunion with delayed healing. Malunion and delayed healing, often a result of insufficient fracture reduction, are not illustrative of normal fracture healing processes. Rat “a” in figure 3.2 had a persistent smooth and sclerotic fracture line at nine weeks post-fracture which is widely accepted as early radiographic signs of hypertrophic nonunion, hypertrophic given the presence of callus [106]. As this study intends to evaluate the stages of normal healing, nonunion is highly undesirable.

It is hypothesized that the main reason for the nonunion and delayed union in these rats is the lack of rotational stability provided by simple pin fixation. Histing et al. performed ex vivo testing of rotational stiffness of various rodent femoral fixators and found that, to no surprise, the conventional pin provides absolutely no rotational stability [80]. Bone growth potential is influenced by both biological factors (blood supply, hormones, growth factors) as well as the biomechanical conditions at the fracture site [107]. It is recognized that inter-fragmentary motion promotes callus formation and strength. Claes et al. quantified this and found that strains less than 5% lead to intramembranous ossification, strains between 5% and 15% lead to endochondral ossification. However strains larger than 15% resulted in fibrous cartilage with no bony union [107]. Pursuing this further, Augat et al. separated axial micromotion from rotational (shear) micromotion with a specially designed fixator in sheep [108]. They found that shear movement considerably delayed union in comparison to axial movement which seemed to promote callus formation and healing. To this end, the detrimental effect of rotational instability between fracture fragments has been repeatedly shown in the literature. Of note, Grundnes and Reikeras found impaired healing with rotational and telescoping instability in intramedullary pinned rat femoral osteotomies [109]. The authors hypothesized the reason to be a prolonged maturation of external callus. Additionally, a study by Molster on varying levels of instability in rat femoral osteotomies with intramedullary nailing found delayed healing in unlocked intramedullary pins with lower mechanical properties throughout the healing process [110]. Moreover and in accordance to the findings in this pilot study, Hietaniemi et al. and Kokubu et al. use transverse osteotomy with simple intramedullary pin for a nonunion model [111, 112]. Ultimately, a simple unlocked intramedullary pin provides an uncontrolled biomechanical situation which is incomparable to that which occurs in humans. When evaluating healing progression, it is desired to mimic the osteosynthesis techniques that would occur in human fractures [84].

Success is more commonly reported when intramedullary pinning occurs with closed fracture models. There are two reasons that can be hypothesized to explain this: lim-
Chapter 3. Pilot Work: Establishing a Fracture Model

...ited soft tissue disruption and slight comminution of the fracture. First, evidence has been found that diaphyseal fractures created with a blunt guilotine treated with open intramedullary nailing healed slower than that treated with closed intramedullary nailing [83]. Second, a slightly comminuted or oblique fracture has the added benefit of providing rotational stability. In one paper, Molster discusses that an oblique osteotomy has a similar effect as locking the ends of the nail. However, such oblique osteotomies are impractical if mechanical testing is to be performed as measurement of resistance to rotation of the fracture callus is less controllable [110].

While masked by stories of success, the evidence of the failure of this fixation method is obvious in the literature by the many attempts to provide rotational stability to intramedullary pinning. One of such methods was attempted using cadaveric femurs. Grundnes and Reikeras as well as Molster studied different levels of rotational instability by locking the ends of an intramedullary pin with cement composite [109, 110]. This method of locking was attempted however it proved logistically challenging. Namely, bone cement should be close to cured when it is applied, however it was not possible to inject the viscous cement through a needle making cement placement difficult. Additionally, the bone cement failed to lock the nail in a portion of the samples therefore the added surgical difficulty was not worth the result. Another method of locking the intramedullary pin was proposed by Holstein et al. [98]. The authors flattened the proximal and distal tips of an injection needle in an orthogonal fashion in an attempt to prevent rotation. Alternatively, Schoen et al. utilized two screws on both sides of the fracture to apply perpendicular compression on the nail as is seen in figure 3.4 [113]. Garcia et al. added an extramedullary clip in addition to the intramedullary nail [114]. While these methods, all illustrated in figure 3.4, are successful in their respective studies, they lack adoption in the literature which suggests a lack of reproducibility. One system with well founded standardization and recommendation in the literature is the RIsystem locking nail for mice and rats [115]. The interlocking pins provide rotational stability and the surgical guide and tools available offer high standardization [84]. However, as a result, the surgical procedure is highly complex and the system cost is significant.

In conclusion, an unlocked intramedullary pin provides insufficient rotational stability to a femoral osteotomy to match outcome of osteosynthesis techniques that occur in humans. A reproducible fracture model is desired for this study that provides rotational stability, allows views of the cortex in orthogonal radiographs and has a simple standardized surgical procedure.
Figure 3.4: Various methods proposed in the literature for locking of intramedullary pinning in rats and mice. a) RLs system RatNail locking intramedullary nail [116]. b) Injection needle with flattened orthogonal proximal and distal tips [98]. c) Compression cortical screws [113]. d) Extramedullary pin clip fixation [114].
Chapter 4

Materials and Methods

4.1 Experimental Design

The experimental protocol is illustrated in figure 4.1. A group of 24 rats underwent femoral osteotomy with a non-critical defect of 1 mm. The rats were assigned an endpoint of either 5, 6, 7, or 8 weeks at which point the fractured and intact femur were harvested. The temporally varied endpoints allowed for samples from various stages during healing. Prior to harvesting, the fractured bone was radiographed laterally and anteroposteriorly. Two fellowship-trained orthopedic surgeons, one orthopedic surgery fellow and one orthopedic surgery resident scored the bone’s orthographic radiographs using RUST and modified RUST. The main outcome of this experiment was biomechanical strength of the healing bone as it indicates the progression towards regaining functional capability and structural integrity of the bone. Both the fractured bone and the contralateral bone were subjected to destructive torsional testing to measure bone stiffness and strength. Biomechanical tests are most often indicative of structural characterizations [50]. In order to get an insight into the material characterizations of the callus, prior to mechanical testing, the fracture callus was imaged using μ-CT to determine callus mineralization and geometric properties.
Chapter 4. Materials and Methods

Figure 4.1: Flowchart illustrating the key steps involved in this study.

4.2 Fracture Fixation

As a result of the pilot work described in chapter 3, it was decided to discontinue the use of intramedullary nail fixation due to lack of reproducibility, stabilization and, equivalence to osteosynthesis techniques in humans. Previous experimental work has utilized bridge plating as an effective means of stabilizing an osteotomy. Many studies have utilized bridge plating to evaluate various aspects of fracture healing with little reported fixation complication with critical sized defects [117, 118, 119, 120, 121]. However, bridge plating is also used for smaller defect sizes such as the in the study by Wildemann et al. [122]. The success and wide adoption of extramedullary plates is likely due to consistent restoration in length, alignment and rotation of the fracture fragments. In fact, Histing
et al. demonstrated that femur stabilization with a locking plate resulted in a rotational stiffness almost similar to the intact femur [80].

One disadvantage of plate fixation is the highly invasive nature of surgical technique as it requires full bone exposure. In this study, however, bone exposure is already necessary due to the osteotomy and will not be an added detriment. A more critical disadvantage is that bone plates are often made of titanium or stainless steel for their strength and biocompatibility. The negative repercussion of such metallic plates is that they are radiopaque and thus occlude any cortex adjacent to the plate in X-ray imaging. Seeing as the RUST score requires two orthogonal views, any cortical occlusion does not allow for the scoring to be made. In humans, this is less an issue as bone plates are often thinner than the bone’s diameter thus allowing visibility of four cortices and assignment of RUST [33, 72]. Contrarily, the small diameter of rat bones does not allow for metal plates that do not occlude the cortex.

The solution to plate occlusion adopted for this study is to use a radiolucent plate made of polyether ether ketone (PEEK). PEEK’s high thermal resistance allows it to be sterilized by autoclave. Additionally, Williams et al. showed that PEEK implanted in rabbits elucidated no negative biological response confirming its biocompatibility [123]. PEEK bone plates used in rats have also been previously reported in the literature [124, 125]. One such study by Poser et al. was used as a base for the design of the custom PEEK plates used in this study. The plate dimensions are illustrated in the design drawing figure 4.2. Due to the lesser stiffness of PEEK in comparison to stainless steel (Young’s modulus of 3.6 GPa compared to 190 GPa), the PEEK plates were designed thicker than their stainless steel counterpart. The plates were created by Computer Numerical Control (CNC) machining by Platech Inc., Concord, Canada. Figure 4.3 shows a regular picture of the PEEK plate next to the traditionally used stainless steel plate as well as a radiograph of the two plates illustrating the radiolucency of the PEEK plate.

It should be noted that primary bone healing can occur with bone plating due to the anatomical reduction of the fracture fragments coupled with rigid fixation. However, bridge plating relies on relatively flexible fixation at the bone defect location to promote secondary bone healing [126, 127]. Therefore in order to ensure that primary healing will not occur, a non-critical gap of 1 mm is to be created: similar to that reported by Wildemann et al. [122].
Figure 4.2: Design drawing of the PEEK plates used in this study.

Figure 4.3: Regular photograph (left) and radiograph (right) of stainless steel plate (left in each image) and PEEK plate (right in each image).
4.3 Animal Model

Adult male Fisher-344 rats weighing 250 to 300 grams were used in this model. The rapid growth of the traditionally used Sprague-Dawley rat caused bone conformational changes which is hypothesized to have contributed to the lack of fixation in the pilot study: the rats doubled their weight within six weeks. Fisher-344 rats have a significantly slower growth curve providing additional control over the bone size and fracture fixation.

4.4 Surgical Procedure

All surgical procedures were performed in accordance with the guidelines provided by the Animal Care Committee at the Keenan Research Centre in St. Michael’s Hospital. The animals were anesthetized with isoflurane inhalation (5% induction and 2% maintenance). A dose of buprenorphine analgesic (0.05 mg/kg) was given to the rat subcutaneously prior to the surgery and two doses were given per day over the 48 hours after surgery. The right thigh area was shaved and disinfected with providone-iodine prior to being draped. A lateral incision was made and the Tensor Fascia Lata was spread to expose the entire femoral diaphysis. With the bone still intact, the PEEK plate was placed on the lateral cortex and holes were drilled into the cortex through the four holes on the plate using a 1.1 mm drill bit under saline irrigation. The holes were tapped and titanium cortical screws (1.5 mm diameter) were inserted. By placing the plate on the bone prior to fracture, it allowed for easier re-alignment and proper reduction of the fragments that resulted from the osteotomy. The three most proximal cortical screws were removed and the most distal screw was loosened such that the plate could be rotated off of the bone. Two marks were made on the bone 1 mm apart at the midpoint between the two middle screw holes. An oscillating saw was used to create a complete transverse osteotomy at one of the marked points. Saline irrigation was used to prevent heat necrosis at the site of the osteotomy. A second cut was made at the second marked line under saline irrigation. The plate was rotated back into place and the three cortical screws were put back in. The osteotomy was checked visually to ensure that the cuts were not oblique. All soft tissue layers were closed using Vicryl 5-0 sutures and the skin was sutured intracutaneously with Vicryl 5-0 sutures. Rats were recovered in a bedding-free cage with a diaper pad on a warming blanket until fully conscious. Immediately after the operation, full weight bearing and cage activity was allowed.
4.5 In vivo monitoring and Radiographic Assessment

The fractured femora was imaged weekly post osteotomy using a GE AMX 4 Plus mobile xray machine set to a peak kilovoltage (kV) of 64, 2 milliampere-seconds (mAs) and tube-to-leg distance of 25 inches. Under anesthetic, the animals were placed in two positions as seen in figure 4.4 to procure an anteroposterior a lateral radiograph. The orthogonality of the radiographs was verified by the angle of the radiopaque screws. The orthographic radiographs were taken weekly, however only the radiographs on the day of sacrifice (5, 6, 7 or 8 weeks post-op) were assigned a RUST score.

Figure 4.4: Images demonstrating the positions in which the rats were placed in order to get anteroposterior (left) and lateral (right) radiographs.

Four independent and blinded readers assigned RUST and modified RUST scores to the set of orthogonal X-rays through a series of online forms. The readers included two fellowship-trained orthopedic surgeons, one orthopedic surgery fellow and one orthopedic surgery resident. They were given table 1.2 and table 1.3 to use as a guide. The individual cortex scores were reported to allow for a more in-depth interobserver comparison. Figure 4.5 illustrates an example of what the readers utilized to input their scores for RUST. A similar questionnaire was used for the modified RUST.
4.6 Specimen Harvest

The animals were sacrificed at either 5 (N=4), 6 (N=4), 7 (N=10) or 8 (N=6) weeks. The multiple time points allowed for a range of healing stages with greater influence on later time point, i.e. more advanced healing. The animals were sacrificed by intracardiac injection of T61 euthanizing agent after induction with 5% isoflurane inhalation. The operated femur was dissected with a thin layer of soft tissue around it and care to not disturb the fracture callus. The screws and plate were removed prior to wrapping the bone in saline-soaked gauze. The intact femur was also harvested and wrapped in saline-soaked gauze. Beaupied et al. found that freezing of rat femurs induced no densitometric, microarchitectural or biomechanical detriment [128]. Therefore, the two bones were kept together and placed in a −20°C freezer until µ-CT was performed.

4.7 Computed Tomography

The secondary outcome measures for this study were acquired using µ-CT imaging. Each fractured femur was imaged in the Bruker Skyscan 1174 desk-top micro-CT scanner. For each of the fractured femurs, approximately 0.75 cm of the diaphysis encompassing the full fracture callus was scanned with isotropic voxel size of 8 µm using a voltage of 50
kV and a current of 800 µA. The contralateral femurs were kept at room temperature as the fractured femurs were being scanned in order to control for freeze-thaw cycles.

Prior to each round of scanning, two hydroxyapatite (HA) phantoms were scanned with different concentrations: 750 mgHA/cm$^3$ and 1300 mgHA/cm$^3$. These phantoms were used as a calibration standard to analyze the fracture callus for bone mineral density by linearly correlating the concentrations with scan intensity output. The scans were reconstructed into tomographic images (known as slices) and subsequently analyzed using Bruker software packages (NRecon v.1.6.3.2, CTAn v.1.10.9 and CTVol v.2.3).

Figure 4.6: Example of the ”starting slice” of the region of interest (ROI) of one fracture callus illustrating the first visible break in the cortical ring in red.

A standardized method of analysis was defined as follows based on previous work and suitability for this study. All analyses were performed by the same person for all samples to ensure consistency in judgment. The distal boundary of the region of interest (ROI) was defined as the first image slice in which the cortical ring exhibited an external break such as that in figure 4.6. The ROI was then defined as 250 slices proximal to that slice which resulted in a section of the disphysis of 1.8 mm. This encompassed all callus within and around the defect site as well as a small amount of cortical bone on both proximal and distal ends. Since the osteotomy defects were all of similar size, the amount of cortical bone encompassed in the ROI is relatively constant across samples and should not affect volume measurements. Additionally, using a constant number of
slices allows for a more accurate comparison of the tissue volume without the need to normalize for length.

On each of the 250 2D tomograms in the ROI, semi-automatic segmentation was used to define the outer boundary of the callus and the tissue volume. Contours were hand-drawn around the outer boundary of the bone at every 50 slices and contours were interpolated for the remaining slices. All slices were then reviewed and edited if necessary to ensure that the contour properly defined the tomogram in question. All cavities enclosed by bone were included in the volume whereas those that were not enclosed by bone were omitted from the volume. An example of contouring is demonstrated in figure 4.7.

![Figure 4.7: Example of a contoured tomogram (right) next to the raw image (left). The volume enclosed in red is the tissue volume (TV).](image)

A custom processing module was used to smooth, filter, de-speckle, threshold and remove noise from the ROI prior to calculating desired geometric and material characterizations. A fixed global threshold of 55-255 on the greyscale which corresponds to a mineral density of approximately 786 mgHA/cm$^3$ was used to distinguish mineralized bone from poorly mineralized tissue such as soft callus. The threshold was decided based on previous research as well as the visual inspection of the tomograms such that it encompassed the majority of cortical bone.

Morgan et al. described critical fracture callus properties to be assessed using $\mu$-CT [129]. These include total callus volume (TV, mm$^3$), mineralized callus volume (BV, mm$^3$), callus mineralized volume fraction (BV/TV, %) and bone mineral density (BMD, mgHA/cm$^3$). The latter of the aforementioned $\mu$-CT measures, bone mineral density, is highly affected by the presence of cortical bone as fully developed bone is significantly
higher mineralized than callus. Therefore, a different ROI was defined for this measure. A section of 138 slices, or 1 mm, was defined at the center of the previously defined ROI. This section fell within the osteotomy defect ensuring that only the callus was being included in the bone mineral density measurement and none of the original cortical bone.

Finally, from the 2D tomograms, geometric measurements were taken. By approximating the fracture callus as a circular shaft, two measurements of the radius were recorded for the proximal, distal and middle tomogram in the ROI. An average of the six resulting measurements was used to approximate the fracture callus polar moment of inertia according to equation (4.1) where $r_o$ is the average outer radius.

$$J = \frac{\pi}{2}(r_o^4)$$

### 4.8 Biomechanical Testing

Both the fractured and contralateral femurs were subjected to destructive torsional testing as the primary outcome for this study. All specimens were defrosted from the $-20^\circ$C freezer by placing at room temperature for 12 hours prior to testing. A MTS 858 Bionix Test System (MTS Systems, MN, USA) was used along with a torque cell rated at 1.3 Nm capacity (Futek, CA, USA). The proximal end of the bone was potted in polymethylmethacrylate (PMMA), also known as bone cement, and aligned using a custom holder as seen in a of figure 4.8. Subsequently, the pot was screwed onto the MTS machine and the distal end of the bone was potted in PMMA resulting in the configuration in b of figure 4.8. The distance between the two pots, or the gauge length, was kept constant at 20 mm as it encompassed the full callus as well as a portion of cortical bone. Angular displacement was applied at a rate of 1 degree/second to the proximal end of the bone while the distal end remained static. The loading rate was chosen based on previous work by Tinsley et al. and Li et al. [117, 125]. Angular displacement was applied up to a maximum displacement of 50° at which point the callus is considered fibrous nonunion. Torque was measured at a frequency of 100 Hz.

After fracture, the location of fracture was measured with a caliper. For the contralateral specimens, the outer and inner radius was also measured. The resulting angular movement and torque data were analyzed according to the graphical interpretation and equations described in section 1.3.3. All biomechanical properties for the fractured femurs were expressed as a percentage of the contralateral femur.
Figure 4.8: Images of biomechanics torsion testing setup. a) A contralateral femur potted in a custom made alignment holder. b) A fractured femur potted proximally and distally in the MTS prior to testing.

4.9 Statistical Analysis

The RUST and modified RUST scores assigned by each radiographic reviewer for each cortex were evaluated for statistically significant differences (N=96, all scores included). The traditionally used ANOVA for comparison of means was not possible as the Shapiro-Wilk test for normality failed and ANOVA is not appropriate for ordinal variables such as the RUST score. Comparison of mean cortex scores was therefore performed with an analysis of variance using the non-parametric Kruskal-Wallis H test.

Agreement among radiographic reviewers was performed for each cortex as well as the overall score using intraclass correlation coefficients (ICC) using model 2 (each subject is assessed by each rater and the raters have been randomly selected) and single-measurement form. Absolute agreement with single measure was reported in order to include possible systematic differences among raters. Landis and Koch suggest the following interpretation of kappa which is consistent with ICC: 0-0.20 represents “slight agreement”, 0.21-0.40 “fair agreement”, 0.41-0.60 “moderate agreement”, 0.61-0.80 “substantial agreement” and above 0.80 is almost “perfect agreement” [130].

Since the methods used in this study did not allow for control over RUST score
group size, comparative analysis between RUST scores and imaging and biomechanics properties was not possible. The difference in sample size caused statistically significant heterogeneity in variance between score groups according to the Levene test of equality of variances. Instead, the strength of relationship of the score to healing parameters was measured. Since RUST is an ordinal variable and the relationships assumed to be monotonic, a Spearman’s rank correlation coefficient was calculated between the specimen’s radiographic scores and, imaging and mechanical properties. The average RUST and modified RUST scores from the four reviewers, including decimals, were used when evaluating the Speaman’s rank correlation coefficient in order to account for the variation and uncertainty in the actual score. It should be noted that the RUST score is integer based and scores with decimals are not clinically relevant.

IBM SPSS v.20.0.0 was used to compute all statistics and all cases were considered significant using a p-value of 0.05 with a two-tailed analysis.
Chapter 5

Results

5.1 Fracture Model and Sample Size

Of the 24 animals that underwent osteotomy and plate fixation, one animal showed signs of deep infection and hardware loosening via radiograph: the animal was excluded from the study. The remaining animals showed no signs of infection or hardware loosening.

Two specimens were broken while handling after μ-CT and during biomechanics. Both are therefore included in the μ-CT results (N=23) but excluded from the biomechanics results (N=21).

Figure 5.1 illustrates the weekly radiographs of a representative femur as the healing progressed until 7 weeks after the osteotomy.

5.2 Radiographic Scoring

The 24 animals in this study that were sacrificed at 5 (N=4), 6 (N=4), 7 (N=10) or 8 (N=6) weeks post osteotomy were radiographed prior to bone dissection. An average RUST and modified RUST score was assigned to each rat based on the scores given by four independent reviewers referred to as Reviewer 1 through 4 (randomly assigned). Scoring agreement among all four radiographic reviewers was determined by intraclass correlation coefficients for all cortex scores as well as the overall score as outlined in table 5.1.

The overall agreement among the four reviewers falls into what is considered the “moderate agreement” category. Such a moderate agreement among scores does not allow for high confidence in the bone’s actual score on which the impending correlations will be based. In fact, a statistically significant difference was found between the individual
Figure 5.1: Representative anteroposterior and lateral radiographs of one animal every week until 7 weeks after osteotomy.
Table 5.1: Intraclass correlation coefficients, ICC(2,1), and 95% confidence interval (CI) for each cortex and overall RUST and modified RUST score according to the four radiographic reviewers in this study. N=24.

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<th>RUST</th>
<th>Modified RUST</th>
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<tr>
<td></td>
<td>ICC (95% CI)</td>
<td>ICC (95% CI)</td>
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<tr>
<td>Lateral Cortex</td>
<td>0.424 (0.155-0.669)</td>
<td>0.422 (0.103-0.690)</td>
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<tr>
<td>Medial Cortex</td>
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<td>0.620 (0.297-0.818)</td>
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<tr>
<td>Posterior Cortex</td>
<td>0.344 (0.130-0.582)</td>
<td>0.292 (0.070-0.545)</td>
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<tr>
<td>Anterior Cortex</td>
<td>0.462 (0.255-0.673)</td>
<td>0.641 (0.379-0.819)</td>
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<td></td>
</tr>
<tr>
<td>Sum</td>
<td>0.535 (0.157-0.780)</td>
<td>0.477 (0.108-0.745)</td>
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rater’s RUST scores according to a Kruskal-Wallis H test ($\chi^2 (3)=26.512$, $p<0.001$). Similarly, a statistically significant difference was found between raters’ modified RUST scores ($\chi^2 (3)=32.562$, $p<0.001$).

As such, the systematic error introduced by one or multiple of the radiographic reviewers must be eliminated. The use of one reviewer is undesired due to the possibility of additional systematic error. In order to evaluate which group of reviewers produced the most reliable scores, agreement ICCs among Reviewers 1 to 4 for their assigned RUST and modified RUST scores are listed in table 5.2 for all combinations of 2 or more reviewers.

Table 5.2: Intraclass correlation coefficients, ICC(2,1), and 95% confidence interval (CI) for each combination of two or more reviewers. N=24.

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<th>RUST</th>
<th>Modified RUST</th>
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<td></td>
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<td>ICC (95% CI)</td>
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<tr>
<td>1,2,3,4</td>
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<td>0.477 (0.108-0.745)</td>
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<tr>
<td>2,3,4</td>
<td>0.545 (0.080-0.805)</td>
<td>0.462 (0.033-0.755)</td>
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<td>1,3,4</td>
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<tr>
<td>1,2,3</td>
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<td>0.418 (0.014-0.727)</td>
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<tr>
<td>1,2</td>
<td>0.637 (0.044-0.862)</td>
<td>0.595 (-0.092-0.869)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,3</td>
<td>0.281 (-0.063-0.667)</td>
<td>0.222 (-0.047-0.602)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,4</td>
<td>0.852 (0.681-0.934)</td>
<td>0.831 (0.649-0.923)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,3</td>
<td>0.514 (-0.034-0.795)</td>
<td>0.535 (-0.022-0.807)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,4</td>
<td>0.803 (0.185-0.936)</td>
<td>0.631 (-0.083-0.881)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3,4</td>
<td>0.360 (-0.068-0.741)</td>
<td>0.298 (-0.073-0.680)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The highest agreement for RUST and modified RUST scores was in the group of
Figure 5.2: Distribution of the average RUST scores assigned by two fellowship-trained orthopedic surgeons to each of the 24 animals in this study, indexed by number of weeks after osteotomy.

Reviewer 1 and Reviewer 4. Both of these reviewers are trauma fellowship-trained orthopaedic surgeons. Due to their expertise and the very high agreement among the two independent reviewers, it was decided to move forward with only the scores assigned by two reviewers. If the two reviewers agreed on the score, that score was used as the definitive RUST score. The two reviewers disagreed in 16/24 cases in which they never differed by more than one point. In such case of disagreement, the score assigned by Reviewer 2 (the orthopaedic surgery fellow) was consulted as this reviewer showed substantial agreement with the two chosen reviewers. The definitive RUST score was chosen as the score assigned by one of the two fellowship-trained orthopaedic surgeons that lay closest to the score assigned by the orthopaedic fellow. This method avoided the use of average RUST scores with clinically irrelevant decimal points while ensuring an accurate and agreed upon score. As such, for the remainder of this document, the RUST and modified RUST scores will refer to scores that result from the aforementioned method.

The resulting score frequency distribution is illustrated in figure 5.2 and figure 5.3 for the RUST score and the modified RUST score respectively.

The RUST and modified RUST scores can be used to evaluate the fracture model. All animals showed signs of bridging callus at 5 weeks and different levels of healing at their assigned endpoints. A Kruskal-Wallis H test was performed to determined that there was a statistically significant difference between the mean cortex RUST scores of all animals ($\chi^2 (3)=25.697$, $p<0.001$). Post-hoc analysis further demonstrated that the average RUST score for the posterior cortex is statistically significantly lower than the three other cortices as is illustrated in figure 5.4. The modified RUST score exhibited...
Figure 5.3: Distribution of the average modified RUST scores assigned by two fellowship-trained orthopedic surgeons to each of the 24 animals in this study, indexed by number of weeks after osteotomy.

statistically significant differences for the posterior and anterior cortices (Kruskal-Wallis H test ($\chi^2 (3)=19.089$, p<0.001) as is illustrated in figure 5.5.
Figure 5.4: Average of two fellowship-trained orthopaedic surgeons’ RUST scores for the lateral, medial, posterior and anterior cortices. Statistically significant differences illustrated with * (Kruskal-Wallis H test, p<0.05).

Figure 5.5: Average of two fellowship-trained orthopaedic surgeons’ modified RUST scores for the lateral, medial, posterior and anterior cortices. Statistically significant differences illustrated with * (Kruskal-Wallis H test, p<0.05).
5.3 Computed Tomography

The measured values for total callus volume (TV), mineralized callus volume (BV), total to mineralized callus volume ratio (BV/TV) and bone mineral density are plotted against the sample’s RUST and modified RUST scores in figure 5.6. Spearman’s rank correlation coefficients were computed for each of the aforementioned parameters and are outlined in table 5.3. Statistically significant correlations were found for total callus volume, mineralized callus volume and total to mineralized callus volume ratio.

Table 5.3: The Spearman correlation coefficients ($r_s$) and statistical significance (p) for the relationships between $\mu$-CT parameters and, RUST and modified RUST. N=23.

<table>
<thead>
<tr>
<th></th>
<th>RUST</th>
<th>Modified RUST</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r_s$</td>
<td>(p)</td>
</tr>
<tr>
<td>Mineralized Callus Volume (BV)*</td>
<td>0.820</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>Total Callus Volume (TV)*</td>
<td>0.779</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>BV/TV*</td>
<td>0.472</td>
<td>(0.024)</td>
</tr>
<tr>
<td>Bone Mineral Density (BMD)</td>
<td>0.278</td>
<td>(0.188)</td>
</tr>
</tbody>
</table>

* signifies a statistically significant relationship (p<0.05).
Figure 5.6: Graphical representation of \( \mu \)-CT parameters and, RUST and modified RUST. N=23.
5.4 Biomechanics

Destructive torsional testing was performed on all fractured femurs and contralateral femurs. Due to the two samples which fractured during handling and the one sample that exhibited deep infection, a total of 21 samples were tested (21 fractured femurs and 21 contralateral femurs). All fractured legs failed at the osteotomy location and all contralateral femurs exhibited spiral fractures. Three of the 21 samples tested did not show a clear failure (i.e. no sharp decline in measured torque) up to the maximum applied angle of 50°: they scored RUST of \{5,5 and 6\} and modified RUST of \{5,6 and 7\}. The remaining specimens had a clearly discernible maximum torque taken as the ultimate torque.

The measured values for ultimate torque, angle at failure, stiffness, shear strain, torsional strength and shear modulus of elasticity for each sample as a function of the contralateral leg is plotted against the sample’s RUST and modified RUST scores in figure 5.7. Spearman’s rank correlation coefficients were computed for each of the aforementioned parameters and are outlined in table 5.4.

Table 5.4: The Spearman correlation coefficients ($r_s$) and statistical significance (p) for the relationships between biomechanical parameters of the fractured femur as a function of the contralateral femur and, RUST and modified RUST. N=23.

<table>
<thead>
<tr>
<th></th>
<th>RUST</th>
<th>Modified RUST</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r_s$</td>
<td>(p)</td>
</tr>
<tr>
<td>Ultimate Torque*</td>
<td>0.911</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>Angle at Failure</td>
<td>0.420</td>
<td>(0.058)</td>
</tr>
<tr>
<td>Stiffness*</td>
<td>0.774</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>Maximum Shear Strain*</td>
<td>0.557</td>
<td>(0.009)</td>
</tr>
<tr>
<td>Torsional Strength*</td>
<td>0.817</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>Shear Modulus of Elasticity</td>
<td>0.300</td>
<td>(0.186)</td>
</tr>
</tbody>
</table>

* signifies a statistically significant relationship (p<0.05).
Figure 5.7: Graphical illustration of biomechanical parameters of the fractured femurs as a percentage of its contralateral femur.

For illustrative purposes, one sample for each RUST score from 6 to 11 was chosen as a representative sample which was used in the µ-CT 3D reconstruction of the callus as well as the resulting biomechanical graphs of the fractured leg and contralateral leg in figure 5.8.
<table>
<thead>
<tr>
<th>RUST</th>
<th>Modified RUST</th>
<th>Micro-CT</th>
<th>Biomechanics</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>7</td>
<td>BV = 3.80 mm$^3$&lt;br&gt;TV = 8.67 mm$^3$&lt;br&gt;BV/TV = 43.87 %&lt;br&gt;BMD = 1.18 g/cm$^3$</td>
<td>$T = 0$ Nmm&lt;br&gt;$\alpha = 0$ deg&lt;br&gt;$k = 0$ Nmm/deg&lt;br&gt;$\gamma = 0$ rad&lt;br&gt;$\tau = 0$ GPa&lt;br&gt;$\sigma = 0$ GPa</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>BV = 4.96 mm$^3$&lt;br&gt;TV = 10.10 mm$^3$&lt;br&gt;BV/TV = 49.11 %&lt;br&gt;BMD = 1.19 g/cm$^3$</td>
<td>$T = 52.5$ Nmm&lt;br&gt;$\alpha = 7.7$ deg&lt;br&gt;$k = 7.1$ Nmm/deg&lt;br&gt;$\gamma = 9.2e-3$ rad&lt;br&gt;$\tau = 14.2$ MPa&lt;br&gt;$\sigma = 1.5$ GPa</td>
</tr>
<tr>
<td>8</td>
<td>10</td>
<td>BV = 5.16 mm$^3$&lt;br&gt;TV = 9.77 mm$^3$&lt;br&gt;BV/TV = 52.74 %&lt;br&gt;BMD = 1.20 g/cm$^3$</td>
<td>$T = 82.7$ Nmm&lt;br&gt;$\alpha = 6.3$ deg&lt;br&gt;$k = 14.5$ Nmm/deg&lt;br&gt;$\gamma = 7.0e-3$ rad&lt;br&gt;$\tau = 26.2$ MPa&lt;br&gt;$\sigma = 3.8$ GPa</td>
</tr>
<tr>
<td>9</td>
<td>12</td>
<td>BV = 8.34 mm$^3$&lt;br&gt;TV = 18.42 mm$^3$&lt;br&gt;BV/TV = 45.26 %&lt;br&gt;BMD = 1.20 g/cm$^3$</td>
<td>$T = 163.4$ Nmm&lt;br&gt;$\alpha = 10.4$ deg&lt;br&gt;$k = 15.2$ Nmm/deg&lt;br&gt;$\gamma = 17.1e-3$ rad&lt;br&gt;$\tau = 17.1$ MPa&lt;br&gt;$\sigma = 1.0$ GPa</td>
</tr>
<tr>
<td>10</td>
<td>15</td>
<td>BV = 11.13 mm$^3$&lt;br&gt;TV = 21.84 mm$^3$&lt;br&gt;BV/TV = 50.89 %&lt;br&gt;BMD = 1.16 g/cm$^3$</td>
<td>$T = 257.7$ Nmm&lt;br&gt;$\alpha = 12.9$ deg&lt;br&gt;$k = 21.3$ Nmm/deg&lt;br&gt;$\gamma = 20.8e-3$ rad&lt;br&gt;$\tau = 26.0$ MPa&lt;br&gt;$\sigma = 1.25$ GPa</td>
</tr>
<tr>
<td>11</td>
<td>14</td>
<td>BV = 10.20 mm$^3$&lt;br&gt;TV = 23.40 mm$^3$&lt;br&gt;BV/TV = 43.60 %&lt;br&gt;BMD = 1.16 g/cm$^3$</td>
<td>$T = 347.6$ Nmm&lt;br&gt;$\alpha = 15.5$ deg&lt;br&gt;$k = 24.6$ Nmm/deg&lt;br&gt;$\gamma = 27.5e-3$ rad&lt;br&gt;$\tau = 30.7$ MPa&lt;br&gt;$\sigma = 11.2$ GPa</td>
</tr>
</tbody>
</table>
Figure 5.8 *(preceding page)*: Representative samples of each RUST score from 6 to 11. From the $\mu$-Ct imaging, a 3D render is illustrated of the fracture callus along side the mineralized callus volume (BV), total callus volume (TV), mineralized to total callus volume ratio (BV/TV) and bone mineral density (BMD). From biomechanical testing, a graph illustrating the angle vs. torque relationship for the fracture callus (purple) and the contralateral femur (green) is shown. Additionally, the ultimate torque (T), angle to failure ($\alpha$), stiffness (k), shear strain ($\gamma$), torsional strength ($\tau$) and shear modulus of elasticity (G) of the fractured bone.
Chapter 6

Discussion

There is currently a lack of evidence-based assessment tools to gauge the progression of bone healing. With approximately 1-5% of all skeletal fractures resulting in nonunion, such an assessment tool is crucial to aiding clinicians in predicting and managing complications and to improve patient outcome. Additionally, an objective parameter to gauge bone healing can aid in the comparison of research studies and clinical trials.

At present, clinicians and researchers use a multitude of fracture healing assessment tools including physical examination, imaging, biomechanics and serological markers. The most common method utilized by orthopaedists remains plain X-ray radiography due to its low cost and easy availability. For this reason, studies have proposed various radiographic tools with limited success. The Radiographic Union Score for Tibial fractures (RUST) and its modified counterpart were born from the need for an assessment tool as well as radiographic parameters that showed promise in the literature: cortical bridging and fracture line visibility. The scoring system is outlined in section 1.4. The RUST and modified RUST scores have been gaining popularity in the literature and have been evaluated for interobserver agreement. These previous studies confirming interobserver reliability have analyzed the precision of the score but they have not evaluated its accuracy. For this, the score must be compared to current gold standards in fracture healing assessment [66].

As such, the present study aims to evaluate the relationship of the RUST and modified RUST scores to computed tomography and biomechanical parameters of a healing bone.
6.1 Fracture Model

A PEEK bridge plate placed on the lateral cortex with a non-critical 1 mm defect osteotomy was utilized as the fracture model for this study. The time to heal with this fracture model was unknown; therefore 4 different timepoints were used for endpoints with an emphasis on later timepoints in an attempt to increase the number of animals with higher scores (more advanced in healing). Advanced healing was desired in order to select a RUST score that could represent a clinically healed bone.

It was found that the posterior cortex scored statistically significantly lower than the other cortices and the anterior cortex scored statistically significantly higher than the other cortices with the RUST and modified RUST scoring systems respectively. This suggests that the fracture model favoured the healing of the anterior cortex and hindered the healing of the posterior cortex. Bridge plating has been shown to exhibit asymmetric gap closure with the least amount of callus formation expected adjacent to the plate due to decreased interfragmentary motion in proximity to the plate [131, 132]. Contrary to the interfragmentary motion theory, in this study, the lateral cortex was adjacent to the bone plate and did not show significant differences in RUST or modified RUST scores. Figure 6.1 is a tomograph resulting from $\mu$-CT of a representative case. This tomogram shows the location of the plate, the original cortical bone as well as the location of callus formation.

Since radiographic visibility of all four cortices is uncommon for bridge plating of
rat femurs, the literature on the topic is limited. The cortical discrepancy in callus formation may be explained biomechanically. The PEEK plate may be stress shielding the proximal cortex due to its increased moment of inertia in the thickness plane of the plate. Another possible explanation may lie with the weight bearing pattern of the rat during gait. Ultimately, conclusions on the matter warrant further study of the fracture model and the resulting stress distributions at the fracture site.

While it is unexpected for the femur, such asymmetric healing is common place for the tibia. Vascularization is one of the main factors involved in the achievement of bony union. In particular, after fracture, a bone may rely on extraosseous blood supply by surrounding soft tissues for nutrients essential to initiating the healing process. However, the tibia’s anatomical location is only sparsely covered by soft tissue anteriorly and will therefore result in asymmetrically favoured cortices when healing [133]. The RUST scoring system, having been originally designed for the tibia, evaluates each cortex separately due to the possibility of cortical discrepancies in healing. This discrepancy could be considered analogous to that observed with the fracture model used in this study. However, more conclusive testing into the reasoning behind the discrepancy in cortical healing with this fracture model should be established before this can be affirmed.

6.2 Agreement in Radiographic Scoring

The animals in this study which were sacrificed at either 5, 6, 7, or 8 weeks post osteotomy resulted in a RUST distribution of 6 to 11 with the most common scores being 6, 9 and 10. No animals reached RUST of 12. Accordingly, the modified RUST resulted in a distribution of 6 to 14 with the most common scores being 7, 13 and 14. No animals reached modified RUST of 15 or 16. The animals that scored highest varied in their time-points post osteotomy. In fact, neither RUST nor modified RUST showed a statistically significant correlation with time ($r_s=0.152$ $p=0.478$ and $r_s=0.116$ $p=0.590$ respectively). This is likely due to differences in osteogenic potential between rat specimens as well as slight variations in surgical technique (i.e. some animals exhibited significant bleeding during surgery which may have caused delays in healing).

The interobserver reliability of the RUST and modified RUST scores were quantified with intraclass correlation coefficients (ICC) for each of the cortices as well as the overall scores assigned by each of the four radiographic reviewers which consisted of two fellowship-trained orthopaedic surgeons, one orthopaedic surgery fellow and one orthopaedic surgery resident. The ICC for RUST was slightly higher than that for the modified RUST for all but one cortex. Overall, the ICC for the RUST score was higher
than for the modified RUST score (ICC=0.535 and 0.477 respectively). This contradicts the literature by Litrenta et al. and Tornetta et al. which showed greater interobserver reliability for the modified RUST when evaluating tibial and femoral fractures with nails and plates [33, 56].

Both RUST and modified RUST values for all four reviewers fall within the “moderate agreement” category. Previous assessments of interobserver reliability of the RUST score using tibial fractures of human and rat fixated with intramedullary nailing demonstrated excellent agreement with ICC values greater than 0.80 [66, 68, 69]. The lower interobserver ratings found in this study are more similar to those in the study by Litrenta et al. that evaluated RUST scores for bones with plates [33]. Litrenta et al. reported an ICC of 0.53 for femoral fractures treated with plates versus 0.67 for those treated with intramedullary nails. This difference is likely a product of the differences of healing processes. While plate fixation will still result in secondary healing, there is less need for the same robust callus that is produced with intramedullary nailing to provide additional fracture stabilization. The lowest ICC and lowest RUST scores were observed for the posterior cortex in this study which further illustrates the relationship between interobserver reliability and lack of callus presence. Additionally, Tornetta et al. found lower ICC values for RUST scoring using radiolucent intramedullary nails when compared to radiopaque intramedullary nails in sheep [56]. It is hypothesized that the radiopaque nail acts as a clear delineation between cortices in radiographs which may aid in scoring agreement. Such delineation is not present with radiolucent nails or plates.

The main reason that is hypothesized to have caused the lower ICC values in this study in contrast to that reported in the literature, is that there was a statistically significant difference measured between the individual raters’ scores according to a Kruskal-Wallis H test ($\chi^2 (3)=26.512, p<0.001$ and $\chi^2 (3)=32.562, p<0.001$). By testing the overall RUST and modified RUST score for consistency among the four reviewers instead of absolute agreement, we get ICC (95% CI) of 0.796 (0.664-0.894) for RUST and 0.791 (0.656-0.891) for the modified RUST score. Both of which then fall at the top of the “substantial agreement” category. While this may confirm that the reviewers are consistent with their scores, it brings forth the possible lack of objectivity of definitions used in the score such as “fracture line visibility”. In particular, the orthopaedic resident was shown to be the only reviewer to have scored significantly differently from all other reviewers. With respect to this study, this introduces the possibility of a systematic error with the RUST and modified RUST scores. The lack of agreement among reviewers creates uncertainty as to what is the actual score for the radiograph. If the score is uncertain, it is then not possible to evaluate its relationship to clinical healing parameters such as
µ-CT and biomechanics. Although the RUST and modified RUST scores are intended to be used by all clinicians, experienced and inexperienced alike, the purpose of this study requires a level of confidence in the score assigned to the radiographs. In order to achieve such confidence, it was decided to assess the agreement among combinations of two or more reviewers. It was found that the absolute agreement ICC among the two fellowship-trained orthopaedic surgeons was the highest among all reviewers: 0.852 (0.681-0.934) for RUST and 0.831 (0.649-0.923) for modified RUST. It can therefore be stipulated that the lack of objectivity of the definitions may be a result of experience and training. In response to this finding, it was decided to utilize only the scores provided by the two experienced fellowship-trained surgeons to evaluate all relationships with healing outcomes. The scores from the orthopaedic surgery fellow were consulted to resolve any disagreement among the two fellowship-trained orthopaedic surgeons.

6.3 Computed Tomography

The fracture callus was imaged using µ-CT to determine callus mineralization and geometric properties. It was found that both the RUST and modified RUST statistically significantly correlated with mineralized callus volume, total callus volume and mineralized to total callus volume ratio. Mineralized callus volume and total callus volume showed higher correlations than the mineralized to total callus volume ratio. The RUST score is fundamentally based on the radiographic presence of callus. The high correlation between the RUST and modified RUST scores with mineralized callus volume and total callus volume confirm that the identification of callus on a 2D plain radiograph can predict the actual presence of callus at the fracture site. A possible implication of the predictive value of 2D plain radiography in predicting computed tomography parameters is that it reduces the need for CT scanning which is financially costly and exposes the patient to a high dose of radiation. Further research should be conducted to develop a numerical relationship between RUST and, total callus volume and mineralized callus volume which may allow for the clinical assessment of such parameters without CT scanning, rather two simple orthogonal radiographs. Interestingly, the goal of the modified RUST is to further qualify the amount of callus present by defining the callus as bridging or not bridging. Indeed, the modified RUST showed a slightly greater correlation between the mineralized callus volume and the total callus volume in comparison to RUST. This may confirm that the additional breakdown of scores does in fact better characterize the fracture callus.

The lower correlation between BV/TV is likely due to a simultaneous increase in tissue
and calcification of tissue resulting in a relatively slow growth of bone percentage. This is especially likely to be true at the early stages of callus formation which is evaluated in this study due to the resulting RUST scores (no specimens with RUST above 11 or modified RUST above 14). It is expected that above a RUST score of 11, as the fracture line begins to occlude, the remodeling stage will commence and the percentage of mineralized tissue (or bone volume) will increase.

Mineralization is a crucial and complex step in fracture healing. The bone mineral density (BMD) was measured of a section of callus in the middle of the osteotomy with reference to hydroxyapatite phantoms. The resulting BMD in this study did not correlate significantly with the RUST nor the modified RUST score. On the other hand, there was a strong significant correlation between BMD and the age of the animal at sacrifice \( r_s = 0.718 \ p < 0.001 \). Accordingly, Banu et al. showed an increase in bone mineral content in intact F344 rat femurs with age up to 24 months [134]. The relationship of bone mineral density to a healing callus is less certain. Cattermole et al. showed an increase in bone mineral density at the site of fracture over time in human tibial fractures [135]. However, evidence of a non-monotonic relationship has been found in healing fractures in rats. Korkusuz et al. proposed that the bone mineral density peaks at one point early in healing after which it decreases [136]. The authors suggest that such a non-monotonic relationship should not be used as a definitive tool of quantitative evaluation. Similarly, Kaspar et al. found extensive mineralization of the intramedullary cavity at an early phase post fracture [137]. Such findings have also been confirmed elsewhere [138, 139]. This, in combination with the varied timing of the rat’s healing patterns, could explain the lack of correlation between the RUST and modified RUST with BMD. It should also be noted that rat bone’s onset of rapid mineralization in the middle phase of healing is not representative of the early phase of healing in large animals or humans which generally experience significant mineralization in later stages of healing (i.e. hard callus formation) [137]. It is therefore difficult to conclude whether the RUST or modified RUST will correlate with bone mineral density in humans or large animals. Given the possibility to measure BMD non-invasively via CT or DEXA (dual-energy x-ray absorptiometry), the relationship between the score and bone mineral density should be undertaken in a clinical setting. Ultimately, the lack of correlation between BMD and RUST indicates that the scoring system shows no relationship to callus material properties at the early stages of healing. A correlation may present itself at the higher RUST scores when the fracture callus begins to remodel itself. Further research into the late stages of healing is required to confirm such a relationship. At any rate, the relationship of BMD to bone mechanics seems uncertain in the literature with some claiming a strong
correlation and others claiming that BMD is only a minor player in a bone’s mechanics [135, 129, 140].

6.4 Biomechanics

The restoration of biomechanical strength is the pillar of fracture healing [31]. Once a bone has been restored to its mechanical strength, a patient can return to daily living. It was therefore crucial to evaluate whether the RUST score and the modified RUST score correlate with biomechanical strength in order to confirm the scores’ future applicability as a predictor of healing.

Destructive torsion testing was used to calculate the ultimate torque, angle at failure, stiffness, shear strain, torsional strength and shear modulus of elasticity of both the fractured femur and the animal’s contralateral femur. The outcome parameters of the fractured femur were reported as a percentage of the contralateral femur in an effort to normalize for age and weight discrepancies between rats at time of sacrifice.

Of all specimens tested, three did not show any sign of failure at the allotted maximum angular displacement of $50^\circ$ suggesting that the fracture callus was fibrous with no bony union. These specimens scored RUST of [6,6,6] and modified RUST of [7,8,8]. Such scores require at least two cortices to present callus formation. These findings indicate that although callus formation is visible on the radiograph, this does not imply mineralized bony union is present. Referring to the biomechanical stages of union proposed by White et al. and described in section 1.3.3, these fractures would be considered to be in stage 1 of healing, with low stiffness [40]. The remaining specimens exhibited clear spiral fracture patterns through the osteotomy location. This indicated that, up to RUST scores of 11 and modified RUST of 14, fractures fall within stages 2 and 3 of biomechanical healing.

Ultimate torque, stiffness and torsional strength showed strong statistically significantly correlation with RUST and modified RUST. This finding suggests that the radiographic scores have a strong relationship with biomechanical healing and, in turn, provides a basis on which to confirm the accuracy of the score. Ultimate torque, the maximum torque that can be sustained by the bone prior to fracture, when compared to intact bone can be interpreted as the return of the bone’s ability to bear weight. In this study, the ultimate torque showed the highest correlation with both RUST and modified RUST ($r_s=0.911$ and 0.844 respectfully). While the differences in sample size and heterogeneity in variance of the score groups do not allow for accurate statistical comparison between RUST score groups, visual inspection of the relationship between ultimate torque and RUST in figure 5.7 show a linear pattern with moderately consistent
differences between score groups. Table 6.1 and table 6.2 outline the sample size, mean ultimate torque and standard deviation of the RUST and modified RUST score groups.

In one study, Litrenta et al. found that over 90% of radiographic reviewers assigned union to bones that score a RUST of 10 and a modified RUST of 13 [33]. A RUST of 10 requires a minimum of two fully healed cortices (callus with no fracture line) with a maximum of one unhealed cortex (no callus). In this study, the average ultimate torque at a RUST of 10 was 66.8% of the contralateral leg and 57.6% for a modified RUST of 13. It is unclear how much of the original strength must be restored in a bone before it is considered healed or safe for weight bearing to be returned. Clinical outcome measures such as pain at the fracture site and ability to weight bear may return prior to the complete restoration of biomechanical properties. To this end, Garcia et al. proposed that the osseous bridging of the whole circumference is not necessary for the determination of bone union [84]. Additionally, Cecik and colleagues found that the RUST score correlated highly with various physical function and pain scores assigned by clinicians during follow-up visits after fracture [141]. Therefore, the percentage of restored strength at various RUST scores may be an indication of how much strength must be restored before the bone is considered healed. It should be noted that the differentials between score groups are simply postulated based on visual inspection. The large standard deviation and sample size of RUST and modified RUST score groups makes statistically accurate comparison difficult.

The aforementioned study by Litrenta et al. based their proposed score of 10 and modified score of 13 on radiographic inspection by clinicians [33]. Quantitatively, it is difficult to confirm this finding in this study due to the lack of plateau observed in healing parameters. It was originally hypothesized that the mechanical parameters of the fractured bones would plateau at higher RUST scores, showing large differentials between lower RUST scores which decrease as the score rises. Such a phenomenon has been observed in previous studies on fracture callus mechanics [48]. Moreover, Kaspar et al. found that rat femurs at 56 days post osteotomy (0.4 mm wide) regained up to 200% of the contralateral ultimate torque likely due to the increased polar moment of inertia from the large callus formation [137]. The attainment of over 100% of contralateral strength was not observed in this study. The lack of plateau and the lack of increase greater than 100% of contralateral strength in this study may indicate that a RUST of 11 (the highest observed RUST score in this study) is attained prior to the bone completing the full stages of histological healing. That said, clinical healing (i.e. the return of weight bearing capability) may occur before histological stages of remodeling are complete. Clinical healing may then align with a RUST of 12 or lower. It should be
noted that the low number of samples at RUST of 11 and 12 (2 and 0 respectively) do not allow for a complete analysis of the progression of biomechanical strength at higher scores. Further specimens should be evaluated in future research.

Table 6.1: Sample size and, mean ultimate torque and standard deviation of ultimate torque as a function of the contralateral leg for RUST score groups. One outlier is not included (at RUST of 8).

<table>
<thead>
<tr>
<th>RUST</th>
<th>N</th>
<th>Mean Ultimate Torque</th>
<th>Standard Deviation</th>
</tr>
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<tbody>
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<tr>
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<td>52.7</td>
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<tr>
<td>11</td>
<td>2</td>
<td>91.0</td>
<td>5.7</td>
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</table>

Table 6.2: Sample size and, mean ultimate torque and standard deviation of ultimate torque as a function of the contralateral leg for modified RUST score groups. One outlier is not included (at modified RUST of 11).

<table>
<thead>
<tr>
<th>Modified RUST</th>
<th>N</th>
<th>Mean Ultimate Torque</th>
<th>Standard Deviation</th>
</tr>
</thead>
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<tr>
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<tr>
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<td>—</td>
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</tr>
<tr>
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<td>9.5</td>
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<td>1</td>
<td>19.9</td>
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</tr>
<tr>
<td>9</td>
<td>3</td>
<td>32.6</td>
<td>18.9</td>
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<tr>
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<td>1</td>
<td>23.3</td>
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</tr>
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<tr>
<td>14</td>
<td>4</td>
<td>77.1</td>
<td>16.5</td>
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</table>

Stiffness, the extent to which bone can resist deformation, is a clinically relevant parameter as it can be measured in vivo with some limitations. In fact, Richardson et al. suggest a stiffness of 15 Nm/degree as a definition of union for tibial fractures [142]. Such a single stiffness value to reflect healing in all patients seems simplified but it does imply the successful use of stiffness in defining bone healing. In this study, stiffness correlated highly with the RUST and modified RUST scores further illustrating the scores’ ability to be used in clinical practice. Upon examination of the stiffness graphs in figure 5.7,
there is one clear outlier at RUST of 8 with close to 0% of the contralateral stiffness. Investigation into this specimen showed a slight obliqueness in the radiograph that was scored by the reviewers creating slight obscurity of the fracture gap and tendency towards erroneously high scores. This outlier highlights the influence of slight irregularities, such as angulation or imperfect orthogonality, on the resulting RUST and modified RUST. This may present a limitation in the score’s clinical use as repeat x-rays are not always possible due to increased radiation exposure, cost and time.

The shear modulus of elasticity and the angle at failure were the two biomechanical properties that were not statistically significantly correlated with the RUST nor the modified RUST score. The shear modulus of elasticity is a distinct material property that represents the resistance to deformation of a material. It has been shown that the Young’s modulus (a similar elastic modulus to the shear modulus of elasticity) was related to bone mineralization [143]. The lack of correlation of shear modulus with the RUST scores may therefore be related to the lack of correlation between scores and bone mineral density as was reported by $\mu$-CT. Similarly, bone mineral density has been shown to relate to a materials brittleness which, in turn, is related to the angle at failure [144]. It can be speculated that the RUST and modified RUST cannot discriminate between the mineralization and resistance to deformation of the callus but rather callus presence and strength.

Other works have attempted to correlate radiographs with biomechanics. In contrast to the strong correlation between biomechanical properties and RUST and modified RUST, past attempts have not shown positive results. One such study by McClelland et al. attempted to correlate stiffness to radiographic assessment using “general assessment” and “number of cortices bridged by callus” [35]. They found poor prediction of healing based on such measures and suggested using caution when interpreting fracture healing on radiographic assessment. Further, Claes et al. found that clinical healing occurred at an earlier time than what was reported by radiographic evaluation of three bridging cortices [145]. Similarly, the Hammer scale outlined in section 1.3.2 was shown to have very little correlation with the mechanical stage of union [36, 37]. It is postulated that these aforementioned attempts to rate radiographs failed due to the scoring criteria used. Some are over-simplified (e.g. “general assessment” or “number of bridged cortices”) which may overlook the finer nuances of fracture healing while some are over-complicated (e.g. the hammer scale’s “discernible” versus “barely discernible” fracture line) which may introduce subjectivity. The RUST and modified RUST’s high correlation with healing are an indication that the criteria used (“callus presence” and “fracture line visibility”) properly characterize a bone mechanical integrity.
It should be noted that although the bimechanical results are highly correlated in this study, error is inherent for biomechanical testing and should be taken into consideration. For example, Wehner et al. performed a sensitivity study on a rat femoral osteotomy model and found that when varying the gap size by 0.1 mm, fixator offset by 0.5 mm and bodyweight by 25 g, their callus stiffness range increased by up to 77% [146]. Additionally, a healing bone’s stiffness and ultimate torque will often rise above 100% of the contralateral bone and subsequently return to approximately 100% as the bone remodels. Such a non-monotonic relationship makes it difficult to predict the bone’s progress in healing [84]. Considering this study evaluated relatively early stages in fracture healing, effects of whole bone mechanics likely did not play a role in measurements. However it should be considered that Chen et al. numerically found that once the shear modulus of the callus reaches 15% of intact bone, the whole bone stiffness rises to 90% of the intact bone and becomes less sensitive to changes in callus stiffness [49]. While significant variation in biomechanical measurements is common, biomechanics remains the best method to evaluate a bone’s recovery in strength. Consistency in measurement technique and statistical significance help mitigate error and validate biomechanical results.

6.5 RUST vs. Modified RUST

Both the RUST and modified RUST score have shown statistical correlations with mineralized callus volume, total callus volume, mineralized to total callus volume ratio, ultimate torque, stiffness, maximum shear strain and torsional strength. The questions of which scoring system is superior remains. Comparing Spearman’s rank correlation coefficients, the modified RUST score is superior in all $\mu$-properties as well as the biomechanical parameter stiffness. The RUST score is superior in the remaining parameters in this study. All noted differences are minimal and all correlation coefficients fall within the same “high positive” or “very high positive correlation” interpretations proposed by Mukaka [147]. It is therefore difficult to identify which score is better correlated with $\mu$-CT and biomechanical healing parameters. Looking at agreement among reviewers, the RUST score showed higher intraclass correlation coefficients for the sum score as well as all cortices with the exception of the anterior cortex with all four reviewers. Similarly, the two fellowship-trained orthopaedic surgeons showed higher agreement for RUST than modified RUST. It is hypothesized that the additional criteria proposed in the modified RUST score introduce additional subjectivity as did many of the more complex scoring systems such as the Hammer scale. While one may be inclined to state that the RUST has therefore superior agreement among reviewers, the literature contradicts this finding.
with two studies having found that the modified RUST resulted in higher agreement [33, 56].

The purpose of the modified RUST is to further breakdown callus presence into visible and bridging. It was noted that the modified RUST score correlated better with callus volume as measured by $\mu$-CT than the RUST which supports the modified RUST’s purpose. Overall, the RUST score had minor advantages over the modified RUST in correlation with ultimate torque, shear strain and torsional strength as well as higher agreement among observers. Further research with increased sample size in each scoring group is required to confirm whether the RUST score is truly superior to the modified RUST score.

### 6.6 Study Limitations

The nature of this study brought about one fundamental limitation in its accuracy. The independent variable on which the correlations with imaging and biomechanics was based, be it RUST or modified RUST, is not independent. There was large variation in the scores assigned by each of the four radiographic reviewers which introduces a level of uncertainty in the independent variable in question. It is assumed that the larger the number of radiographic reviewers, the more accurate the RUST and modified RUST scores will be. Unfortunately, the logistics of a large number of reviewers was not possible in this study. Instead and in order to minimize this error, it was decided to use exclusively the scores from two fellowship-trained orthopaedic surgeons that showed high levels of agreement after scoring radiographs independently. With this in mind, it must be acknowledged that there may still be some error within the RUST and modified RUST scores which limits the ability to precisely manipulate or predict healing parameters from the RUST scores given that the two reviewers did not agree perfectly in all cases. However, the purpose of this study was to determine the presence and relative strength of various healing parameters with respect to the score. By using an average score from two expert reviewers who show high agreement, the determination of such a relationships was indeed possible.

A second limitation in this study was the heterogeneity of variance and sample size for each of the score groups. By assigning each animal a time endpoint, there was little control over the resulting number of specimens in each score group. It is recommended that for future studies, the radiographs are scored weekly and RUST scores are used as endpoints. Animals will be sacrificed and femurs dissected only when the assigned RUST score has been attained. This presents logistical challenges that were not faced in this
study. The main challenge being that the radiographic reviewers would have to score the radiographs weekly which may be difficult with their busy schedules. One possible workaround would be to use one willing reviewer to score the radiographs weekly and subsequently use a group of reviewers to confirm the scores after sacrifice.

A third limitation of this study was that none of the specimens reached RUST scores above 11 or modified RUST scores above 14. This limits the conclusions that can be deducted for late stages in the healing process. This study has shown that 8 weeks post osteotomy is not sufficient time for an animal to attain a RUST of 12. Future studies should be prepared to have later endpoints.

Possible error and challenges with the methodology are also addressed here. Of note, the semi-automatic segmentation process used during µ-CT presents elements of subjectivity which may affect the measured values for total callus volume and mineralized callus volume. The segmentation process requires hand-drawn contours around what is suspected to be bone on each tomogram. Variation in “non-bone” space included in the contour will affect the measured values. Two samples were repeated twice in order to test for the repeatability of the contouring procedure and a difference of 0.01 mm$^3$ was noted in the total callus volume which was deemed insignificant. Even though that test was successful, from sample to sample, there may be variation in “non-bone” space based on indentations and low mineralized tissues. Additionally, the polar moment of inertia was estimated using a two-radius technique assuming approximately elliptical geometry. This is not the optimal method for doing so as the callus symmetry was asymmetric and non-homogeneous over its length. This may have created error in the values for material properties (strength and shear modulus of elasticity). Instead, a method such as that outlined by Morgan et al. [129] could be used in future research which involved the use of computational methods to interpolate over the cross-sectional area on each tomogram.
Chapter 7

Conclusions and Future Directions

7.1 Conclusions

Bone fracture healing assessment is crucial to orthopaedic clinical care and research studies on the topic. Current tools for fracture healing assessment are either subjective, such as patient reported outcome and physical examination; or impractical, such as computed tomography and in vivo biomechanics. Radiographic means of fracture healing assessment remain the most popular method for orthopaedic evaluation. The RUST and modified RUST scores provide radiographic assessors an objective tool with which to evaluate radiographs. The precision of the scores has been previously evaluated in the literature. This study aimed to compare the radiographic score with µ-CT and biomechanical healing outcomes. The following conclusions were drawn from this study:

1. RUST and modified RUST assigned to rat femoral osteotomies fixated with radiolucent plates showed lower agreement among radiographic reviewers in multiple levels of clinical practice than previously reported in the literature for intramedullary nail and plate fixated fractures in human and rat long bones. RUST showed a slightly higher agreement among reviewers than the modified RUST. **The contradicting results from this study and the literature suggest that the reviewer agreement of RUST and modified RUST should be reevaluated.**

2. RUST and modified RUST correlate highly with the mineralized callus volume, total callus volume and mineralized to total callus volume ratio. All imaging parameters correlated slightly higher with modified RUST in comparison to RUST. **Both RUST and modified RUST show strong relationships with the amount of callus present at the fracture site; modified RUST showing a slightly higher correlation.**

3. RUST and modified RUST correlated highly with ultimate torque, stiffness, maxi-
mum shear strain and torsional strength. All correlated slightly higher with RUST with the exception of stiffness which correlated higher with modified RUST. Both RUST and modified RUST show strong relationships with the biomechanical integrity of a healing bone.

4. There is no significant superiority between RUST and modified RUST in terms of strength of relationship with $\mu$-CT or biomechanical parameters. Modified RUST shows no superiority to RUST in correlation with healing measures, and preliminary results indicate that its further subdivision does better quantify callus volume but not biomechanical strength.

5. Neither RUST nor modified RUST correlated with bone mineral density, angle at failure or the shear modulus of elasticity of the fracture bone. Neither RUST nor modified RUST have a relationship with the callus material properties.

### 7.2 Future Directions

Several questions remain about the accuracy of the RUST and modified RUST scores which require future study to answer. In order to answer some of these questions, this study will have to be repeated with modifications. First, a large enough sample size should be used to provide statistical power to compare the means of $\mu$-CT and biomechanical parameters for each score group in both the RUST and modified RUST scales. Second, additional time should be given to specimens in order to attain higher RUST and modified RUST scores in order to evaluate the accuracy of the score at later points in healing. Third, the effect of fracture fixation on the relationship of RUST and modified RUST with healing outcomes should be evaluated by repeating this experiment using alternate fixation techniques such as a locking nail. In particular the effect of differences in osteosynthesis techniques, such as the formation of periosteal callus with intramedullary nails, should be evaluated. Should similar relationships be found with alternate fixation methods, the validity of RUST predictive ability will increase.

Once a complete and comprehensive profile of the relationship between RUST and mechanical parameters can be defined, the score can begin to be used for prediction of healing clinically. With the confirmation of strong correlation between RUST and biomechanical integrity of a bone, orthopedists will be able to use RUST to assess a healing bone’s mechanical integrity. Further, assigning RUST (and/or modified RUST) scores to average key time points in healing can allow for orthopedists to predict whether a fracture will heal or whether intervention will be necessary. Preliminary studies have begun using such a system. Van Houten et al. found that for each 1-point increase in
their version of the RUST score at 6 weeks, there was a decrease in the odds for nonunion of 0.19 [79].

Ultimately, this study was able to provide a first step in analyzing the accuracy of the RUST and modified RUST scores and provides a basis for future work on the matter.
Bibliography


[116]


