**BIOMECHANICAL DEVELOPMENT AND ANALYSIS OF A NEW CARBON-
FIBER/FLAX/EPOXY PLATE FOR REPAIRING FEMUR FRACTURES**

by

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Abstract

Biomechanical Development and Analysis of a New Carbon-fiber/Flax/Epoxy Plate for Repairing Femur Fractures

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Femur fracture at the tip of a total hip replacement (THR), commonly known as Vancouver B1 fracture, is mainly treated using rigid metallic bone plates, which may result in "stress shielding" leading to bone resorption and implant loosening. To minimize "stress shielding", a novel carbon fiber (CF)/Flax/Epoxy composite plate with a "sandwich structure" has been developed and characterized mechanically, biologically and biomechanically compared to a standard clinical metal plate. The CF/Flax/Epoxy composite material showed no cytotoxicity with no negative influence on the expression level of bone formation genes at all incubation time. The results of mechanical tests revealed a considerably high ultimate strength in tension (399.8 MPa), flexion (510.6 MPa) and fatigue loading (200-220 MPa). The composite plate showed higher elastic modulus in bending tests (57.4 GPa) compared to tension tests (41.7 GPa). The dynamic modulus (E^*) was found to stay almost constant versus the number of cycles, which can be related to the contribution of both flax/epoxy and CF/epoxy laminae to the stiffness of the composite. The results of biomechanical assessment of the plate showed that the bone beneath the CF/Flax/Epoxy plate was the only area that had a significantly higher average surface stress (fractured = 2.10 ± 0.66 MPa; healed = 1.89 ± 0.39 MPa) compared to bone beneath the metal plate (fractured = 1.18 ± 0.93 MPa; healed = 0.71 ± 0.24 MPa) ($p < 0.05$), cause less "stress

shielding” effect. The CF/Flax/Epoxy plated femur had comparable axial stiffness (fractured = 645 ± 67 N/mm; healed = 1731 ± 109 N/mm) to the metal plated femur (fractured = 658 ± 69 N/mm; healed = 1751 ± 39 N/mm) ($p=1.00$). These results confirm that the new CF/Flax/Epoxy material could be a potential candidate for bone fracture plate applications as it shows good biocompatibility and considerably higher strength in tension, flexion and fatigue than actual clinical load level experienced by femur during daily normal activities. Moreover, it can simultaneously provide similar mechanical stiffness and lower “stress shielding” (i.e. higher bone stress) compared to commercially-used metal bone plates.

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Units Used

MPa Mega Pascal

GPa Giga Pascal

g/m³ Surface Density

W/mK Thermal Conductivity

Kg Kilogram

kW Kilowatt

N Newton

g/cm³ Density

Hz Hertz(One cycle per second)

List of Abbreviations

ANOVA	Analysis of variances
BMP	Bone morphogenetic protein
BW	Body weight
CA	Contact angle
CAD	Computer aided design
CCD	Charged-coupled device
CF	Carbon fiber
CT	Computerized tomography
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl sulfoxide
FBS	Fetal bovine serum
FEA	Finite element analysis
HA	Hydroxyapatite
HCFS	High cycle fatigue strength
IR	Infrared
IRT	Infrared thermography
MRI	Magnetic resonance imaging
MTT	Methyl tetrazolium
PMMA	Poly methyl methacrylate
RT-PCR	Reverse transcription polymerase chain reaction
RUNx	Runt-related transcription factor
SEM	Scanning electron microscopy
TCP	Tissue cultured plastic plates
THR	Total hip replacement
TSA	Thermoelastic stress analysis
UTS	Ultimate tensile strength

CHAPTER 1: Introduction

1.1. Background Motivation

Femur fracture at the tip of total hip replacement is known as Vancouver B1 fracture, which compromises 75% of periprosthetic fracture. According to the American Academy of Orthopaedic Surgeons, a fractured femur takes about 3 to 6 months to heal fully; however healing can begin to occur as early as 6 weeks [1]. This is much longer than for most other human bones. Such a long healing period leads to a high chance of misalignment. Therefore the fractured femur requires an aid to enhance its healing process and to provide proper alignment. Bone plate, i.e. plating, is the standard modern treatment for long bone fractures in the vicinity of a total joint replacement, since nailing become physically impossible [2, 3]. Most of the commercially available bone plates are made of metallic materials such as titanium, titanium alloys, cobalt alloys or stainless steel [2-4], because of their well-known material properties and developed manufacturing methods. The elastic modulus of metal is about 5 to 10 times higher than that for human bones. Practical experiences, however, revealed that such materials are much stiffer than bone, which results plate carrying a higher percentage of the load compared to the femur, i.e. stress-shielding[2-4]. It was reported that stress shielding results in a considerable reduction of the compressive stress in the affected bone. Also, stress shielding can cause the density in an affected bone to be lower than it was pre-operatively. Consequently, the risk of re-fracture in weakened bone increases once the bone plate implant is removed. In one study, about 3% of patients re-fractured their femurs after the bone plate was removed following a visible re-union of the injured femur[5]. Therefore, bone fixation methods must be mindfully selected in order to minimize the risk of re-fracture by optimizing the compressive stresses transferred to the femur in order to maintain healthy bone density. Although primary bone healing needs a stiff fixation plate in the short term immediately after surgery, bone loss may result from a stiff plate in the long term. As a fracture heals over time, the need for a highly stiff plate is diminished. Thus, the stiffness of a fracture plate should ideally decrease over time to allow bone to carry more of the load in the long term. As such, there has been an increased interest in using plates with lower stiffness in order to reduce stress-shielding, with improved bone healing and bone strength as a long term effect[6]. Stress shielding is not very sensitive to the bending stiffness of the plate, however, it is very sensitive to the relative axial stiffness of the plate with respect to the

bone[2]. Hence, plates using two materials have been considered to reduce the axial stiffness while maintain the adequate bending stiffness[2].

Additionally, there are some other complications accompanied by using metallic biomaterials. First of all, during the monitoring process of an injured bone using computerized tomography (CT) and magnetic resonance imaging (MRI), these metallic materials appear white in color, which limits the visibility of bone, which also appears white in color. In addition, the fatigue strength of metallic materials is highly dependent on how quickly they can repassivate when oxide layers are removed by fatigue cracks [7, 8]. The inability of metallic implants to repassivate quickly exposes unprotected regions to corrosion, which immediately results in corrosion-fatigue. This can cause a considerable drop in fatigue endurance limit, which is one of the main reasons for bone plate failure [7, 8]. To address these drawbacks, a new generation of biomaterials, i.e. composite materials, has been introduced [9]. Polymer-matrix composite materials are resins reinforced by fibers, which have diverse applications in different industries; such as marine, aerospace, construction and transportation [9]. Composite materials were initially introduced because they have potential to be manipulated to have combinations of properties that cannot be obtained by conventional materials [9-11]. For medical imaging applications, some benefits of using carbon fiber reinforced composite implants compared to metal implants is that composites are radiolucent, i.e. they appear dark on exposed radiographic film, and are compatible with modern medical imaging like MRI (magnetic resonance imaging) and CT (computerized tomography) scans, making clinical monitoring easier[12]. Yet, the complications of using composite materials include reduction of properties over time, degradation of the properties in humidified atmosphere, the risk of fiber breakage and [9]. Additionally, there are potential risks to surrounding biological tissue associated with generation of carbon debris from carbon-based composite implants, such as inflammation (i.e. soft tissue thickening), osteolysis (i.e. bone cell resorption), and cytotoxicity (i.e. slowed or halted bone cell proliferation), which might eventually lead to implant loosening and/or failure [13-16]. Also, unlike metallic plates, contouring of composite plates is not feasible sometimes, especially for composite plates based on thermosetting polymers. This may cause “pre-stresses” on the plate or bone which may introduce additional clinical problems. However, composite materials have been considered for biomedical applications, since they have mechanical and biological

similarities to the human tissue being replaced [17-20]. The applications of composite materials in orthopaedics can be traced back to the 1980s, when they were initially used for fracture fixation and joint replacement in cases of trauma [11, 18, 19, 21-23]. Studies have considered composite materials for various biomedical applications: spinal cage for degenerative discs [24, 25], filling bone defects for anchoring into bone [26-31], and also orthopaedic trauma implants [11, 18, 19, 21-23]. These composite materials were mainly reinforced using one type of synthetic fiber (i.e. carbon and glass fibers), which can accompany the emission of pollutants and result in numerous environmental and occupational health issues during the fabrication process[32]. Therefore, natural fibers have recently gained attention for biomedical applications [10, 33]. In the case of using composite materials as bone plates, a hybrid composite may need to be used to have a combination of properties with acceptable performance in different directions which may not be achieved by one type of fiber. Many investigations have been done regarding hybrid composites [34-38], but few studies involve hybrid composites reinforced with natural fibers and carbon fibers [39, 40], and no studies have considered hybrid composites with a “sandwich structure” for bone fracture plate applications. For that reason, a unique hybrid CF/Flax/Epoxy composite plate with potential application as fracture bone plates has been developed in the current study. The plate has a sandwich structure with perfect bonding at the interface of Flax/Epoxy and CF/Epoxy laminas. However, no prior studies exist which have assessed and characterized this unique CF/Flax/Epoxy hybrid composite for biomedical applications as a long-bone fracture plate.

1.2. Current Thesis Outline

This thesis contains eight main chapters. Chapter 1 provides the motivation behind the need for doing this research as well as the goals this thesis sets out to accomplish. The present work is comprised of a series of studies previously published or currently under-review by academic journals as part of the author’s doctoral dissertation.

Chapter 2 consists of a comprehensive literature review including an introduction to the anatomy of the human limb, clinical and biomechanical aspects of femur fracture, surgical and non-surgical methods for femur fracture fixation, a summary of the studies proposed less-rigid materials for manufacturing bone plates as well as thermography and finite element analysis for

biomechanical assessments, which play a role in the current research.

Chapter 3 will explain the lack in the literature on composite materials developed for bone fracture plate applications. The reason for the material selection as well as plate structure will be discussed in this chapter.

Chapter 4 will determine the biological properties of the developed CF/Flax/epoxy composite plate to be used in orthopaedic trauma applications. This chapter will discuss the plate's in-vitro effect on the level of bone formation gene as well as cell viability in comparison to a medical grade metal material with actual clinical use in fabrication of bone plate.

Chapter 5 will discuss the mechanical properties of the developed composite plate. The effectiveness of the developed composite material for fabrication of bone plates will be determined in this chapter considering its mechanical properties such as tension, three-point bending, and Rockwell hardness.

Chapter 6 will analyze the fatigue performance of the developed composite material, because cyclic loading is one of the main types of loads carried out by a femur fracture plate during daily normal activities. This chapter explores the plate's fatigue properties using conventional fatigue test and thermography approach.

Chapter 7 will explore the success of the proposed composite plate in fixing Vancouver B1 fracture. This chapter compares the amount of "stress shielding" and stability caused by the proposed composite plate with that of a medical grade metal plate for fixing Vancouver B1 fracture.

Chapter 8 concludes the important finding of the current study and also highlights the in-vitro evaluation of this novel CF/Flax/epoxy composite material for bone fracture plate applications.

1.3. Research Objectives

The purpose of this thesis is to develop a new CF/Flax/epoxy hybrid composite material with potential biomedical engineering applications as a long bone fracture plate. This new CF/Flax/epoxy composite material is manufactured using a compression molding facility; and its

biological, mechanical properties and its biomechanical performance are characterized. The main research question was: Can the current CF/Flax/Epoxy plate provide promising mechanical and biological properties as well as adequate overall stiffness for a repaired and healed femur, while also allowing for greater bone stresses (i.e. less “stress shielding”) than a traditional clinical metal plate? To achieve these objectives, the following tasks were carried out.

- a. Manufacturing of the CF/Flax/Epoxy composite plate
- b. Perform osteogenesis and cytotoxicity tests for the CF/Flax/Epoxy composite plate
- c. Evaluate the mechanical properties of the CF/Flax/Epoxy composite plate
- d. Assess the biomechanical performance of the developed composite plate implanted on the femur to fix Vancouver B1 fracture.

CHAPTER 2: Literature Review

2.1. The Hip Joint

The hip joint is one of the largest and most stable joints in the body. It is a ball-and-socket system. Hip motion takes place in all three planes of the body: sagittal (flexion and extension), coronal (abduction and adduction), and transverse (external and internal rotation) [41, 42] (Figure 2.1). Coronal and sagittal planes both pass through the body longitudinally which, in turn, divide into front/back and left/right. The transverse plane, perpendicular to both sagittal and coronal planes, divides the body to upper and lower parts.

Figure 2.1. Anatomical planes of the human body [41, 42]

The hip joint consists of two components, namely, the femoral head and the acetabulum of the pelvis, which are convex and concave components of the ball-and-socket configuration, respectively. The acetabulum, covered with articular cartilage predominantly in lateral side, has a smaller diameter than that of the femoral head when it is unloaded. But upon application of load it deforms elastically in which it conforms to the femoral head and produces contact via its anterior, posterior and superior articular surfaces. The construction of this stable joint allows normal motion in the performance of daily activities. The hip joint is responsible for two functions. First, it allows for static posture like standing as it carries the weight of one's body.

Second, it allows the femur head to rotate in the acetabulum; hence it makes one's body able to experience dynamic motion like walking or running. Deformation and disease of the hip joint cause changes in the stress distribution in the joint cartilage and bone, resulting in degenerative arthritis[2, 3].

2.2. Gait Cycle

A process of human movement on about two limbs is known as bipedal locomotion or gait. Lower joint has the ability to simultaneously bear weight and provide three unique functions of locomotion necessary for one's movement. Each limb in gait cycle experiences two phases including stance and swing phase (Figure 2.2). Walking as a simplest form of daily normal activity is produced by repeating gait cycles. According to the gait cycles introduced by Perry, gait cycle of walking consists of stance and swing phases which comprise 62% and 38% of the gait cycle, respectively[43].

Figure 2.2. Human walking gait cycle [43]

As it is shown in Figure 2.2, the stance phase consists of 4 different stages including loading response, midstance, terminal stance and pre swing. Assuming that subject stepped forward with his right foot, the different stages of walking is described here. The loading response stage starts when the right heel contacts the ground and lasts when the left foot leaves the ground [43, 44]. During the loading stance phase, load is transmitted to the left foot once the right foot touches

the ground in a way it gets a flat position and thus the left (contralateral) foot becomes able to stance on its toe[43, 44]. As a result, at this stage of the walking gait cycle, human body would experience double limb stance[43, 44]. Following the loading response stage, the mid-stance starts when the left toe leaves the ground and terminates when the center of gravity shifts towards the load bearing foot, this is the right foot in this example[43, 44]. After mid-stance stage, the terminal stance phase begins and ends when the left foot comes to complete contact with the ground. These two phases are mostly performed with single limb stance[43, 44]. Pre-swing, as the last stage of the stance phase, begins at the initial contact of the left toe with the ground and terminates when the right toe leaves the ground. Unlike the mid-stance and terminal stance stages, the human body would experience mainly double limb stance in pre-swing stage. The swing phase can be divided to 3 stages including initial swing, mid-swing and terminal swing. The initial swing stage is between the moment when the right toe leaves the ground and when the right knee reaches its maximum flexion at approximately 60°. The mid-swing stage starts follows initial swing and terminates when the tibia gets perpendicular to the ground. The terminal swing as the last stage ends when the right foot touches the ground. Table 2.1 summarized the above-mentioned walking gait cycle [45].

Table 2.1.Summary of walking gait cycle stages [45].

Gait Cycle	Stance phase 0-62%	Loading response 0-10%	Initial contact to contralateral toe-off
		Midstance 10-35%	Contralateral toe-off to when the body CG is directly above the reference foot; weight loading begins
		Terminal stance 35-52%	CG directly above the reference foot to contralateral initial contact; weight loading ends and heel of the reference foot leaves ground
		Preswing 52-62%	Contralateral initial contact to toe-off
	Swing phase 62-100%	Initial swing	Toe-off to maximum knee flexion
		Midswing	From maximum knee flexion to when the tibia is perpendicular to the ground
		Terminal swing	Tibia perpendicular to the ground until initial contact; knee reaches maximum extension just before initial contact

While during the stance phase, the single limb stances are carried out by the initially load bearing leg, which is the right leg in the example above, they are carried out by the contralateral leg, i.e. the left leg in this example, during the swing phase.

2.3. Motion of the Hip

Hip joint motion during walking is tri-axial in 3 different planes: Coronal, Sagittal and Transverse. Flexion-extension takes place about mediolateral axis in the sagittal plane, abduction-adduction happened in the coronal plane about anteroposterior axis and internal-external rotation occurred about a longitudinal axis in the transverse plane [46](Figure 2.3).

Figure 2.3. Typical hip motion range [46]

Hip joint experienced the maximum motion in the sagittal plane with a range of 0-140° for flexion and 0-15 ° for extension. Alternatively, hip motion becomes less in coronal plane, with the range of 0-30 ° for abduction and 0-25° for adduction. External rotation (eversion) ranges between 0-90° and internal rotation (inversion) ranges between 0-70°. The range of hip rotation during extension is restricted because of the soft tissues attached to the joint.

It was reported that at the beginning of the stance phase the joint is fully extended as the body moves forward, whereas at the late stage of the swing phase the joint is completely flexed as the

limb moves forward for the heel strike [46]. Maximum extension would be achieved at heel-off. During the swing phase, the joint would turn around to the flexion to the maximum of 35-40°, before the heel-strike (Figure 2.4) [47].

Figure 2.4. Hip joint flexion and extension range during gait cycle [47]

Aging is one of the determining factors for the range of hip joint motion, where elderly people usually have shorter strides with a limited hip motion range [47]. A minimum flexion of 120° with 20° abduction and external rotation are reported to be mandatory for doing daily normal activities with any barrier.

2.4. Range of Forces on Hip Joints during Routine Activities

Studies have been done on the substantial forces act on the hip joint during daily normal activities [47-49]. The range of typical peak forces on the hip during routine activities is shown

(Table 2.2). For patients with prosthesis, the peak resultant forces during gait ranged from 1.8 to 4.36 times the body. In general, hip forces peak at two stages: initially at early stance and again at late stance.

Table 2.2. Peak forces on the hip joint due to routine activities (BW= body weight)

Activity	Reported Peak Forces (BW)	References
Walking	2.7-4.3	
Ascending Stairs	3.4-5.5	Bergmann et al., 1993
Descending Stairs	3.9-5.1	
Walking	1.8-3.3	Rydell, 1966
Walking	4.9-7.0	Murray, 1967
Walking	4.5-7.5	Crowninshield et al., 1978
Walking Slow Speed	2.7	English et al., 1979
Walking Slow Speed	2.6	
Ascending Stairs	2.6	Davy et al, 1988

2.5. Human Femur

The femur is the longest and strongest bone in the skeleton, and it is almost entirely cylindrical (Figure 2.5). It is approximately 480mm in length and 23.4mm in diameter for an average adult male [50]. Each femur undergoes typical loading of up to 2 times the weight of an adult body [50]. The lower end of the femur joins the tibia to form the knee joint. The upper end is rounded into a ball which is also known as the “head” of the femur, which fits into a socket in the pelvis to form the hip joint. In order to bring the knee-joint near the line of gravity of the body, the femur is separated above from an opposing femur by a substantial interval, which corresponds to the width of the pelvis. The breadth of the pelvis influences the degree of femur inclination. As an example, due to the greater breadth of the pelvis in the human female femur, this inclination is

greater in the female than the male femur. Like other long bones, the femur is divisible into a body and two extremities.

Femur is mainly made of 5 protuberances (head, greater trochanter, lesser trochanter, medial lateral condyles) and a shaft (Figure 2.5). The head and the greater trochanter are placed at the proximal end of the femur in the coronal plane laterally and medially, respectively. The lesser trochanter, inferior to both the head and the greater trochanter, is projected from the anterior side. Both medial and lateral condyles are located at distal end of the femur which got jointed with tibia to form the knee joint.

Figure 2.5. The human femur seen in 4 different views [51]

The femur, characterized with its long shaft, is among the longest bone in body. The shaft (diaphysis) is nearly cylindrical in form and a little wider above than in the center. To some extent, it is arched, so as to be convex in front. The distal portion is fairly cuboid in the form and is larger in comparison with the proximal portion [52]. The long shaft of the femur, i.e. diaphysis, is made of compact or cortical bone with a hollow internal canal known as medullary

cavity [53]. The two protuberances at both proximal and distal ends are known as epiphysis which is mainly composed of spongy bone, i.e. cancellous bone, which is softer, weaker, less dense and less stiff than the compact or cortical bone. Metaphysis is the transitional zone between the diaphysis and epiphysis (Figure 2.6) [53].

Figure 2.6. General Bone Anatomy of (a) long bone [53] and (b) femur (posterior view)[54]

2.5.1 Femoral Head

The proximal region of the femur is composed of femoral head and neck. The femoral head, which is spherical and forms somewhat more than a hemisphere, is directed upward, medialward, and a little forward. The head surface is smooth and coated with cartilage. However, the coverage of the femoral head with cartilage is not uniform, where articular cartilage is thicker in

the medial-central surface and it becomes thinner towards the periphery. This dissimilarity causes variations in the stiffness and strength of femoral head regions. It was reported that the loading pattern of the femoral head highly depends on the magnitude of the applied load, where the load bearing area is either concentrated at the central lunate at high loading magnitude or anterior/posterior horn at low loading magnitude [48, 55, 56]. During daily normal activities, the majority of load is transmitted by the medial and anterior lunate [48, 55, 56].

2.5.2. Femoral Neck

The neck connects the head with the body of femur. The orientation of neck in the coronal and transverse plane is a determining factor for force relationship in the hip joint. The angle inclination of the femoral neck to the shaft is normally around 125° in healthy adults. The force relationship in the hip joint would get altered for angle smaller than 125° , commonly known as coxa vara ($<125^\circ$), or for angle bigger than 125° , i.e. coxa valga ($>125^\circ$) (Figure 2.7).

Figure 2.7. Femoral neck orientation in the coronal plane [57]

Another important angle is the angle between the projections of the femoral head with the transverse axis of the femoral condyles in the transverse plane, which is usually equal to 12° in

normal adults. However, condition of anteversion ($>12^\circ$) or retroversion ($<12^\circ$), common in children, can cause external or internal rotation during daily normal activities, respectively (Figure 2.8).

Figure 2.8. Femoral Neck orientation in transverse plane [58]

The structure of femoral neck is internal cancellous bone with trabeculae system (Figure 2.9). The direction of joint reaction force in the femoral head is parallel to the medial and lateral trabeculae system in the neck region, thus indicating the importance of this arrangement for supporting the applied force [59]. Osteoporosis, as a common disease in elderly people, makes bone vulnerable to fracture specially from the neck region [59].

Figure 2.9. Lateral and medial trabeculae system of the femoral neck[60]

2.6. Material Properties of Femur

Femur like other bones has anisotropic materials properties, in which it shows dissimilar properties when it is loaded in different directions. As an example, femur demonstrates superior strength when it is loaded in longitudinal direction rather than transverse direction [2]. The material properties of the femur for both cortical and cancellous bone are summarized (Table 2.3) [2].

Table 2.3. Average values for the material properties of the human femur achieved for an entire bone, representing healthy bone from a young population [61]

Bone Type	Direction & Load type	Density (g/cm ³)	Ultimate Strength (MPa)	Modulus of Elasticity (GPa)
Cortical	Longitudinal tension	1.85	133	17.7
	Longitudinal compression	1.85	193	17.7
	Longitudinal Shear	1.85	68	3
	Transverse tension	1.85	51	12.8
	Transverse compression	1.85	33	12.8
Cancellous	Compression	0.31	6	0.76

As explained earlier, the femur is made of cancellous and cortical bone. Cortical bone is stronger than the cancellous bone for both strength and modulus of elasticity, thus it can keep bone structure in place. Cancellous bone shows much lower density compared to that of the cortical bone, thus it acts as a shock absorber. Since femur is habitually loaded in longitudinal direction during daily normal activities, the contribution of both cancellous and cortical bone allows the body to carry one's body weight. In general, femur is stronger in compression rather than tension especially when it is loaded in longitudinal direction. The ultimate compressive strength is almost 6 times bigger in longitudinal direction rather than transverse, indicating the weakness of the femur to the compressive loads particularly in the transverse direction[2].

Moreover, the femur can withstand almost 31% higher loads in compression compared to that for tension when it is loaded longitudinally [2].

2.7. Femur Fractures

As the femur is one of the strongest bones in body, it needs to undertake a considerable amount of compressive load before it fractures. Femur fractures are one of the main reasons for hospitalization, and injured femurs also may disrupt blood supply from thigh muscles and cause extensive blood loss [2, 62]. Femur fractures may occur because of bone illness or physical impact. One of the main bone diseases that significantly increase the risk of femur fracture is osteoporosis, when the bone mineral density (BMD) is reduced to a critical value, which is related to factors such as aging, heredity, nutrition, lifestyle, medication and other illnesses. Biomechanically, low and high-energy trauma in patients with osteoporosis frequently causes femur fracture. Femur fractures can be considered to 3 main types including proximal, shaft and distal fractures, which will be discussed in details below [62].

Proximal fractures are mostly common in elderly patients with osteoporosis, and it takes place at the upper end of the femur. This type of fracture, which is usually accompanied by hip joint failure or hip fracture, can be subdivided to 3 different categories: femoral neck fracture, intertrochanteric femur fracture and subtrochanteric femur fracture (Figure 2.10).

Figure 2.10. Different types of proximal fractures: a) subcapital neck fracture b) transcervical neck fracture c) basicervical neck fracture d) intertrochanteric fracture and e) subtrochanteric fracture [63, 64]

Femoral neck fractures happens when the femoral head is fractured off the femur, which can be categorized to 3 different types, namely subcapital, transcervical, and basicervical neck fractures (Figure 2.10) [2, 3]. For subcapital fracture, the fracture line is exactly beneath the femoral head on the neck and caused by stress riser regions resulted by sharp reduction in the cross sectional area. Transcervical fractures took place at mid-neck region where is naturally lower in cross sectional area resulting in high mechanical stresses. The fracture line for the basicervical neck fractures generally runs along the top of the greater trochanter to the lesser trochanter where fracture occurs exactly at the base of the neck. The intertrochanteric femur fracture cuts through the materials between greater and lesser trochanter. And finally, subtrochanteric femur fracture, as the least common proximal fracture, occurs below the lesser trochanter and may sometime extend down to the femoral shaft [62].

Femur shaft fractures are generally caused by high-energy impact or ill-fit orthopaedic implants acting as stress riser. Based on the mode of loading, femur fractures can occur with different fracture patterns. Five main types of loading modes that may produce a fracture are tension, compression, torsion, bending and a combination of bending and compression (Figure 2.11).

Figure 2.11. Five modes of force that fracture the femoral shaft: (a) tensile force, (b) compressive force, (c) torsional force, (d) bending force, (e) combination of bending and compressive forces [65]

Simple transverse fractures mainly caused by tensile stresses applied to the both end of the femoral shafts if the magnitudes of the applied stresses at both ends are equal. No debris or particle produced in such type of fractures [66]. It should be noted that transverse fracture occurs along the plane with the highest tensile stress normal to the direction of applied load (Figure 2.11.a) [66]. High compressive stress produces high shear stress along the plane oblique to the direction of applied load and may cause fracture along this plane. In other words, the compressive force produces a fracture along the planes of high shear stresses (Figure 2.11.b) [67]. Torsional forces mainly cause oblique spiral fractures. This type of fracture initiates on the surface of the femoral shaft parallel to the axis of the specimen in the plane with the highest torsional stress and continues to growth through the bone in a spiraling manner within the planes where tensile stress exists (Figure 2.11.c) [67]. The fracture caused by bending forces is called butterfly fracture. Bending moment introduces compressive force to one side of the femur and tensile force to the other side. Thus the fracture pattern would be the combination of those observed for tensile and compressive force, which would be, in turn, transverse and oblique. Two types of oblique fractures typically are observed on the compression side, which result in comminution called butterfly fragments (Figure 2.11.d) [67]. The fracture pattern produced by the combination of bending and compressive forces would be something similar to that for compressive force alone. However, the butterfly fragment would involve more than 50% of the cortex, thus the contact between distal and proximal fragments would be less than 50%. In this case, increasing compressive force would produce more than one butterfly fragments [67] (Figure 2.11.e).

Distal femur fracture happened at the bottom portion of femur and usually caused by either low-energy injuries in patients with total knee replacement or high-energy trauma where it is transmitted to the flexed knee and causes distal fracture [68]. In general, patients with the ill-fitting knee prosthesis are at high risk for distal femur fracture. The distal femur fracture can be divided to 4 main types: supracondylar fracture, intercondylar fracture, condylar fracture and distal femoral epiphyseal fracture [69] (Figure 2.12). The supracondylar fracture which breaks off the distal portion of the femur from the rest of it in a transverse direction, happened in the plane perpendicular to the femur long axis just above the epiphysis where a change in the cross-sectional area is observed (Figure 2.12.a). The intercondylar fracture grows along the medial and

lateral condyles and would have divided the femur into at least 3 components if the magnitude of the applied load is not that severe (Figure 2.12.b). The condylar fracture causes an oblique cut through the epiphysis. In this type of fracture one of the condyles remains unaffected (intact) while the other would experience an oblique crack (Figure 2.12.c). The characteristic of the distal femoral epiphyseal fracture is the presence of a crack across the epiphysis in which parts of the epiphysis are intact with the femoral shaft, whereas the rest of it shows a transverse crack with respect to the femoral long axis (Figure 2.12.d).

Figure 2.12. Different types of femoral distal fractures a) supracondylar fracture, b) intercondylar fracture, (c) condylar fracture, (d) distal femoral epiphyseal fracture [69].

2.8. Femur Fracture Fixation

Femur fracture can be repaired either with non-surgical or surgical techniques, which depends on the maturity degree of the patients' skeletal system, the amount of displacement at the fracture site, fracture type and the existence of the associated injuries [70]. Commonly known non-surgical treatments are casting and tracing, whereas external fixations, intramedullary nailing and plating are known as the most popular methods for surgical treatments.

2.8.1. Non-surgical Treatment Methods for Femur Fracture Fixation

One of the traditional non-surgical treatment methods for femur fracture fixation is traction. In this method, the fractured leg first is placed inside a cast. Then pins are inserted into the cast all the way to the fractured bone. After that, the free ends of the pins are connected to a series of weights using strings. In this method, the healing process of the bone should be closely monitored to make adjustment necessary for perfect alignment as regularly as possible (Figure 2.13). Although traction is an effective method for simple fracture, surgeons prefer other methods because of the long healing process accompanied with this technique [70].

Figure 2.13. Illustration of traction [68]

Another common non-surgical technique for femur fracture fixation is casting. Patients treated by casting method should be lighter than 50-60 lbs, as casting cannot withstand any of the body's weight of heavier bodies. This requirement restricted the age of the patients who can be treated by casting method to 8 years or younger as the elderly people would be heavier than the specified body's weight. Moreover, this type of treatment is not applicable for more complicated fractures produced any comminution because casting cannot remove the fragments from the injured site, thus this can add more complication due to the infection introduced at the fracture site [1].

2.8.2. Surgical Treatment Methods for Femur Fracture Fixation

Among all surgical treatments available for femur fracture fixation, external fixation, intramedullary nailing (IM) and plating are the most favorable ones. Physicians mainly choose nailing or plating unless the patient is expected to have a serious complication during operation or at non-ambulatory status.

For external fixation method, pins are placed through the lateral tight muscle to provide the proper alignment to the fractured femur (Figure 2.14). These pins are connected to a ring-shaped frame, which could be used to adjust the position and the location of the pins. The benefit of this technique is that it can compress the fractured femur without introducing any additional changes in its dimension. Moreover, this technique does not cause any external wound, thus it could be beneficial for patients suffer from damaged soft tissue or contamination at the fractured site. Additionally, this method is recommended when serious complications would be expected during an operation [1].

Figure 2.14. (a) illustration of external fixation method[71], (b) x-ray picture showing fractured fixed with external fixator [72]

Although this technique is beneficial for patient with damaged soft tissue, external fixation method is accompanied with several disadvantages. First, the design of the ring-shaped frame, which holds the pins, is really bulky. Secondly, over 30% of patients experienced infection at the site of pin insertion, thus several factors have to be guaranteed such as meticulous pin preparation prior to the operation, careful infection preventions and appropriate antibiotics. Thirdly, complete healing would not achieve using this technique alone and once the patient's condition improves other treatments should be applied. Fourthly, patients cannot perform their daily normal activities, as the restriction of muscles caused by the inserted pins would limit the movement of the knee joint. Lastly, any displacement of pins may cause rupture within neurovascular systems, muscles and tendons [71].

Figure 2.15. Nailing operation illustration [73]. The femur is reamed from the distal end for proper sized canal and a rod is inserted inside the canal.

Intramedullary nailing, which is also known as intramedullary fixation, is currently the most favorable treatment method as it is the least aggressive treatment method with no further soft tissue injuries that can provide proper alignment. This technique is more common for femoral diaphysis fracture. For this method, the femur is reamed from either the proximal or distal end for proper sized canal. After passing a guidewire through the canal, then a rod is inserted inside the canal and fixed using lateral screws (Figure 2.15). Since the internal structure of femur is composed of medullary cavity and spongy bone at epiphysis, a rod can be placed inside the femur without removing material from the core bone. Also, early movement is possible using this technique as nailing has load-bearing capabilities [70].

The insertion technique depends on the type and the location of fracture. The insertion techniques can be categorized to retrograde and antegrade nailing. For patient with proximal or shaft femoral fracture, retrograde nailing is recommended. In this method, nailing initiates from the medial condyle and passes all the way through the proximal region (Figure 2.16). Due to the relatively easy access to the distal femur, this method is promising for obese and pregnant patients [68].

Despite retrograde nailing, antegrade nailing starts at the proximal region of femur either at the piriformis fossa or the greater trochanter as shown (Figure 2.17). Antegrade nailing is more difficult than retrograde nailing because access to the starting point is sometimes problematic particularly for obese patients. However, it was reported that different starting points would not alter the healing process or infection at the insertion points [68]. This type of fixation treatment could be used for a wide range of fracture locations starting from just below the lesser trochanter to about 7cm above the knee joint. Intramedullary fixation techniques, i.e. retrograde and antegrade, showed high healing rate between 90% and 95% [68]. Nonetheless, this technique is mostly recommended for patients who have attained completely mature skeletal systems since some evidence of damaging of the growth plate of the femur, during nailing operation, exists in the literature.

Although intramedullary fixation method has a high rate of healing, this technique like other methods has its own drawbacks. First, intramedullary nailing may cause rotational deformity as it is passed through the medullary cavity to make a contact between two fractured components at

the injured site. This complication may be further fixed by additional operation that can add more difficulties. Second, this method is not feasible for more complicated fracture resulting in more fractured bone segments or comminutions at the fracture site, since nailing cannot gather all the fractured bone segments into alignment. Third, the overall length of the injured leg may be shortened upon union, which could be fixed surgically either by shortening the contralateral leg or lengthening the injured one. This side effect produces unequal leg length that restricted performing daily normal activities.

Figure 2.16. Retrograde nailing, a) Illustration of complete nailing insertion b) Retrograde nailing X-ray picture [73].

Fixation of the aforementioned side effect needs further operation, which can be sometimes very costly and time consuming for suitable union. Fourth, reaming process necessary for rod insertion inside femur may add additional difficulties especially for antegrade nailing with the start point close to the neck region, either at greater trochanter or piriformis fossa, and can cause fracture at the femoral neck. Moreover, the medullary cavity is not uniform in its diameter, so the

inserted rod would only contact the narrowest portion of the medullary cavity, which introduces the bending moment to the rod's free end. Reaming is required here to increase the diameter of the medullary canal for perfect implantation as well as providing the stability to the inserted rod. However, this may cause excessive damage to the periosteal supply, which can result in the permanent failure of revascularization [75]. Lastly, implantation of a rod inside the femur can damage the nutrient artery. Despite the fact that revascularization can start by the small vessels in approximately 6 weeks following surgery, regeneration of nutrient artery requires more time up to almost 6 months after operation [75].

Figure 2.17. X-ray images of antegrade nailing started at (a) piriformis fossa entry, (b) greater trochanter entry [74] .

Another common method of femur fracture fixation is plating. Bone plate fixation occurs when the injured area is surgically opened and a plate is placed laterally over the fractured bone. The plate can be fixed to the bone using screws, cables or a combination of them. Locking plates provide superior engagement between the threaded screws and the threaded holes of the plate,

compared to non-locking plates which only have non-threaded holes in the plate. The 2 main types of bone plates are neutralization plate and compression plate [75].

Neutralization plate is mainly used to fix short oblique fracture, butterfly fracture and fracture with mild fragment. Using bone screws, this plate is fixed to the healthy bone component below and above the fracture line without applying preload to the site of fracture. In order to provide the intra-fragmentary compression to the site of failure, these plates are usually used with lag screws (Figure 2.18).

Figure 2.18. Neutralization plate secured with regular screws as well as two lag screws [75].

Compression plate applies compressive preload to the fracture site, which prevents the fracture surface from opening up and inhibits inter-fragmentary fractures under torsional or shear loading condition, thus can reduce the normal healing period. This plate is fixed to one side of the fracture surface and then it is pulled to reach the desired location on the other end (Figure 2.19). Once the plate is attached to the free end using screws, the external tensile applied load, used for pulling the plate, would be removed. Therefore, compressive preload is introduced to the side of fracture and results in squeezing the two bone halves together [75].

Figure 2.19. Compression plate secured with bone screws and pulled by tensioner [75].

Bone plating has numerous advantages and it can be used when application of intramedullary nailing (IM) is not feasible. As an example, femur fracture at the proximal end of the femur is so common after total hip arthroplasty. The inserted hip replacement at the proximal end of the femur makes antegrade nailing impossible. Second, unlike IM nailing, bone plates can be used when there are many fractured bone segments or in case of large amount of comminutions at the fracture site. Third, the risk of shortening of the injured leg upon union is less when it is fixed with bone plates compared to IM nailing. Because bone plate can keep the appropriate distance between the fractured bone segments, thus after full recovery, the length of the injured leg would be almost same as the contralateral one. Fourth, bone plates avoid lateral rotation and subsequent deformity, as it is directly applied to the lateral surface of the injured bone. Lastly, plating could be used for pediatric patients since it does not need cutting epiphysis, nearby growth plate and reaming. However, plating can result in soft tissue damage during implantation and removal. Therefore, IM nails are still very popular for general fracture repair as it avoids soft tissue damage and removal.

Most commonly used bone plates are made of metallic material, like Stainless Steel, Al alloys and Co alloys, because of their well-known material properties and developed manufacturing methods. However, such materials are much stiffer than the human cortical bone, which results in the plate carrying a higher percentage of the load compared to the femur, i.e. stress-shielding. Table 2.4. Mechanical properties of metallic materials commonly used in orthopaedic applications and the human femur representing young population [76-79] shows a comparison of the mechanical properties of the femoral shaft compared to commonly used bone plate materials [76-79] (Table 2.4).

According to Wolff's Law of stress related bone remodeling, when the loading on bone decreases, bone becomes weaker [70, 75]. Hence, bone becomes weak and less dense, which is also known as bone resorption, even after the full union of the fracture site, which increases the possibility of refracture after plate removal [75]. Therefore, an ideal bone plate should permit bone to carry an essential amount of load, while being stiff enough to maintain proper alignment of the fractured fragments [75].

Despite the aforementioned advantageous for femur fracture fixation with bone plates, this method has its own shortcomings. First, the risk of infection is higher for plating method compared to IM nailing method. The reason is that many apparatus are used for plating, like cables and screws. All of them have to be carefully sanitized and sterilized prior to insertion to minimize the risk of infection. Moreover, the risk of failure for screws and cables is higher than the IM nailing. If screws and cables fail close to the fractured surface, it may cause misalignment resulting in nonunion.

Table 2.4. Mechanical properties of metallic materials commonly used in orthopaedic applications and the human femur representing young population [76-79]

	Density (g/cm³)	Ultimate Strength (MPa)	Modulus of Elasticity (GPa)
Human Bone	1.85	133	17.7
Titanium Alloys	4.5	220	116
Cobalt Alloys	8.80	225	211
Stainless Steel	7.81	870	190

2.9. Vancouver Fractures

Periprosthetic femur fractures, occurring around pre-existing hip implant, are known as Vancouver type fractures. The Vancouver A fracture is a fracture either at greater or lesser trochanter. The Vancouver B1 injury is a femur fracture at or near the distal tip of a total hip replacement (THR) with a stable hip stem, it represents about 80% of all periprosthetic femur fractures, and it is commonly fixed using metal plates (Figure 2.20). In contrast, B2 fractures have a loose hip stem, whilst B3 fractures have a loose hip stem surrounded by insufficient bone stock [80]. The Vancouver C fracture is a diaphyseal fracture well distal to the tip of a total hip replacement. Although uncommon, femoral fractures occur in approximately 0.1% to 6% of all total hip arthroplasty cases [81, 82]. These types of femoral fracture are mostly found in osteopenic elderly women [81, 83]. The factors that mostly predispose patients to periprosthetic femoral fractures include cracks or defects generated intra-operatively, prior hip surgery, and stress risers at the tip of the hip stem. The Vancouver B1 fracture is classified as the most complex fracture to manage and has been reported to comprise as many as 75% of all types of

periprosthetic fracture cases. Various treatments are available including operative and non-operative interventions depending on the age of the patient, the location of fracture, and the general medical condition of the patient [84]. These types of fractures experience non-union in 25-42% of cases treated non-operatively and are best managed surgically. Spica cast immobilization and traction, as non-operative measures, have been used successfully but it can be accompanied with an unacceptable rate of complications [85].

Figure 2.20. Vancouver B1 fracture in an 86-year-old woman [86].

There are several repair methods clinically available, such as various combinations of cables, bicortical and unicortical screws, allograft struts, metal plates and cerclage wires. In 1976, Ogden and Rendall [87], introduced a repair construct which consists of a metallic bone lateral plate which is proximally and distally secured by Parham bands and bicortical screws, respectively. This technique gained attention because of its minimal morbidity, decreased

difficulties and immediate stable fixation. Nevertheless, there is still no ‘gold standard’ technique for the B1 fracture that is widely accepted by most investigators. Long term implant stability and immediate fracture union are the main purposes of periprosthetic femoral fracture treatment. The optimal surgical repair method and the most appropriate treatment still remain as a point of controversy. Hence, many studies in the literature have qualitatively and quantitatively investigated the mechanical properties of repair constructs that are clinically used. The main objectives for designing an optimal fracture fixation plate is that it should be stiff enough to prevent tensile stress at the fracture site and allow the non-fractured sites of bone to be stressed under normal loading conditions to prevent stress shielding.

2.10. Using Less Stiff Materials for Making Bone Fracture Plates

The conventional compression bone plates are made of metallic materials, which have a 5-10 times higher modulus than cortical bone. Thus if a plate with a high modulus of elasticity is attached to bone, a greater percentage of load is carried by the plate compared to the underlying bone, leading to stress-shielding. Subsequently, this phenomena leads in localized osteopenia of cortex beneath the plate, changes the diameter of the cortex, prohibits normal bone remodeling, and then increases the possibility of bone refracture during loading and unloading [27]. Moreover, during the monitoring process of an injured bone using computerized tomography (CT) and magnetic resonance imaging (MRI), these metallic materials appear white in color, which limits the visibility of bone which also appears white in color. Finally, the fatigue strength of metallic materials is highly dependent on how quickly they can repassivate when oxide layers are removed by fatigue cracks [7, 8]. The inability of metallic implants to repassivate quickly exposes unprotected regions to corrosion, which immediately results in corrosion-fatigue. This can cause a considerable drop in fatigue endurance limit, which is one of the main reasons for bone plate failure [7, 8]. Consequently, researchers are giving more attention to the stimulation of stress at the fracture site using two new concepts to eliminate “stress shielding”, namely, stress-relaxation or less-rigid devices [4]. In the late 1980s, the concept of a stress-relaxation bone plate was initiated by applying minimal changes in traditional rigid fixation systems [20]. Studies have been done on sliding plates made of stainless steel [88], placing a silicon rubber between a bone plate and host bone [89, 90], and using coelastic washers capable of creep in the screw holes [91]. Gangesh et al [6] developed stiffness-graded materials (SGMs) for fracture-

fixation plates and did preliminary compression tests between stainless steel plates and SGMs plates for bone healing using finite element analysis. They reported that stiffness-graded plates allow bone away from the fracture site to take more stress compared to stainless steel plates; subsequently allowing bone to retain its strength properties. Similarly, Benil et al [92], developed a new plate consisting of a combination of low-stiffness metal plate with low Young's modulus and stainless steel plate with high stiffness ($E = 7.4 \text{ GPa}$). The finite element analysis showed that the low-stiffness metal plate applied more compressive load to newly formed callus, as a result accelerating callus formation compared to titanium alloy plates and stainless steel plates. However, the application of metallic implant for biomedical purposes is still a point of controversy due to inherent corrosion fatigue and radiopaquency [7, 8]. To address the aforementioned drawbacks of metallic biomaterials, a new generation of biomaterials, i.e. composite materials has been introduced as a potential candidate to replace metals for biomedical applications due to their inherent advantageous of non-corrosiveness, tailorability, flexibility, and radiolucency [4, 93]. Composite materials can be defined as materials that are made of two or more components with superior properties, which cannot be achieved using each component alone. For the purpose of this study composite materials are considered to be consisted of reinforcing phase distributed in continues phase, i.e. matrix [9]. By altering the types and the distribution of the reinforcement agent, it is possible to achieve a wide range of mechanical and biological properties, and thus to optimize their structure in order to improve their interaction with surrounding tissue. As an example, for biological materials some type of composite materials can be used to demonstrate specific properties whilst reducing their metabolic cost, size and weight. This makes composite materials attractive solution for various biomedical applications particularly orthopedics, where they can be designed in a way to be more compatible mechanically with the adjacent bone and other tissue having lower elastic modulus while maintaining their strength and durability. This possibility led to widespread interest in using composite materials for various non-load bearing and load bearing orthopaedic applications such as spinal cage for degenerative discs [24, 25], filling bone defects for anchoring into bone for dental implants [26-28], intramedullary implants for fixing subtrochanteric defects [94], long bone fracture repair plates [95-99], high tibial osteotomy [100], and total hip replacements [101, 102] (Figure 2.21).

In the case of using bone fracture fixation plate, Woo et al [9] introduced composite materials made of carbon fiber. These plates were found to be more flexible than comparable metallic components, thus would increase the load on the fractured bone resulting in faster healing process (Figure 2.22 and Figure 2.23).

CF: carbon fibers, C: carbon, GF: glass fibers, KF: kevlar fibers, PMMA: Polymethylmethacrylate, PS: polysulfone, PP: Polypropylene, UHMWPE: ultra-high-molecular weight polyethylene, PLDLA: poly(L-DL-lactide), PLLA: poly (L-lactic acid), PGA: polglycolic acid, PC: polycarbonate, PEEK: polyetheretherketone; HA: hydroxyapatite, PMA: polymethylacrylate, BIS-GMA: bis-phenol A glycidyl methacrylate, PU: polyurethane, PTFE: polytetrafluoroethylene, PET: polyethyleneterephthalate, PEA: poltethylacrylate, SR: silicone rubber, PELA: Block co-polymer of lactic acid and polyethylene glycol, LCP: liquid crystalline polymer, PHB: polyhydroxybutyrate, PEG: polyethyleneglycol, PHEMA: poly(20hydroxyethyl methacrylate)

Figure 2.21. Various applications of composite biomaterials [9]

Figure 2.22. Carbon fibre reinforced epoxy fracture plates [9].

Figure 2.23. X-ray images of a mid-shaft tibial fracture, after plating with a carbon fibre reinforced epoxy bone plate for 37 weeks [9].

In the case of using composite materials to fabricate hip arthroplasty, the performed attempts were less successful due to the complicated design issues, required high strength and durability and costly manufacturing process. Christel et al. performed the first clinical study on composite hip implant in 1987 [102]. Another successful example of composite hip implant, made of carbon fiber reinforced thermoplastic polymer, developed by Orthodesign with elastic modulus approximately almost one-fifth of commercially used hip implants made of stainless steel materials. Studies until 1992 confirmed early post-operative recovery and bone stock retention [9] (Figure 2.24).

Figure 2.24. Carbon fibre reinforced thermoplastic hip prosthesis, with and without hydroxyapatite coating, shown alongside conventional titanium equivalent [9].

As it was mentioned earlier, new materials proposed for bone fracture plate applications need to have proper stiffness with adequate fatigue strength comparable to those of stainless steel material. As bone would experience high cyclic loading during daily normal activities, the amount of strain inserted to an injured bone should be minimized by bone plate to avoid subsequent non-union or misalignment. Composite materials that have been suggested for bone fracture plate application can be mainly grouped to non-resorbable, partially-resorbable and fully-resorbable. Non-resorbable composite materials could be considered either as thermoset and thermoplastic polymer composite. Although thermoplastic polymer composite showed biocompatibility and durability, it was very difficult to fabricate long fiber reinforced composite

using thermoplastic polymers with sufficient strength for load-bearing applications earlier. As the technology for manufacturing good quality thermoplastic polymer composite became available later, many studies have been done developing non-resorbable polymer composite bone plates such as CF/polymethylmethacrylate (PMMA) [20], CF/polypropylene (PP) [102], CF/polysulfone (PS) [103-105], CF/ polyethylene (PE) [106], CF/nylon [107] and CF/ polyetheretherketone (PEEK)[15, 95, 96, 100, 108]. Another benefit of using thermoplastic composite materials for manufacturing bone fracture plate is that, similar to metallic implants, they could be formed in certain condition to adopt the curved geometry of femur. Some few examples of thermoset polymer composite materials are CF/epoxy and Glass/epoxy composite [13, 23, 103, 109]. Although some evidence of toxicity exists in composite materials with partially cured epoxy [110], composite materials with properly processed epoxy resin showed not only excellent biocompatibility but also the capability to be manufactured with a very sophisticated geometry [111]. New bone growth and apposition around an epoxy pin coated with hydroxyapatite confirms the excellent in-vivo behavior of the epoxy resin (Figure 2.25) [9].

Figure 2.25. Section through an epoxy pin with coated with hydroxyapatite after 21 days implantation in the distal femur of the rabbit, showing new bone growth and bone apposition the implanted pin. Phase contrast microscopy, showing bone (top), coating (central band) and pin (bottom) [9].

Thermoset composite biomaterials are supposed to stay in-vivo condition possibly without a change in their properties, i.e. stiffness. However, for fracture bone plate applications, it is desirable that the stiffness decreased slowly as the bone heals. Therefore, researches give interest in designing composite biomaterials using resorbable polymers for this application. Composite plate made of resorbable polymers are capable of degradation in their mechanical properties overtime and thus would allow the underlying bone to carry a greater portion of load as it heals. Several studies have suggested partially-resorbable composite which consisted of resorbable polymers reinforced with non-resorbable fibers including carbon fiber [112-114], and polyamide fibers [115]. In addition, Tormala et al. [116] and Choueka et al. [117] proposed reinforcing resorbable polymers using resorbable fibers such as calcium phosphate based glass fibers and poly (L-lactic acid)(PLLA) fibers. These composites are called as fully resorbable composites. Nevertheless, the complication of using such composite was that it was difficult to achieve desirable mechanical properties, specially strength and stiffness. Hence, their application was limited to non-load bearing purposes. Moreover, they only could retain their properties only for few weeks. Also, synchronizing the degradation rate of such composites with healing rate is reported to be not feasible. According to Zimmerman et al.[118], CF/PLA composite lost their properties too rapidly in-vivo because of delamination, even though it poses superior properties before implantation. In addition, the histological abnormalities introduced by the degradation product, the control of the degradation speed and initial fixation are still problems that have not yet been solved.

Among all above-mentioned studies only a few of them reaches pre-clinical or clinical stages because the developed composite introduced a new problem whilst attempting to solve the existing one. Along the problems associated with composite materials in-vivo durability, one of the reported difficulties of using composite biomaterials as bone plates is that they may break, especially in severe bending loading condition normally exists in vicinity of bone gap at the fracture site. It is still hard to convince surgeons to use composite biomaterials since there is lack in their clinical data and their in-vivo stability, whereas metallic biomaterials have been used for this purpose for quite a long period of time with sufficiently strong clinical data background. Therefore, there is still a need to develop new composite material and optimize their design for various biomedical applications.

2. 11. Testing Methods in Biomechanical Analysis

Experimental and computational analysis are the two common test methods for biomechanical assessment [58-60]. Experimental approaches can be performed either in-vivo or in-vitro. In-vivo experiments can provide clinically reliable data since tests are performed on human subjects and physiological condition. Nonetheless, there are some complications accompanied with using in-vivo approach and most importantly data acquisition may be very difficult sometimes especially for joints. Therefore, in-vitro research has been done for decades on human cadaver or synthetic bone. Since accurate replicating the physiological load, which is resulted by the interaction of both hard and soft tissues, experimentally is not feasible, most of biomechanical studies have considered mechanical properties of the human femur and metallic materials commonly used in orthopaedic applications in-vitro [76-79] (Figure 2.26).

Figure 2.26. Loading under axial compression, torsion and 4-point bending loading conditions [119].

Cadaveric bone showed many drawbacks in experimental analysis. Their mechanical properties may degrade by time, where axial stiffness decreased by 30% over several months [120]. Although it is possible to keep mechanical properties almost constant by embalming them, specimens need to be embalmed for several months. In addition, the inter-specimen variability in

the geometry and material properties of cadaver cause discrepancy in the results and make comparison to the control difficult. Papini et al. showed that axial and torsional stiffnesses for intact human femurs, in turn, varied by 3.3 and 3.2 [121]. Synthetic longbones are gaining more widespread use due to advantages they provide over cadaveric specimens. The use of artificial or synthetic femurs has a sufficiently established history in biomechanics studies [45, 81, 122-127] because of their similar axial vs. torsional stiffness ratio[128], cancellous and cortical screw pullout shear stress [129, 130], and identical failure mechanism under axial compression compared to human femurs [131].

One of the most popular techniques for bone evaluations and designing implants is finite element analysis (FEA). This technique is much less expensive and can be done more rapidly compared to clinical researches. Also, it is capable of providing consistency in the modeled tested specimen and simulated loading conditions, which is beneficial especially where identical testing conditions are required. Biomechanical analysis done with FEA technique can be traced back to 1970s. The early FEA biomechanical analysis was limited to two-dimensional geometries. By further advancement in computer software such as computer aided design (CAD) software, the design of three-dimensional human model with more accurate geometry became feasible. Since, computational modelings are all based on some postulations which may not be the exact scenario in clinical condition; they need to be validated experimentally. Measuring strain at some certain spots on femur using strain gauges is a common approach to validate most FEA biomechanical models [127]. Strain gauges are inexpensive, easy to use and can provide relatively stable results. However, the accuracy and reproducibility of the results obtained via strain gauges can be highly affected with their position. Additionally, in order to achieve the best possible strain detection, gauges need to typically be placed on flat and smooth surfaces. Therefore, the detected results out of the curved surface tend to be unstable because the reading is averaged over an arc length. Furthermore, the main complication of using strain gauges is their limited resolution; therefore the ability to evaluate the overall stress behaviour of the longbone or implant is not feasible.

To address the aforementioned drawbacks of using strain gauges for recording bone surface strain and/or stress, a new approach, i.e. thermography approach, has been introduced recently [132, 133]. Thermography approach is a non-destructive technique that can be used to detect

temperature variations on the surface of an object. This technique has sufficiently established background for almost half a century measuring temperature on inaccessible, moving and very small objects [124, 133-136]. The thermography camera works based on thermoelastic effect of an object during cyclic loading condition. When a material loaded cyclically, its volume would change and can cause temperature variation. In other words, an object would experience alternate cooling and heating process during cyclic loading condition, which is directly proportional to the sum of principle stresses of the materials and is synchronies with the loading frequency. An infrared thermography camera is capable of recording the temperature variations of the object if there was a link between the camera and loading machine. The schematic set-up for the technique is shown (Figure 2.27).

Figure 2.27. Schematic of setup for lock-in thermographic detection [134]

CHAPTER 3: Current Study

Although there are several studies in the literature proposing composite materials for medical applications as bone fracture plates, there is no composite plate available in the market for human use. Moreover, none of the previous studies have considered the hybrid composite with “sandwich structure” reinforced by carbon and flax fibers for bone fracture plate applications. Therefore, in the current study, a new CF/Flax/Epoxy composite plate with potential biomedical application as a long-bone fracture plate was manufactured. The reason for the use of Carbon Fibers rather than, say, glass or tricalcium phosphate fibers, was that it has a much greater tensile and compressive strength, while producing an implant that is very light in weight. The reason for the use of an Epoxy matrix rather than, say, PEEK or acrylate matrix, was that the prepreg sheets available contained Epoxy, thus eliminating the financial costs associated with obtaining these alternate materials. Moreover, Carbon Fibers in an Epoxy matrix has a sufficiently established research history in bone plate applications to justify its current use [13, 18, 19, 23]. The experiments on this composite material were designed in a way to characterize its biological and mechanical properties as well as its biomechanical performance to figure out how it can act in comparison with a clinically-used metal plate with actual clinical use for the same purpose. For that reason, this study was conducted based on 4 separate chapters entitled as below.

Chapter 4- Osteogenesis and Cytotoxicity of a New Carbon/Flax/epoxy Composite Material for Bone Fracture Plate Applications.

Chapter 5- Biomechanical Static Properties of an Advanced New Carbon/Fax/Epoxy Composite Material for Bone Plate Applications.

Chapter 6- Biomechanical Fatigue Analysis of an Advanced New Carbon Fiber/Flax/Epoxy Plate for Bone Fracture Repair using Conventional Fatigue Tests and Thermography.

Chapter 7- Biomechanics of a New CF/Flax/Epoxy Plate versus a Traditional Metal Plate for Vancouver B1 Femur Fractures: An Experimental and Computational Study

CHAPTER 4: Osteogenesis and Cytotoxicity of Carbon/Flax/epoxy Composite

4.1. Introduction

One of the most important key factors in selecting a material for medical application is being biocompatible, i.e. the interaction of the medical device with the human body would not negatively affect the clinical success of the biomaterial. The aim of this chapter was to determine this new CF/Flax/Epoxy composite material's cytotoxicity and its effect on the osteogenesis (i.e. new bone formation by osteoblast cells) process in comparison with a medical grade metal material obtained from a commercially-used clinical bone fracture plate. It was hypothesized that this new CF/Flax/Epoxy composite material would show good biocompatibility without any negative influence on the osteogenesis process.

4.2. Materials and Methods

4.2.1. General Approach

CF/Flax/Epoxy specimens were compared to metal specimens manufactured from a fracture plate commonly used clinically. In addition, CF/Epoxy, Flax/Epoxy, and CF/Flax/Epoxy composites were tested for their wettability properties by contact angle (CA) and water absorption tests. The aim was to determine whether the CF/Flax/Epoxy composite material had acceptable biological properties based on the current in-vitro test, to be used for biomedical engineering applications as orthopaedic long bone fracture plates.

4.2.2. Specimens

The CF/Flax/Epoxy composite was manufactured using 16 layers of Flax/Epoxy laminae, as well as 2 layers of CF/Epoxy laminae added to the outer surface of the composite resulting in a “sandwich structure”. The CF/Flax/Epoxy composite was manufactured in-house using a compression-mold machine at 500 kPa and 150°C for 1 hr. The Flax/Epoxy laminae (Lineo NV, Dendermonde, Belgium) had a density of 1.3 g/cm³, a volume fraction of 58-60%, and a unidirectional fiber orientation, whereas CF/Epoxy laminae (Hexcel® Composites, Stamford, CT, USA) had a density of 1.56 g/cm³, a volume fraction of 57%, and unidirectional fiber orientation. To see the effect of each material on the cell behavior separately, Flax/Epoxy and

CF/Epoxy specimens were also manufactured using compression molding and autoclave techniques, respectively. The final composites were cut using a circular saw to create 15 mm long X 5 mm wide X 4 mm rectangular specimens. The metal specimens (316L stainless steel) were obtained from a commercially-available clinical bone fracture plate (Zimmer, Warsaw, IN, USA) and were cut to the same dimensions as the composite specimens.

4.2.3. Sterilization and Cell Cultures

All specimens were prepared for culture by autoclave (Heidolph, USA) sterilization using 121°C at 200 kPa for 45 min. Tissue cultured plastic plates (TCP), used as a standard dishes for cell growth, were cut to the same dimensions as other specimens and were sterilized using high power microwave sterilization for 15 min [137]. Following sterilization, specimens were handled according to standard sterile culture protocols. A rat osteoblast-like cell line, UMR-106 osteosarcoma (American Type Culture Collection, USA) was used, since this type of cell allows for the study of certain common cellular functions like viability, membrane state, proliferation, and attachment which are factors associated with basal cytocompatibility of the biomaterial[138, 139]. In addition, this type of cell is widely employed as a human osteoblastic model [138, 139]. Cells were grown at 37°C in a 5% CO₂ humidified atmosphere in Dulbecoo Minimal Essential Medium (DMEM), and supplemented with 10% fetal bovine serum (FBS), 1% penicillin streptomycin (PS), and 2mM-glutamine at 5% CO₂ and 37°C.

4.2.4. Assay Preparation and Testing

Extract Preparation.

Each specimen was immersed in DMEM as an extracting media with an extraction medium volume to surface area ratio of 1.25 cm²/ml at 5% CO₂ and 37°C [140]. Extraction was completed over 24 hrs, at which point the extraction media was removed and centrifuged before being applied to cell cultures (Figure 4.1).

Figure 4.1. Extract preparation for cytotoxicity test by immersing each sample in DMEM as an extracting media. A, B and C are Flax/epoxy, CF/epoxy and metal samples, respectively.

MTT Assay-Indirect Test.

The cytotoxicity of Flax/Epoxy, CF/Epoxy, and CF/Flax/Epoxy composite extracts was evaluated against UMR-106 osteoblast-like using MTT assay in 96-well plates. The MTT is a salt that can be enzymatically converted by living cells, resulting in a color change from yellow, 3-(4, 5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide, into purple formazan crystal. The intensity of the color produced is directly proportional to the number of viable cells and can be measured by reading absorbance at 590 nm on an enzyme-linked immunosorbent assay plate reader. Briefly, UMR-106 cultured cells were seeded at 1×10^4 cells/ml in a 96-well plate and allowed to adhere for 24 hrs at 37°C in a 5% CO₂ humidified atmosphere, before being media aspirated. DMEM supplemented with FBS was added to cultures every second day. Extraction cultures were maintained at 37°C in a 5% CO₂ atmosphere. Control samples consisted of UMR-106 cells grown on TCP supplemented with complete DMEM that was not exposed to extracts. After 24 hrs, 48 hrs, and 72 hrs of incubation, extracts were removed and each well was treated with MTT solution at 5% CO₂ and 37°C for 4 hrs. The medium was removed and then 100 μ L of

dimethyl sulfoxide (DMSO) was subsequently added to each well, and the plate was shaken for 5 min before reading the optical density at 590 nm with a corrected wavelength of 690 nm. Cytotoxicity was calculated as the percentage of control cell viability (Figure 4.2).

Figure 4.2. The image of 96-well plate ready for ELISA microplate reader machine. a) Flax/Epoxy, b) CF/Epoxy, c) Control, d) CF/Flax/Epoxy, e) Metal.

MTT Assay-Direct Test.

A quantity of 5000 cells/cm² was seeded to the surface of each sterilized specimen contained in the wells of a 12-well polystyrene culture plate (Figure 4.3). To ensure cells would attach to the specimen surface (rather than the culture plate) and to prevent any cell suspension slipping, 100 µl of cell suspension was carefully overlayed on the surface of the specimens. Cells were allowed to seed for 8 hrs for all tested materials, except for Flax/Epoxy. This was done because it was observed that a seeding time greater than 4 hrs resulted in media soaked into Flax/Epoxy

composite and shorter seeding time less than 8 hrs for the other specimens would have resulted in inadequate cell adherence. Following seeding, 1.5 ml of complete phenol-red free DMEM was added. Cultures were maintained at 5% CO₂ at 37°C. A total of 4 specimens x 3 measurements were obtained for each type of material (Figure 4.4.b).

Figure 4.3. Direct cell seeding by overlaying cell suspension on the surface of each sample. A, B and C are Flax/epoxy, CF/Epoxy, and metal samples, respectively.

After 24 and 96 hrs, cell viability/cell proliferation rate was assessed by MTT assay. Culture wells containing the samples were gently rinsed with phosphate-buffered saline (PBS) followed by the addition of 1.5 ml of 0.5 mg/ml MTT solution in phenol-red free DMEM. After incubation for 4 hrs in 5% CO₂ at 37°C, the MTT solution was aspirated. Specimens were inverted in 0.6 ml of dimethyl sulfoxide (DMSO) and gently shaken for 5 min to dissolve insoluble formazan crystals, in order to accurately record the number of surviving cells on the

surface of the specimen. Triplicate aliquots of 100 µl per specimen were moved to a 96-well plate. The plate was read at 590 nm with a corrected wavelength of 690 nm.

Figure 4.4. Flowcharts showing the process of testing for MTT assay using (a) Indirect Test and (b) Direct test.

4.2.5. Osteogenetic Induction.

Approximately 1×10^5 UMR-106 cells were seeded in a 6-well culture dish and allowed to adhere for 24 hrs at 37°C in a 5% CO₂ humidified atmosphere before old media was discarded and specimen extracts were added to each well. The UMR-106 cells were cultured in CF/Flax/Epoxy composite extracts, as this composite was the final selection for fabrication of the bone fracture plate. The positive and negative controls were, in turn, cells exposed to metal specimen extract and cells that were not exposed to any conditioned media. The medium of the cultured cells was replaced every other day. Identification of osteoblast markers and gene

expression (RT-PCR) were done after 24 hrs, 48 hrs, and 72 hrs by analyzing the results of electrophoresis gel (Figure 4.5).

Figure 4.5. Image of PCR electrophoresis gel

RT-PCR was used to determine the mRNA expression of Runx2, Osterix, and BMP2 after 24 hrs, 48 hrs, and 72 hrs. RNA was extracted by Trizol (Invitrogen, USA) from the UMR-106 cells exposed to the conditioned media and the control cells. RNA (0.5 $\mu\text{g}/\mu\text{l}$) was used to synthesize cDNA using random primers following SuperScript® First-Strand Synthesis System kit for RT-PCR (Cat no: 11904018, Invitrogen, USA). Then, the relative expression of Runx2, BMP2, and Osterix genes was determined using semi-quantitative polymerase chain reaction (RT-PCR). The results of PCR electrophoresis gel were analyzed using ImageJ software (National Institute of Health, USA), and expression levels of all genes were normalized to the internal control hypoxanthine phosphoribosyltransferase HPRT House-keeping gene.

4.2.6. Wettability.

The water absorption was performed by immersing the CF/Epoxy and Flax/Epoxy samples in a distilled water bath at room temperature according to ASTM standards for moisture absorption properties of polymer matrix composite materials [141]. The samples were weighed to the nearest 0.1 mg each day for 45 days. The contact angle (CA) at the liquid-solid surface of each specimen was measured using the sessile drop technique [142]. Briefly, a drop of water from a syringe was placed onto the surface of the samples, and the digital images of the drops were taken by a Charged-Coupled Device Camera (CCD-Camera). The CA test was performed on 6 specimens for each test group. For comparison, the same wettability tests were conducted on a CF/Flax/Epoxy composite as well, to study how an external layer of carbon fiber would affect the behavior of the material.

4.2.7. Statistical Analysis.

For each of the test groups in the cytotoxicity assay and osteogenesis tests, 4 specimens were used from which 3 measurement readings were taken. Data are represented currently as mean $\pm$ standard deviation. All data were combined into a single one-way ANOVA to compare the cell viability and osteogenesis marker induction of the test groups at each time period, and also to compare the effect of time on a given test group. Tukey's Honestly Significant Difference method was used for all post hoc tests to determine exactly which pair-wise comparisons generated the statistical differences between test groups at the same time point or for the same test group between times. Statistical significance was set at $p < 0.05$. For wettability tests, 6 specimens were used for each test group, and a similar one-way ANOVA was used for CA and water absorption results. Two-tailed post hoc power analysis was done to determine if there were enough specimens per test group to detect all the statistical differences that may have been actually present, i.e. to avoid type II error.

4.3. Results

4.3.1. MTT Assay-Indirect Test

For each time point, comparisons between the 5 test groups are summarized (Figure 4.6). After 24 hrs, viability of cells grown in Flax/Epoxy, CF/Epoxy, CF/Flax/Epoxy, and metal

extracts was comparable for all tested groups and remained at around 100% ($p=0.254$). After 48 hrs, the viability of cells exposed to Flax/Epoxy extract significantly decreased to 70% when compared to that of the control ($p<0.001$), but was not different from that of CF/Epoxy ($p=0.08$). On the other hand, a reduction in cell viability exposed to CF/Epoxy and CF/Flax/Epoxy extracts were not significantly different from metal ($p=0.897$, $p=0.750$). After 72 hrs, the viability of cells exposed to extract of all tested materials was significantly different when compared to control ($p<0.001$). Viability of cells grown in Flax/Epoxy extract decreased significantly compared to metal samples and reached 68% of the control ($p<0.001$). While the viability of cells exposed to Flax/Epoxy was significantly reduced when compared to CF/Epoxy composite extracts ($p<0.001$), there was no difference observed between CF/Epoxy and CF/Flax/Epoxy versus metal ($p=0.259$, $p=0.167$).

For each test group, changes over time are shown (Figure 4.6). Generally, the cell viability decreased with increasing incubation time for all extracts. As mentioned earlier, cell viability stayed around 100% for all test groups that were exposed to extracts for 24 hrs. Thus, the 24-hr mark was selected as a reference point. For Flax/Epoxy, with respect to the 24-hr mark, after 48 and 72 hrs of incubation time the cell viability dropped to almost 70% ($p<0.001$). For CF/Epoxy, with respect to the 24-hr mark, cell viability at 72 hrs significantly decreased to approximately 80% ($p<0.001$). For CF/Flax/Epoxy and metal, the level of cell viability was not significantly different at 24 hrs versus 48 hrs ($p=0.06$, $p=0.056$), but it became significantly different for 24 versus 72 hrs ($p=0.003$, $p=0.008$). Moreover, the morphology of UMR-106 cells exposed to 4 conditioned media is given (Figure 4.7).

The images show that the number of viable cells exposed to CF/Flax/Epoxy specimens was comparable to metal at all incubation times. However, more cells were observed at 72 hrs compared to 24 and 48 hrs for all conditioned media. This can be related to the doubling time of UMR-106 cells which was reported to be around 24 hrs [169].

Figure 4.6. Effect of specimen extracts on the viability/proliferation rate of rat osteoblast-like cells at 24 hrs, 48 hrs, and 72 hrs. Results are expressed as % of control and are the mean $\pm$ SD of 4 independent experiments. Symbols (&, \$, ~, #, @, *, **, %, %%, ^, ^^, +, ++, “, =, `) indicate pairwise comparisons that were statistically different ($p < 0.05$), whereas all other comparisons were not different ($p \geq 0.05$).

4.3.2. MTT Assay-Direct Test.

For a given time point, comparisons between the 5 test groups are illustrated (Figure 4.). After 24 hrs, Flax/Epoxy data were not different than CF/Epoxy ($p=0.527$), metal ($p=0.240$), CF/Flax/Epoxy ($p=0.320$), or control ($p=0.825$). The viability of cells grown onto CF/Epoxy and CF/Flax/Epoxy were not different than metal ($p=0.920$, $p=0.960$). After 96 hrs, the number of viable cells grown onto Flax/Epoxy was lower compared to metal and control ($p < 0.001$). Similarly, the difference between Flax/Epoxy and CF/Epoxy was significant ($p=0.039$). However, no significant difference between cell viability was determined on CF/Epoxy and CF/Flax/Epoxy versus metal ($p=0.194$, $p=0.200$).

Figure 4.7. Images of UMR-106 cells cultures' viability subjected to five conditions at 10X magnification. (Control = Normal fresh media + Cells, Flax/Epoxy, CF/Epoxy, CF/Flax/Epoxy, and metal plate) at all incubation time.

Figure 4.8. Effect of specimens on the viability and/or proliferation rate of rat osteoblast-like cells at 24 hrs and 96 hrs. Control samples consisted of cells grown on tissue culture plastic supplemented with complete DMEM. Results are expressed as % of control and are the mean $\pm$ SD of 4 independent experiments. Symbols (&, \$, ~, *, **, %, ^, +, ++, “, #, @) indicate pairwise comparisons that were not statistically different ($p \geq 0.05$), whereas all other comparisons were different ($p < 0.05$).

For a given test group, changes over time are illustrated (Figure 4.8). Cell growth onto Flax/Epoxy composite for 96 hrs reached 52% of its original value ($p < 0.001$), whereas cell growth onto CF/Epoxy and CF/Flax/Epoxy composite for the same amount of time reached approximately 75% of their initial levels, respectively ($p < 0.001$). Cell growth onto metal for 96 hrs dropped to 85% of its starting value ($p < 0.001$).

4.3.3. Osteogenesis Results.

The osteogenesis results of 3 incubation times after exposure of UMR-106 cells to the conditioned media of CF/Flax/Epoxy in comparison to the control are given (Figure 4.9). The expression level of Runx2 stayed around 100% at 24 hrs ($p = 0.896$), whereas after 48 hrs the expression level from 3 CF/Flax/Epoxy media was significantly more than that from metal

extract ($p=0.026$). After 72 hrs, the difference in the expression level of Runx2 was not significant in all tested groups ($p=0.700$). For Osterix, no significance difference in the expression level was observed at all incubation times ($p\geq 0.05$). The difference in the expression level of BMP2 gene from the cells exposed to CF/Flax/Epoxy extract was not significant at all incubation times in comparison to that from metal extract (24 hrs, $p=0.987$; 48 hrs, $p=0.578$; 72 hrs, $p=0.437$).

Figure 4.9. Expression levels of bone formation gene markers. (a) Runx, (b) Osterix, and (c) BMP2 were examined at 24, 48, and 72 hours. Symbol (*) indicate pairwise comparisons that were statistically significant ($p < 0.05$), whereas all other comparison were not significantly different ($p \geq 0.05$).

4.3.4. Wettability Tests.

The water absorption behavior of the composites is shown (Figure 4.10). The maximum water uptake for CF/Epoxy, Flax/Epoxy, and CF/Flax/Epoxy composite specimens after 45 days

reached almost 1.25 %, 17.2 %, and 8.3 %, respectively ($p < 0.001$). Although the best fit curve was logarithmic for CF/Epoxy and Flax/Epoxy, it was polynomial for CF/Flax/Epoxy with R^2 values equal to 0.90, 0.98, and 0.98, respectively (Figure 4.10). In addition, the CA for CF/Epoxy, Flax/Epoxy, and CF/Flax/Epoxy, in turn, was equal to $93.22^\circ \pm 1.95^\circ$, $68.07^\circ \pm 2.05^\circ$, and $83.39^\circ \pm 2.37^\circ$ ($p < 0.001$). The Flax/Epoxy and the CF/Flax/Epoxy revealed a hydrophilic character because their CA was smaller than 90° [142, 170]. However, CF/Epoxy showed a hydrophobic character, since its CA was larger than 90° [142, 170].

Figure 4.10. Water absorption results. The equations for the best fit curves for Flax/Epoxy, CF/Flax/Epoxy, and CF/Epoxy data, respectively, were $Y = 2.929 \ln(X) - 2.668$, $Y = -9 \times 10^{-6} X^2 + 0.016 X + 0.371$, and $Y = 0.196 \ln(X) - 0.193$, with R^2 values equal to 0.90, 0.98, and 0.98, respectively. Error bars are 1 standard error of the mean.

4.3.5. Post Hoc Power Analysis.

For indirect MTT tests, average powers for all comparisons at a given time point were 18% (24 hrs), 67% (48 hrs), and 90% (72 hrs), whereas average powers for comparison between time points for a given test group were 100% (Flax/Epoxy), 77% (CF/Epoxy), and 51% (metal). For direct MTT tests, average powers for all comparisons at a given time point were 33% (24 hrs) and 99% (96 hrs), whereas average powers for comparisons between time points for a given test group were 100% (Flax/Epoxy), 100% (CF/Epoxy), and 99% (metal). For osteogenesis tests,

average powers for all comparisons were 18% (BMP2), 17% (Osterix), and 36% (Runx). Average post hoc powers obtained for CA comparison were 100%, whereas average powers for water absorption comparisons were 99%. A minimum power of 80% is generally considered to indicate a good study design, such that all statistical differences present are detected.

4.4. Discussion

4.4.1. General Findings.

The current study is the first to assess the *in-vitro* biological compatibility of a new CF/Flax/Epoxy composite for potential application as bone fracture plates. Comparisons were made to stainless steel because of this material's common use in fabricating clinical fracture plates. Cell survival and growth onto CF/Flax/Epoxy versus metal showed no appreciable differences for all of the measurements taken. Moreover, CF/Flax/Epoxy would not negatively affect the common process of osteogenesis necessary for bone healing in comparison with metal specimens commonly-used clinically. This novel CF/Flax/Epoxy composite material had promising biological properties, thereby warranting further investigation for potential use to fabricate orthopaedic implants.

4.4.2. Comparison to Prior Studies.

Present Flax/Epoxy data had similar trends to prior studies examining cytotoxicity of flax components individually or as reinforcements for composites. Skorkowska-Telichowska et al [171] assessed cytotoxicity of 3 different flax components (fiber, seedcake extract from transgenic flax, and oil emulsion) obtained from a genetically engineered flax plant in a culture of mice fibroblast. They found that increasing incubation time caused no significant changes in the total number of viable cells treated with different flax components, except for those obtained from the plant material. Similarly, in the current study significant changes in osteoblast-like cell proliferation rate and viability were observed by increasing incubation time for the extract containing flax material in the cytotoxicity test.

Current CF/Epoxy results were generally comparable to reported data from other studies in which CF composites showed acceptable biocompatibility [13, 172]. Dimitrievska et al [172] reported considerable mice fibroblast and osteoblast proliferation rates, as well as no cytotoxicity

on CF/Polyamide specimens. Ali et al [13] showed the biocompatibility of CF/Epoxy based on small animal implantation and tissue cultures with no obvious macrophages thickening or chronic inflammation. Similarly, the present novel CF/Epoxy also showed no cytotoxicity (Figure 4.6) and good biocompatibility (Figure 4.8).

The first component of an implant that comes into contact with the cells and biological fluids is the surface of the biomaterials [173]. Thus, surface characteristics, particularly the wettability, would affect the material's biocompatibility. It has been long assumed that the high wettability evidenced by low CA would result in better biocompatibility for specific applications, such as dental impression and intraocular lenses [174, 175]. In contrast, studies on blood-contacting devices and tissue engineering substrate suggest that materials with a moderate balance of hydrophilic and hydrophobic surfaces would demonstrate better biocompatibility [176, 177]. In the present study, CF/Epoxy composite with the hydrophobic surface showed a better compatibility, as per the cytotoxicity assay results, compared to Flax/Epoxy composite with the hydrophilic surface, which can absorb more media and release many particulates compared to other studied groups; it is possible that this had a greater effect on the cells.

4.4.3. Practical Implications.

Flax/Epoxy currently reduced cell viability percentages to almost 68% and 52% after 72 hrs (Figure 4.6) or 96 hrs (Figure 4.8). No considerable difference for cell viability was found between CF/Epoxy and metal at each time in the testing period. This may be related to the hydrophilic and hydrophobic surface of Flax/Epoxy and CF/Epoxy, respectively. The low CA (smaller than 90°) on Flax/Epoxy composite means that the material has a high surface energy to absorb moisture and water, whereas CF/Epoxy composite had a hydrophobic surface, caused by its large CA (larger than 90°) [142, 170]. For that reason, the Flax/Epoxy composite absorbed water up to 17.2%, but CF/Epoxy composite only absorbed water up to 1.25% (Figure 4.10). Therefore, the Flax/Epoxy composite, due to its hydrophilic nature, may “draw” or “suck” the media from the cells and thus contribute to cell death.

The osteogenesis process, which is caused by osteoblastic differentiation, results in bone formation necessary for fracture healing. The two most important transcription factors that required for osteoblastic differentiation are, in turn, Runx2 and Osterix [178-180]. Bone

morphogenetic proteins (BMPs), like BMP2, are known to act as key growth factors in skeletal differentiation [179, 181-183]. It was reported that the osteogenesis process is directly related to the activity of the transcription (Runx2, Osterix) and growth (BMPs) factors, where antagonization of BMPs results in inhibition of osteogenesis process [183]. Based on the current results, the normal process of bone formation resulted by osteoblastic differentiation would not get harmed at all in vicinity of the CF/Flax/Epoxy composite material. In other words, the expression levels of all examined markers subjected to CF/Flax/epoxy were found to be statistically equal to those cultured with clinically-used metal specimens, except for Runx2. Particularly, the expression level of Runx2 gene, the central regulator of bone formation, upregulated from the cells cultured with the CF/Flax/Epoxy extracts compared to those from metal extracts, indicating the superiority of CF/Flax/Epoxy over metal for osteogenesis process (Figure 4.9).

4.4.4. Addressing Potential Limitations.

Firstly, specimens were cut in rectangular shapes, rather than circular shapes. For further direct cytotoxicity studies, it is optimal to have specimens with the same dimensions and geometry as the flask container to minimize any cell adherence to plastic. This geometric effect was present in all test groups, whose relative performance compared to one another would likely have been the same if circular samples were used.

Secondly, the higher amount of particulate release generated by the Flax/Epoxy most likely had an effect on the cells reflected by MTT assay results. Flax/Epoxy specimens had less seeding time compared to other test groups, which may have resulted in errors.

Thirdly, although there were numerous statistical differences noted, it is not clear whether the corresponding absolute values for changes in cell survival for the Flax/Epoxy and CF/Epoxy test groups would be of clinical or practical importance. A future *in-vivo* animal study for a prolonged period of time may be able to address this issue.

Finally, the current study was low in statistical power for 7 of 16 measurement categories as a result of limited sample size, i.e. $n = 4$ for cytotoxicity tests, for each test group. In order to have an acceptable statistical power of more than 80% for all measurement categories, more than 500

samples would have been needed, which was not practically or financially feasible. However, the statistical differences that were detected for cytotoxicity study still indicated clearly the inferiority of the Flax/Epoxy versus the CF/Epoxy material, relative to the metal and control test groups.

4.5. Summary

This chapter confirms the biocompatibility of the developed CF/Flax/Epoxy composite material for biomedical application by showing comparable cell viability as well as no negative influence on bone formation gene expression level at all incubation times to a medical grade stainless steel material with actual clinical use in manufacturing orthopaedic implants.

CHAPTER 5: Biomechanical Static Properties of CF/Flax/Epoxy Composite

5.1. Introduction

The purpose of this chapter was to determine the mechanical properties of the developed CF/Flax/Epoxy plate having a sandwich structure. Specifically, the plate was tested in uniaxial tension and three-point bending to simulate the main loads experienced by orthopaedic femur fracture plates, as well as Rockwell hardness tests. It was hypothesized that the CF/Flax/Epoxy composite would have properties similar to that of human cortical bone, thereby having potential application as a long bone fracture plate.

5.2. Materials and Methods

5.2.1. General Study Design

Mechanical testing, include tension, three-point bending, and Rockwell hardness tests, were performed on CF/Flax/Epoxy composite specimens to define its mechanical properties, such as longitudinal strength, Young's modulus, flexural strength, flexural modulus, and hardness. The aim was to determine the mechanical properties of the composite plate and the strength of bonding at the interface of CF/Epoxy and Flax/Epoxy laminae under clinical-type axial and bending loading conditions. Also, the failure mechanism of the specimens was characterized using scanning electron microscopy (SEM).

5.2.2. Composite Material Structure

Manufacturing of the CF/Flax/Epoxy composite plates was done using 16 layers of Flax/Epoxy prepreg sheets (Lineo NV, Dendermonde, Belgium), with a density of 1.3 g/cm^3 , a volume fraction of 58-60%, and unidirectional fiber orientation, as well as 2 layers of CF/Epoxy prepreg sheets (Hexcel® Composites, Stamford, CT, USA) with a density of 1.56 g/cm^3 , a volume fraction of 57%, and unidirectional fiber orientation (Figure 5.1).

These sheets were inserted into an in-house custom-made stainless-steel mold with CF/Epoxy sheets added as outside layers, resulting in a sandwich structure which makes this composite unique compared to previous ones for orthopaedic bone plate applications [10, 13, 18, 19, 23,

118, 143]. The mold was then heat-pressed in a compression molding machine (Carver Press, Wabash, IN, USA) at 150 °C and 500 kPa pressure for 60 min. The final composite material sheet was 260 mm in length, 250 mm in width, and 3.6 mm in thickness. There is no study in the literature considering hybrid composites reinforced by natural fibers and carbon fibers with a sandwich structure for bone plate applications.

Figure 5.1. Drawing of the CF/Flax/Epoxy specimens showing the novel “sandwich structure”.

5.2.3. Tension Tests.

Composite plates were cut from the sheet using a table saw, yielding plates with a length of 250 mm and a width of 15mm based on the recommended dimensions from ASTM standards for tensile properties of polymer matrix composite materials [144]. The experiments were conducted on a tensile machine (STM series, United Calibration Corp., Huntington Beach, CA), with a testing speed of 2 mm/min and a 50 kN load cell (Figure 5.2.a). An extensometer (United Calibration Corp., Huntington Beach, CA, USA) was placed at the center of the specimens and used to measure elongation. The specimen was positioned in the grips with the long axis in-line with the force direction (Deluxe-Action Wedge Grips, United Calibration Corp., Huntington Beach, CA, USA) so that 45 mm of the samples at each end was gripped. The test was performed on 6 samples loaded to failure under quasi-static loading conditions. The ultimate strength and ultimate strain were measured just before the failure. The modulus of elasticity, i.e. slope of the curve, was obtained by applying linear regression to the linear domain of the experimental stress-vs-strain curve. The fractured surface of the specimens was characterized under Scanning Electron Microscopy (JEOL, JSM-6380, Tokyo, Japan), with a high resolution of 3.0 nm, to

better understand the fracture mechanism. The specimen was coated with a thin layer of gold in preparation for SEM observation.

5.2.4. Bending Tests.

The three-point bending test was performed on 6 specimens with a length of 140 mm and a width of 13 mm based on the recommended dimensions from the ASTM standard for bending properties of polymer matrix composite materials [145]. The three-point bending test is accurate enough for solid specimen. However, for plates with holes 4-point bending test is recommended [145]. All specimens were loaded to failure under quasi-static loading conditions. The experiments were conducted on the same testing machine used for tension tests, in which each specimen was supported on a fixture, with the span length of 108 mm, depending on the specimen thickness (span-to-thickness ratio equal to 30:1) (Figure 5.2.b). The reason a large span-to-thickness ratio was chosen was to minimize the effect of out-of-plane shear in calculation of bending properties[145]. Both loading nose and supports had circular contact surfaces of radius 3 mm. The testing speed was 12.54 mm/min, and a 5000 N load cell was used in all tests. Using beam theory, the ultimate strength and ultimate strain were measured just before the failure. Also, the flexural modulus of elasticity, which is the ratio of stress to strain at any point on the stress-vs-strain curve, was calculated using equation 1[145],

$$E_f = \frac{L^3 m}{4bh^3} \quad (1)$$

where E_f is the flexural modulus of elasticity (MPa), L is the support span (mm), b is the width of the beam (mm), h is the thickness of beam (mm), m is the slope of the force-vs-deflection curve (N/mm).

The scanning electron microscope (JEOL, JSM-6380, Tokyo, Japan) had a high resolution of 3.0 nm and was finally used to observe the appearance of the fractured surface of the specimens. A thin layer of gold was coated on the surface of the specimen, in order to make the specimen conductive.

5.2.5. Rockwell Hardness Tests.

Rockwell E hardness tests were performed on 9 square-shaped specimens each with surface area equal to 1 in² (i.e. 645 mm²), based on the recommended dimensions in the ASTM standard for Rockwell hardness test of plastics and electrical insulating materials [146]. The reason is that there is no ASTM standard for specifically testing the hardness of polymer-matrix composite materials. Rockwell hardness test was chosen instead of Shore (durameter) hardness test, another commonly used hardness test for plastics, because it is more generally used for harder plastics such as nylon, epoxy, and polycarbonate. However, Shore hardness is the preferred method for rubbers/elastomers and is also commonly used for softer plastics such as polyolefins, fluoropolymers, and vinyls.

Figure 5.2. Mechanical tests set up: a) tension test, b) bending test, and c) Rockwell hardness test.

All samples were visually tested for parallel flat surfaces to eliminate deflection caused by poor contact between samples and the anvil of the hardness tester machine. A large steel ball (rather than a cone-shaped indenter) was chosen in order to distribute the load more evenly, since fibers may influence the penetration of the indenter and could result in variations in data [146]. Tests were done at ambient room temperature using a Rockwell hardness tester (LR300TD, LECO Corporation, St. Joseph, MI, USA), which forced a steel sphere with a 1/16 inch diameter at a load of 100 kg (HRE) (Figure 5.2.c). The total time taken from the initial indentation, to load dwell time, and finally to the recording of the measurement was 10 sec, which was controlled entirely by the hardness tester. For each sample, 15 readings were made directly from the testing device, and the mean value of these readings was reported as the hardness of each specimen.

5.2.6. Statistical Analysis.

All data for elastic modulus results from tension and bending tests were combined into a single one-way ANOVA to determine if the two test methods yielded statistically different results. A similar one-way ANOVA was used for ultimate failure strength results to compare tension vs. bending test results. A one-tailed post hoc power analysis was done to determine if the numbers of specimens per test group were enough to avoid type II error and detect all the statistical differences. Statistical significance was set at $p < 0.05$.

5.3. Results

5.3.1. Tension Test Data

The tensile behavior of the specimens is shown by plotting stress vs. strain (Figure 5.3). All specimens experienced brittle fracture with linear behavior up until failure. The mean $\pm$ SD for Young's modulus and ultimate strength were 41.7 ± 1.6 GPa and 399.8 ± 8.2 MPa, respectively. Failure for all specimens took place inside the bottom grip with catastrophic failure accompanied by an abrupt decrease in stress after reaching a peak value. None of the specimens showed edge delimitation or long splitting until failure (Figure 5.4). The SEM images showed fiber breakage along with fiber bundle and/or fiber pull-out at the fractured surface (Figure 5.5). Most fibers exhibited smooth tensile failure surfaces caused by propagation of the crack in the composite.

5.3.2. Bending Test Data.

The behavior of the specimens in the bending test is shown by plotting force vs. displacement (Figure 5.6). All specimens showed linear behavior in the force-vs-displacement curve up until failure. The overall average for flexural modulus and ultimate flexural strength were 57.4 ± 1.8 GPa and 510.6 ± 66.3 MPa, respectively. In all specimens, failure occurred at the outer surfaces due to cracking along the tensile surfaces (failure 1 in Figure 5.6) followed by progressive delamination and buckling of flax plies (failure 2 in Figure 5.6). A typical failed specimen in three-point bending is depicted (Figure 5.7). The SEM images also exhibited CF failure at the outer surfaces with Flax ply-level delamination and/or buckling (Figure 5.8).

Figure 5.3. Tension test results for CF/Flax/Epoxy specimens.

Figure 5.4. A typical failed specimen in tension. The specimen underwent brittle fracture caused by fiber breakage at about its mid-length.

Figure 5.5. SEM images of fractured surface in tension at different magnification. a) Fracture surface at 30-times magnification b) Fractured surface at 100-times magnification (failure caused by fiber bundle and/or fiber pull-out).

5.3.3. Tension vs Bending Data Comparison

The results for ANOVA analysis revealed that the Young's modulus (from tension tests) and flexural modulus (from bending tests) were significantly different ($p < 0.001$). Also, there was a significant difference in ultimate tensile strength (from tension tests) and ultimate flexural strength (from bending tests) ($p = 0.003$). Average post hoc powers obtained for Young's

modulus vs. flexural modulus comparisons were 100%, whereas average powers for ultimate tensile strength vs. ultimate flexural strength were 99.2%. A good study design is considered to have a minimum power of 80% to avoid type II statistical error.

5.3.4. Rockwell Hardness Test Data

The overall average for Rockwell E hardness of CF/Flax/Epoxy composite was 72.43 ± 3.87 HRE. All specimens showed circular-shaped indentations at their tested surfaces (molded surfaces), perpendicular to the testing direction (parallel to the molding pressure).

Figure 5.6. Bending test results for CF/Flax/Epoxy specimens.

Figure 5.7. A typical failed specimen in bending. Cracking along the tension surface results in specimen failure.

5.4. Discussion

5.4.1. General Findings

The current study is the first to assess the mechanical properties of the new CF/Flax/Epoxy hybrid composite for potential biomechanical application as a long bone fracture plate. The composite material has mechanical properties that make it attractive for several applications, such as bone fracture plates experiencing clinical-type axial loading or bending. The SEM images showed perfect bonding at the interface of CF/Epoxy and Flax/Epoxy adjacent layers, as no debonding or delamination between these adjacent layers occurred up until failure.

Figure 5.8. SEM images of fractured surface in bending. a) Fracture surface at 15-times magnification b) Fractured surface at 50-times magnification. Failure occurred due to CF breakage followed by flax ply-level buckling and/or delamination.

5.4.2. Comparison to Prior Studies

There are scattered results in the literature on the mechanical properties of composites reinforced by natural fibers and carbon fiber. The results of some prior studies on carbon fiber

composites, flax fiber composites, and hybrid composites reinforced by natural fibers and carbon fiber, are summarized in comparison to the present study. The results of the current study do not perfectly correlate with available results in the literature, due to different carbon or flax fiber orientation, dissimilar fiber or matrix volume fraction, and various manufacturing methods. However, the present composite material showed higher mechanical properties compared to the previously-studied hybrid composites, which were reinforced by natural fibers and carbon fiber, in both tension and bending properties [39, 40], as the presence of the flax fibers in core region moves CF/epoxy layer away from the neutral axis and results in better bending properties .

5.4.3. Practical Implications

Orthopaedic long bone fracture plates with lower axial stiffness can reduce the negative effect of “stress shielding” introduced by high-stiffness metallic materials [13, 17, 19, 20, 95, 96, 118]. Such plates should have lower axial stiffness to allow the underlying bone to carry a considerable amount of applied load, while having sufficient bending stiffness to prevent gross motion at the fracture site [2]. The current CF/Flax/Epoxy composite, with moduli of elasticity (41.7 and 57.4 GPa) much closer to that of cortical bone (7-25 GPa) [2, 93], may allow more load sharing compared to titanium and stainless-steel plates that have much higher moduli of elasticity, respectively, of $E = 110$ GPa [184] and $E = 220$ GPa [185]. The present ultimate tensile strength (399.8 MPa) and ultimate flexural strength (510.6 MPa) are high enough to immobilize adjacent bone fragments and carry clinical-type loads, for example, on the human femur (2.5 to 3 times body weight) [2, 3].

The current CF/Flax/Epoxy composite showed a difference between its tensile properties (i.e. Young’s modulus and tensile strength) vs. flexural properties (i.e. flexural modulus and flexural strength). Theoretically, if the effects of geometry were fully accounted for using the current ASTM standards, then tension and bending tests should have yielded the same elastic modulus and ultimate strength. However, this was not the case. The reason was that flexural properties of composite materials depend on the stacking-sequences of each lamina, whereas the tensile properties of such materials are independent of stacking-sequence [144, 145]. In other words, the superior flexural properties compared to tensile properties of the current CF/Flax/Epoxy composite were caused by the “sandwich structure effect”, i.e. two strong facesheets of

CF/Epoxy layer were attached to the flexible core material of Flax/Epoxy. The composite plate had a CF/Epoxy outer layer, which was much stiffer than the core Flax/Epoxy. It was this outer layer that mainly experienced tension and compression during bending tests, whereas all the inner and outer layers experienced approximately the same stress load during uniaxial tension tests. Therefore, composite materials with a sandwich structure may be an ideal candidate for manufacturing long bone plates, as they can simultaneously demonstrate lower axial stiffness and adequate bending stiffness.

Table 5.1. Mechanical properties of the current CF/Flax/Epoxy material, human cortical bone, carbon fiber composites, flax fiber composites, and hybrid composite reinforced by natural and carbon fibers, where E_L is Young's modulus, E_f is flexural modulus, σ^l_U is ultimate tensile strength, and σ^f_U is ultimate flexural strength.

Materials	E^L (GPa)	E^f (GPa)	σ^l_U (MPa)	σ^f_U (MPa)	Ref.
Current CF/Flax/Epoxy	41.7±1.6	57.4±1.8	399.8±8.2	510.6± 66.3	-
Human Cortical Bone	7-25	-	50-150	-	[93]
Sisal/CF/polyester	1.99-2.78	6.52-11.33	84.44-107.51	140.89-169.14	[40]
CF/Flax/Epoxy	5.09-6.48	14.41-23.84	172.4-288.3	85-160.42	[39]
Flax/Epoxy	20	-	200	-	[186]
UD CF/Epoxy	50-300	-	-	-	[187]
2D CF/Epoxy	20-30	-	-	-	[187]
3D CF/Epoxy	10-20	-	-	-	[187]
Flax/Epoxy	20-30	-	-	-	[188]
Flax/Epoxy	12.98	-	148.1	-	[189]

The current CF/Flax/Epoxy composite material showed brittle catastrophic failure, in which stress abruptly dropped from a peak value, in both tension and flexural tests (Figure 5.3 and 5.6). These types of fractures showed that the CF/Flax/Epoxy composite is a brittle material with no plastic deformation prior to failure. This may be further explained due to the brittle nature of thermosetting epoxy resin and unidirectional fiber orientation [35]. Also consistent with brittle behavior, the current SEM images showed fiber breakage and fiber pull-out at the fractured surfaces with some matrix residue on the fiber surfaces (rough fiber surfaces), indicating perfect bonding between fibers and matrix (Figure 5.5 and 5.8). As the current CF/Flax/epoxy showed brittle behavior, it may have a high notch sensitivity which is usually associated with brittle materials. Therefore, the presence of a surface inhomogeneity such as a notch, a sudden change in section, a scratch or a crack may increase the sensitivity of the material to failure.

5.4.4. Addressing Potential Limitations

Firstly, only one type of CF/Flax/Epoxy structure was used. For future comprehensive studies on this material, it would be necessary to determine the effect on mechanical properties of other variables, such as fiber volume fraction, fiber orientation, lay-up direction, and manufacturing process [186-189]. However, to obtain the present material's maximum tensile and bending properties, its unidirectional fiber orientation was adequate. In order to improve the properties of the proposed plate for transverse loading from screws used to secure it, the composite plate structure should be optimized by including 90° layers in its lay-up.

Secondly, all tests were performed under quasi-static loading conditions to determine the basic mechanical properties of the composite. For future studies, it is recommended to apply cyclic loading with a large number of cycles, in both tension and bending tests, in order to assess the fatigue resistance of the developed composite material under fatigue loading. This would simulate a more clinically-realistic loading regime, for instance, in the case of femur fracture repaired with a plate [123, 158].

Thirdly, this study only evaluated the mechanical properties of the composite material in tension and flexural loading conditions. The performance of the material under shear and compression loading needs to be further investigated, as these stresses may be the main type of stress applied to the composite material for some specific applications. For instance, in the case

of femur fracture plating, clinical loading regimes typical in biomechanical studies include axial compression, lateral bending, and torsion, which could induce some degree of shear and compression [123, 158, 159]. Moreover, all tests were performed on solid composite plates without considering the effect of drilled holes. For future comprehensive studies, the effect of drilled/molded holes of the properties of the composite plate should be considered.

Fourthly, the square dimension of the specimens for Rockwell hardness tests was chosen based on the ASTM recommended dimensions for Rockwell hardness test of plastic materials. The reason was that there is no current ASTM standard for Rockwell hardness testing specifically for polymer-based composite materials. Consequently, no known correlations exist of polymer composite hardness values versus ultimate tensile strength and/or Young's modulus. Future researchers may wish to consider doing tests on a series of composite materials to obtain such a correlation.

Fifthly, it could be argued that viscoelastic effects may have influenced Rockwell hardness results, since tests were accomplished over a 10 sec timeframe from initial indentation to data recording. However, this effect was not expected to be substantial because the composite was a thermosetting polymer (i.e. thermoset) that was tested at ambient room temperature, whereas viscoelasticity is often more of a concern for a thermosoftening polymer (i.e. thermoplastic) at higher temperatures [190, 191]. Moreover, the stress vs. strain graphs for this composite (Figure 5.3) displayed purely linear elastic, rather than curved viscoelastic, behavior.

Finally, a limited number of samples, i.e. 6 samples, were used for tension and bending tests. This choice was due to the limited amount of composite material available to complete the series of studies planned in an ongoing project to develop a composite long bone fracture plate. Even so, mechanical testing yielded largely consistent results with coefficients of variations (i.e. SD / Mean) smaller than 10% for 5 out of 6 measured properties. Also, the post hoc power analyses showed values well above 80%, indicating that there were enough specimens per test group to detect all statistical differences between results from tension vs. bending tests.

5.5. Summary

The chapter confirms that the developed composite material has a considerably high failure stress to carry clinical-type forces as experienced in bending or axial compression during daily normal activities. Furthermore, the composite material demonstrated higher mechanical properties in bending compared to tension, which make the CF/Flax/Epoxy composite material attractive for bone fracture plate applications.

CHAPTER 6: Biomechanical Fatigue Analysis of CF/Flax/Epoxy Plate using Conventional Fatigue Tests and Thermography.

6.1. Introduction

The purpose of this chapter was to determine the fatigue behavior of the developed CF/Flax/Epoxy composite with both conventional experimental fatigue tests and infrared thermography analysis. It was hypothesized that the composite plate would have high cycle fatigue strength (HCFS) that is much higher than the main loads experienced by a femur bone fracture plate during normal daily activities, such as running and walking.

6.2. Materials and Methods

6.2.1. General Approach

Fatigue tests were performed on CF/Flax/epoxy composite plates to determine the fatigue strength using both the conventional S-N (Stress-Number of Cycles) approach and thermography analysis. The purpose was to evaluate the bonding strength at the Flax/epoxy and CF/epoxy interfaces, as well as the fatigue strength of the composite plate under clinical-type cyclic loading conditions. The application of thermography analysis has been proven previously as a reliable non-destructive technique for quick determination of the fatigue strength for metals [147-150]. However, to date, only a few studies have used this technique for composite materials, and no one has considered the thermographic approach to determine the fatigue life of composite materials for medical application [135, 151]. It should be noted that composite materials, unlike most metallic materials, do not have a clear fatigue limit or endurance limit. Thus, the concept of high cycle fatigue strength (HCFS) in composite materials conveys the same meaning as the fatigue limit in metallic materials, but while also considering the damage accumulation process during the entire fatigue life of the composite material [135, 151].

6.2.2. Composite Materials Structure.

Sixteen layers of unidirectional Flax/Epoxy prepreg sheets (Lineo NV, Dendermonde, Belgium) with a density of 1.3 g/cm³ and a volume fraction of 58-60%, and two layers of unidirectional CF/Epoxy prepreg sheets (T700/GC/M21, Hexcel Composites, Stamford, CT, USA) with a density of 1.56 g/cm³ and a volume fraction of 57%, were used to manufacture the

current CF/Flax/Epoxy composite.. The CF/Epoxy prepregs are composed of carbon fibers treated with epoxy-type sizing agent that promotes the covalent bonding between the fibers and the epoxy matrix [152]. The Flax/Epoxy and CF/Epoxy laminae, with [0] unidirectional ply orientation, were placed into an in-house custom-made stainless-steel mold in which CF/epoxy laminae were added to each of the outer surfaces, resulting in a “sandwich structure”. The combination of two types of natural and synthetic fibers (i.e. Flax and Carbon) for the fabrication of the current composite, as well as its “sandwich structure”, make this composite unique compared to what has been done previously for orthopaedic bone plate applications [10, 13, 19, 21-23, 26, 28]. Then, the mold was heat-pressed in a compression-molding machine (Carver Press, Wabash, IN, USA) for 1 hour at 150 °C and 500 kPa. The resulting composite sheet had a length of 260 mm, a width of 250 mm, and a thickness of 3.6 mm.

6.2.3. Conventional Fatigue Tests.

The test setup for conventional fatigue experiments is given (Figure 6.1).

Figure 6.1. Experimental setup of the plates under cyclic loading for conventional fatigue tests and thermographic analysis.

The test plates were cut individually from the above composite sheet in a rectangular-beam shape using a diamond wheel with a length of 250 mm and a width of 15 mm, according to recommended dimensions from ASTM standards for tension-tension fatigue of polymer matrix composite materials [153]. All tests were performed on a servo-hydraulic materials testing system (Model 322.21, MTS System Corporation, Eden Prairie, MN USA), with 100 kN load cell capacity. The plates were mounted in the grips (Model 2743-402, Illinois Tool Works Inc (ITW), Norwood, MA, USA) using a 50 pound.inch (i.e. 5.6 N.m) torque with its long axis in-line with the force direction, in which 45 mm of the plates at each end were gripped. An extensometer (Model 634.12F-24, MTS System Corporation, Eden Prairie, MN, USA) was placed at the center of the plate to record strains. Five loading levels ranging from 0.5-0.8 of UTS (ultimate tensile strength) were used, and four plates were tested at each load level. All fatigue tests were conducted under load control at ambient room temperature (23 °C) with a constant sinusoidal wave form at a frequency of 5 Hz and a loading ratio of $R = 0.1$ ($R = S_{\min} / S_{\max}$), where S is the applied load. Tests were stopped either at plate failure or at 6×10^6 cycles in order to produce the S-N curve.

6.2.4. Thermography Tests.

The thermography technique was used to more rapidly determine the fatigue strength because the conventional fatigue tests are time consuming and also require a large number of specimens thus could be very expensive [135, 147-150]. Also, the thermographic approach is a non-destructive technique which works based on thermoelastic stress measurements caused by small temperature variations (on the order of 0.001 °C) when a material is in its elastic region and can be used for quick determination of fatigue strength [135, 147-150]. The same plate dimensions, mechanical testing machine, and loading regime were used as described above. Starting from 30% of UTS as the applied stress amplitude, the plate was subjected to cyclic loading for 7000 cycles, which is enough to reach the stabilization temperature [135]. The applied stress amplitude was incremented stepwise, and the plate was retested for another 7000 cycles. This procedure was repeated until the plate failed. The temperature variations were recorded at each applied stress amplitude using an infrared thermography camera (Silver 420, FLIR Systems Canada, Burlington, ON, Canada). The camera had a spatial resolution of 320 pixels X 240 pixels and a temperature resolution of 20 m°K. The camera was synchronized to the controller of

the mechanical test machine in order to trigger image recording at the highest stress level in each loading step and to eliminate the effect of any temperature fluctuations due to thermoelastic heating [135].

6.2.5. SEM Tests.

The cross-sectional areas of three fatigue-tested plates were inspected using scanning electron microscopy (SEM) (JEOL, JSM-6380, Tokyo, Japan) to evaluate the damage progress during fatigue loading. Pre-failure and post-failure microscopic structures were analyzed using 3 specimens, which had been loaded for 1/3, 2/3, and the entire fatigue life at 70% of UTS. Also, the cross-sectional area of the plate loaded at 60% of UTS was monitored to evaluate the microstructural defects that occurred after 2 million fatigue cycles. A fatigue life equal to 1 million cycles at high stress cyclic loading is considered to be a safe level for bone fracture plate applications [154]. After the sectioning process close to the center of the plates, the samples were then embedded in an epoxy resin (LECOSET 7007, Leco Corporation, St Joseph, MI, USA) for mounting purposes and then polished. Prior to the SEM tests, all samples were coated with a thin layer of gold to make the surface conductive. The crack density at the cross-sectional area of all inspected samples was calculated using ImageJ software (National Institute of Health, Manhattan, NY, USA).

6.3. Results

6.3.1. Conventional Fatigue Tests.

The S-N curve was generated using fatigue life values vs. the applied stress amplitude (Figure 6.2). The raw stress-vs-strain behavior of the composite for a typical test (70% of UTS) is given (Figure 6.3). The plot is linear in log-scale with a gradual downward slope with an increasing number of cycles. The current composite does not have a true fatigue limit, since the S-N curve does not become horizontal. Thus, the fatigue properties were characterized by considering fatigue strength at an arbitrary number of cycles (HCFS), i.e. 6×10^6 , which was approximately found to be in the range of 50-55% of UTS.

No failure was observed until 6×10^6 cycles in those plates loaded at an applied stress amplitude smaller than HCFS. The slope of the linear region in the stress vs. strain curve at each cycle was

used to calculate the dynamic modulus (E^*), which was then plotted vs. normalized number of cycles to failure (Figure 6.4). E^* stayed almost constant with the number of cycles and remained close to its initial value (Region A in Figure 6.2 and Figure 6.3) up until failure, where it drastically decreased (Region C, Figure 6.2 and Figure 6.3).

Figure 6.2. S-N curve for the CF/Flax/Epoxy composite. Load Ratio $R=0.1$, frequency=5 Hz, and UTS=400 MPa.

6.3.2. Thermography Tests.

For each stress level, the corresponding temperature variations recorded with the thermographic camera as a function of the number of cycles is shown (Figure 6.5). The line of best fit curve was found to be exponential at all applied stress amplitude. At each loading step, the same location on the plate was imaged, which was where the ultimate failure took place. The

temperature distribution map at each applied stress, after the temperature stabilized, is shown (Figure 6.6). The temperature distribution at the surface of the plates was uniform until 50% of UTS. However, for stress amplitudes above 50% of UTS, the temperature was no longer uniform across the surface with some obvious spots where the temperature was considerably higher, i.e. hot zones. The temperature rise versus the applied stress amplitude is also plotted using each matching stabilization temperature (Figure 6.7). The data had bilinear behavior, i.e. the slope of temperature rise increased clearly at the specific stress amplitude. The fatigue strength was determined by intersecting the two linear regions of the graph, which was found to be approximately 51% of UTS (Figure 6.7).

Figure 6.3. Typical stress (σ) versus strain (ϵ) curve for one plate loaded at 70% of UTS. Black (A), blue (B), and red lines (C), in turn, stand for constant, increasing, and decreasing value of E^* .

Figure 6.4. Dynamic elastic modulus E^* versus normalized number of cycles (N/N_f). N = number of cycles. N_f = number of cycles at failure. Black, red, blue, and green lines represent the dynamic modulus E^* at 55%, 60%, 70% and 80% of UTS, respectively. A, B, and C stand for constant, increasing, and decreasing value of E^* , respectively.

6.3.3. SEM Tests.

The SEM images for the core regions of plate samples loaded at 70% of UTS for 1/3, 2/3, and the entire number of fatigue life cycles are given (Figure 6.8). At 1/3 of N_f , the crack initiated inside the bundle of flax fibers (Region 1, Figure 6.8.a) when the pectin failed causing delamination between the adjacent flax fibers (Region 2, Figure 6.8.b). At 2/3 N_f , the initiated crack inside the flax fiber bundles propagated between the bundles (Region 3, Figure 6.8.b) with some degree of bundle/matrix debonding (Region 4, Figure 6.8.b). At failure, the degree of bundle/matrix debonding became more evident with both microcrack and deep crack formation at the bundle/matrix interface (Region 5 and 6, Figure 6.8.c).

Figure 6.5. Thermal variations versus number of cycles for maximum applied stress. R^2 = coefficient of determination for the line of best fit; y_0 = room temperature; A_1 , A_2 , t_1 , and t_2 are empirical constants specific to each curve.

Figure 6.6. Thermographic temperature distribution map after temperature stabilization. The center portion of the test plate is shown. UTS = ultimate tensile strength.

Figure 6.7. Thermographic results showing average stabilized temperature versus the maximum applied stress. R^2 = coefficient of determination for the straight line of best fit.

The interfaces of Flax/Epoxy and CF/Epoxy laminae loaded at 70% of UTS for 1/3, 2/3, and the entire fatigue life are depicted (Figure 6.8.d, Figure 6.8.e, and Figure 6.8.f). The CF/epoxy and Flax/epoxy laminae interfaces showed solid bonding until failure (Region 7, Figure 6.8.d and Figure 6.8.e). However, the failed plate showed some degree of debonding at this interface (Region 8, Figure 6.8.f). The SEM images for the plates loaded at 60% of UTS for 2 million fatigue cycles are given (Figure 6.9). There was some crack initiation and propagation inside the flax fiber bundles (Region 1, Figure 6.9.a) with solid bonding at the CF/Epoxy and Flax/Epoxy laminae interfaces (Region 2, Figure 6.9.b). The calculated crack density using Image software for SEM images are summarized (Table 6.1).

Figure 6.8. SEM images of the cross-sectional area of the composite plate samples showing formation of microcracks at 70% of UTS for different damage levels. (a) core region at $1/3 N_f$, (b) core region at $2/3 N_f$, (c) core region at N_f , (d) CF/Epoxy and Flax/Epoxy interfaces at $1/3 N_f$, (e) CF/epoxy and Flax/epoxy laminae' interface at $2/3 N_f$, (f) CF/Epoxy and Flax/epoxy interfaces at N_f . N_f = number of cycles at failure.

Crack density was low at the beginning of cyclic loading because at this stage the crack nucleation took place. Once the cracks initiated, they would propagate quite rapidly, which caused a considerable rise in the crack density value at the termination of cyclic loading. The crack intensity increases clearly in 3 stages as usually described for composite materials. A steep increase of the crack intensity occurred in the early stages of the fatigue life, followed by another steady increase which remained for a relatively long number of cycles. Acceleration in damage took place at the final stages of the fatigue life. This indicated that the current CF/Flax/Epoxy composite would have a stable cyclic performance.

Figure 6.9. SEM images at 60% of UTS at 2 million fatigue cycles. (a) core region, (b) CF/epoxy and Flax/epoxy interface.

Table 6.1. Crack density for the current composite. UTS = ultimate tensile strength. N_f = number of cycles at failure.

% UTS	Number of Cycles	% Of crack
70	$1/3 N_f$	0.54 ± 0.15
	$2/3 N_f$	1.66 ± 0.03
	N_f	2.20 ± 0.53
60	2 million	0.48 ± 0.03

6.4. Discussion

6.4.1. General Findings.

This is the first study which assessed the fatigue properties of this new CF/Flax/Epoxy composite material using conventional fatigue tests and thermography analysis. Based on the current preliminary results, the composite showed promising fatigue properties that make it a potential candidate for orthopaedic applications as a long bone fracture plate where cyclic loading would be experienced during normal daily activities. Also, it retained its mechanical stiffness during its entire fatigue life. SEM images showed solid bonding at Flax/Epoxy and CF/epoxy laminae up until failure. Finally, the fatigue strength obtained using thermography was in accordance with the HCFS obtained using conventional fatigue test methods, suggesting that the thermography technique may be successfully used for rapid determination of HCFS for composite biomaterials.

6.4.2. Comparison to Prior Studies.

Current data are compared to the results of prior studies on the fatigue properties of carbon fiber-based composites, natural fiber-based composites, and human femoral cortical bone (Table 6.2) [13, 108, 135, 192-194]. Present results are in the range of some reported prior results for carbon fiber-based and natural fiber-based composites, but not for those addressing the same applications. Any differences can be explained by the different composite structures which used various fiber orientations, various fiber/matrix volume fractions, diverse manufacturing processes, and dissimilar tests methods. The current composite showed a HCFS (200-220 MPa) much above the actual loads experienced by a human femur during normal daily activities with a fatigue life of more than 6 million cycles below its HCFS.

6.4.3. Practical Implications.

The current CF/Flax/Epoxy composite showed a fatigue life of more than 1 million cycles when it was loaded at 60% of UTS (i.e. 240 MPa). Considering that a person walks an average of 1 step every second for only several hours per day, it is reasonable to estimate that an average patient with an orthopaedic implant would experience 1 million cycles annually [7, 154]. According to ASTM F382 for evaluating bone plate fatigue strength, 1 million cycles seems to

be conservative, as no bone plate is supposed to withstand more than 1 million high stress fatigue cycles in clinical conditions [154].

Table 6.2. Fatigue properties of the current CF/Flax/Epoxy material, human femur cortical bone, carbon fiber composite, and flax fiber composites from prior studies.

Materials	Testing Condition	Applied Stress Amplitude	Changes in Stiffness	Type of Loading	Final Number of Cycles (N_f)
Current CF/Flax/epoxy	Ambient atmosphere	50-80% UTS	0%	Tension	3×10^4 - 6×10^6
Human femur cortical bone[58]	Physiological condition	40-80 MPa	-	Tension	10 - 10^6
		60-120 MPa	-	Compression	100 - 10^6
		15-30 MPa	-	Shear	10 - 10^6
CF-PEEK[65]	Room Temperature	>60% UTS	-	Bending	10^6
Compact Bovine Bone[194]	37 °C	65-108 MPa	20% loss	Tension	-
CF/epoxy[13]	-	6 Nm	-	Bending	10^6
CF/polyamide[135]	Room Temperature	50-80% UTS	-	Tension	2000 - 6×10^6
Flax/epoxy[192]	23°C	40-80% UTS	2% rise	Tension	2058-1151224

Additionally, the applied loading magnitude equal to 60% of UTS (approximately 13 kN) is far above clinical-type loads on the human femur during normal daily activities which are 2.5 to 3 times body weight [2, 3]. The fatigue strength of the current composite was in a range of 50-55%

UTS (200-220 MPa), which is comparable to 316L stainless steel materials that are clinically used (i.e. around 200 MPa and 300 MPa for cast and forged 316L stainless steel, respectively)[8]. The dynamic modulus E^* obtained for the current CF/Flax/Epoxy composite was almost constant versus the number of cycles and stayed around its initial value. For most other composite materials (like CF/epoxy lamina and glass/epoxy), E^* usually decreases with the number of cycles [135, 192, 195]. This can be explained by the hardening behavior of Flax/Epoxy laminae caused by progressive reorientation of the cellulose microfibrils towards the loading direction. The contribution of both Flax/Epoxy and CF/Epoxy laminae of the composite during the fatigue loading helped to maintain an almost constant level of stiffness up until failure. The effect of Flax/Epoxy laminae on the dynamic modulus of the CF/Flax/Epoxy composite became more dominant at the termination of the cycles (Region B, Figure 6.3 and Figure 6.4). Such a hardening effect was reported previously on the fatigue behavior of flax/epoxy composites [192].

The current composite stiffness remained constant around its initial value during its entire fatigue life, without any stiffness decrease. This is important for estimating the fatigue life of a composite material at a given service load, since it is not sufficient to only consider the number of cycles that the material can survive without failure. Changes in stiffness during fatigue cycling may indicate substantial fatigue damage, even though the polymer matrix composite may appear to be structurally intact overall. Consequently, one of the main reported complications of using composite biomaterials is the gradual loss of mechanical properties, i.e. stiffness, during cyclic loading caused by fatigue damage [13-16]. This eventually generates wear debris which necessitates an immunological response via blood clotting, macrophages, and leukocytes [13-16]. Moreover, the calculated percentage for crack density of the plate loaded at 60% of UTS after 2 million cycles was found to be only 0.48%. This suggests that the current composite may be a potential candidate for fabrication of a long bone fracture plate because it can retain its stiffness for more than 1 million cycles at 60% of UTS with negligible fatigue damage, which is enough time for complete union of fractured bone thus bone would act as load bearing component. Consequently, this would reduce the stress applied to the plate. However, in most cases, bone fracture plates stay in the body permanently and are only removed if there is breakage, infection, pain, soft tissue damage, or fracture non-union [196-198]. Therefore, future

studies may wish to investigate the low stress fatigue behavior of this composite for very high number of cycles considering its long term durability and debris formation. This composite's "sandwich structure" has previously shown similar static properties in tension and flexion compared to human femoral cortical bone, thus minimizing stress shielding [33].

The temperature rise versus applied stress had bilinear behavior (Figure 6.7). This can be explained by microscopic changes in the structure of the plate during fatigue loading. Although the plate was loaded in the elastic region, there may have been some stress fluctuation at the microscopic level in the vicinity of defects. This may eventually cause matrix deformation, debonding at matrix and fiber interfaces, and fiber breakage. When a material is subjected to cyclic loading, this microscopic phenomenon can develop and finally cause heat emanation, which can cause temperature rise in a material [135, 199]. Below the fatigue strength of a composite material, the temperature rise caused by heat dissipation has a constant slope. However, when a material reaches its fatigue strength, the temperature rises significantly since the microscopic phenomenon could be accelerated in both initiation and growth. Additionally, current thermographic analysis has been shown to be a successful tool for the quick determination of fatigue strength of a composite biomaterial. The HCFS from thermographic analysis was determined by intersecting two linear regions [135, 147-150, 199] and was in a good accordance with the range obtained for the HCFS using the conventional test methods.

The composite material examined can be considered a brittle material. Several current results confirmed this observation. The fracture line in the failed plates was perpendicular to the fiber orientation, which is a classic criterion for differentiating between brittle and ductile materials [200]. In addition, the stress vs. strain graphs for displayed purely linear elastic behavior with no plastic deformation prior to failure, in which stress abruptly decreased from a peak value (Figure 6.3). This can be related to the brittle nature of the thermosetting epoxy resin and the unidirectional fiber orientation [39].

6.4.4. Addressing Potential Limitations.

First, the main limitation of the current study is that the fatigue properties of the CF/Flax/Epoxy composite were only studied in an ambient air environment. The interaction of body environment with composite materials, due to their non-corrosiveness, would not have a

chemical mechanism to cause corrosion-fatigue. This becomes problematic when using metallic biomaterials, since chloride ions are the main components of body fluids and accelerate fatigue crack propagation by shifting the electrochemical potential applied to the specimen[7, 201-203]. Yet, long-term exposure of polymeric-based composite plates to humid atmosphere may be detrimental to their mechanical behavior. Thus, for future comprehensive studies on the biomedical application of this material, it is recommended to consider the effect of physiological fluids (e.g. blood, synovial fluid, water, etc) to simulate a more clinically-realistic environment. For example, a future study could involve soaking the composite plate in a bath of blood and/or synovial fluid for different timeframes (baseline, 3 months, 6 months, etc), then cleaning it of residue, and finally immersing the entire plated femur into a water tank for thermographic stress imaging [204]. However, the current thermographic system could not be used to image the composite plate if it was covered in blood or synovial fluid because this would introduce surface roughness elements that would create image artifacts [124, 133].

Second, only one type of composite structure with unidirectional fiber orientation was used. The effect of other variables could be evaluated for this composite material, such as fiber orientation, lay-up direction, manufacturing process, and fiber volume fraction [95, 186, 188]. In addition to the inherent material properties of the composite plate (e.g. Young's modulus, ultimate tensile strength, etc.), in a clinical context the inherent design parameters of the bone plate can also influence its mechanical performance regarding overall femur-plate construct stiffness and strength. This would include factors such as the total number of screw holes in the plate, the relative distance and position (i.e. in-line or staggered) of the plate's screw holes, the total number of screws used by the surgeon (which may or may not equal the total number of screw holes available), and the final shape of the plate in order to match the femur geometry which is achieved using a plate bender by surgeons.

Third, all tests were performed under tension-tension conditions. It would also be necessary eventually to determine the fatigue properties of this material under cyclic bending, cyclic compression, and cyclic torsion tests with a large number of cycles which may occur clinically [122, 158, 159]. However, during the extremely common activity of walking, the lateral side of the femoral shaft on which fracture repair plates are placed, mainly experience bending resulting in tensile stress [127]. Thus the current test methodology was a good approximation.

Fourth, it could be argued that the bonding mechanism between bone and the composite plate may have an effect on the load sharing phenomenon for long term stability in case the plate has to stay in body permanently. Although the initial connection between the plate and bone would be achieved using screws and cables, there are other potential ways for bonding improvement like treating the surface of the composite plate by coating it with hydroxyapatite (HA) [9] or bioactive glass granules [94]. Moreover, the inherent surface texture of the current composite material, i.e. roughness, may also allow for better cell attachment and could facilitate any additional coating processes using HA or glass granules.

Finally, although it is beyond the present scope, a future study could involve manufacturing a flax-only plate and/or CF-only plate for comparison to the current CF/Flax/Epoxy and 316L steel plates. In part because of the unique sandwich structure of the current composite plate, it is anticipated that flax-only and CF-only plates would have less optimal mechanical properties than the current CF/Flax/Epoxy plate when compared to human bone.

6.5. Summary

The fatigue strength of the developed composite plate was found to be high enough to carry clinical-type cyclic forces that occur in normal daily activities. The developed composite retained its mechanical stiffness up until failure, which makes it appropriate for applications that require specific mechanical properties in a given period of time, i.e. healing for orthopaedic fixation applications. Moreover, thermographic analysis has been shown to be a reliable tool for the quick determination of the fatigue limit of composite biomaterials.

CHAPTER 7: Biomechanics of the CF/Flax/Epoxy Plate versus a Traditional Metal Plate for Vancouver B1 Femur Fractures: An Experimental and Computational Study

7.1. Introduction

As the proposed composite plate is supposed to fix bone fracture eventually, the aim of the current chapter was to determine the biomechanical performance of the current composite used to fix Vancouver B1 fracture in comparison with traditional clinically-used metallic bone plate. Since the current CF/Flax/Epoxy plate has adequate bending stiffness with lower axial stiffness [27], it was hypothesized that it would provide comparable mechanical fixation, yet lower “stress-shielding” effect compared to that of a clinically-used metallic bone plate.

7.2. Materials and Methods

7.2.1. General Approach.

Six artificial femurs were cyclically loaded in axial compression and monitored using an infrared thermography camera. Finite element analysis (FEA) was then done to mimic the experiments, but under static conditions. Experiments and FEA were performed for 4 different stages: Stage 1 (intact femur); Stage 2 (after THR); Stage 3 (after plate fixation of a simulated Vancouver B1 femoral midshaft fracture gap); and Stage 4 (after fracture gap healing). For fracture fixation, one group was fitted with the CF/Flax/Epoxy composite plate, while the other group was repaired with a traditional metal plate. Stiffnesses and stresses of the constructs were compared to evaluate plate performance.

7.2.2. Experimental Analysis

Plate Specifications.

A composite plate and metal plate were used (Figure 7.1). The composite plate was made from pre-impregnated 16 sheets of Flax/Epoxy (Lineo NV, Dendermonde, Belgium) with a density of 1.3 g/cm^3 and a volume fraction of 58-60%, as well as two layers of unidirectional CF/Epoxy prepreg sheets (Hexcel Composites, Stamford, CT, USA) with a density of 1.56 g/cm^3 and a volume fraction of 57%.

Figure 7.1. Photographs of composite plate and metal plate on the lateral surface of the femur showing proximal fixation with 4 unicortical screws and distal fixation with 4 bicortical screws.

The composite was manufactured using a 5 bar (500 kPa) pressure and a 150 deg temperature with a compression-molding technique, in which CF/Epoxy laminae were added to its outer surface resulting in a “sandwich structure”. Plates were cut from the final composite sheet using a diamond wheel with a length of 246 mm and a width of 16.8 mm. None of the other studies producing composite materials for orthopaedic bone fracture plate applications used the combination of natural and synthetic fibers with a “sandwich structure” [10, 13, 20, 107, 118, 143, 155-157]. Thus, the current composite material is unique and novel for its “sandwich structure” combining 2 types of fibers [33]. This composite plate was already black in color so no further dying stage was required for perfect infrared emission during thermography testing. The metal plate was an 8-hole non-locking cable plate (Zimmer, Warsaw, IN, USA) with a length of 246-mm. The application of this metal plate is well documented in the literature for femur fracture treatments in biomechanical investigations and clinical settings [122, 127, 158, 159]. To boost the infrared emission during infrared themography testing the plate, which was originally gray in the color, was painted in black.

Femur Properties.

Six large left adult artificial femora (Model 3406, Sawbones, Vashon, WA, USA) were used in this study, which were used effectively in a variety of biomechanical studies [122, 131, 133, 136, 158-160]. To obtain the maximum infrared emission from the femur, the femur were injected with a black dye called 6836 Black (BJB Enterprises Inc, Tustin, CA, USA) [161]. The artificial femurs consist of a composite of glass fibers and epoxy resin to simulate “cortical” bone (density=1.64 g/cm³) which covered a polyurethane foam core that simulated “cancellous” bone (density = 0.20 g/cm³). The artificial intact femurs had a length of 485-mm, an intramedullary canal diameter of 16-mm, and a medio-lateral midshaft diameter of 32-mm. The densities of the artificial cortical and cancellous material overlapped with densities of cadaveric human femur cortical and cancellous bone (human cortical density = 1.6 to 2.1 g/cm³, human cancellous density = 0.1 to 1.0 g/cm³) [2-4]. Likewise, the moduli of elasticity of the artificial femur (cortical, E = 16.7 GPa; cancellous, E = 0.155 GPa) were in the range of those of human femurs (cortical, E = 11.4 to 19.7 GPa; cancellous, E = 15 to 200 MPa) [2-4].

Specimen Preparation.

Stage 1 was an intact femur alone. Femoral condyles were augmented using a band saw on the lateral and medial sides and placed vertically into hollow steel cubes (88 mm wide X 88 mm deep X 75 mm high) with their long axis aligned in the frontal and sagittal planes. Anchoring cement (Flow-stone, King Packaged Materials Company, Burlington, ON, Canada) was used to fill the steel cubes.

Stage 2 was an intact femur with a THR. Femurs were assigned to 2 statistically equivalent groups of 3 femurs based on Stage 1 mechanical test results. Each femur received a THR, which was an Exeter metal hip implant with a 28-mm diameter cobalt chrome molybdenum ball (Stryker, Mahwah, NJ, USA). Each THR stem were inserted into the medullary canal and then secured with Simplex P polymethyl methacrylate bone cement (Stryker, Mahwah, NJ, USA).

Stage 3 replicated a post-operative Vancouver B1 comminuted fracture. This was done by transversely osteotomizing. Stage 2 specimens with a 5-mm gap just below the THR cement mantle at 200 mm above the cement potting cube. The femurs in one group then received the

composite plate, whereas the femora in the other group received the metal plate, on their lateral surfaces. Four unicortical and 4 bicortical screws were used to secure the plates proximally and distally, respectively. The screws were made of 316L medical grade stainless steel (Synthes, Paoli, PA, USA), had a diameter of 4.5-mm, and were tightened using a torque wrench to 3.38 Nm (30 lb.in).

Stage 4 mimicked a fully healed Vancouver B1 fracture, which was based on Stage 3 specimens prior to the creation of the fracture gap. Chronologically in this investigation, Stage 4 specimens had to be prepared and mechanically tested before Stage 3. Although the “real world” clinical scenario demands plate repair following fracture, this reversal in the present order allowed optimal fracture segment alignment, anatomic reduction, and fracture plate application for Stage 3 and 4 specimens. All specimens had an overall working length of 410 mm from the top of the femoral head (or ball) to the top of the cement potting cube.

Loading Regime.

All tests were executed on an 800 LE machine (TestResources, Shakopee, MN, USA) (Figure 7.2). The specimens were secured distally by an industrial swivel vice and were orientated at 15 deg of adduction to simulate anatomical single-legged stance phase of walking [3, 125]. Proximally, specimens contacted a smooth steel cup in which the femur head was able to freely rotate to mimic the natural frictionless hip articulation. After application of 100 N vertical compressive preload to the femoral head to eliminate any “mechanical slack”, specimens were then cyclically loaded in axial compression at an average load of 750 N (min = 350 N, max = 1150 N) with a frequency of 5 Hz using a sinusoidal waveform for 1000 cycles. The sub-clinical load for this study prevented any possible damage to the specimen during cyclic testing [121, 127, 133, 136]. The dynamic stiffness of each specimen was the slope of its force vs. displacement curve. Stage 1 intact femora were tested in order to obtain baseline values for axial compression stiffness, so that 2 statistically equivalent groups could be created for Stage 2 to 4 testing. Each test was repeated 3 times to obtain an average value of stiffness and surface stress for each specimen.

Figure 7.2. The experimental set up, at anterior view, for cyclic loading tests for femurs in 15 deg of adduction.

Thermographic Imaging.

The thermography camera used was a Silver 420 (FLIR Systems Canada, Burlington, ON, Canada) (Figure 7.3), with an image resolution of 1 MPa, an image size of 320 x 256 pixels, a built-in auto focusing 27 mm lens, and frame rate of 5 to 170 Hz. Previous studies validated its application for measuring surface stresses by linear and rosette strain gages [133]. The camera was used to image the proximal portion of the femur instead of the entire femur length because of the proximal region's proneness to clinical neck failure, as well as significance for hip arthroplasty [125, 127, 131, 133]. Stage 1 and 2 specimens were imaged only anteriorly to provide baseline values, while Stage 3 and 4 specimens were imaged anteriorly, laterally, and medially for a more comprehensive assessment of the effect of the composite vs. metal plate on “stress shielding” [89, 162]. The temperature images were captured after 1000 cycles to ensure

reaching adiabatic conditions where the temperature change is stabilized. Also, the loading frequency should exceed 3 Hz to maintain steady state conditions [163].

Figure 7.3. Photograph of experimental setup used for infrared thermographic imaging.

For the best quality images, the image capturing frequency was synchronized with the loading frequency at 5 Hz. Using dedicated camera software called Altair-Li (Cedip Infrared Systems, Croissy-Beaubourg, France), the acquired temperature changes on the surface of the femurs were converted to stress changes using the following thermo-elastic equation:

$$\Delta\sigma = \frac{\Delta T}{KT} \quad (2)$$

where $\Delta\sigma$ is the sum of the principle stresses, K is the thermoelastic coefficient of the material being tested, ΔT is the temperature change detected by the camera, and T is the ambient temperature. The user-inputted K values for the software were 1.16×10^{-5} / MPa for artificial

cortical bone [133, 136], 1.95×10^{-7} MPa for the composite plate [134, 136], and 3.31×10^{-6} /MPa for the metal plate [136]. Since the K-value, representing thermal conductivity of a material, is different for metal plate vs. composite plate, surface temperature as well as surface stress on these two plates would not be similar. Previous studies also applied a similar test set-up to thermographically image an artificial femur, a polymer-based composite hip implant, a metal hip implant alone, and a metal hip implant inserted into an artificial femur [124, 133, 164-167].

Statistical Analysis.

One-way analyses of variance (ANOVA) were performed on all data for stiffness and thermographic surface stresses by considering a significance level of $p < 0.05$. When ANOVA yielded $p < 0.05$, post hoc pairwise multiple comparisons were done by using either Tukey's honestly significant difference method when homogeneity of variances was maintained (i.e. equal sample size in each group) or Tamhane's T2 method when homogeneity was not maintained (i.e. unequal sample size in each group). Two-tailed post hoc power analysis was performed to find out if there were enough specimens to detect all the statistically significant differences that may have been really exist.

7.2.3. Computational Analysis

CAD Models.

For Stage 1, a CAD model of the intact femur was created using a large, left, fourth generation composite femur (Model 3406, Sawbones, Vashon, WA, USA) which had computerized tomography (CT) images obtained every 0.5 mm along the length. The intramedullary canal was then created by cutting away bone using the cut function in SolidWorks. For Stage 2, the proximal section was removed by cutting at an angle of 55 deg, with respect to horizontal axis, along the greater trochanter. A portion of the proximal cancellous bone was also removed to simulate surgically-generated bone loss for a THR. In addition, the Exeter hip stem (size 2, offset 37.5 mm) was inserted into the proximal end of the femur, and the space between the hip stem and the bone was filled by a 1 mm to 20 mm layer representing bone cement. For Stage 3, a 5-mm bone fracture gap was created using the cut function in Solid Works software, and a 246-mm

length plate with 8 screws was applied to the lateral cortex of the femur to mimic the fracture fixation construct. For Stage 4, the same CAD model of Stage 1 was used. CAD models of the femoral ball, fixation plates, and screws were generated based on the specimen geometry in the experimental trials using Vernier caliper measurements of the geometries, while the geometry of the THR stem was developed based on laser scans every 0.5 mm of its outer surface using NextEngine 3D scanner (NextEngine, Inc., Santa Monica, CA, USA).

Elements and Meshing.

Each assembly was exported to ANSYS Workbench 12.1 program for FE analysis. The contact between the assembled surfaces was automatically generated via the WorkBench “simulation” module. CONTA174 contact elements were located on a deformable surface of a 3D solid element that contacted and slid on a target surface, i.e., TARGE170 in the current study. This contact element had 8 nodes with 3 degrees-of-freedom at each node and matching geometric characteristics as the solid element surface to which it was attached. The contact types at the interfaces of THR-PMMA, PMMA-cancellous bone, cancellous bone-cortical bone, and screw-bone were set to fully bonded. However, the contact between the fracture plate and the bone was considered as a No-Separation contact. Previous studies also used similar assumptions for their FE models of femur fractures and THRs [126, 127, 168]. These assumptions are similar to the clinical condition in which bone remodels itself, which simulated complete adhesion of PMMA cement onto the THR, full bony ongrowth around the cement mantle into which the THR was implanted, full bony ongrowth and sufficient compression at the femur-plate edge, and perfect interdigitation of cortical bone around the threads of the screws. Tetrahedral elements were employed for meshing the femur and all implants. Mesh sensitivity was set to 90% mesh relevance because only 1% difference was detected between stress and strain values of a femur model meshed with 90% mesh relevance compared to those for 100% mesh relevance. “Relevance” is a Workbench mesh tool indicating the minimum (coarse mesh, 0% relevance) to maximum (very fine mesh, 100% relevance) number of elements. Based on the mesh relevance study, the number of nodes and elements was 492347 and 325865 (Stage 1), 455422 and 297380 (Stage 2), 419976 and 268433 (Stage 3), and 471700 and 305995 (Stage 4) (Figure 7.4).

Figure 7.4. FE model mesh of the synthetic femur showing Stage 1 (intact femur), Stage 2 (intact femur with total hip stem), Stage3 (fracture repair with plate), and Stage 4 (complete fracture union). The anterior view is shown.

Materials properties.

Except for composite fixation plate ($E_x = 42$ GPa, $E_y = 5.9$ GPa, $\nu_{xy} = 0.33$, $\nu_{yy} = 0.4$)[33] which was modeled as orthotropic, all other materials modeled for FEA were assumed to be linearly elastic and isotropic having known material properties for synthetic cortical bone ($E = 16.7$ GPa, $\nu = 0.26$) [127, 168], synthetic cancellous bone ($E = 0.155$ GPa, $\nu = 0.3$)[127, 168], THR ($E = 210$ GPa, $\nu = 0.31$)[168], metal fixation plate ($E = 96$ GPa, $\nu = 0.36$) [127], screws ($E = 193$ GPa, $\nu = 0.3$)[127], and the cement mantle around the THR ($E = 2.45$ GPa, $\nu = 0.38$)[127]. It should

be noted that the properties of the composite plate were considered for an intact specimen without holes. However, the presence of the drilled holes on the plates for screw insertion may change composite plate material properties.

Finite element analysis.

To avoid rigid body motion, the displacement of the femur's distal end was restrained by assigning displacement restrictions on the distal faces of the femur at a height similar to the cement cube in experiments. A static force of 750 N was applied over a surface area at the superior end of the intact femur head or the femoral ball with an angle of 15 deg with respect to the femur's longitudinal axis. Using FEA, the sum of the principal stresses was mapped to be consistent with the experiment results as the thermography camera measures the sum of principle stresses.

7.3. Results

7.3.1. Experimental Results

Average axial cyclic stiffnesses for Stage 1 to 4 are given (Figure 7.5). Neither femora nor implants experienced gross permanent damage during the testing by remaining in their linear elastic region with an average coefficient of determination of $R^2 = 0.95$. Stiffnesses of composite vs. metal plated femurs for Stage 3 (composite = 645 ± 67 N/mm, metal = 658 ± 69 N/mm, $p=1.00$) or Stage 4 (composite = 1731 ± 109 N/mm, metal = 1757 ± 39 N/mm, $p=1.00$) were not statistically different. However, composite and metal plated femurs comparing Stage 3 vs. 4 were significantly different (p for composite plated femurs < 0.01 , p for metal plated femurs < 0.01).

Typical thermographic stress maps for Stage 1 to 4 specimens are presented for the anterior view only for illustrative purposes (Figure 7.6). All specimens underwent tensile stress on the lateral shaft surface, while experiencing compressive stresses on the medial shaft surface, particularly below the lesser trochanter. For Stage 1, the 2 groups of 3 intact femora were probed at 5 points on lateral and medial surfaces, yielding statistical equivalence for stress ($p=0.99$ to 1.00) (Figure 7.7.a). For Stage 2, the 2 groups of 3 femurs each with THRs were also equivalent regarding surface stresses ($p=0.99$ to 1.00) (Figure 7.7.b). Therefore, one group of 3 femurs was

used for composite plating in Stage 3 and 4, whilst the other group of 3 femurs was used for metal plating in Stage 3 and 4, without introducing any *a priori* statistical bias.

Femur thermographic stresses were extracted from corresponding images for anterior, medial, and lateral views for statistical comparison (Figure 7.7.a, Figure 7.7.b, and Figure 7.7.c). In the anterior view, there were statistical differences in composite vs. metal plating groups for Stage 3 (60%) and Stage 4 (43%) for bone directly under the plate, i.e. level 3, 4, and 5 ($p < 0.05$). However, for lateral or medial views at any level, composite vs. metal plating groups showed no difference in bone surface stress at Stage 3 ($p = 0.07$ to 1.00) or Stage 4 with ($p = 0.05$ to 1.00).

Figure 7.5. Stiffness values for Stage 1, Stage 2, Stage 3 with composite plate, Stage 3 with metal plate, Stage 4 with composite plate, Stage 4 with metal plate. Symbols (*, **, #) indicate pairwise comparisons that were statistically different ($p < 0.05$), whereas all other comparisons were not different ($p \geq 0.05$).

Plate thermographic stresses were extracted from corresponding images for each view and statistically compared (Figure 7.7.d). For Stage 3 fractured femurs, both composite and metal plate experienced almost equal stresses at all levels ($p = 0.08$ to 0.29). For Stage 4 healed femurs, the composite plate carried 70% less stress vs. the metal plate ($p = 0.00$ to 0.01). Both metal and

composite plates themselves carried substantially higher stresses at Stage 3 vs. Stage 4 ($p=0.00$ to 0.02).

A minimum power of 80% is generally considered to indicate a good study design, such that all statistical differences present are detected. The average post hoc powers with $\alpha=5\%$ two-tailed for all pairwise comparisons yielded 96% (anterior view, lateral surface), 92% (anterior view, medial surface), 46% (medial view), 66% (lateral plate), 87% (lateral bone), and 56% (stiffness).

Figure 7.6. Thermographic stress maps for femurs A) Stage 1 B) Stage 2 C) Stage 3 D) Stage 4. The anterior view is shown. M=Metal plate, C=Composite plate.

Figure 7.7. Thermographic stress values for femurs and plates above the fracture gap for a) anterior femur, b) medial femur, c) lateral femur, and d) lateral plates data. Specific points at thermographic images shown by white circles (o) were used to extract the stress values. Symbols (&, \$, ~, #, @, *, %, =, +, -) indicate pairwise comparisons that were statistically different ($p < 0.05$), whereas all other comparisons were not different ($p \geq 0.05$).

7.3.2. Computational Results.

Axial stiffness computed with FE analysis were found to be 2238 N/mm (Stage 1) and 2508 N/mm (Stage 2), 709 N/mm for composite plated femurs and 1211 N/mm for metal plated femurs (Stage 3), and 2716 N/mm for composite plated femurs and 2815 N/mm for metal plated femurs (Stage 4). FEA stress patterns of the anterior view only are given for illustrative purposes (Figure 7.8), whilst FEA stress levels for anterior, medial, and laterals views are extracted and graphed (Figure 7.8).

For the femur itself in the anterior view (Figure 7.9.a), differences between composite plated vs. metal plated specimens became more noticeable for bone beneath the plate, yielding an average difference of 13% (Stage 3) and 16% (Stage 4), for all levels. For femur stresses at Stage 3, composite plated vs. metal plated specimens showed only small differences at all levels in the medial (0.5%) (Figure 7.9.b) and lateral (0.02%) (Figure 7.9.c) views. For femur stresses at Stage 4, composite plated vs. metal plated specimens yielded small differences at all levels in the medial (2%) view (Figure 7.9.b) with no difference at lateral view (Figure 7.9.c). FE bone mean surface stresses peaked at distal epiphysis for Stage 1 (13 MPa) and Stage 2 (16 MPa), the vicinity of nearest screw just proximal to fracture for Stage 3 (composite = 85 MPa, metal = 129 MPa) and at screw farthest away distally from fracture for Stage 4 (composite = 21 MPa, metal = 24 MPa). Although mean surface stresses were higher on femurs repaired with composite vs. metal plate, the peak stress in the composite plated femur was lower than that of metal plated femurs, which reduced the risk of bone fracture. For the plate itself, there was only a 7% difference in stress between composite vs. metal plates at Stage 3, whereas the difference was 50% at Stage 4 (Figure 7.9.d). For Stage 2 vs. Stage 4, the difference in femur stress was 18% (composite plate specimens) and 32% (metal plate specimens).

Figure 7.8. FEA model of the synthetic femurs showing a) Stage 1 b) Stage 2 c) Stage 3 d) Stage 4. The anterior view is shown. M=Metal plate, C=Composite plate. Numbers represent sum of principle stresses at each probed location.

Figure 7.9. FEA stress values for femurs and plates for a) anterior femur b) medial femur c) lateral femur, and d) lateral plates data.

7.3.3. Experiments vs. Computations.

Although experiments were done under dynamic cyclic conditions and FEA computations were performed for the static scenario, there are some similarities in their trends. Both experimental and computational results showed a significant reduction in surface stress of bone directly under the fixation plate for femurs fitted with metal plate compared to those of composite plate. However, the difference between femur stresses at Stage 3 (experiments, 33%; computations, 13%) was smaller than at Stage 4 (experiments, 60%; computations, 18%). This can be related to the load-bearing capabilities of a plate in the vicinity of bone gap at the fracture site. Specifically, for the fractured Stage 3 condition, the applied load would be transferred to the plate, since there is no bone at the fractured site to transfer load to the distal part of a femur. On the other hand, for the healed Stage 4 condition, the metal plate would decrease femur stresses more than the composite plate (experiments, 60%; computations, 32%). Similarly, the composite plate itself attached to a Stage 4 healed femur carried significantly lower stresses vs. a metal plate (experiments, 70%; computations, 50%), whereas they experienced comparable stresses in Stage 3 fractured specimens (experiments, 9%; computations, 7%).

7.4. Discussion

7.4. 1. General Findings

This is the first study which biomechanically evaluated this new CF/Flax/Epoxy hybrid composite plate for repairing a Vancouver B1 femur fracture vs. a standard clinical metal plate. Stage 3 and 4 specimens stiffnesses were statistically equivalent for composite vs. metal plated femurs, indicating that the current composite plate can provide adequate initial fixation immediately after surgery. Also, the composite plate carried a lower percentage of load compared to the metal plate both during Stage 3 and 4, indicating that the composite plate minimized the effects of “stress shielding.” The bone directly under the plates seemed particularly vulnerable to “stress-shielding” with the metal plate for Stage 3 and 4, which was not the case for the composite plate.

7.4.2. Comparison to Prior Studies.

First, with one exception [24] the prior studies which developed a polymer-based composite for bone fracture plating did not assess their plate for the clinically challenging Vancouver B1 femur fractures in the presence of a THR. These previous studies tested the composite plate alone [10, 13, 95, 205], performed FEA for the plate itself [97] or when it was used to repair tibia fracture[6], did experimental evaluations by attaching the plate to a long bone-like plastic tube [205], or did in-vivo studies in canine or human cadaver bone [18, 19, 205]. Second, The current composite and metal plated specimens had comparable axial stiffness with other reports involving fracture and/or healed femurs with THRs (Table 7.1) [81, 122, 123, 126, 127, 133, 158]. Any differences between current and previous investigations could be related to different loading regimes, varying implants, and dissimilar fixation methods (i.e. cables, screws, and/or strut). Third, only a very recent study used thermography to investigate femurs repaired with a composite vs. metal plate in the presence of a THR [136, 165]; the present experimental results for stiffness and surface stresses were in good agreement with that study. Fourth, from a methodological viewpoint, the other few studies using thermography analysis for biomechanics applications did so for an intact femur [133], a canine femur [206], composite hip implant on its own [124], a metal hip implant along [166], and a metal hip implant in an artificial femur [167].

7.4.3. Clinical Implications.

The medial and the lateral surfaces of a femur repaired with either composite or metal plates may be most susceptible to injuries, as evidenced by the current peak compressive and tensile stresses, respectively, just below the lesser trochanter and adjacent to the plate (Figure 7.6). This is consistent with prior studies on Vancouver B1 fractures repaired with metal plate showing a mediolateral fracture line [122], but higher stresses on medial and lateral sides far from the fracture gap [168]. This can be explained using basic beam theory, namely, that the bone primarily underwent in-plane bending in the coronal plane. Thus, a vertical force would introduce tensile stresses along the femur's lateral surface, while compressive stresses were dominant along the femur's medial surface.

Table 7.1. Stiffness of the current CF/Flax/Epoxy composite plated femurs, current metal plated femurs, and femurs repaired with a metal plate from prior studies.

Sample	Load (N)	Testing method	Femur type	Stiffness (N/mm)	Ref
Current CF/Flax/epoxy plate +screws				645± 67	
Current Metal plate + screws	750	Experimental	Synthetic	658±69	
Current CF/Flax/epoxy plate +screws		FEA		709	
Current Metal plate + screws				1211	
Non-locking plate and screws	3000	FEA	Synthetic	973	[127]
Non-locking plate and cables				768	
Non-locking plate and screws	1000	FEA	Synthetic	1023	[168]
Non-locking plate with cables+screws				1102	
Plate with cables				1105	
Plate with cables + screws	500	Experimental	Synthetic	923	
Plate with screws				4835	[81]
Allograft struts with cables				3010	
Non-locking plate with screw + cables	1000	FEA	Synthetic	601-1359	[126]
Locked plate with screws	400	Experimental	Embalmed	420	[123]
Non-locking plate with screw + cables				304	
Locked plate				700	
Non-locking plate and medial strut	700	Experimental	Synthetic	1100	[158]
Non-locking plate and medullary strut				1400	
Locked plate with screws				951	
Locked plate with cables + screws	100	Experimental	Synthetic	993	[122]
Non-locking plate with cables+screws				1110	
Non-locking plate cables + strut				1526	

Bone healing is a natural process which includes different stages: callus formation, callus proliferation, bony bridging, callus consolidation, and eventual bone remodelling [2]. Although

the amount of callus formed depends on the interfragmentary strain (i.e. movements at the fracture site), in which larger strain results in more callus formation, gross motion at the fracture sites leads to non-union. However, micromotion would be beneficial for callus stimulation results in eventual bone healing [2-4]. Fixation of a fracture with a rigid bone plate not only limits micromotion at the fracture site and prolongs the healing process, but also maintains the most load-carrying capabilities and causes “stress-shielding” [2, 3]. On the other hand, a plate should be rigid enough to provide sufficient stability to the fracture site, especially for bending loads at unstable comminuted fracture sites [2, 4]. Therefore, an ideal bone fracture plate should provide perfect immobilization in bending but partial fixation in compression to concurrently stabilize fracture site while allowing micromotion compulsory for callus formation. Consequently, a selective “stress shielding” approach could be used to design fracture fixation plates, which could be achieved by using 2 or more types of materials [2, 4], like the current CF/Flax/Epoxy.

The current CF/Flax/Epoxy composite vs. metal plate specimens had higher femur stresses in Stage 3 fractured (experiments, 33%; computations, 13%) and Stage 4 healed femurs (experiments, 60%; computations, 18%) (Figure 7.7 and Figure 7.9). Thus, the composite plate might more easily allow the micromotion necessary for bone healing by minimizing “stress shielding” [127]. Moreover, the composite plate specimens showed no difference in stiffness to the metal plate for Stage 3 and 4 in experimental results, thus it is capable of providing the stability needed to hold the fracture fragments in apposition as the bone heals. This distinctive behaviour (i.e. lower “stress shielding” and adequate stiffness) of the current CF/Flax/Epoxy composite can be related to its unique “sandwich structure” using 2 fibers (i.e. CF and Flax). This composite has been previously shown to yield a similar E modulus (tensile test, 42 GPa; 3-point bending test, 57 GPa) [33] but greater strength (tensile test, 400 MPa; 3-point bending test, 511 MPa) [33] vs. human cortical bone (E, 7–25 GPa; strength, 50–150 MPa). Not surprisingly, it currently provided sufficient stiffness mandatory for fracture site fixation by showing comparable stiffness to metal plated femurs and reduced “stress shielding” by allowing the underlying bone to carry a substantially larger portion of applied stress to that of metal plated femurs.

7.4.4. Addressing Potential limitations

Artificial femurs were used instead of cadaveric bones. The reason was that application of a black dye, which is mandatory for proper infrared emission from femurs during thermography testing, cannot be done using human cadaver femurs because of bone texture, residual soft tissue, and blood. Moreover, the use of artificial femurs has a sufficiently established history in biomechanics studies [121, 122, 131, 133, 136, 158, 168] because of their similar axial vs. torsional stiffness ratio [121], cancellous and cortical screw pullout shear stress [129, 130], and identical failure mechanism under axial compression [131] compared to human femurs. Thus, current trends would likely be similar to in vivo patient conditions during normal daily activities and in vitro experiments on human femurs.

Thermography tests were performed at 5 Hz of cycling, as the camera required a minimum working frequency range of 3-5 Hz to maintain the adiabatic conditions and consequently capture good quality images. However, the usual range of walking frequency simulated in biomechanical studies is between 1-3 Hz [158, 207, 208]. It should be noted that the hip forces are directly proportional to the walking gait frequency [209]. Thus, if the current tests were performed at 1-3 Hz, the cyclic forces would be lower and thus result in a drop in the absolute values of the measured stresses. Nonetheless, the present general trends and relative performance between plate types would be retained.

Cortical bone properties were inputted into the thermography camera software to the exclusion of the cancellous bone, which was expected to be sufficient to represent femur behaviour. One prior study with FEA showed that removing all cancellous bone from a femur would alter its axial and torsional stiffness only by 1% compared to a femur with both cortical and cancellous bone [121]. Also, another recent experimental study reported that the axial, torsional, and lateral stiffness remained virtually unaffected unless there was a 6-fold change in cancellous bone density [131]. Moreover, cortical bone properties alone were previously used for the same thermography method on an artificial femur and were validate against the well-established method of strain gages [25].

The effect of the abductor muscle group was neglected for experiments and FEA. A portion of abductor muscle, acting as a counteractive force, would minimize tensile stresses along the

superior aspect of the femur neck and greater trochanter. This phenomenon would affect absolute values of the current stresses, but not the relative performance of the compared specimen. Moreover, during the onset of walking, the human femur is in axial compression as imitated here, which is a principle employed by other researches using artificial or human femur without considering the effect abductor muscle [121, 126, 131, 158, 168].

Experiments were performed for only 1000 cycles to prevent any permanent damage to the specimens, thus tests could be accomplished for both repaired and healed conditions. However, further tests should be done using a large number of cycles to be able to predict the long-term daily normal activities and fatigue life of specimens [158, 207, 208].

Finally, the current study design was low in statistical power for 3 out of 6 measurement categories as a result of a limited sample size, i.e. $n = 3$. More specimens would have been needed to reach a satisfactory statistical power of more than 80% for all measurements, which was not practically feasible. However, statistical differences that were detected for stress and stiffness still clearly indicated the superiority of the CF/Flax/Epoxy composite plate versus the traditionally used metal plate, providing sufficient stiffness and higher surfaces stresses on the underlying femurs.

7.5. Summary

It was found in this chapter that the only area that underwent “stress shielding” by the metal bone plate is the bone directly beneath the plates. Also, the femur stresses at the healed stages were significantly decreased by the metal plate lateral side of the femur, which could lead to subsequent osteoporosis, while the composite plate did not affected stresses. Although the composite plate provided the same mechanical stability, i.e. stiffness to the femur, as did the metal plate, the large stress differences conducive to reducing “stress shielding”. Therefore, the CF/Flax/Epoxy composite plate can simultaneously reduce stress shielding while providing adequate immobilization at the fracture site.

CHAPTER 8: Conclusion

In this thesis, a novel CF/Flax/Epoxy with a “sandwich structure” and a perfect bonding at CF/Epoxy and Flax/Epoxy laminae has been developed. Its mechanical and biological properties as well as its biomechanical performance when it is used to fix a Vancouver B1 fracture are all characterized in 4 separate stages. The current CF/Flax/Epoxy composite plate showed promising properties for biomedical application as a long-bone fracture plate as described in details below. Moreover, the benefits of the proposed composite plate are not only limited to its unique mechanical properties. The final price of the proposed composite plate is expected to be much cheaper compared to a clinically-used metal plate (i.e. composite plate is priced at \$200 vs. metal plate is priced at \$2000). This is an additional motivation for pursuing further investigations to develop this material to a final product, which can replace metallic bone plates in reality

The set of biological experiments in chapter 4 confirmed the biocompatibility of the current CF/Flax/Epoxy composite material by showing similar cell viability with no negative effect on gene expression levels responsible for bone formation compared to a medical grade stainless steel material which is used widely to manufacture orthopaedic implants.

The set of mechanical experiments in chapter 5 of the current thesis determined the static mechanical properties of the current composite material. The results revealed that the composite material has higher mechanical properties in bending compared to tension, which can be related to the “sandwich structure effect”. This phenomenon makes the material attractive for applications that need adequate bending stiffness while having lower axial stiffness, like long bone fracture plates. Moreover, the failure stress in both tension and bending were high enough to carry clinical-type forces as occur in axial compression or bending.

Chapter 6 of this thesis evaluated the fatigue properties of the CF/Flax/Epoxy composite. Its fatigue strength was high enough to carry clinical-type cyclic forces that occur in normal daily activities. It retained its mechanical stiffness up until failure, which may result in less debris formation and subsequent immune system response. This makes this composite appropriate for applications that require specific mechanical properties in a given period of time, i.e. healing for orthopaedic fixation applications. Moreover, thermographic analysis has been shown to be a reliable tool for the quick determination of the fatigue limit of composite biomaterials.

Chapter 7 of this study assesses the biomechanical properties of the current CF/Flax/Epoxy composite plate for Vancouver B1 fractures in comparison with a standard clinically-used metal plate. The only area that underwent “stress shielding” by the metal bone plate is the bone directly beneath the plates, where a difference of 33% for Stage 3 fractured femurs and 60% for Stage 4 healed were detected for composite vs. metal plated femurs. Also, the metal plate on the lateral side of the femur after Stage 4 healing significantly decreased the femur stresses, which could lead to subsequent osteoporosis, while the composite plate did not affected stresses. Despite the large stress differences conducive to reducing “stress shielding”, the composite plate provided the same mechanical stability, i.e. stiffness to the femur, as did the metal plate.

Based on the current results in this thesis, the new CF/Flax/Epoxy composite materials can be a potential candidate for bone fracture plate applications. However, the composite plate is not ready for the human use yet. Building on the results of this work, following future in-vitro and in-vivo studies are recommended before the plate can be safely and reliably used in humans as described in details below.

Since a clinical plate bender cannot be used on the developed composite plate to match femur geometry, it could be argued that the resulting presence of “pre-stresses” on the plate/bone may become a clinical problem. Future studies may consider molded plates with favourable curvature to maximize the interface between bone and plate. In order to use the plate bender on the composite plate, another solution would be replacing thermosetting epoxy polymeric matrix, used in the proposed plate fabrication, with thermoplastic ones like PMMA. Another possible solution for a better long-term approach for this technology could be considering locking screw design to eliminate the need for contouring. However, the structure of the plate should be modified by including perpendicular fibers to the unidirectional ones in its lay-up, in order to have sufficient strength in transverse loading introduced by locking screws.

Physical activities such as stair climbing or raising from a chair induce high torsional loads. Therefore, future work should consider torsional testing on the composite plate alone as well as femur plate structure for both static and dynamic loading conditions to evaluate stiffness and strength of the developed plate in torsion.

All the tests were performed in ambient air environment. As long term exposure of polymeric-based composite materials to humid atmosphere may have influence on their mechanical properties, future studies may wish to consider the effect of physiological fluids (e.g. blood, synovial, fluid, water, etc.) to simulate a more clinically-realistic environment.

Although the CF layers at the surfaces of the composite plate reduce the water absorption problem considerably, the exposure of flax fibers at the sides and screw holes may cause moisture uptake as a long term effect. Future studies should consider coating the edges as well as screw holes with carbon fibers. Moreover, since flax fibers are hydrophilic in their nature, another solution could be coating them with an amphiphilic agent to solve their moisture uptake problem. Also, fabrication of the proposed plate with vacuum-molding or autoclave techniques would reduce the number of voids produced during the manufacturing process, which eliminate the moisture uptake problem.

Finally, the proposed composite plate with optimized structure should be examined for animal studies in order to simultaneously consider the effect of complicated loading regimes happened in daily normal activities, the immune system response and the possible histological abnormalities. Briefly, a series of rats could be randomly assigned into two groups. After creating a midshaft femur fracture with a 5 mm gap to simulate comminution, one group would be fitted with the CF/Flax/Epoxy composite plate, while the other group would be repaired with a standard clinical metal plate. The contralateral femur in each group would serve as an intact control. The same number of rats ($n=8$) from both groups would be sacrificed at different time points for a period of about 2 years, i.e. 0, 6, 18, 30, 42, 54, 66, 78, 90, and 102 weeks. The operated and control femurs would be harvested and mechanically tested. These time points could be selected in order to have continuous monitoring of bone quantity and quality for the immediate post-operative condition (i.e. 0 weeks), at the start of bone healing (i.e. typically 6 weeks), and throughout the healing process for a typical clinical follow-up period (i.e. 2 years). Moreover, the operated femurs would be imaged using radiography at 2-week intervals for 102 weeks to examine the amount of callus formed, osseous bridging of the gap, and corticalization of the regenerated bone.

Appendices

A1. Chapter 4 - Osteogenesis and Cytotoxicity of Carbon/Flax/Epoxy Composite

A1.1. Procedure for RNA dilution to 500 ng/ μ L

24 hrs

Samples	RNA(ng)	RNA*13	(RNA*13)/500	Added Water(μ L)
Flax/epoxy	979.2	12729.6	25.4592	12.4592
CF/epoxy	656.3	8531.9	17.0638	4.0638
CF/Flax/epoxy	516.3	6711.9	13.4238	0.4238
Metal	473	6149	12.298	-
Ctrl	761.8	9903.4	19.8068	6.8068

48 hrs

Sample	RNA(ng)	RNA*13	(RNA*13)/500	Added Water(μ L)
Flax/epoxy	522.5	6792.5	13.585	0.585
CF/epoxy	999.7	12996.1	25.9922	12.9922
CF/Flax/epoxy	661.8	8603.4	17.2068	4.2068
Metal	603.1	7840.3	15.6806	2.6806
Ctrl	1454.3	18905.9	37.8118	24.8118

72hrs

Sample	RNA(ng)	RNA*13	(RNA*13)/500	Added Water(μ L)
Flax/epoxy	413.6	5376.8	10.7536	-
CF/epoxy	692.5	9002.5	18.005	5.005
CF/Flax/epoxy	599.5	7793.5	15.587	2.587
Metal	854.6	11109.8	22.2196	9.2196
Ctrl	2583.8	33589.4	67.1788	54.1788

A1.2. Flowchart for cDNA and PCR Synthesis

Day 1 → Synthesis of cDNA

Label 6 PCR tubes (Flax/epoxy, CF/epoxy, CF/Flax/epoxy, metal, Ctrl, NT):

Samples	Random Primers	RNA2	DPEC
Flax/epoxy	1	2	9
CF/epoxy	1	2	9
CF/Flax/epoxy	1	2	9
Metal	1	2.114165	8.885835
Ctrl	1	2	9
NT	1	0	11

@ 70 °C 5min

4 °C ∞

For 6 tubes, prepare master mix for 8 tubes:

5X Buffer 4 μL * 8 = 32

RNase Inhibitor 1 μL * 8 = 8

RT 1 * 8 = 8

dNTP(10mM ea) 2 μL * 8 = 16

Vtotal 8 * 8 = 64

Add 8 μL of master mix to each tube.

Run RNA synthesis using the following program:

@ 25 °C 5min

42 °C 60min

70 °C 5 min

4 °C ∞

Day 2 → PCR

Prepare MM1(Housekeeping HPRT)

Reagent	One Rxn(μL)	MML(μL)
10X Buffer	2.5	20
10mM DNTPs	0.5	4
Dream Taq	0.25	2
HPRT-F	0.75	6
HPRT-R	0.75	6
DPEC water	19.25	154
Vtotal	24*8	192

1. Label 6 PCR tubes as follow(Flax/epoxy, CF/epoxy, CF/Flax/epoxy, metal, Ctrl, NT) and add 1 μL of corresponding cDNA/H₂O
2. Add 24 of MM1 to each tube
3. Mix the content of each tube by pipetting up and down and gently
4. After preparation of PCR tubes spin them down place them on ice to move them for PCR machine.

Preparation of MM2 (oxterix)

Reagent	One Rxn(μL)	MML(μL)
10X Buffer	2.5	20
10mM DNTPs	0.5	4
Dream Taq	0.25	2
Oxterix-F	0.75	6
Oxterix-R	0.75	6
DPEC water	19.25	154
Vtotal	24*8	192

1. Label 6 PCR tubes as follow(Flax/epoxy, CF/epoxy, CF/Flax/epoxy, metal, Ctrl, NT) and add 1 μL of corresponding cDNA/H₂O
2. Add 24 of MM2 to each tube
3. Mix the content of each tube by pipetting up and down and gently
4. After preparation of PCR tubes spin them down place them on ice to move them for PCR machine.

Preparation of MM3 (BMP2):

Reagent	One Rxn(μ L)	MML(μ L)
10X Buffer	2.5	20
10mM DNTPs	0.5	4
Dream Taq	0.25	2
BMP2-F	0.75	6
BMP2-R	0.75	6
DPEC water	19.25	154
Vtotal	24*8	192

1. Label 6 PCR tubes as follow(Flax/epoxy, CF/epoxy, CF/Flax/epoxy, metal, Ctrl, NT) and add 1 μ L of corresponding cDNA/H₂O
2. Add 24 of MM3 to each tube
3. Mix the content of each tube by pipetting up and down and gently
4. After preparation of PCR tubes spin those down place them on ice to move them for PCR machine.

Preparation of MM4 (RUNX2):

Reagent	One Rxn(μ L)	MML(μ L)
10X Buffer	2.5	20
10mM DNTPs	0.5	4
Dream Taq	0.25	2
RUNX2-F	0.75	6
RUNX2-R	0.75	6
DPEC water	19.25	154
Vtotal	24*8	192

1. Label 6 PCR tubes as follow(Flax/epoxy, CF/epoxy, CF/Flax/epoxy, metal, Ctrl, NT) and add 1 μ L of corresponding cDNA/H₂O
2. Add 24 of MM4 to each tube
3. Mix the content of each tube by pipetting up and down and gently
4. After preparation of PCR tubes spin those down place them on ice to move them for PCR machine.

48 hrs:

Day 1 → Synthesis of cDNA

Label 6 PCR tubes (Flax, CF, CFF, 316 L, Ctrl, NT):

Samples	Random Primers	RNA2	DPEC
Flax/epoxy	1	2	9
CF/epoxy	1	2	9
CF/Flax/epoxy	1	2	9
Metal	1	2	9
Ctrl	1	2	9
NT	1	0	11

@ 70 °C 5min

4 °C ∞

For 6 tubes, prepare master mix for 8 tubes:

5X Buffer 4 μL* 8= 32

RNase Inhibitor 1 μL*8=8

RT 1*8=8

dNTP(10mM ea) 2 μL*8=16

Vtotal 8*8=64

Add 8 μL of master mix to each tube.

Run RNA synthesis using the following program:

@ 25 °C 5min

42 °C 60min

70 °C 5 min

4 °C ∞

72 hrs:

Day 1 → Synthesis of cDNA

Label 6 PCR tubes (Flax, CF, CFF, 316 L, Ctrl, NT):

Samples	Random Primers	RNA2	DPEC
Flax/epoxy	1	2.417795	8.582205
CF/epoxy	1	2	9
CF/Flax/epoxy	1	2	9
Metal	1	2	9
Ctrl	1	2	9
NT	1	0	11

@ 70 °C 5min

4 °C ∞

For 6 tubes, prepare master mix for 8 tubes:

5X Buffer 4 μL * 8 = 32

RNase Inhibitor 1 μL * 8 = 8

RT 1 * 8 = 8

dNTP(10mM ea) 2 μL * 8 = 16

Vtotal 8 * 8 = 64

Add 8 μL of master mix to each tube.

Run RNA synthesis using the following program:

@ 25 °C 5min

_ 42 °C 60min

70 °C 5 min

4 °C ∞

A1.3. Image of DNA acrylamide gel for HPRT gene for 24 hrs

DNA acrylamide gel showing successful PCR of HPRT (69 bp, Housekeeping gene):
Gel run at 100V for 1 hrs. Stained 1 hrs in Invitrogen's SYBER Safe DNA gel stain, rinsed briefly:

Lane 1= 50bp marker(5 uL loaded)

Lane 2= Flax/epoxy

Lane 3= CF/epoxy

Lane 4=CF/Flax/epoxy

Lane 5= metal

Lane 6=Control

PCR parameters for all reactions (all reactions performed on VertiCycler, 5th floor):

94C for 3 min

(94 C for 30 sec, 60C for 30sec, 725C for 1 min)X 35 cycles

72C for 10 min

4C hold

Acrylamide gel:

1.25mL 10X TAE buffer

7.1 mL ddH₂O

4.17 mL 30% acrylamide/bis-acrylamide solution

62.5 uL 10% APS solution

6.25 uL TEMED

Staining:

1 hrs SYBR safe, made by adding 1uL to 50mL ddH₂O (per gel)

A1.3. Image of DNA acrylamide gel for osterix gene

	Flax/epoxy	CF/epoxy	CF/Flax/epoxy	Metal	Ctrl
24 hrs	1	2	3	4	5
48 hrs	6	7	8	9	10
72 hrs	11	12	13	14	15

A1.4. Image of DNA acrylamide gel for RUNX gene

	Flax/epoxy	CF/epoxy	CF/Flax/epoxy	Metal	Ctrl
24 hrs	1	2	3	4	5
48 hrs	6	7	8	9	10
72 hrs	11	12	13	14	15

1 2 3 4 5 6 7 8 9

10 11 12 13 14 15

A2. Chapter 5- Biomechanics of the CF/Flax/Epoxy Plate versus a Traditional Metal Plate for Vancouver B1 Femur Fractures: An Experimental and Computational Study

A 2.1. Maximum Stress regions for the intact femur (anterior view).

The sums of the principle stresses are shown along the anterior region of the femur (Figure A.1). The sum of principle stress peaked around the neck. The maximum stresses are noticed at points A and B, corresponding to the medial and lateral sides of the femur.

Figure A.1. High stress regions along the anterior side of the femur

A2.2. Dynamic Axial Stiffness

The numerical values for forces and displacements are directly recorded by Instron testing machine based on its coordinate system. Thus, one loading cycle initiated at the top right of the graph with the minimum value of force, continued until peak force was reached at bottom left of the graph (maximum). The slope of force-displacement curve was used to calculate the stiffness using the line of best fit, and R^2 pointed to the degree of linearity and agreement between the line of best fit and the experiment. The force-displacement curve is given for one typical sample for intact femur (Figure A.2), femur with hip implant (Figure A.3), fractured femur healed with composite plate (Figure A.4), fractured femur healed with metal plate (Figure A.5), healed femur with composite plate (Figure A.6) and healed femur with metal plate.

Figure A.2. Dynamic stiffness for intact femur.

Figure A.3. Dynamic stiffness for femur with hip implant.

Figure A.4. Dynamic stiffness for fractured femur with composite plate.

Figure A.5. Dynamic stiffness for fractured femur with metal plate.

Figure A.6. Dynamic stiffness for healed femur with composite plate.

Figure A.7. Dynamic stiffness for healed femur with metal plate.

A2.3. Material Safety Data Sheet Black Dye

6836 BLACK

Date of Preparation: 09/28/2004

Revision: 09/28/2004

Section 1 - Chemical Product and Company Identification

Product Name: 6836 BLACK

Product Class: Pigment

Chemical Type: Non-aqueous colorant

Manufacturer: BJB Enterprises, Inc., 14791 Franklin Avenue, Tustin, CA 92780, Phone (714) 734-8450, Fax (714) 734-8929, (M-Th: 8-4:30, F: 7:30-4), Emergency Phone: Chemtrec (800) 424-9300 or (703) 527-3887

Section 2 - Composition / Information on Ingredients

Ingredient Name	CASRN	% wt
1. Carbon Black	1333-86-4	28

Trace Impurities: N/A

Ingredient	OSHA PEL		ACGIH TLV		NIOSH REL		NIOSH IDLH
	TWA	STEL	TWA	STEL	TWA	STEL	
#1	3.5 mg/m ³	NE	NE	NE	NE	NE	NE

Section 3 - Hazards Identification

☆☆☆☆☆ Emergency Overview ☆☆☆☆☆

Appearance: Black liquid; Odor: Mild. May cause eye and skin irritation. Harmful if inhaled. Use in well-ventilated areas. Emits toxic fumes under fire conditions. This product contains one or more reported carcinogens or suspected carcinogens which are noted NTP, IARC, or OSHA-Z in the other limits recommended column. This product contains pigments which may become a dust nuisance when removed by abrasive blasting, sanding or grinding.

HMIS

H 1

F 1

R 0

PPE†

†Sec. 8

Potential Health Effects

Primary Entry Routes: Eye and skin contact; inhalation of vapors, accidental ingestion. No harmful effects by chronic exposure reported.

Acute Effects:

Inhalation: Heating can generate vapors that could cause headaches, nausea, dizziness, and respiratory irritation if inhaled.

Ingestion: Low oral toxicity.

Eye: May cause minor irritation but no corneal injury.

Skin: Exposure may cause mild skin irritation. Symptoms may include redness and burning.

Carcinogenicity: This product contains carbon black, a substance identified by the International Agency for Research on Cancer (IARC), as a Classification 2b (possibly carcinogenic to humans). Based on the International Agency for Research on Cancer (IARC) conclusion that there is sufficient evidence in experimental animals for the carcinogenicity of carbon black dust and inadequate evidence if carcinogenicity in humans, IARC's overall evaluation is that carbon black dust is possibly carcinogenic to humans (Group B). Consult IARC's Monograph Volume 65. The results of the working group were based on studies involving the inhalation of carbon black and other insoluble fine dust particles. Other routes of entry were not reviewed as part of this study. This dispersion contains carbon black in a "wet out" form and does not pose an inhalation hazard. Good hygiene practices should be followed to minimize exposures to any respirable dusts. The study findings produced results consistent with the massive accumulation of fine dust particles in the lung, which overwhelm the natural lung clearance mechanisms, known as the "lung overload" phenomenon, rather than a specific chemical effect of the dust particle in the lung. Carbon Black has not been listed as a carcinogen by the national Toxicology Program (NTP) or the Occupational Safety and Health Administration (OSHA). The National Institute of Occupational Safety and Health (NIOSH) criteria document on carbon black recommends that only carbon blacks with a PAH level greater than 0.1% be considered suspect carcinogens.

Medical Conditions Aggravated by Long-Term Exposure: None known

Section 4 - First Aid Measures

Inhalation: Not likely. Remove to fresh air environment.

Ingestion: If swallowed, call a physician immediately. Do NOT induce vomiting. Never give anything by mouth to an unconscious person.

Eye Contact: Flush eyes with clean, lukewarm water for 15 minutes. Flush under both upper and lower lids. Obtain medical attention if irritation develops.

Skin Contact: Remove contaminated clothing and wash affected areas well with soap and water. Launder contaminated clothing before use.

Note to Physicians: Treat any ill effects symptomatically.

Section 5 - Fire-Fighting Measures

Flash Point: 200°F (93°C)

Extinguishing Media: CO₂, foam, or dry chemical. Water may be used to keep fire-exposed containers cool until fire is out.

Unusual Fire or Explosion Hazards: Emits toxic fumes under fire conditions.

Fire-Fighting Instructions: Cool fire exposed containers with water spray. Remove containers from fire area if possible. Do not release runoff from fire control methods to sewers or waterways.

Fire-Fighting Equipment: Firefighters should wear positive pressure self-contained breathing apparatus (SCBA) and consider use of unmanned hose holders or monitor nozzles for fighting large fires.

Section 6 - Accidental Release Measures

Spill /Leak Procedures: Provide adequate ventilation and wear personal protective equipment. Eliminate all ignition sources. Prevent product spill from entering sewers, streams or drinking water supplies. Collect liquid or soak up with inert filler or an absorbent, such as dry earth, sand or oil absorbent (sweeping) compound. Collect material into suitable containers for disposal. Wash area with detergent and flush with copious amounts of water.

Containment: For large spills, dike ahead of liquid spill for later neutralization, absorption, clean up and disposal.

Section 7 - Handling and Storage

Handling Precautions: Avoid contact with eyes, skin and clothing. Containers of this material may be hazardous when emptied. Since emptied containers retain product residues (vapor, liquid, and/or solid), all hazard precautions given in the data sheet must be observed.

Storage Requirements: Store in closed, properly labeled containers away from heat, open flames and strong oxidizers.

Shelf life: 24 months from date of shipment under manufacturers recommended storage conditions.

Section 8 - Exposure Controls / Personal Protection

Eye Protection Requirements: Safety goggles or glasses are recommended. Plastic face shield should be worn for complete face protection.

Skin Protection Requirements: Impermeable gloves should be worn. Employees should wash their hands and face before eating, drinking or using tobacco products.

Ventilation/Respiratory Requirements: Exhaust ventilation recommended. An organic vapor cartridge or fresh air supplied respirator (NIOSH certified) may be necessary for certain applications. Consider the type of application, environmental concentrations, and other materials being used concurrently when determining respirator use and selection. Observe OSHA regulations for respirator use (29 CFR 1910.134).

Additional Protective Measures: Safety showers and eye wash stations should be easily accessible to the work area. Training is important. Follow all label precautions.

Section 9 - Physical and Chemical Properties

Physical State: Liquid

Appearance/Odor: Black/Mild

Vapor Pressure: 0.35

Specific Gravity (H₂O=1): 1.11

Water Solubility: NE

% Volatile: None

V.O.C. (ref EPA meth 24): None

Section 10 - Stability and Reactivity

Stability: Stable at room temperature in closed containers under normal storage and handling conditions.

Hazardous Polymerization: Product can undergo hazardous polymerization.

Chemical Incompatibilities: Strong oxidizers such as chlorates, bromates, and nitrates.

Conditions to Avoid: Excessive heat, flame and other possible ignition sources.

Hazardous Decomposition: Carbon monoxide, carbon dioxide, and various hydrocarbons.

Section 11- Toxicological Information

No Toxicological Information Available

Section 12 - Ecological Information

No Ecological Information Available

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Glossary

Abduction	moving of a body part away from the central axis of the body
Acetabulum	the cup-shaped cavity on the lateral surface of the hip bone, receiving the head of the femur
Adduction	moving of a body part toward the central axis of the body
Anterior	towards the front of the body
Biocompatibility	the property of being biologically compatible by not producing a toxic, injurious, or immunological response in living tissue
Cancellous bone	sponge bone tissue contained in inner space of bone
Condyle	a rounded prominence at the end of a bone, most often for articulation with another bone
Contralateral	taking place or originating in a corresponding part on an opposite side
Cortical bone	the compact bone of the shaft of a bone that surrounds the marrow cavity
Diaphysis	the shaft of a long bone, between the epiphyses
Distal	situated farthest from the centre, median line, or point of attachment or origin
Epiphysis	the expanded articular end of a long bone, developed from a secondary ossification center, which during the period of growth is either entirely cartilaginous or is separated from the shaft by a cartilaginous disk
Femur	the longest thickest bone of the human skeleton, articulating with the pelvis above and the knee below
Intercondylar	between two condyles
Lateral	situated or extending away from the median plane of the body
Medial	situated toward the median plane or midline of the body or a structure
Metaphysis	the wider part at the end of a long bone, adjacent to the epiphyseal disk

Osteopenia	reduced bone mass due to a decrease in the rate of osteoid synthesis to a level insufficient to compensate for normal bone lysis
Osteoporosis	A disease characterized by decrease in bone mass and density
Patella	a triangular bone, about 5 cm in diameter, situated at the front of the knee in the tendon of insertion of the quadriceps muscle. Called also knee cap.
Physiologic	relating to the way that living things function, rather than to their shape or structure
Posterior	towards the rear of the body
Prosthesis	an artificial substitute for a missing body part, such as an arm, leg, eye, or tooth; used for functional or cosmetic reasons or both
Proximal	nearest to a point of reference, as to a center or median line or to the point of attachment or origin
Resorption	the loss of substance or bone by physiologic or pathologic means, such as the reduction of the volume and size of the residual ridge of the mandible or maxillae
Sagittal	situated in the direction of the sagittal suture; said of an anteroposterior plane or section parallel to the median plane of the body
Supracondylar	above a condyle
Trochanter	a broad, flat process on the femur, at the upper end of its lateral surface, or a short conical process on the posterior border of the base of its neck

Peer-reviewed Publications Based on this Dissertation

Journal Articles

1. Bagheri ZS, El-Sawi I, Schemitsch EH, Zdero R, Bougherara H, Biomechanical Properties of an Advanced New Carbon/Flax/Epoxy Composite Material for Bone Plate Applications, Journal of the Mechanical Behavior of Biomedical Materials, 2013, 20, 398-406 [Epub 2013 Jan 8], DOI: <http://dx.doi.org/10.1016/j.jmbbm.2012.12.013>.
2. Bagheri ZS, El Sawi I, Bougherara H, Zdero R, Biomechanical Fatigue Analysis of an Advanced New Carbon Fiber/Flax/Epoxy Plate for Bone Fracture Repair using Conventional Fatigue Tests and Thermography, Journal of the Mechanical Behavior of Biomedical Materials, 2014, 35, 27-38 [Epub 2014 Mar 26], DOI: 10.1016/j.jmbbm.2014.03.008.
3. Bagheri ZS, Tavakkoli Avval P, Bougherara H, Aziz MSR, Schemitsch EH, Zdero R, Biomechanical Analysis of a New Carbon Fiber/Flax/Epoxy Bone Fracture Plate Shows Less Stress Shielding Compared to a Standard Clinical Metal Plate, Journal of Biomechanical Engineering, 2014, 136(9), 091002-1-10.
4. Bagheri ZS, El-Sawi I, Giles E, Amleh A, Schemitsch EH, Zdero R, Bougherara H, Osteogenesis and Cytotoxicity of New Flax/Epoxy and Carbon Fiber/Epoxy Composite Materials for Bone Repair, Journal of Materials Science part C, under review.

Conference Transactions

1. Bagheri ZS, Bougherara H, Elsayi I, Zdero R. Biomechanical Fatigue Analysis of a New Carbon Fiber/Flax/Epoxy Composite for Bone Fracture Plate Applications using Infrared Thermography. 16th European Conference on Composite Materials. Seville, Spain, Jun 22-24, 2014, podium presentation.
2. Bagheri ZS, Tavakkoli Avval P, Bougherara H, Aziz MSR, Schemitsch EH, Zdero R, Biomechanical Evaluation using Infrared Thermography and Finite Element Analysis of a New CF/Flax/Epoxy Composite Plate versus a Traditional Metal Plate for Femur Fractures, Breakfast of Our Champions (BOOC) Annual Meeting, St. Michael's Hospital, Toronto, Canada, July 15, 2014, poster.
3. Bagheri ZS, Amleh A, Schemitsch EH, Zdero R, Bougherara H, Bone Formation Gene

Expression in the Vicinity of a New Carbon fiber (CF)/Flax/epoxy Material for Bone Fracture Plate Applications, 13th International Symposium on Bioplastics, Biocomposite & Biorefining (ISBBB), Guelph, Canada, May 19-24, 2014, podium presentation.

4. Bagheri ZS, Aziz MSR, Schemitsch EH, Bougherara H, Zdero R, Biomechanical Evaluation using Infrared Thermography of a New CF/Flax/epoxy Composite Plate versus a Traditional Metal Plate for Femur Fractures, 7th World Congress of Biomechanics, Boston, USA, July 6-14, 2014, poster presentation.

5. Bagheri ZS, Elsayi I, Amleh A, Schemitsch EH, Zdero R, Bougherara H, New Flax/Epoxy and CF/Epoxy Composite Materials for Bone Fracture Plate Applications: a Biological and Wettability Study, Proceeding of the 19th International Conference on Composite Materials, Montreal, Canada, July 28- Aug 5, 2013, poster presentation.