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Smoking and dose-dependent early effects of nicotine on bone mechanical properties and histology

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Smoking and Dose Dependent Early Effects of Nicotine on Bone Mechanical Properties and Histology

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ABSTRACT

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The objective was to study the effects of nicotine and cigarette smoke on mechanical properties of cortical bone. Experiments were conducted for 5 weeks with New Zealand White Rabbits. The first experiment of 18 rabbits studied the effect of nicotine levels delivered via a nicotine patch (5.25, 10.5, 21 ng/ml), measured by different mechanical tests, porosity, and composition. There was no significant difference between the control and the treatment groups.

The second experiment of 26 rabbits studied the effects of nicotine delivered via a nicotine patch (10.5 ng/ml) and via a smoking chamber on fracture toughness and porosity. The rabbits exposed to the smoke for 5 weeks had significantly lower fracture toughness values when compared to the different groups (exposed to smoke for 4 weeks group, nicotine group, and the control group). This suggests that other agents besides nicotine are responsible for the weakening of bone clinically seen in smokers.

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Nomenclature

General:

ASTM:	American Society of Testing Mechanics
a:	major axis
a:	crack length
A:	Crack Area
AW:	Ash Weight
AvPoRd:	Average Pore Radius
b:	minor axis
B:	Thickness
BMU:	Bone Multicelluar Unit
BMD:	Bone Mineral Density
BMC:	Bone Mineral Content
C:	Control
C:	dimensionless constant that depends on geometry and mode of loading
C:	compliance
CDC:	Center of Disease Control
°C:	Celsius
CT:	Compact Tension
DW:	Dry Weight
EPFM	Elastic plastic fracture mechanics
F:	work done by external force
Fx:	Fracture
Fem:	Fem(ur)(oral)
F:	work done by external force
f_{ij}:	dimensionless function
f(a/W):	dimensionless function
g:	gram
G:	Strain energy release rate
G_C:	Critical Strain energy release rate
Hn.Cn.Ar:	Haversian Canal Area
K:	stress intensity factor
K_C:	Critical stress intensity factor
K_I:	Mode 1 opening
K_{II}:	Mode 2 sliding
K_{III}:	Mode 3 tearing
kg:	Kilogram
I_P:	moment of polar inertia
I:	moment of inertia
LEFM:	Liner elastic fracture mechanics
M:	Moment
MMA:	methymethacrlate
min:	Minute
ml:	Milliliter

mm:	millimeters
mg:	Milligram
N:	Nicotine
ng:	NanoGram
n:	number
OVX:	Ovariectomized
P:	load
pa:	pascal
P_Q:	Critical Load
Pr.Po.Ar:	Primary Pore Area
Po:	Porosity
PTH:	parathyroid hormone
psi:	pound per square inch
rpm:	rotation per minute
r:	radius
s:	Second
Sham:	Surgery was done but nothing extra was added to the surgery
T:	Torque
t:	thickness
TrAr:	Total Bone Area
V.Cn.Ar:	Volksmann Canal Area
U:	Strain energy
V:	Volume
W:	length from center of drilled hole to the end of the CT specimen see figure 2.2.5 – 1
WW:	Wet Weight
$?_s$:	energy to break a bond
$?_p$:	plastic work
t :	shear stress
?:	radius of curvature
? :	potential energy
U:	strain energy stored in the body
? :	displacement
µg:	Microgram
σ:	Stress (pa)
σ_A:	Stress at point A
s_{ij}:	stress tensor
s_{nom}:	Stress nominal
s_{ys}:	Yield Stress
?:	angle

Chapter 1 Introductions

1.1 Background on Smoking and Nicotine

About 50 million of the United States adult population smokes. Some risks due to smoking for bone include the increased risk of osteoporosis; increase injuries and stress fractures during basic training, and lower fusion rates of bone healing. According to the CDC postmenopausal women who currently smoke have lower bone density than do women who do not smoke. Also women who currently smoke have an increased risk for hip fracture compared with nonsmoking women. A cigarette has over 4,000 chemicals contained in it. Nicotine is the most well known of these chemicals, however cigarettes have 60 known carcinogens. Nicotine is the agent that causes the addiction to the cigarette. The average smoker takes in 1 to 2 mg of nicotine per cigarette. Nicotine can act as both a stimulant and sedative. Many clinical studies have looked at the effect of smoking on bone.

1.1.1 Smoking Effect on Human Bones

In clinical studies done on the effect of smoking on human bones found that smoking has a negative effect on bone mineral density, and that the cessation of smoking can help recover from the negatives effect of smoking but not completely. Most patients who showed symptoms of osteoporosis before the age of 65 were smokers (Daniell 1972). Gerdhem has found that smoking has negative effect on bone mass independent of difference in weight, but no differences in bone mass were found between former smokers and non-smokers (Gerdhem and Obrant 2002). Daniell, in another study, found results similar to Gerdhem in that smoking has negative effect on bone mass independent

of weight for younger women 40-49, but in older women age 60-69 he found that weight also had an effect on bone mass in that non-obese women had less bone mass than obese women (Daniell 1976). Cornuz and Baron also found that smoking cessation reduces the risk of hip fractures (Cornuz, Feskanich et al. 1999; Baron, Farahmand et al. 2001). Ortego-Centeno (Ortego-Centeno, Munoz-Torres et al. 1997) found that smoking by healthy young males is associated with decreased bone mass. Heavy smokers (greater than 20 cigarettes per day) had lower BMD in all skeletal sites compared to nonsmokers. In meta-analysis done by Ward (Ward and Klesges 2001), studies show smoking increased the risk of vertebral fracture by 13% in woman, and 32% in men; and hip fracture by 31% in woman, and 40% in men, however the cessation of smoking partially reverses these risks. In another meta-analysis done by Law (Law and Hackshaw 1997), studies found that smoking has no effect on premenopausal women, but postmenopausal women have .2% increase of bone mass loss per year due to smoking. One out of eight hip fractures in women are attributable to smoking. In former smokers these effects of bone density and risk fracture were less than current smokers but more than people who never smoked.

Smoking has also been shown to increase the risk of injury and stress fractures. In the army, many injuries happen during basic training. People who smoke 1-10 cigarettes per day have increased risk of injury, while those who smoke more than 10 cigarettes per day have a higher chance of injury compared to those who do not smoke during basic training(Reynolds, Heckel et al. 1994). Lappe (Lappe, Stegman et al. 2001) and Friedl (Friedl, Nuovo et al. 1992) found that female army recruits that had developed stress fractures were more likely to report current smoking or past smoking habits.

Of 160 patients with hind foot fusions, smokers had a higher rate of non-unions than did non-smokers (18.6% to 7.1%) (Ishikawa, Murphy et al. 2002) (Ishikawa 2002). The rate of non-unions for former smokers was lower than current smokers, but higher than people who never smoked.

1.1.2 Studies Done on Rabbits with Cigarette Smoke

Studies were done by Ueng (Ueng, Lin et al. 1999) on rabbit tibia to see if cigarette smoke had an effect on bone mineral density (BMD) and torsional strength. The cigarette smoke was delivered via a smoking chamber for 7 minutes every 30mins for 8 hours a day. An operation was done to lengthen the rabbits' right tibia by 5 mm. Ueng found that BMD of the smoke inhalation group was significantly lower than the control group at 4, 5, and 6 weeks after the operation. Ueng also found that the torsion strength in the smoking group was significantly lower than the control group. Ueng (Ueng, Lee et al. 1997) performed another study which found that torsion strength in healing rabbit tibia was lower for the smoking group at 4 weeks ($P < .01$) , 6 weeks ($p<.01$) and 8 weeks ($p < .05$). A histology study was done which found that new bone formations were higher in the non-smoking inhalation rabbits than those in the smoke inhalation group. These results suggest that smoking delays minerization during the bone healing process. These studies thus far, show that smoking has an impact on bone mass, increased risk to fractures, and delayed bone fusions, however they do not tell which chemicals contained in cigarettes may be responsible for this impact.

1.1.3 Studies Done on Rabbits with Nicotine for Bone Fusions Showing to Have a Negative Effect

All of the studies listed in this section had nicotine delivered via ostmotic minipumps. Many studies have been done to show the effect of nicotine on fusion rates. Silcox (Silcox, Daftari et al. 1995) used New Zealand White rabbits for spinal fusions and found that the nicotine group had a lower fusion rate than the control group. The average nicotine serum levels for the nicotine group was 88.8 ng/ml, and the median was 74.1 ng/ml. The rabbits' weights did not change over time for either group.

In 1998, another study done by Silcox found the effects of nicotine can be overcome with an osteoinductive bone growth factor in an animal model (Silcox, Boden et al. 1998). A dosage of 4.5/µg/kg/minute was used to achieve a dosage of 10-70 ng.ml of nicotine serum level, which is the same as smoking 20 to 30 cigarettes a day.

Daftari (Daftari, Whitesides et al. 1994) found that nicotine inhibits, but does not prevent, the revascularation of cancellous bone grafts. For Daftari study a dosage of 6 ug/kg/min was used to achieve the dosage of 10-70 ng/ml. Also no difference in weight was seen between the two groups after 4 weeks. Riebel (Riebel, Boden et al. 1995), did another study that also showed similar results as Daftari.

Raikin (Raikin, Landsman et al. 1998) found in long bone fracture healing that the nicotine group had a 17.2% lower in callus formation between the control groups, and an increase in non unions in the nicotine group. A 3-point bending test showed the nicotine group to be 26% weaker than control group. The dose given to the rabbit was 6.0 ng/kg/minute. The nicotine serum level average for the 8 weeks was 61 ng/ml. The

nicotine rabbit in this group gained less weight then the control group but it was not significant at p= .065.

Wing (Wing, Fisher et al. 2000), showed that rabbits exposed to nicotine but had been weaned prior to fusion had higher fusion rates than those who had not been weaned, but lower than those who had not been exposed to nicotine. The biomechanical properties between the groups had no significant differences. The nicotine serum level seen during the study was 32 ng/ml.

1.1.4 Studies done on Rats with Nicotine for Bone Strength

A number of studies have been done to see if nicotine is the main agent for causing weakened bone strength by using rats that are subjected to different nicotine doses in healthy bone. It has been shown in rats that nicotine has little or no effect on bone strength, no matter the amount of time, the way the nicotine was distributed, age of the rat, or if the rats were ovariectomized or not.

Fung's (Fung, Mendlik et al. 1998; Fung, Iwaniec et al. 1999) study showed that was no differences were found in histomorphomertic end-points, bone mineral density, bone mineral content and vertebral strength of 7-month-old rats. Fung did find a decrease of the serum 25-hydroxyvitamin D by 30% for a 2 & 3 months period. The nicotine was delivered via ostmotic minipumps for both the 2 and 3 months study. For the 2-month study the average nicotine serum level was 33.1 ng/ml for the 3.0 mg/kg/day group and 55.6 ng/ml for the 4.5 mg/kg/day group. The average nicotine serum levels for the 3 month study was 60 for the 3 mg/kg/day and 85 for the 4.5 mg/kg/day. No significant difference was found in the body weights for the 2 or 3 months studies.

Syversen (Syversen, Nordsletten et al. 1999) used a chamber with nicotine vapor to expose 2-month-old growing rats to nicotine and found no significant difference in ultimate bending moment, ultimate energy absorption, stiffness, or deflection between the two groups. There was no difference found in BMD between control rats or the nicotine exposed rats. This study found that rats exposed to nicotine weigh approximately 10% less then the controls.

Iwaniec (Iwaniec, Fung et al. 2000; Iwaniec, Fung et al. 2001) did a study using 7 month old rats giving them various doses of nicotine for 2 or 3 months and found that the turnover rates in cancellous or cortical tibial bone, femoral density, and bone mineral content did not change when subjected to varying nicotine doses. There was a lower femoral ultimate load and vertebral bone mineral content (BMC) in high dose nicotine group (6.0 mg/kg/day) than in control rats. Not difference was detected in 25-hydroxyvitiamin D serum levels. Iwaniec concluded that nicotine serum level, 2.5 times greater than the average smoker had limited effects on bone. The nicotine serum level for this study is 111 for the 4.5 mg/kg/day and 137 for the 6.0 mg/kg/day. This study also found that rats that received nicotine weighed approximately 7 % less than the controls, but did not lost any weight.

Iwaniec (Iwaniec, Fung et al. 2000) in another study was looking at the effect of serum mineral and calciotropic hmormones levels, bone resorption, bone mass, and bone strength. No difference was detected in the serum mineral levels or hormone concentration for all groups. Also no differences were found in BMD, BMC or bone strength. They concluded that no difference were detect for 2 or 3 months in a growing

rat. The body weights for each group went up over time but no difference were detected. The nicotine serum level for the 3.0 and 4.5 mg/kg/day was 67 and 89 ng/ml.

Akhter (Akhter, Iwaniec et al. 2003) did a study to see if nicotine affected ovariectiomized rats 8 months in age. What he found was that nicotine had no effect on BMC, BMD, or any of the structural and material strength properties in either the OVX or Sham.

1.1.5 Studies done with Nicotine showing positive effects

A study done by Waldum (Waldum, Nilsen et al. 1996) on the long term effect of nicotine, over 2 years, on rats showed that nicotine did not increase mortality, atherosclerorsis, or amount of tumors compared to controls. Heeschen (Heeschen, Jang et al. 2001) has shown that nicotine stimulated angiogenesis both in vitro and in vivoin animal models not involving bone.

Two studies done by France and Norman (France and Norman 2002) found the nicotine groups had higher fusions rates than control groups for rabbits. The nicotine in these studies was delivered via nicotine patch. In another study done by (France and Norman 2003) it was found that a nicotine patch of 5.25 mg/day has a higher fusion rate then the other nicotine groups and control, however this rate was non significant. Also the biomechanical testing and radiographic evaluations showed similar results. The serum level for the 5.25 ng patch is 7.8 ng/ml. The daily average serum level for heavy smokers (10-70 ng/ml) (Benowitz and Jacob 1984; Daftari, Whitesides et al. 1994; Sipe, Buck et al. 2000). An another study done by Mukherjee (Mukherjee, France et al. 2003) found that in rabbit bone marrow derived large dose of nicotine 100 µg/ml significantly increases osteoblastic activity over controls.

1.1.6 Summary of background

Studies in humans suggest that smoking cigarettes increases your risk for osteoprorois, stress fractures and reduces bone healing, however the cession of smoking shows a decrease in these risks. Many studies in rabbits have been done on nicotine effects on bone healing and bone strength. From the studies done with nicotine on bone strength it is shown that nicotine does not have an effect on bone strength. It is debatable if nicotine has any effect on bone healing. Most of the studies show that nicotine has a negative effect on bone healing but recent studies done by France, Norman, and Mukherjee have shown nicotine to help bone healing. In these studies the nicotine was administer via nicotine patches and the increase of osteoblastic activities, which may explain why the nicotine groups having better or equal fusion rates compared to controls.

1.1.7 Bone strength versus Fracture Toughness

Bone is a flawed material microscopically and usually under goes a series of loading and unloading. Another way to measurement bone quality is fracture toughness, which is the mechanical property ability to resist crack initiation and growth (Norman 1991). According to Anderson, Zipoupos and Curry (Anderson 1991; Zioupos and Currey 1998) bone strength and fracture toughness cannot be predicted from one another. To the best of my knowledge and research no one has studied the effects of nicotine and smoking on fracture toughness of bone.

1.2 Objectives

The objective of this research is to study the dose dependent effect of nicotine delivered via patch and from cigarette smoke on bone's mechanical properties. The effect of nicotine and smoking on bone histology will also be investigated.

1.3 Hypotheses

1. Nicotine has a non-debilitating effect on bone mechanical properties where as, smoking does have a debilitating effect on bone mechanical properties.
2. The effects of nicotine and smoking on bone fracture toughness and strength can be explained by changes in bones' histomorphomety and composition.

1.4 Tasks

Using bone taken from rabbits exposed to nicotine via a transdermal patch and cigarette smoke, the following tasks will be performed. Two experiments were done. The first experiment compared different nicotine patch, and the second experiment compared nicotine delivered via a patch and smoke inhalation.

Task 1: Biomechanical testing including fracture toughness, femoral neck loading, 3-point bending test, and torsion test for experiment 1 and fracture toughness test for experiment 2.

Task 2: Histomophometric and compositional analysis of cortical bone from the tibia for experiment 1 and histomophometric for experiment 2.

Task 3: Statistical analysis comparing mechanical and histological properties in each test group.

1.5 Anatomical Background

In this section bone biological properties will be discussed. There are four main functions of bone. The first two are mechanical support and protection of vital structures, but bone is also responsible for Hematopoiesis, which is the production of red blood cells, and Mineral Homeostasis, which is the bodies' primary place to store calcium. Bone is a living organ, which has the ability to adjust to new loads and repair itself after damage by constantly removing and restoring bone through resorption of old bone and formation of new bone.

1.5.1 Macrostructure of Bone

Macrostructure of bone is made up of two main type of bone, cancellous and cortical bone. Cancellous bone is found at the ends of most long bones and in short bone it fills most of the bone, an example of short bone is the spine. Cancellous bone or trabeculear bone is spongy bone with many cavities surrounded by cortical bone as shown in figure 1.5.1 – 1. Cortical bone or compact bone is very dense, is mainly bone matrix with canals, which are called haversian canals as shown in figure 1.5.1 – 2.

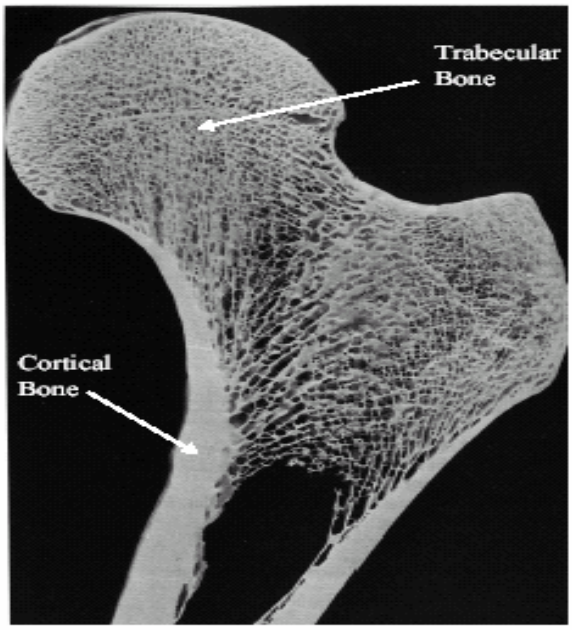


Figure 1.5.1 - 1: The Proximal Femur showing cortical and cancellous bone with labels added (Albright and Skinner 1987)

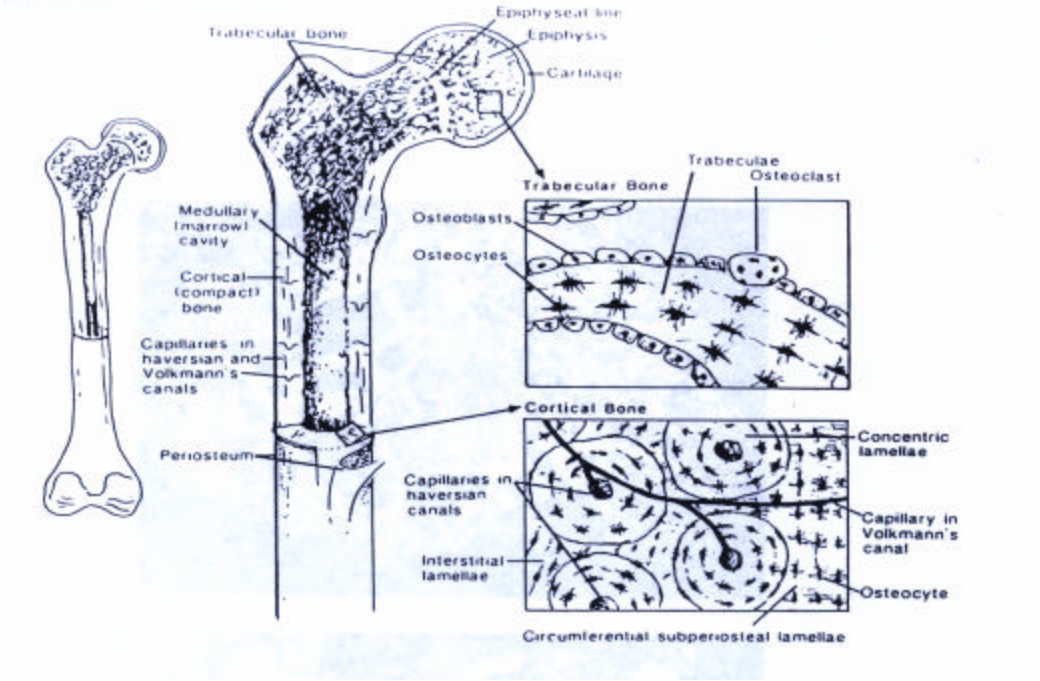


Figure 1.5.1 – 2: Coritcal and cancellous shown in more detail (Mow and Hayes 1991)

1.5.2 Microstructure of bone

Mature bone matrix is normally made up of about 35% organic and 65% inorganic materials. Of the organic material about 90% of it is type 1 collagen. Collagen gives the bone its flexibility, and strength in tension. The 65% of the inorganic material is called hydroxyapatite, which has the molecular formula $3Ca(PO_4)\bullet Ca(OH)_2$. This mineral gives the matrix toughness in compressive strength and stiffness.

Microstructure of bone also is made up of 2 main types of bone, lamellar and woven bone. According to Burr and Martin, woven bone is bone that is irregular in formation, and unorganized pattern of collagen orientation (Martin and Burr 1989). Woven bone can be placed down *de novo* without any hard tissue already there to support it. No other type of bone can be formed *de novo.* In adults woven bone is usually found after a pathologic skeletal process. Main function of woven bone is for skeletal repair and defense. The most common example of woven bone in adults is callus found in bone fractures (Burr and Martin 1989).

Primary lamellar bone requires a surface to be deposited upon, unlike woven, which does not. Primary lamellar is found in both cancellous and cortical bone. This bone collagen orientation is arranged in a circular pattern around the inner (endosteal) and outer (periosteal) circumference of whole bone. This primary lamellar bone can be very dense. Primary lamellar bone is bone found in the first generation of bone, before bone undergoes remodeling. When the bone is the product of bone resorption or remodeling it is called secondary lamellar bone.

1.6 Bone Modeling and Remodeling.

Secondary lamellar bone comes from remodeling of bone by Basic Multicellur Unit (BMU). First, a signal is needed to begin bone remodeling. According to Albright and Skinner, this is done by the parathyroid hormone (PTH), which is emitted from the parathyroid gland (Albright and Skinner 1987). When blood calcium levels get too low the secretion of PTH happens. This stimulates osteoclast (see figure 1.5.2 – 2) activity. Osteoclast is a cell that is responsible for removing or absorption of bone. After the osteoclast is done there is lag time between removal and new formation of bone. Because of the lag time and reversal in formation a cement line forms. According to Wang cement lines are less stiff than bone, which allow them to arrest crack propagation (Burr, Schaffler et al. 1988; Wang 1995). After a short period of time bone formation begins by osteoblast (see figure 1.5.2 – 2). Osteoblasts are much smaller than osteoclasts, and do not refill the canal completely. Once an osteoblast is surrounded by bone matrix it becomes an osteocyte (see figure 1.5.1 – 2). Osteocytes are thought to be signaling cells in bone, and detect mechanical stimulation. The remaining osteoblast become cell lining for the haverisan canal. The haversian canal is surround by the secondary bone that is lamellar in nature. The entire secondary osteon is about 200 to 300 μm in diameter. Cells seen around the center of the haversian canal are osteocytes and are in circular patten to ensure maximum supply of nutrients to more bone tissue.

The resoprtion and formation of normal bone is usually in balance. Both cortical and cancellous bone under-goes remodeling. Bone remodeling starts at about the seventh week of embryonic life continuing till death. Bone undergoes remodeling to repair damaged bone and to adjust the bone structure for new load supports. This balance is

important if there is too much remodeling then the bone becomes too porosis, if there is not enough remodeling then cracks can grow causing factures. When osteoclast become more active and osteoblast become less active or stays the same, the skeletal mass decreases thus increasing the risk for fractures.

1.7 Animal Model

These studies will utilize bone from a different study that investigated the effect of nicotine and cigarette smoke on spine fusion. The first group will be a study comparing the effect of different nicotine doses via patch on bone. The second test group will be the nicotine given via patches versus inhaled cigarette smoke. In this study the nicotine was administrated though a nicotine patch, (Habitrol, Parsippary, NJ) and via a chamber containing cigarette smoke. The type of cigarette used in this study was Marlboro (Phillip Morris, Richmond, VA**)**. It has been shown that nicotine levels are higher with nicotine delivered by a patch and the concentration of nicotine stays in the blood longer (Sipe, Buck et al. 2000). The rabbits that were used are New Zealand White rabbits, which are male retired breeders, and are skeletally mature. The rabbit's age is about 24 to 29 months. These rabbits were obtained from Covance (Denver, PA).

Figure 1.7 – 1 shows the rabbit skeleton. The bones used in this study were the femur, and tibia. All the bones were retrieved during dissection. Figures 1.7 – 2 and 1.7 – 3 show the anatomy for the femur and tibia. The proximal end is the end of the bone closest to the midpoint of the body. The mid-shaft is in the middle bone between the proximal end and the distal end. The distal end is the part of the bone closest to the ventral side or in this case near bottom.

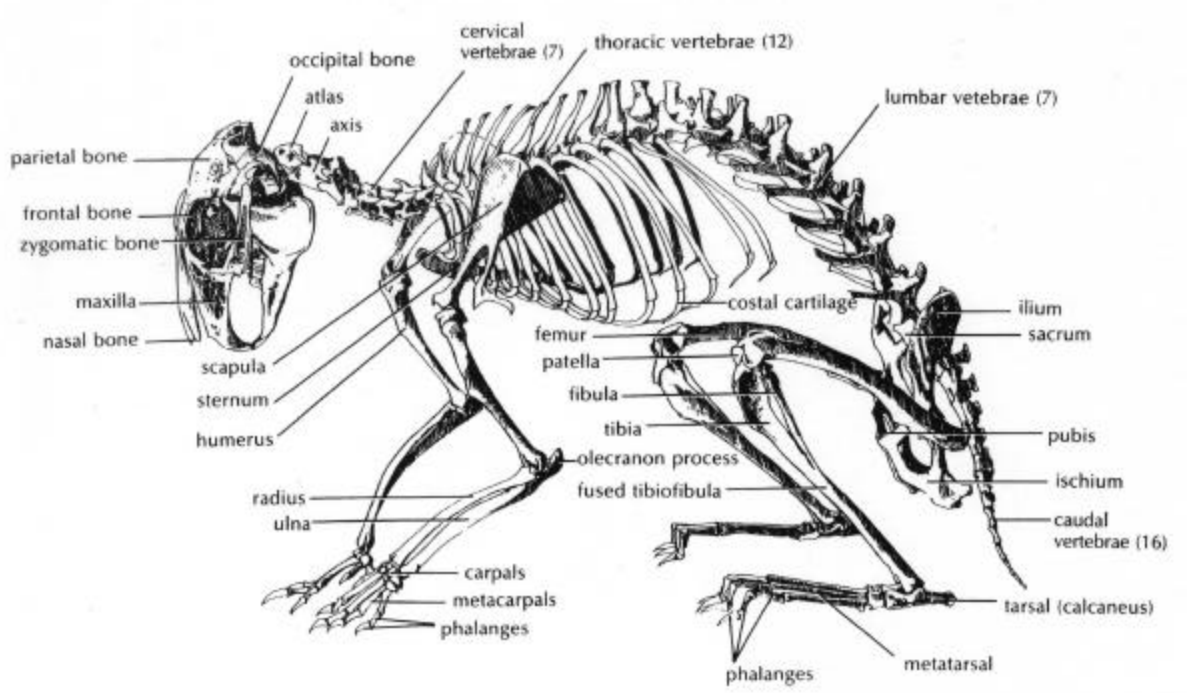


Figure 1.7 – 1: Diagram of rabbit skeleton (Wingerd and Stein 1985)

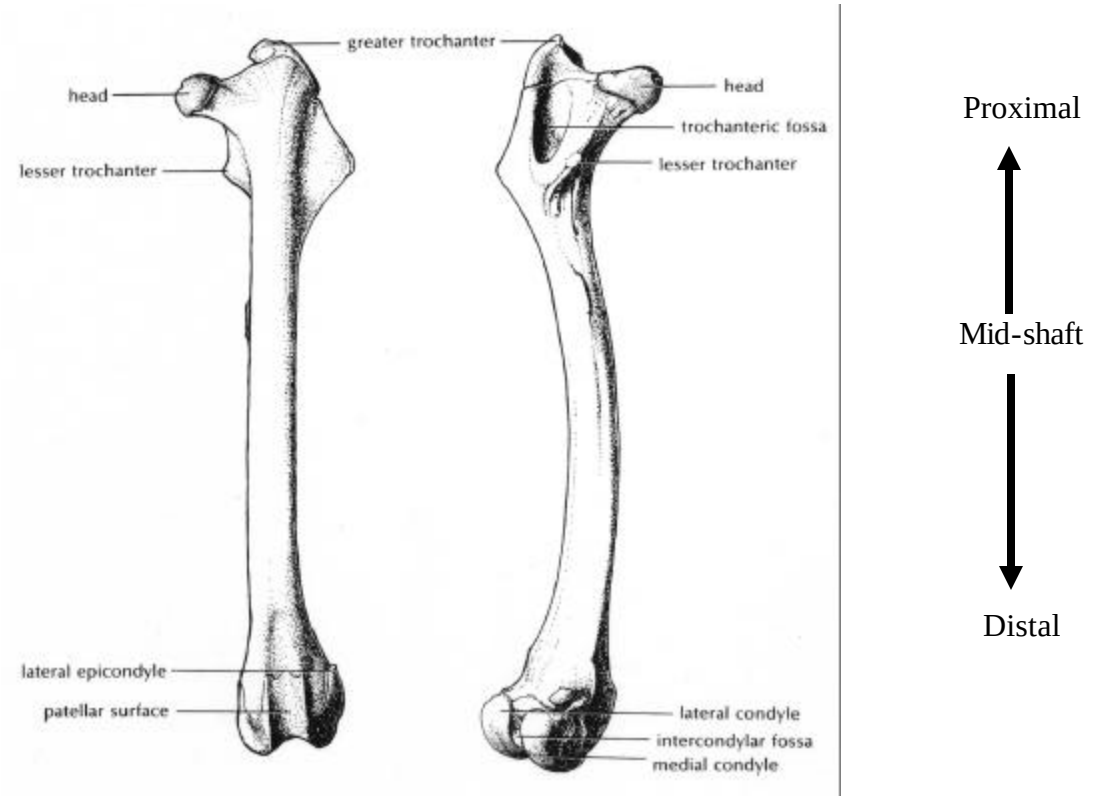


Figure 1.7 – 2: Definitions of locations and Anatomy of the Femur (Wingerd and Stein 1985)

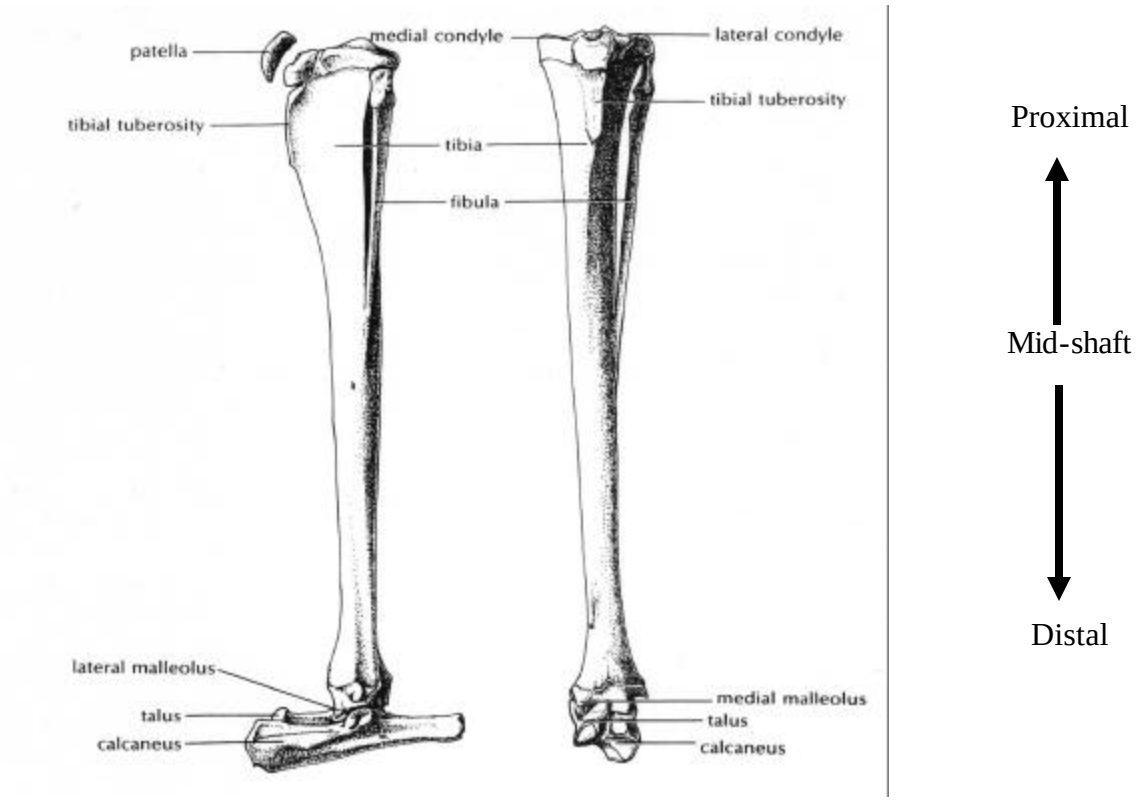


Figure 1.7 – 3: Definitions of locations and Anatomy of the Tibia (Wingerd and Stein 1985)

Chapter 2 Mechanical Testing

2.1 Bone as an Engineering Material

Bone is anisotropic material. Bone is unique engineering material in that it has the ability to heal. Bone is also considered to be a composite material having fiber and matrix. The fibers are the osteons, and the remaining bone being the matrix.

2.2 Introduction to Fracture Toughness testing

A study done by Leonardo de Vinci with similar iron wires and found that the longer wire could not hold the same weight as the short wire. The theory was that the longer wire had a greater chance of having flaws thus weakening the material. Flaws cause higher localized the stresses. If the stress around the flaw is high enough, the material could fail well be before the ultimate strength is researched. Fracture mechanics is the study of unexpected failure in material due to preexisting flaws (Anderson 1991; Boresi and Schmidt 2003). Fracture toughness is a material property, which measures the ability to resist crack initiation and growth. Linear elastic fracture mechanics (LEFM) applies to materials that obey Hook's Law, and has a small plastic zone compared to the thickness. To ensure that LEFM theory can apply one must make sure that the plastic zone is contained and is not too large. Once the plastic zone becomes too large one must look at different theories to measure the stress at the crack tip. Elastic plastic fracture mechanics (EPFM) applies to material that shows time-independent and nonlinear behavior (plastic deformation). For most brittle materials fracture will occur at a critical stress. Most ductile materials plastic deformation will occur before fracture. In an ideally brittle material, a crack forms by breaking the atomic bonds, where $?_s$ the amount

of energy it is needed to break a bond (see figure 2.2 – 1a). A Quasi-brittle elastic plastic material shows both brittle and ductile behavior. The amount of work needed for a crack to grow in this material is both $?_s$ and $?_{p,}$ where $?_p$ is the plastic work (see figure 2.2 – 1b) and is the area right in front of the crack tip known as the plastic zone. In a real crack growth brittle material, occurs by meandering and branching. The amount of work it takes for the crack to grow is $?_s$ (True area/Projected area) (see figure 2.2 – 1c).

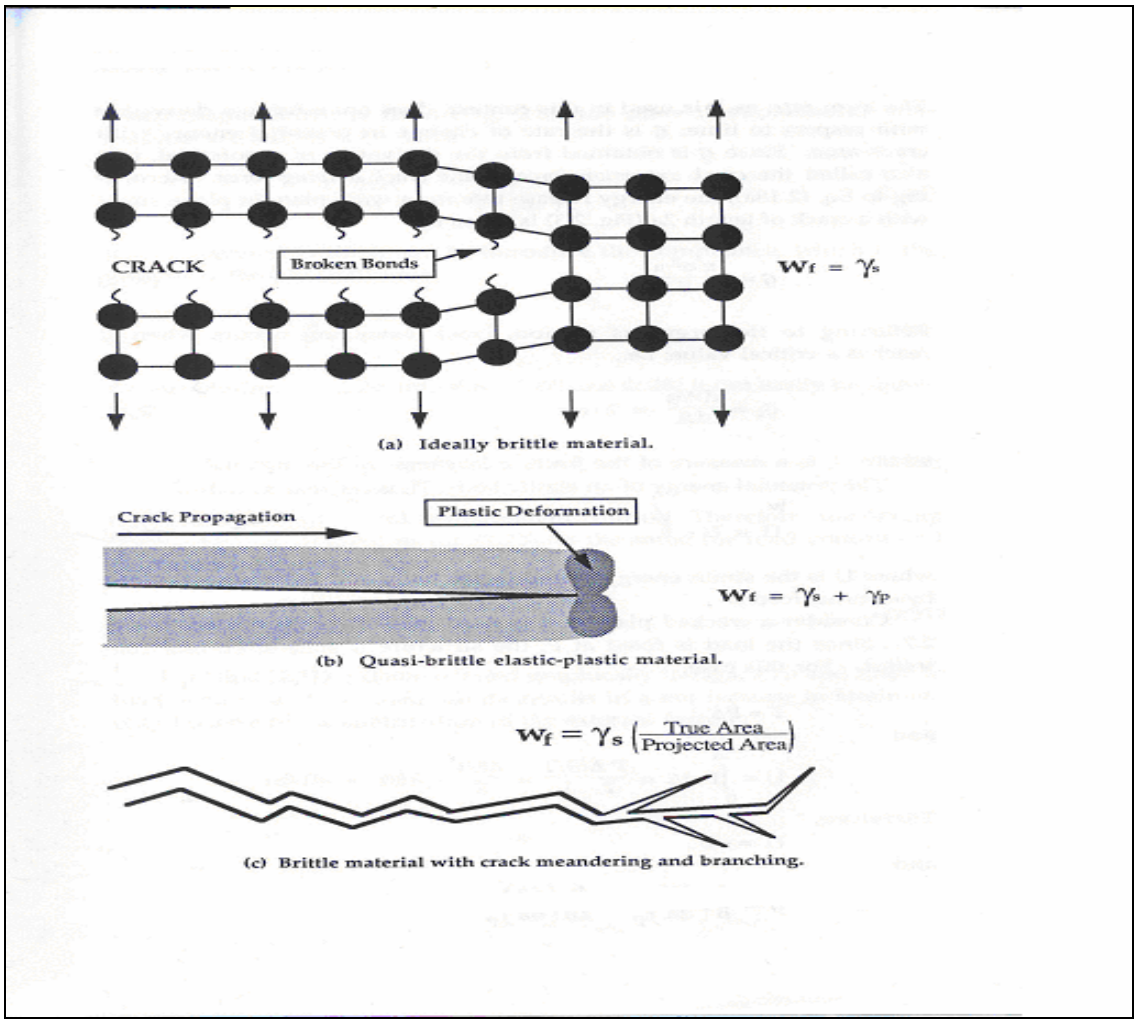


Figure 2.2 – 1: Crack growth in different type of materials (Anderson 1991)

2.2.1 Stress Concentration Factor

The stress concentration factor is the elevated stress at a point divided by the remote normal stress equation 1, and is a measure of the effect of the hole (defect) on the stress state. Stress concentration factor is found around the edge of a hole in a plate, with the hole having a finite radius (see figure 2.2.1 – 1).

$$K = \frac{s_a}{s_{nom}} \quad (1)$$

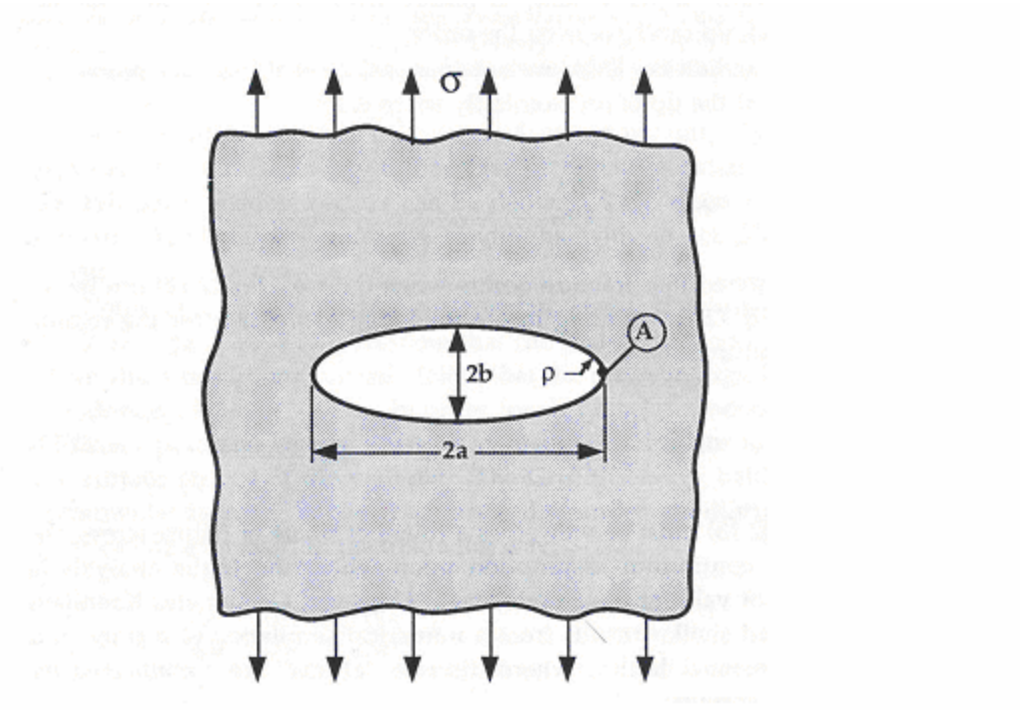


Figure 2.2.1 – 1: Elliptical hole in a flat plate (Anderson 1991)

To find the stress concentration factor around a hole at point A on figure 2.2.1 –1 in a plate the following equation is used.

$$s_A = s\left(1 + 2\sqrt{\frac{a}{r}}\right) \quad (2)$$

Where in figure s is the stress applied, *a* is the major axis, *b* is the minor axis, and ρ is the radius of curvature. This will give higher stress concentration at point A.

When the radius of the hole approaches 0 thus forming a crack, the stress concentration factor will results in the stress around the crack tip to be infinite. This result is not useful because no material can withstand a stress of infinite. Therefore, this method does not give a usable number to determine the stress around the crack tip. A new method needs to be developed to determine the stress around a crack tip. The new method is fracture mechanics (fracture toughness). There are two ways to measure fracture toughness, the strain energy release rate and the stress intensity approach.

2.2.2 Strain Energy Release Rate

Griffith introduced the concept of strain energy release rate, G. The strain energy release rate is the amount of strain energy lost by members per unit area of the newly formed crack as the crack propagates i.e.

$$G = -\frac{d\Pi}{dA} \quad (3)$$

where ? is the potential energy of an elastic body, and A is the crack area. The potential energy is defined as

$$\Pi = U - F \quad (4)$$

where U is the strain energy stored in the body, and F is the work done by external force. Figure 2.2.2 – 1 shows that the external work done is the load P multiplied by the displacement ?

$$F = P\Delta \quad (5)$$

The strain energy is the area

$$U = \int_0^{\Delta} P d\Delta = \frac{P\Delta}{2} \quad (6)$$

Thus the potential energy become

$$\prod = -U \quad (7)$$

The strain energy release rate thus become

$$G = \frac{1}{B} \cdot \left(\frac{dU}{da}\right)_P = \frac{P}{2B} \left(\frac{d\Delta}{da}\right)_P \quad (8)$$

Compliance is the inverse of the plate stiffness.

$$C = \frac{\Delta}{P} \quad (9)$$

Substituting compliance into (9) for P results in the strain energy release rate equation (10).

$$G = \frac{P^2}{2B} \cdot \frac{dC}{da} \quad (10)$$

Where P is the load, B is the thickness, C is the compliance, and $\underline{a}$ is the crack length. Rate in this content is not dependent on time but change of potential energy with crack area. Crack growth occurs when G reaches a critical value G_{IC}.

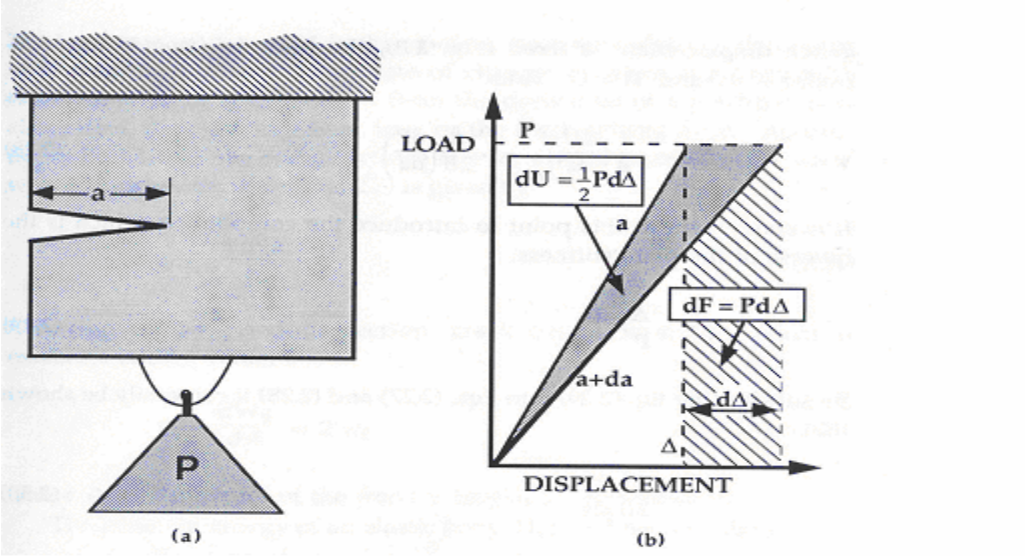


Figure 2.2.2 –1: Cracked plate with a fixed load P (Anderson 1991)

2.2.3 Stress Intensity Factor

The stress intensity factor is mechanical property predicts when a crack will self propagate. The higher the stress intensity factor value the higher the load is needed to get the crack to self propagate. To measure the stress intensity factor the specimen has to be in a state of plane strain.

The equation to determine the stress around the crack is

$$s_{ij} = \left(\frac{k}{\sqrt{pr}}\right) \cdot f_{ij} + other\ terms \quad (11)$$

where s_{ij} is the stress tensor, r and ? are defined by figure 2.2.3 – 1, k is the proportionality constant and f_{ij} is dimensionless function. When k is replaced by the Stress intensity factor K.

$$k = \frac{K}{\sqrt{2p}} \quad (12)$$

When k is placed in equation 11 it becomes equation 13.

$$K_{(I,II\ or\ III)} = C \cdot s\sqrt{pa} \quad (13)$$

Where C a is dimensionless constant that depends on geometry and mode of loading, σ is the stress applied, and $\underline{a}$ is the crack length. As with G when K reaches its critical value, crack growth occurs and $K = K_C$.

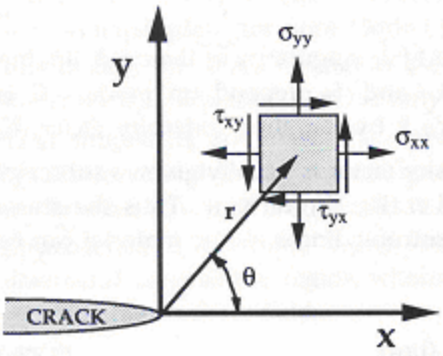


Figure 2.2.3 – 1: Coordinate definition in front of the crack tip (Anderson 1991)

The stress intense factor and the strain energy release rate can be related by the follow equation

$$G = \frac{K^2}{E} \quad (14)$$

There are three different type modes of crack growth, which are mode 1 (opening mode), mode 2 (sliding mode) and mode 3 (tearing mode) (figure 2.2.3 – 2). In this study we will be dealing with mode 1 (K_{IC}).

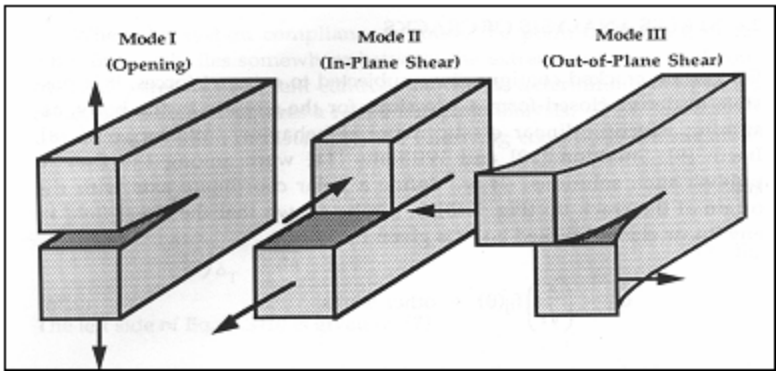


Figure 2.2.3 – 2: Three different testing modes (Anderson 1991)

The test specimen used to test for the fracture toughness in mode 1 is known as the compact tension (CT) specimen (figure 2.2.3 – 3).

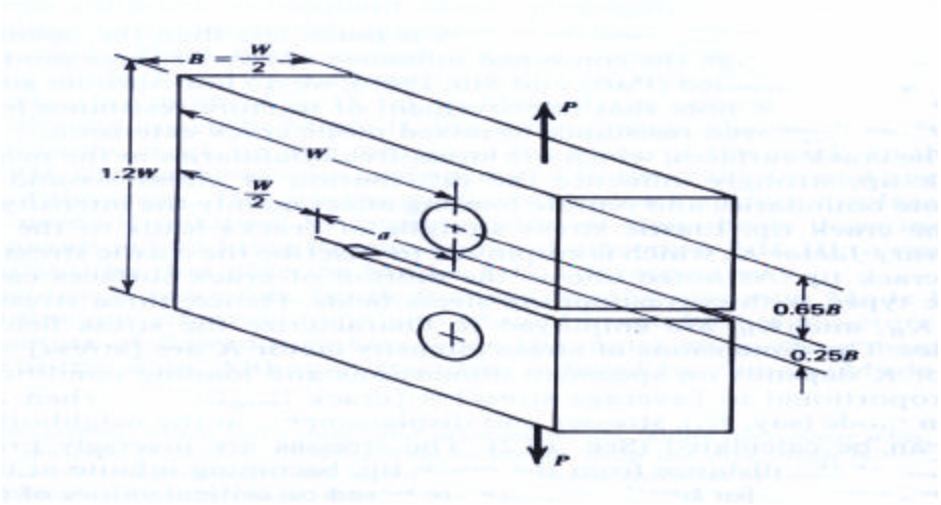


Figure 2.2.3 - 3 CT specimen and dimensions

The equation used to calculate the fracture toughness of a material in mode I crack growth is

$$K_{IC} = \frac{P_Q}{B \cdot \sqrt{W}} \cdot f(a/W) \quad (15)$$

where P_Q is the critical load, B is the thickness, W is the length between the applied load and the end of the specimen and f(a/W) is a dimensionless function of a/W. The equation for f(a/W) is

$$f(\frac{a}{W}) = 29.6 \cdot (\frac{a}{W})^{.5} - 185.5(\frac{a}{W})^{1.5} + 655.7(\frac{a}{W})^{2.5} - 1017(\frac{a}{W})^{3.5} + 638.9(\frac{a}{W})^{4.5} \quad (16)$$

If K equals K_C then the crack will propagate. Tests can be done for each material to determine the critical value for K known as fracture toughness, or K_{IC}, for mode I loading. The American Society of Testing Mechanics (ASTM) standard E-399 gives guidelines on how to make the compact tension specimen. Certain dimensions have to be meet for the test to be valid. To make a compact tension specimen, size requirement have to be met to make sure the specimen is in the plane strain regions (see figure 2.2.3 – 4). K has been shown to linearly increase with increasing width of the specimen up to a certain thickness during the Plane Stress stage 1. Once a certain thickness has been reached a transitional stage 2 begins, where K begins to decrease. Once the K begins to level off then you enter the Plane Strain stage 3 when K is a constant equals K_{IC}.

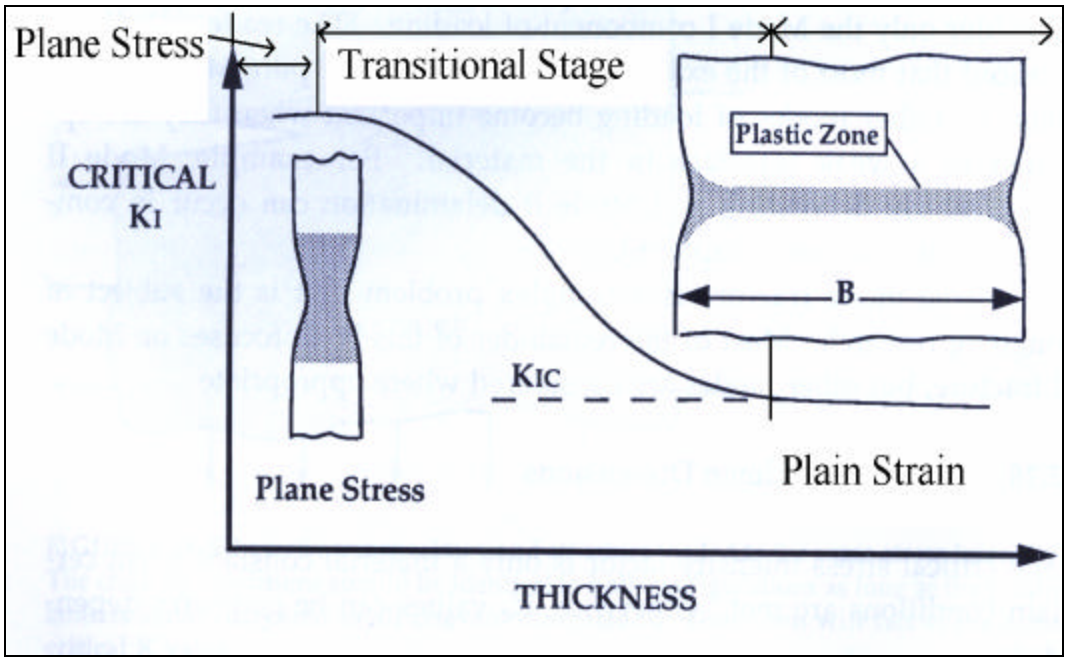


Figure 2.2.3 – 4: Regions for K_{IC} of plain strain and stress (modification made) (Anderson 1991)

Most cracks are in 3-dimension however, in a compact tension specimen assume the crack propagates is in 2-dimensions. In the plane strain case all the values of strain in the z direction are equal to zero and the plastic zone is small compared to the thickness. The plane stress stage is where the stress values in the z direction are zero. The Plane stress exists if the plastic zone is in the same order as the thickness. To ensure that the specimen is in plane strain mode the thickness of the specimen must be equal to half of W, or if K_{IC} is given the thickness must be greater than or equal to thickness found in equation (15).

$$a, B, (W - a) \geq 2.5 \cdot \left(\frac{K_{IC}}{s_{ys}} \right)^2 \quad (17)$$

Other requirements for compact tension specimen is that the a/W must be between

$$0.45 = a/W = 0.55. \quad (18)$$

Norman found in 1991, that for CT specimen of 17.5 mm by 16.8 mm the minimum thickness needed to obtain plane strain of bovine bone is 7 mm, which is difficult to do in most animals and humans (Norman 1991). The correct thickness needed for these specimens to ensure plan stress state is 2.25 mm but this can not be obtained because most rabbits bone are about 1 mm thick. The thickness of this studies specimen will be .5 mm, while the W will be 5.5 mm. These specimens may not satisfy the requirements for plain strain fracture toughness, but this does not mean that comparison within groups is not valid.

The plastic zone correction factor takes into effect for mode I test by P_{max} must be

$$P_{max} = 1.10\ PQ \quad (19)$$

this is to allow for corrections for the plastic zone effects and deviations from the linearity in the load displacement curve. To obtain PQ for the equation, find it on a load displacement curve. There are three different load-displacement curves for the fracture toughness test shown in figure 2.2.3 – 4.

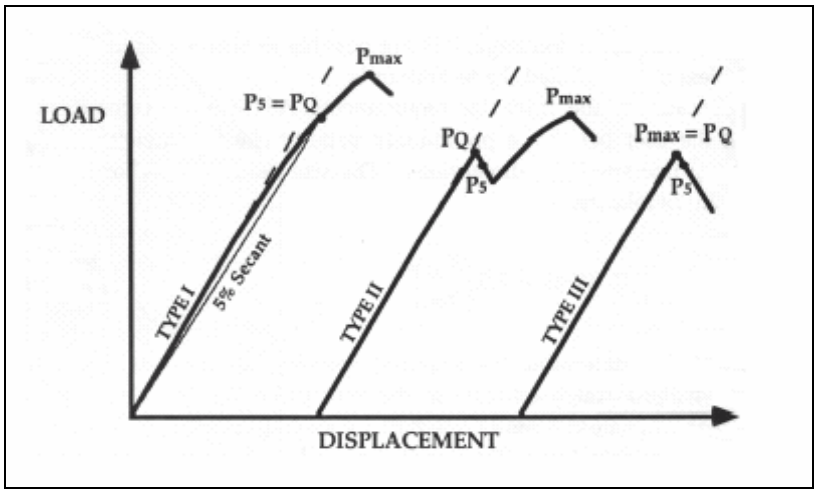


Figure 2.2.3 – 5: Three different loads versus displacement curves for fracture toughness test with the 5% secant line. (Anderson 1991)

Once the linear portion for the load displacement curve is found and it is multiplied by .95. The new load displacement curve is plotted onto the original curve and used to find the PQ shown in figure 2.2.3 – 5. The high noise seen in figure 2.2.3 – 6 is due the to the small displacement.

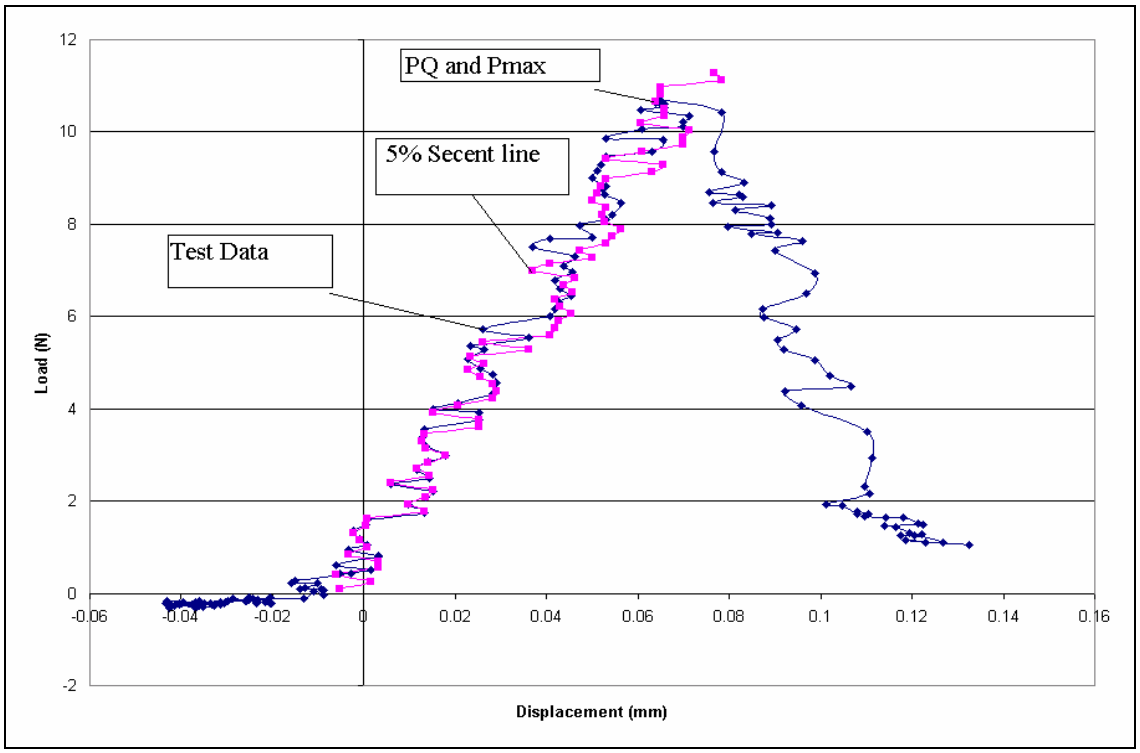


Figure 2.2.3 – 6: Fracture toughness result with the 5% secant line plotted

Fracture toughness values are dependent on material microstructure. For an isotropic material specimen orientation doesn't matter. Since bone is considered to be a transversely isotropic, fracture toughness does depend on specimen orientation i.e. direction that a crack is propagated the fracture toughness value will depend on (Behiri and Bonfield 1989). The different direction that cracks can be propagated is shown in figure 2.2.3 – 7. The direction that these specimens will be tested is C-L.

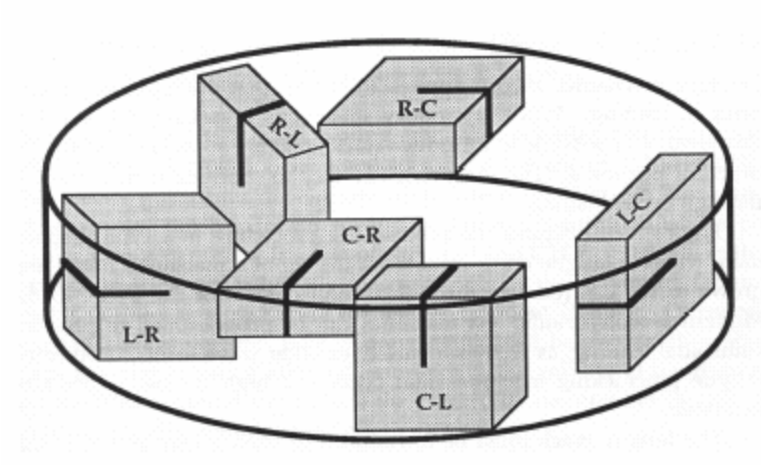


Figure 2.2.3 – 7: ASTM notation for specimen obtained from a disks and hollow cylinders (Anderson 1991)

2.2.4 Fracture Toughness of bone

Several studies have been performed to elucidate how microstructural features of cortical bone influence clinical fractures. Barth (Barth, Williams et al. 1992) found that people who fracture the hip have larger haversian canals and lower osteon density than the other groups. Crabtree (Crabtree, Loveridge et al. 2001) found that in hip fracture cases that people who fracture their hip have the same amount of cancellous bone left, as do non-fractures, but the amount of cortical bone is different by 15%. This show that the cortical bone is an important factor in hip fracture cases.

Responding to these studies, several studies were performed to correlate clinical finding to mechanical behavior to measure fracture fragility i.e. fracture toughness. Yeni, Brown, and Norman (Yeni, Brown et al. 1997; Yeni, Brown et al. 1998; Yeni and Norman 2000) conducting a number of studies to find the effects of bone morphology, composition, influence of microdamage, and bone location on bones fractures toughness. The following in Table 2.2.4 – 1, is a fracture toughness values chart for many different

tests done in the past years, and table 2.2.4 – 2 is a summary of different studies fracture toughness.

The bone morphology effects are the following. The higher the osteon density the higher strain energy release rate and the higher the % of porosity the lower the strain energy release rates.

The bone composition effects are the following. Also found was that the strain energy release rate is affected by the apparent density, amount of H_2O, and age of the bone. The strain energy release rate increased with wet or dry density increase. The strain energy release rate also decreased with an increase in water content.

The microdamage effects are the following. Microdamage of bone also had an effect on the strain energy release rate; as the microdamage density went up the strain energy release rate went down. In addition, as the micocrack length increased the strain energy release rate went down.

The bone locations are the following. Fracture toughness was also found to depend on where the specimen is taken. The fracture toughness values for femoral neck were the highest while the lowest fracture toughness values was in the femoral shaft (Brown, Yeni et al. 2000).

Table 2.2.4 – 1: Different variable effect on fracture toughness.

Variable	Increase	Effect on fracture toughness or Strain energy release rate
microdamage density (#/mm^2)	increase	decrease
microdamage surface density (1/mm)	increase	decrease
Average micorcrack length (mm)	increase	decrease
porosity %	increase	decrease
Age	increase	decrease except for the femural neck
dry density	increase	increase
% H20	increase	decrase in mode I
osteon density (#/mm^2)	increase	increase femur only
wet density	increase	increase

Table 2.2.4 – 2: Stress Concentration Factors for the Literature for the Tibia (Yeni 1998)

Author(s)	Species	Experiment	Measurement	Direction	Loading Rate
Bonfield & Datta (1974)	Bovine	Center Notched Shock Tube	Kc=0.23	Longitudinal	~ 7 s^-1
Bonfield & Datta (1976)	Bovine	Single-edge Notched	Kc=2.2 – 4.6	Transverse	3e-3 s^-1
Behiri & Bonfield (1980)	Bovine	CT	Kc = 4.46 – 5.38	Longitudinal	0.0102 - 1.02 mm/min
Behiri & Bonfield (1982)	Bovine	CT	Kc = 3.3 – 5.7	Longitudinal	
Behiri & Bonfield (1984)	Bovine	CT	Kc = 2.8 – 6.3	Longitudinal	0.01 - 50 mm/min
Bonfield et al. (1985)	Human	CT	Kc = 2.1 – 4.7	Longitudinal	0.504e-3 mm/min
	Canine	CT	Kc = 3.2 – 6.5	Longitudinal	0.0102 mm/min
Moyle & Gavens (1986)	Bovine	Single-edge Notched	Kc = 11.2	Transverse	0.45 mm/min
Behiri & Bonfield (1989)	Bovine	CT (grooved)	Kc = 3.2	Longitudinal	0.0198 mm/min
Norman et al (1991)	Human	CT	Kc = 4.48	Longitudinal	0.5 mm/min
Norman et al (1991)	Human	CT (corrected for 7 mm thickness)	Kc = 3.68	Longitudinal	0.5 mm/min
Norman et al (1992)	Bovine	CT	Kc = 5.3 – 9.4	Longitudinal	0.5 mm/min
Norman et al (1992)	Bovine	CT (grooved)	Kc = 5.2 – 9.3	Longitudinal	0.5 mm/min
Valishth et al (1994)	Bovine	CT	Kc = 4 - 7.6	Longitudinal	0.5 mm/min
Valishth et al (1994)	Human	CT	Kc = 1.6 – 2.5	Longitudinal	0.5 mm/min
Norman et al (1995b)	Bovine	CT	Kc = 4.68 – 6.73	Longitudinal	2.6 mm/min
Norman et al (1995b)	Human	CT	Kc = 4.05 – 4.32	Longitudinal	2.6 mm/min
Feng & Salzmann (1995)	Bovine	CT	Kc = 2.55	Longitudinal	0.2 mm/min
Norman et al (1996)	Human	CT	Kc = 2.12	Longitudinal	0.2 mm/min

2.2.5 Methods fracture toughness preparation

The first testing group of eighteen New Zealand white rabbits were used in this study group I. The rabbits were divided up into 4 groups. The first groups was a control group with n=3, the 5.25 mg group had n=4, 10.5 mg group had n= 5, and the 21 mg group had n= 6. The specimens where randomly tested. The patches were placed on the inside of the rabbits' ear and changed every day.

The second testing group of twenty-six rabbits and where divided up into controls n = 5, nicotine delivered via patches n = 9 (10.5 ng/ml), nicotine delivered via cigarette n = 6 (Group II) for 4 weeks in the chamber, and nicotine delivered via cigarette n= 6 (Marlboro filter cigarettes) in the chamber for 5 weeks. The 10.5 ng/ml patch was used in this study because it gave the most constant levels of nicotine. The smoking chamber could only hold 6 rabbits. The first groups of rabbits only got 4 weeks of cigarette smoke which they started a week after there surgery. This was due to moving the chamber around to place where it could be used. This group also only got second hand smoke for 3 weeks and for the last week got both first and second hand smoke. The second group was in the chamber for the full 5 weeks. This group got both first and second hand smoke for all 5 weeks. The rabbits that received the cigarette smoke stayed in the smoking chamber created here at the lab, for 6 hrs a day 5 days a week. The specimens where randomly tested. The rabbits patches where changed every day and the rabbits in the chamber were constantly monitor every day while the rabbits where in the chamber.

During the 5 weeks after their surgery the rabbits' nicotine level was measured at the 1, 3, and 5 week interval. The daily average nicotine serum level for heavy smokers is 10-70 ng/ml (Benowitz and Jacob 1984; Daftari, Whitesides et al. 1994; Sipe, Buck et

al. 2000). In appendix A is the nicotine level for each rabbit at 1, 3, and 5 weeks, and average nicotine level. All test specimens, at all times, were keep in a .9% saline solution during all stages of specimen preparation and testing. Fracture Toughness test were done using the right tibia. A procedure was developed for the machining procedure that will be used for this project (Smith 2003). The specimen was machine down to the dimensions in figure 2.2.5 – 1. The specimen is taken from the flat lateral aspect of the proximal tibia adjacent to the fibula figure 2.2.5 – 2.

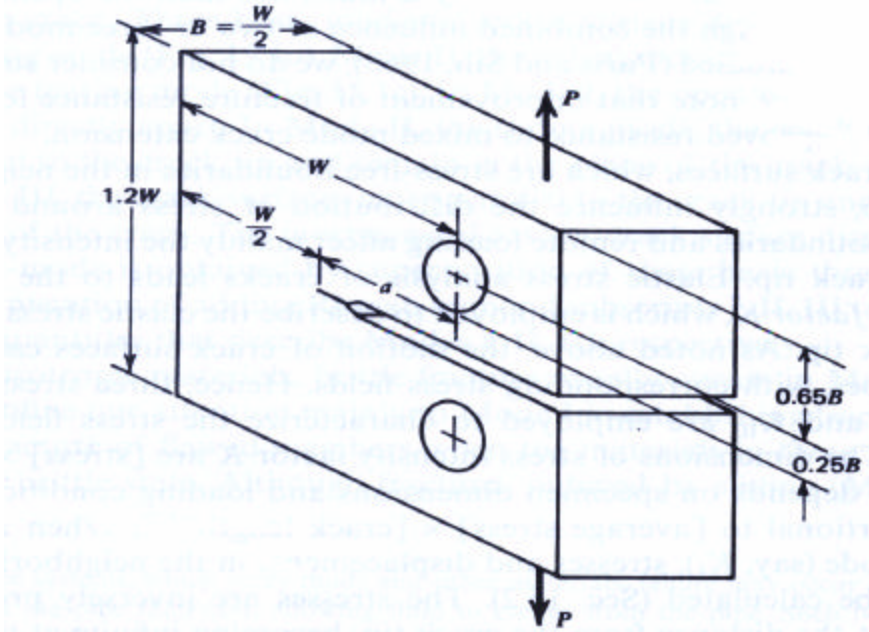


Figure 2.2.5 - 1: Dimensions of the fracture toughness specimen (Boresi and Schmidt 2003) W equals 5.5 mm

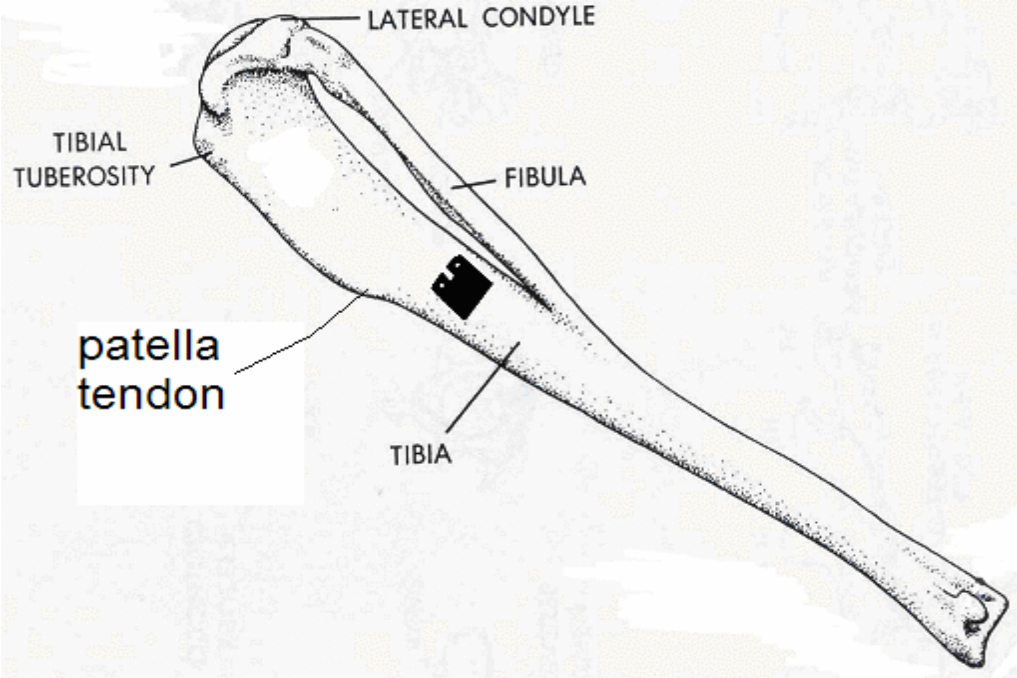


Figure 2.2.5 - 2: Location of the fracture toughness specimen on the tibia with adaptation with CT specimen (Wingerd and Stein 1985)

First, the proximal end of the tibia has been cut off just below the patella tendon attachment (see figure 2.2.5 – 2). These cuts were done by using a band saw/sander 12' blade (Sears/Craftmen). The band saw was used to cut the tibia down the center of the long axis of the bone and then cut once more to remove the specimen from the tibia. Next the specimen's width is trimmed to about 6 mm using the band saw, and then is trimmed down even more by using sandpaper to achieve 5.5 mm. After that the specimen external cortex is flattened by sandpaper. Once the external cortex side is flattened down the specimen is placed into a fixture (Smith 2003) that will hold the specimen while it is being milled on slow speed (260 rpm) to the correct thickness. The milling is done on a Bridgeport milling machine (Bridgeport Machine Inc., Bridgeport, CT).

After the specimen is milled to the uniform thickness, holes are drilled into it using a 1 mm drill bit at high speeds on the mill (2300 rpm) (see figure 2.2.5 – 3). While in the mill, pencil marks are placed on the specimen to mark length, which is 6.8 mm (see figure 2.2.5 – 3). After the marks are in placed on the specimen they are cut using an Isomet Low Speed Saw (Buehler LTD, Evanston, IL). Next the specimen have a chevon notch (see figure 2.2.5 – 3) placed in the center between the two drilled holes using the Isomet Low Speed Saw. Finally after the chevon notch is cut a precrack (see figure 2.2.5 – 3) will be placed in the specimen by a razor blade.

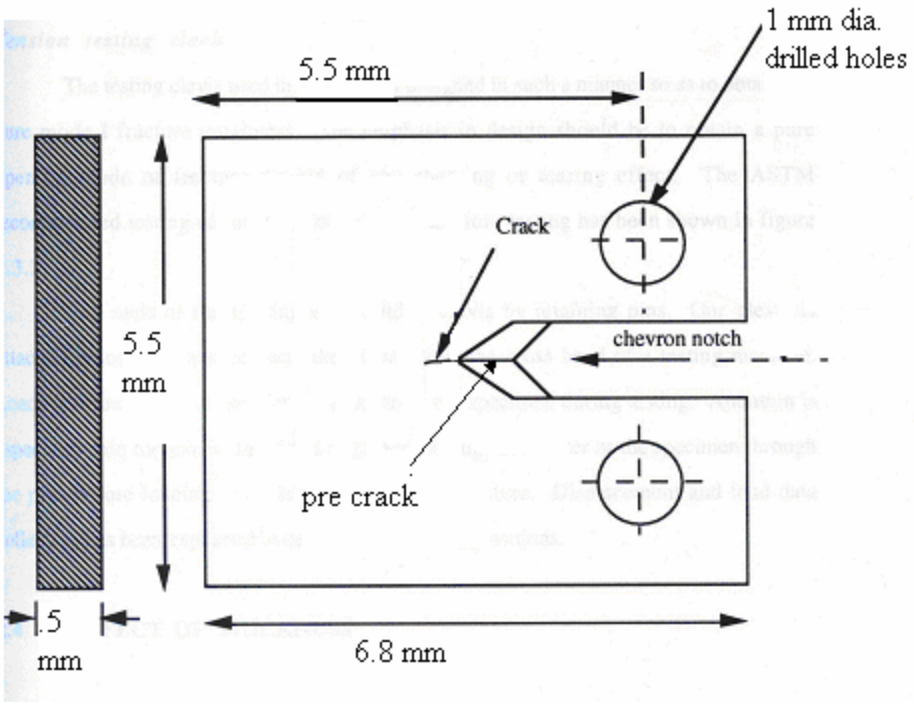


Figure 2.2.5 – 3: CT specimen made with dimension added (Vashishth 1991)

The specimen is then tested on the MTS machine model 812.21 (MTS Systems Corporation Minneapolis, MN). The specimen is loaded using a rate of .2 mm/min. The data is collected an Analog to Digital board converter model PC-CARD-DAS 16/16-AO

(Computer Measurement Corp, Middleboro, MA) and uploaded to a Labtech NOTEBOOK pro software (Laboratory Technologies Corporation, Wilmington, MA) on a Dell model 3500 Inspiriron Computer (Dell, U.S.A). The data that will be collected from this test is the load to failure.

2.2.6 Smoking Chamber

The smoking chamber is a BioClean, DuoFlo, model H 5500, Lab Product Inc (figure 2.2.6 – 1).

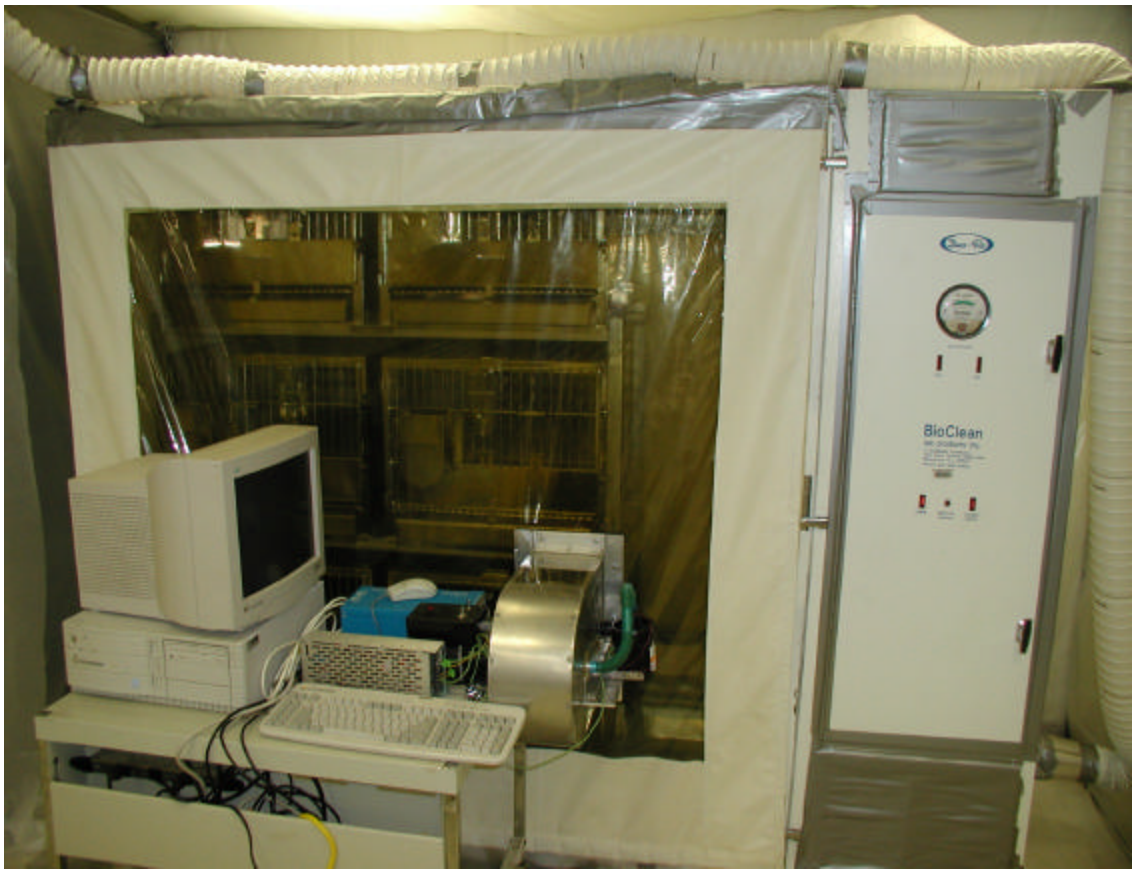


Figure 2.2.6 – 1: Smoking Chamber

The inside of the chamber measured was 1.92X1.92X.097 m (3.58 M^3) and in this study could hold six rabbits. The rabbits were rotated clockwise in the cage to ensure uniform dosage.The rabbits were exposed to sidestream smoke (second

hand smoke) from Marlboro filter cigarettes. The device used to light the cigarettes and was used to vent smoke into the chamber (figure 2.2.6 – 6). This device lit 4 cigarettes every 15 minutes for 6 hrs a day. A fan was used to mix the smoke in the chamber. The CO levels were monitored and keep to an average of about 50 part per million. The smoking chamber was model after Hutchison study (Hutchison and Reitz 1997).

Figure 2.2.6 – 2: Device to light the cigarettes.

2.3 Introduction to 3 point bending test

This test measures the strength and stiffness of the femur (figure 2.3 – 1).

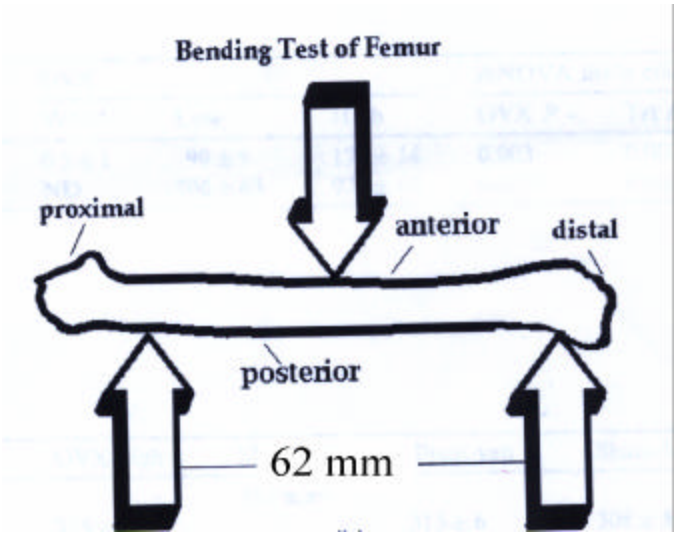


Figure 2.3 – 1: 3-point bending test set up (Akhter, Iwaniec et al. 2003)

To calculate the bone area was assumed to be a hollow ellipse. To find the cross section area of bone it had to be embedded and then stained. This will be explained in more detailed in sections 3.3.1 and 3.3.3. Once the bone is stained some measurements were taken using a microscope and the program optimums.

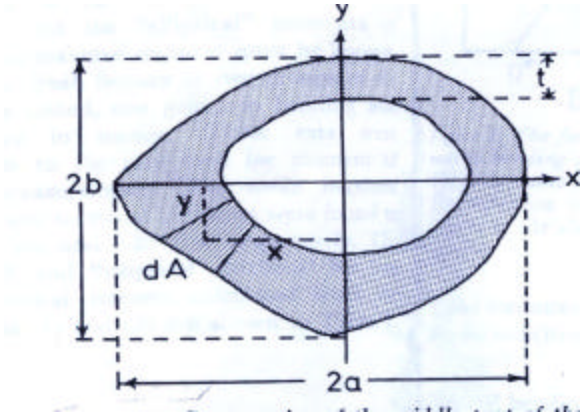


Figure 2.3 – 2: Cross sectional area of the middle part of the tibia or femur (Engesaeter, Ekeland et al. 1978).

The bending stress was found using the follow equation

$$s = \frac{M \cdot b}{I} \quad (20)$$

where, the follow equation was used to find the area moment of inertia

$$I = p \cdot (ab^3 - (a-t)(b-t)^3) \quad (21)$$

Where the major axis a and minor axis b along with the thickness in 4 places shown in figure 2.3 – 2. In a study done by Engesaeter it was found that if bone is estimated hollow ellipse that there is 11% error in the area moment of inertia and 2% in polar moment of inertia (Engesaeter, Ekeland et al. 1978).

Three point bending test uses the right femur. First the outside area and length was measured. The MTS machine will be used to apply a load in the center of the femur. The unsupported length is 62 mm. The loading rate was 3 mm/min (Akhter, Iwaniec et al. 2003). The bone was loaded till failure, while the force and displacement data were collected.

2.4 Introduction to Femoral Neck Testing

The femoral neck is common area for fractures in osteoporoistic patients. As stated earlier, one out of eight hip fracture in women are attributed to smoking, according to Law (Law and Hackshaw 1997). This test is done to test the strength for femoral neck to see if there are any structural changes in the bone due to nicotine. Rabbit femurs are quite different from human femur, but they are still testable.

Femoral neck fracture test used the right femur. First, the distal end will be cut off at the middle of the femur. Next the specimen will be plotted in Corallite Doz-all, and then re-hydrated for an hour. The MTS will be used to test the specimen with

loading rate of 4.91 mm/ 200 s. The load will be applied on the femoral head as shown in figure 2.3 – 1. The load is applied in Newtons, and the data will be collected in the same way the fracture toughness was.

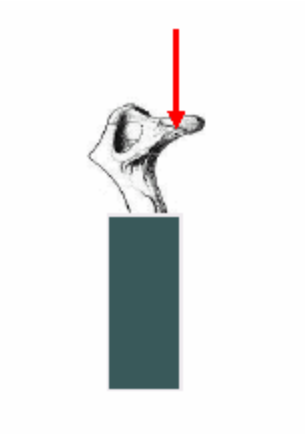


Figure 2.4 – 1: Location of where the load is applied for the femoral neck test (Smith 2003)

2.5 Introduction to Torsion test

The Torsion test was done to see if nicotine had any effect on shear stress. Ueng (Ueng, Lee et al. 1997) used the torsion test on rabbit tibia to see if smoking affected bone healing of the tibia. This test measures (figure 2.5 – 1) the amount of torque it takes for each bone to break, the angle at which it will break, and the amount of shear stress in each bone.

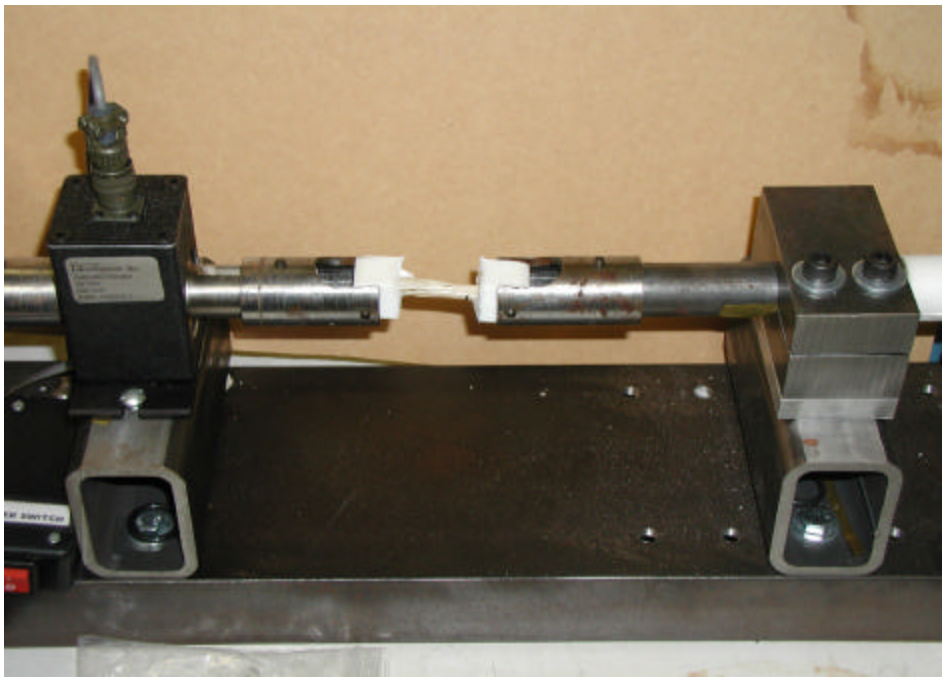


Figure 2.5 – 1: Torsion Test set up

Once the polar moment of inertia was found the shear stress could be found using the equation

$$t = \frac{T \cdot b}{I_P} \quad (22)$$

where the polar moment of inertia the following equation was used.

$$I_P = p\,(ab^3 + a^3 b - (a-t)(b-t)^3 - (a-t)^3(b-t)) \quad (23)$$

The left tibia was used in the torsion test. First, the proximal end has been cut off perpendicular to the axis. Next each specimen was plotted in Corallite Doz-all. Then after specimens dried they were placed in saline for an hour to re-hydrate. Before each test the outside area, and the unsupported length was measured using a caliber. Finally the specimen was placed in the torsion testing device made by Vincent Kish, tested at a rate of 1 degree per second (Cain 2003). The data was collected in the same manner as the fracture toughness test.

Chapter 3 Histomorphometry and Composition

3.1 Introduction to Histomorphometry

Histology is the study of anatomy that deals with the minute structure of animals and plants by using a microscope. Lexicon is the abbreviations of words commonly used histomorphometric measurements of bone. An example is osteon area, which is, OnAr. This abbreviation has became standardized by Parfitt (Parfitt, Drezner et al. 1987). This help when reporting measurements by abbreviations to reduce the size and ease of the report. In table 3.1 – 1 is an example of Lexicon abbreviations.

Table 3.1 – 1: Lexicon abbreviations (Parfitt, Drezner et al. 1987)

A	Apposition(al)		m	Maturation
Ab	Absolute		N	Number of profiles or structures
Ac	Activation		n	Number of sampling units
Aj	Adjusted		O	Osteoid
Ar	Area (2D)		Ob	Osteoblast(ic)
a	Activ(e)(ity)		Oc	Osteoclast(ic)
B	Bone		On	Osteon(al)
BMU	Basic Multicellular Unit		Ot	Osteocyt(e)(ic)
Ca	Canal(icula)(r)		P	Period
Cd	Corrected		Pm	Perimeter (2D)
Cn	Cancellous		Po	Por(e)(ous)(osity)
Ct	Cortical		Ps	Periost(eal)(eum)
d	Double		Pt	Point
E	Ero(ded)(sion)		Q	Quiescent
EX	External		R	Rate
F	Formation		Rd	Radi(al)(us)
Fb	Fibro(sis)(us)		Rf	Referen(ce)(t)
Fr	Front		Rm	Remodeling
f	Frequency		Rs	Resorption
G	Grow(th)(ing)		S	Surface (3D)
H	Haversian		Sa	Sample
Hp	Hypertrophic		Se	Section
Ht	Height		Sn	Spongiosa
Hz	Horizontal		St	Structur(e)(al)
I	Interface (3D)		s	Single
Ic	Intercept		Tb	Trabecula(r)
Il	Initial		Th	Thickness (3D)
In	Internal		Tt	Total
Ir	Inter		t	Time
i	Intersection		U	Unit
L	Label(led)		V	Volume (3D)
Lc	Lacuna(r)		Vd	Void
Le	Length		Vk	Volkmanns
Lm	Lamella(r)		Vt	Vertical
Ln	Line		W	Wall
Lo	Longitudinal		Wi	Width
l	lag		Wo	Woven
M	Mineral(iz)(ing)(ation)		Z	Zone
Me	Medullary			
Ml	Modeling			

3.2 Static Histomorphometry

Static histomorphometry is the microscopic measurement of the surface and structure. A number of measurements will be taken. The reason we look at the bone microstructure is because it is usually where changes occur first as the most sensitive measure of bone alteration. These measurements are static because the bone is not living and a slide is snapshot of what was happening to the bone at the time of death. These measurements have been done in terms of the total area viewed. Some important measurements are listed below.

Total area (TtAR)

Is the total area of the image. This measurement is used for many of the other measurements made.

Porosity (Po)

The pores in the bone, which are darker, then the bone matrix where measured under the microscope (figure 3.2 – 1). These pores are haversian, and volkmann canals, and any other space in the bone. This area is then divide by the total area measured.

$$Po = \frac{Hn.Cn.Ar + V.Cn.Ar + \Pr.Po.Ar}{Tt.Ar} \quad (24)$$

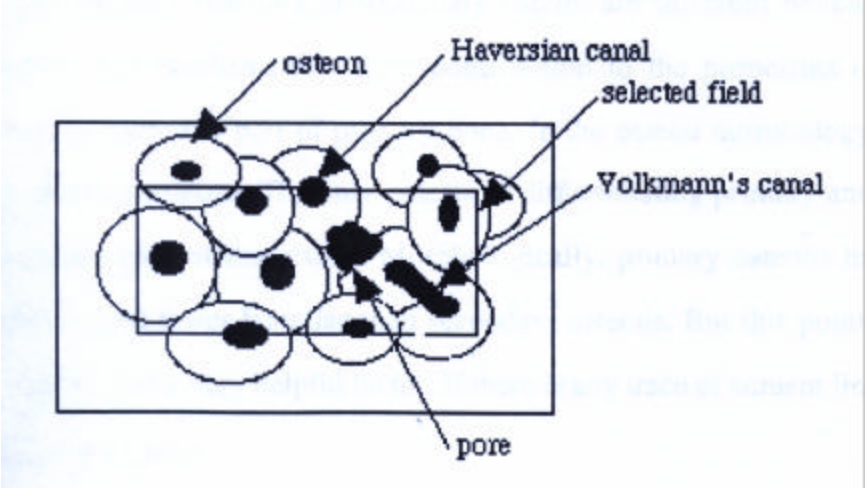


Figure 3.2 –1: Example Porosity Measurements (Wang 1995)

Average Pore Radius (AvPoRd)

The haverisan canals are most circular in shape and this equation finds the average radius of each haverisan canal.

AvPoRd = sqrt(Haverisan Canals Area/(# of Haverisan Canals PI())) (25)

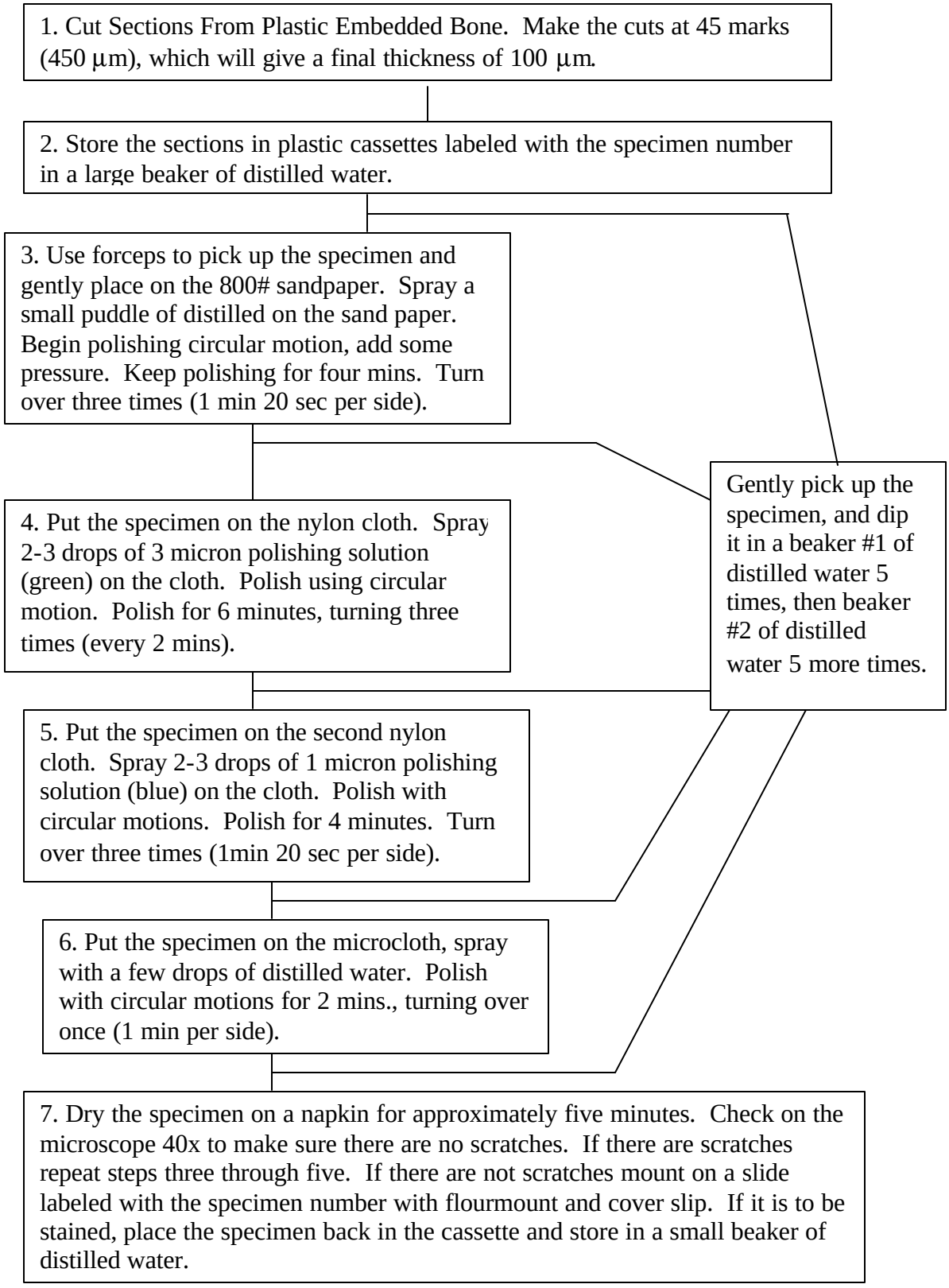
3.3 Histomorphometric Analysis

To perform static histological analysis, it is bone cross section need to be stained and mounted on slides for microscope viewing. Because bone is fragile material, it cannot be cut to thin slices by it self, and therefore is needs to be embedded in plastic (Section 3.3.1). Once in embedded the specimens have been cut on the diamond wire saw to about 100 µm. After the specimen has been sliced it then is polished to remove any scratches (Section 3.3.2) (Wang 1995). After the specimen has been polished it will need to be stained (Section 3.3.3). Once this has been done histology measurements of the specimen were conducted.

3.3.1 Embedding Procedure

1. Specimen are placed in 10% neutral buffered formalin for about a week
2. The specimen are placed in hypercenter XP tissue processor (Shandon, Pittburgh, PA) for processing which includes:
    a. Dehydration in alcohol
    b. Clearing with Xylene
    c. Inflitration with methymethacrylate (MMA)
3. Specimens are embedded in MMA (100 ml MMA to .2 g Perkadox)
4. Specimen are left in a vacuum oven for 1-2 hours to remove air bubbles
5. Specimen are placed in water bath (36°C) overnight for polymerization

3.3.2 polishing procedure



3.3.3 Staining Procedure

Protocol was used for staining of the bone slices. First the section was placed into a container, then the section is rinsed in water and is placed in Harris' Hematoxylin for 15 minutes at 95 rpm on platform shaker. After the 15 minutes in the platform shaker the specimen is placed in running water for 5 minutes, in which it is then dipped in to acid alcohol for 20 dips, then it is placed in running water again for 8 minutes. The specimen is then dipped into ammonia water for 15 dips. Again it is placed in running water for 15 mins. After that it is placed in eosin for 6 minutes then is dipped in 80% alcohol for 10 dips, then 95% alcohol, 100% alcohol, 100% alcohol, and Xylene for 15 dips. Once this is done the specimen is removed from the container and is placed on a slide and is covered and left to set for the night.

3.4 Compositions

This is the procedure used to measure the compositions of bone.

1. Take dimensions of the specimens and calculate their volume
2. Weigh and record crucible weights
3. Hydrate specimens overnight in saline
4. Blot specimens dry, weigh and record wet weights of the specimens.
5. Defat specimen by placing them in acetone and agitating overnight (platform shaker at 160 rpm)
6. Weigh and record defatted weight

7. Place in vacuum oven, containing desiccant at 60° C (2.2) and 20 psi vacuum pressure overnight. Remove and place in desiccator for one hour to permit return to room temperature.
8. Repeat step 8 until a constant weight is obtained.
9. Place specimen in crucible and ash in muffle furnace at 600° C (60 on the dial High) for 24 hours.
10. Remove specimen from furnace. Place in desiccator until room temperature is attained (1 hour). Weight and record weight of crucible and ashed specimen.

Equations used

Wet Density = WW/V

Dry Density = DW/V

% Mineral, %Min = (AW/DW)*100

% Organic (Wet) = [(DW-AW)/WW]*100

%Organic (Dry) = [(DW – AW)/DW]*100

%Ash = (AW/WW)*100

$\%H_2O$ = [(WW-DW)/WW]*100

Chapter 4 Statistical Methods

Statistical comparison between groups was done by JMP (version 3.2.1 SAS Institute, Inc, Cary, NC). Significance was tested using ANOVA, student t-test, and Tukey-Kramer HSD. Statistical significance was set $p<.05$ (95%).

Chapter 5 Results

5.1 Nicotine serum levels and Weight for Experiment 1

All individual rabbit data for each test is in Appendix A for Group 1.

Terminology for Results

Average Nicotine serum level is the average of measurement of the nicotine level measure during weeks the 1, 3 and 5.

Weight Difference is the measured final weight minus the starting weight, which is the weight measurement right after the surgery.

Figure 5.1 – 1 is average nicotine level seen during the 5-week period after surgery for each group, and table 5.1 –1 is the values for figure 5.1 – 1 and the p values for each group. Figures and table 5.1 – 2 & 3 are for weight and nicotine level over the 5-week period for each group. Figure 5.1 – 4 & 5 are for weight difference versus nicotine level during the 5 weeks.

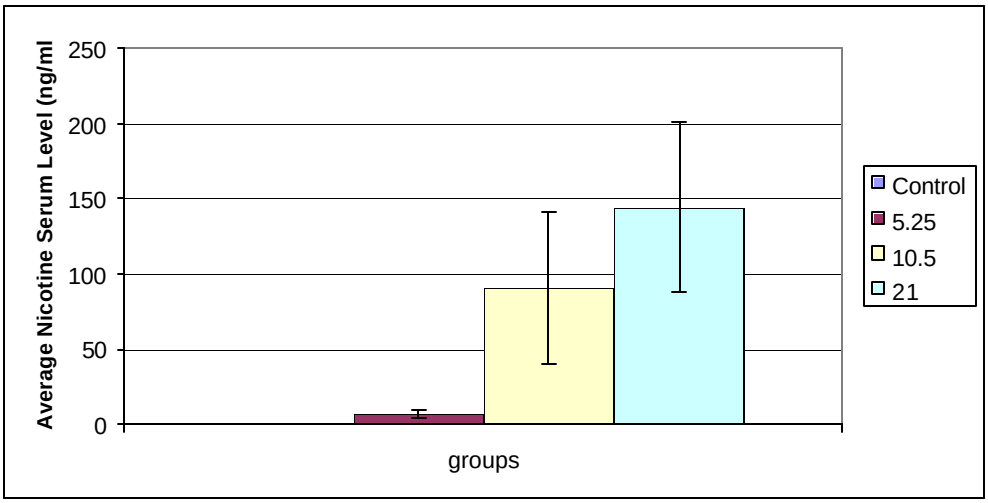


Figure 5.1 –1: Average nicotine level for each group

Table 5.1- 1: Values for figure 5.1-1 and p values for each group

Group	Nicotine Level (ng/ml)	Standard Deviation
Control	0	0
5.25	7.45	2.549437
10.5	90.68	50.81941
21	143.9833	56.39678

groups	P values
All	0.0004
all	0.0794
5.25 & C	0.1787
10.5 & C	0.0645
21 & C	0.0145
5.25 & 21	0.0015
10.5 & 21	0.1374

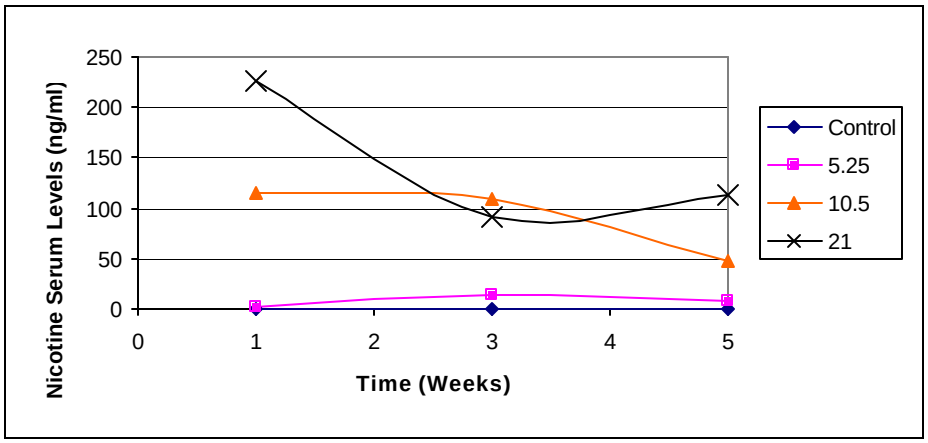


Figure 5.1 – 2: Nicotine levels during the 5 weeks period

Table 5.1 – 2: Values for figure 5.1 – 2

Group	Week 1 Nicotine Level (ng/ml)	Week 3 Nicotine Level (ng/ml)	Week 5 Nicotine Level (ng/ml)
Control	0	0	0
5	1.75	13.475	7.125
10.5	115.44	109.2	47.4
21	227	91.45	113.5

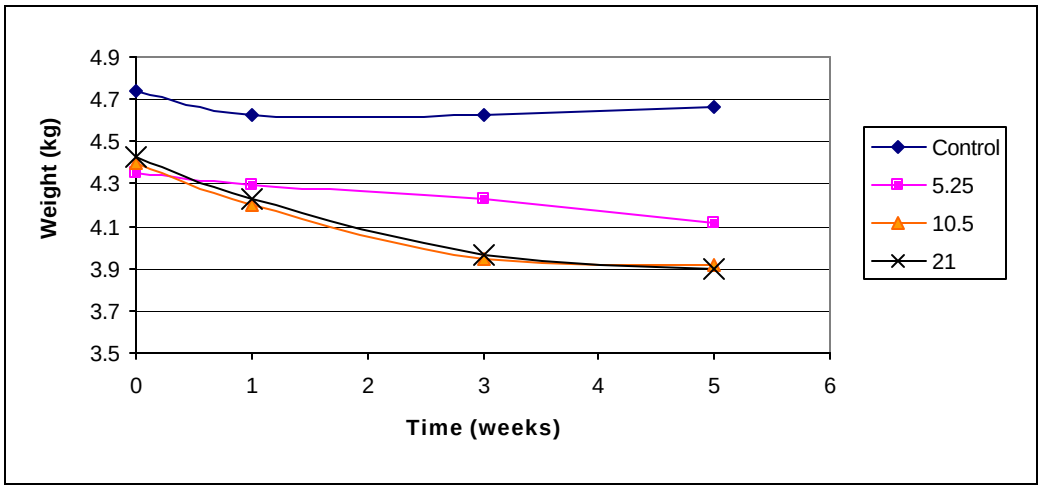


Figure 5.1 – 3: Average Weight of the rabbits during the 5-week period

Table 5.1 – 3: values for figure 5.1 – 3

Group	Starting Weight (kg)	Week 1 Weight (kg)	Week 3 Weight (kg)	Week 5 Weight (kg)
Control	4.743333	4.63	4.63	4.666667
5	4.3525	4.2925	4.225	4.1125
10.5	4.398	4.198	3.944	3.912
21	4.43	4.225	3.963333	3.898333
Group	**Standard Deviation**	**Standard Deviation**	**Standard Deviation**	**Standard Deviation**
Control	0.127017	0	0	0.247049
5	0.339252	0.39424	0.413642	0.443199
10.5	0.184716	0.213588	0.390167	0.35968
21	0.275536	0.335067	0.34691	0.294239

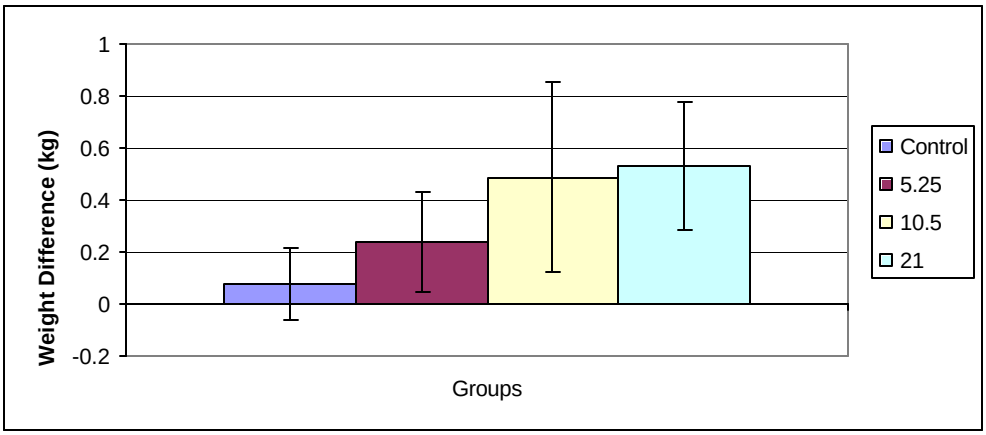


Figure 5.1 – 4: Weight difference for each group

Table 5.1 – 4: Values for figures 5.1 – 4 and p values for each group

Groups	Weight difference	Standard Deviation
Control	0.076667	0.138684
5.25	0.24	0.191311
10.5	0.486	0.365828
21	0.531667	0.243509

Groups	Prob>F
all	0.0924
5.25 & C	0.2694
10.5 & C	0.1199
21 & C	0.0217
5.25 & 10.5	0.2663
5.25 & 21	0.0799
10.5 & 21	0.8096

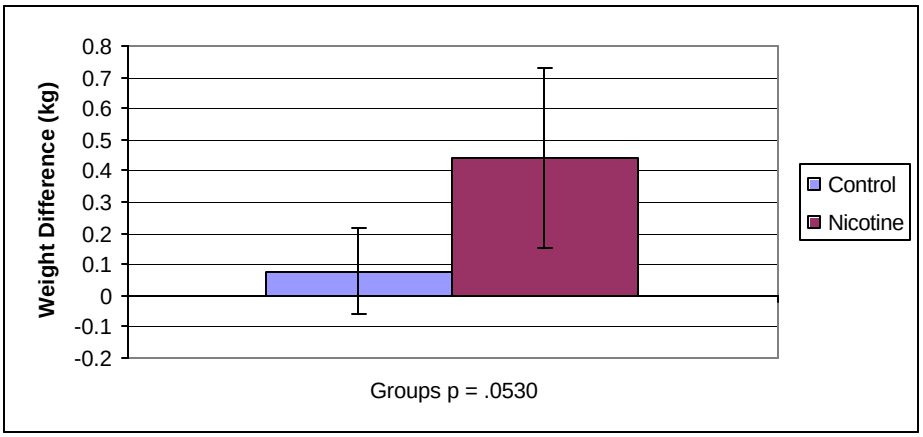


Figure 5.1 – 5: Weight Difference for control and nicotine groups as a whole

Table 5.1 – 5: Values for figure 5.1 – 5

Groups	weight difference	Standard Deviation
Control	0.076667	0.138684
Nicotine	0.438667	0.288168

5.2 Fracture Toughness Testing for Experiment 1

Figure 5.2 – 1 is fracture toughness values for each group and table 5.2 – 1 is the values for figure 5.2 – 1 along with the p values for each group. Figure 5.2 –2 and table 5.2 is fracture toughness values as consider the nicotine and controls group as a whole. Table 5.2 – 3 is the dimensions for fracture toughness specimens groups.

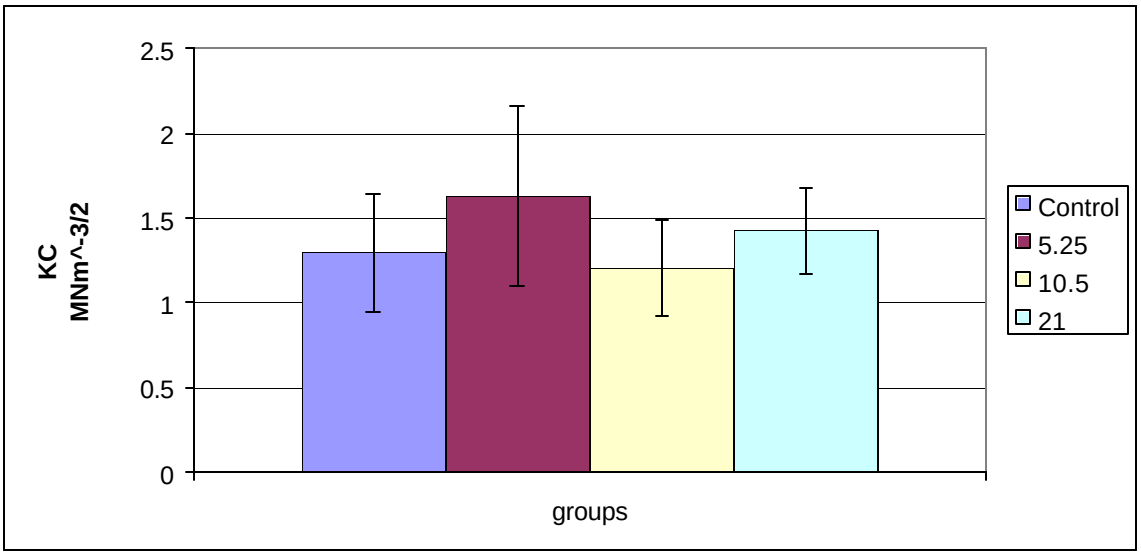


Figure 5.2 – 1: Fracture toughness values for each group

Table 5.2 – 1: Value for figure 5.2 –1 and p value for each group

Nicotine groups	Fracture Toughness (MNm^-3/2)	Standard Deviation
Control	1.294559	0.346752
5.25	1.63341	0.529539
10.5	1.206556	0.281598
21	1.425818	0.25425

Groups	Prob>F
all	0.3483
5.25 & C	0.384
10.5 & C	0.7063
21 & C	0.5339
5.25 & 10.5	0.1618
5.25 & 21	0.4237
10.5 & 21	0.2077

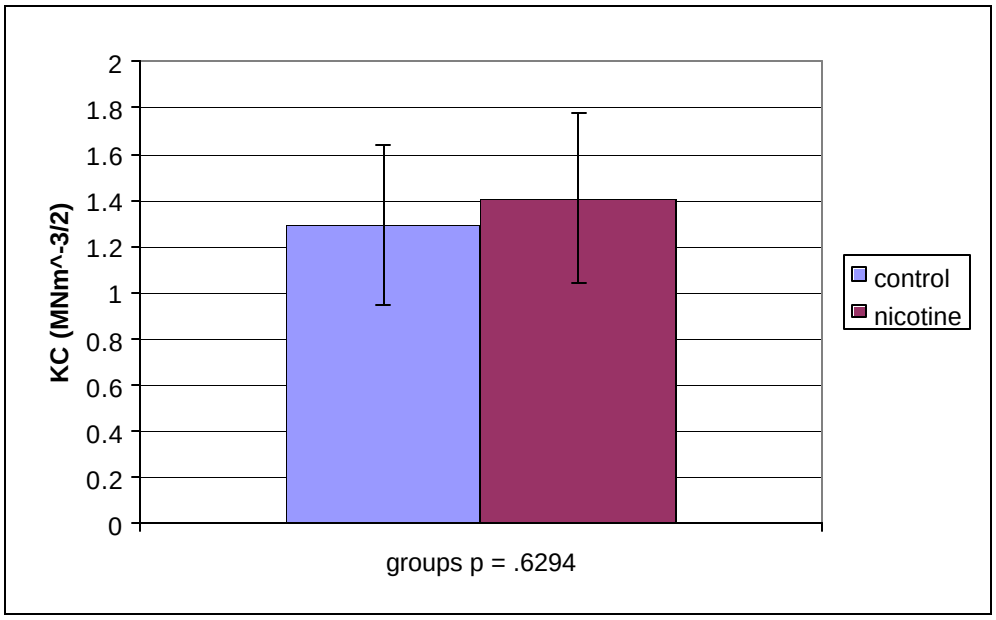


Figure 5.2 – 2: Fracture toughness of nicotine and control groups as a whole

Table 5.2 –2: Value for figure 5.2 – 2

Nicotine groups	Fracuture Toughness (MNm^-3/2)	Standard Deviation
control	1.294559	0.346752
nicotine	1.408088	0.367393

Table 5.2 – 3: Dimension for fracture toughness specimens groups

group	thickness	a	W	a/W	Load
Control	0.000617	0.0027	0.005203	0.518473	5.560433
5.25	0.000533	0.002598	0.005299	0.491512	7.084
10.5	0.000604	0.002508	0.005147	0.487858	5.74026
21	0.000588	0.002592	0.005366	0.482685	6.757855
group	**Standard Deviation**	**Standard Deviation**	**Standard Deviation**	**Standard Deviation**	**Standard Deviation**
Control	0.000087	0.000303	0.000191	0.047365	1.337118
5.25	0.000043	0.000161	0.000219	0.048047	3.324273
10.5	0.00009	0.000377	0.00034	0.075883	1.874261
21	0.000099	0.000219	0.000343	0.019239	1.894408

5.3 3-Point Bending Testing for Experiment 1

Figure 5.3 – 1 and table 5.3 – 1 are the bending stress for each group along with the values and p values for each group. Figure 5.3 – 2 and table 5.3 – 2 are bending stress values for nicotine and control groups as a whole.

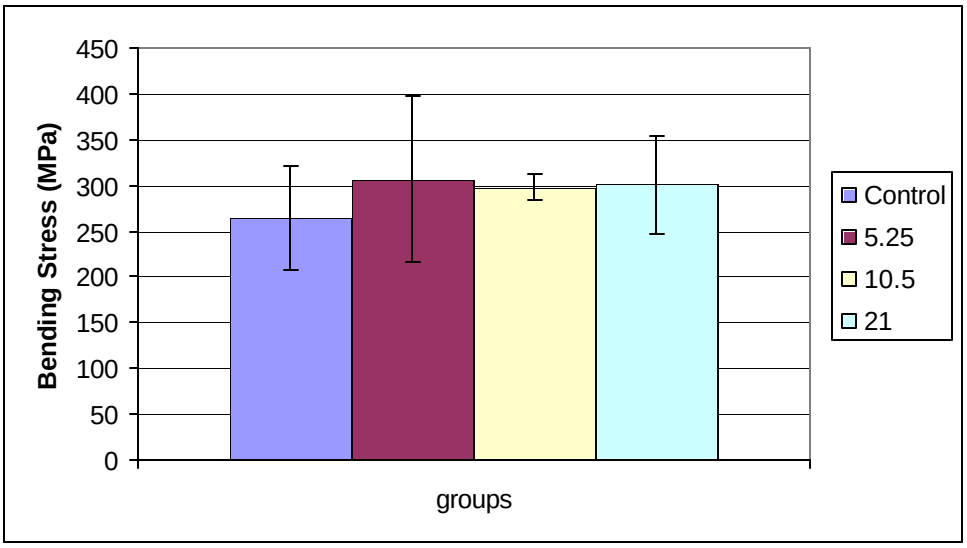


Figure 5.3 – 1: Average Bending Stress for each group

Table 5.3 –1: Values for figure 5.3 –1 and p value for each group

groups	Bending Stress (MPa)	Standard Deviations
Control	264.5452	57.44951
5.25	306.9135	89.84725
10.5	297.6781	14.39813
21	300.7559	53.99466

groups	p values
All	0.7816
5.25 & C	0.5114
10.5 & C	0.2448
21 & C	0.3828
5.25 & 10.5	0.8246
5.25 & 21	0.8944
10.5 & 21	0.9049

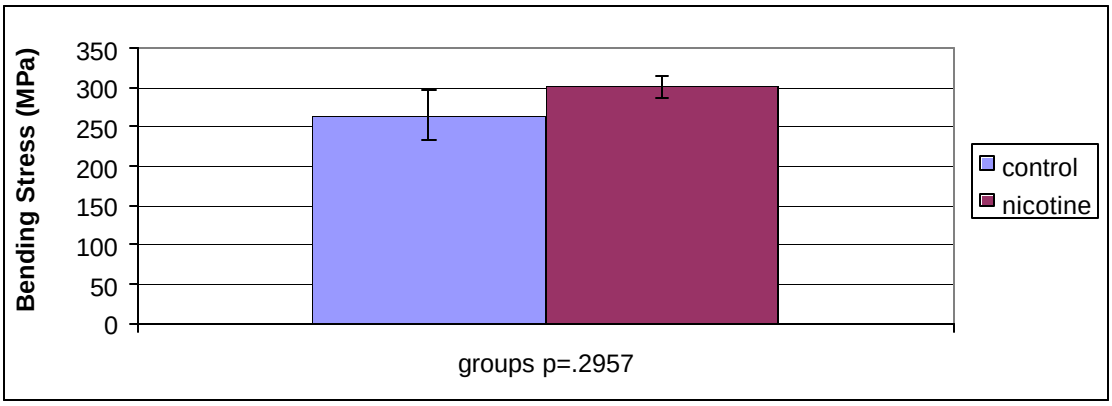


Figure 5.3 –2: Bending Stress for Nicotine and control group as a whole

Table 5.3 – 2: Values for figure 5.3 -2

groups	Bending Stress (Mpa)	Standard Devation
control	264.545	31.097
nicotine	301.372	13.907

5.4 Femoral Neck Testing for Experiment 1

Figure 5.4 – 1 and table 5.4 – 1 are for femoral neck load along with the value and p values for each group. Figure 5.4 – 2 and table 5.4 – 2 is the femoral neck load for nicotine and control groups as a whole

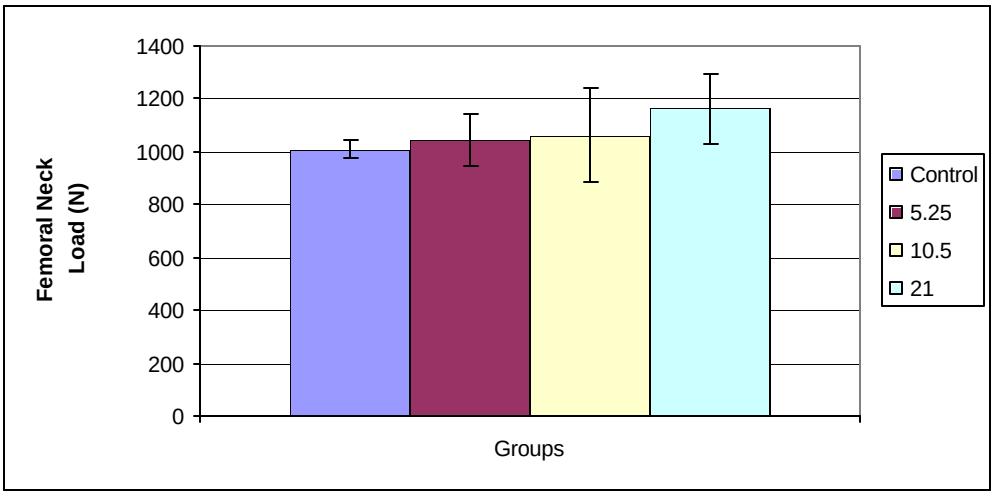


Figure 5.4 – 1: Femoral Neck Load for each group

Table 5.4 –1: Values for figure 5.4 – 1 and p values for each group

Groups	Femoral Neck load (N)	Standard Deviation
Control	1007.286	33.55777
5.25	1043.511	97.31283
10.5	1061.407	179.7679
21	1165.185	132.3397

Groups	Prob>F
all	0.3292
5.25 & C	0.5712
10.5 & C	0.6345
21 & C	0.0893
5.25 & 10.5	0.864
5.25 & 21	0.1561
10.5 & 21	0.2982

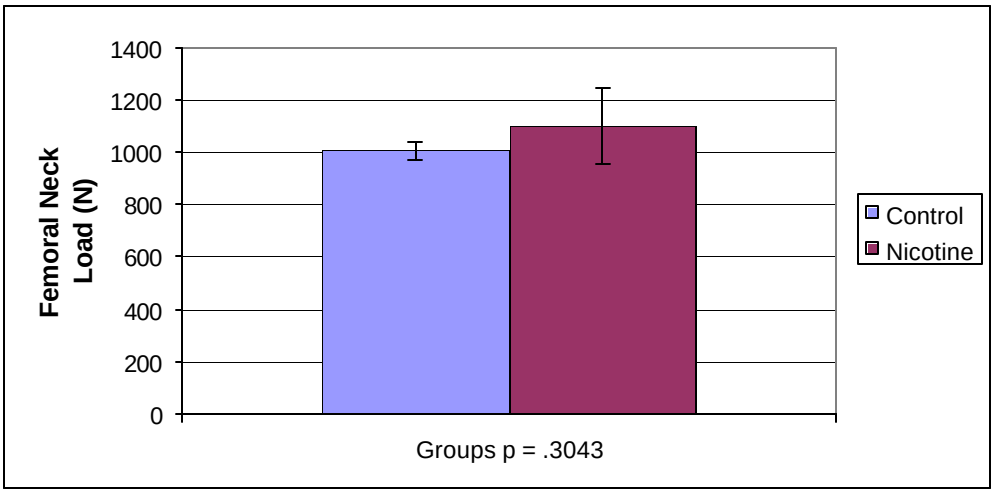


Figure 5.4 – 2: Femoral Neck load for nicotine and control groups

Table 5.4 – 2: Value for figure 5.4 – 2

Groups	Femoral Neck load (N)	Standard Deviation
Control	1007.286	33.55777
Nicotine	1098.146	144.1473

5.5 Torsion Testing for Experiment 1

Shear stress for each group is shown in figure 5.5 – 1 and values for figure 5.5 – 1 are the values shown in table 5.5 – 1 for each group. Figure 5.5 – 2 is comparing the nicotine group as a whole versus the control group, and table 5.5 – 2 is values for figure 5.5 – 4.

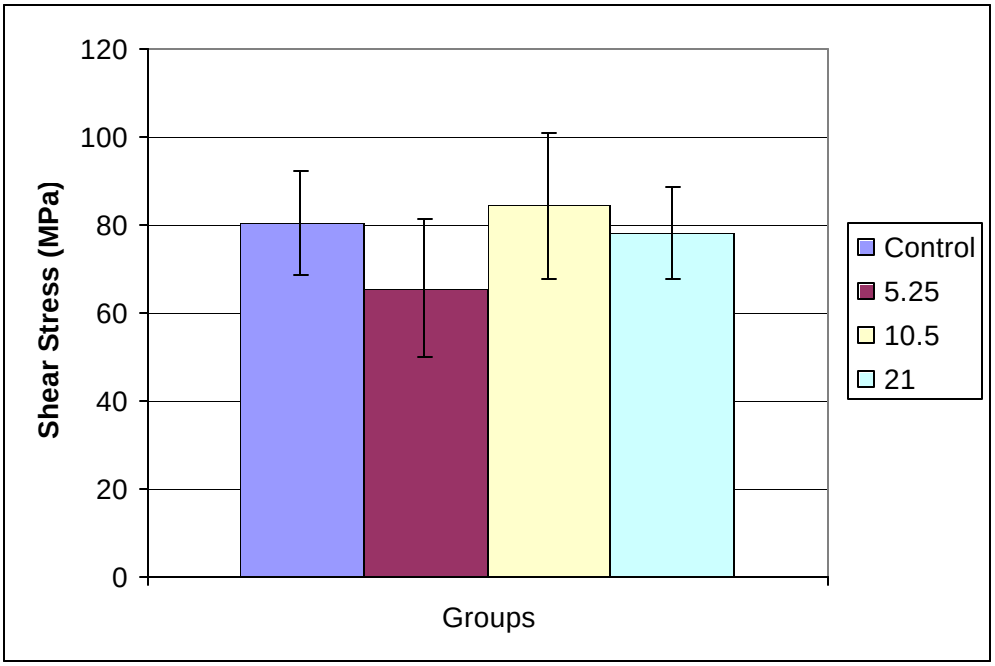


Figure 5.5 – 1: Shear stress for each group

Table 5.5 – 1: Values for figure 5.5 – 1 and p values for each group

Groups	Shear Stress	Standard Deviations
Control	80.28573	11.85253
5.25	65.61587	15.62457
10.5	84.43872	16.55057
21	78.19596	10.45718

Groups	Prob>F
all	0.2676
5.25 & C	0.2352
10.5 & C	0.7203
21 & C	0.7936
5.25 & 10.5	0.1261
5.25 & 21	0.1618
10.5 & 21	0.4649

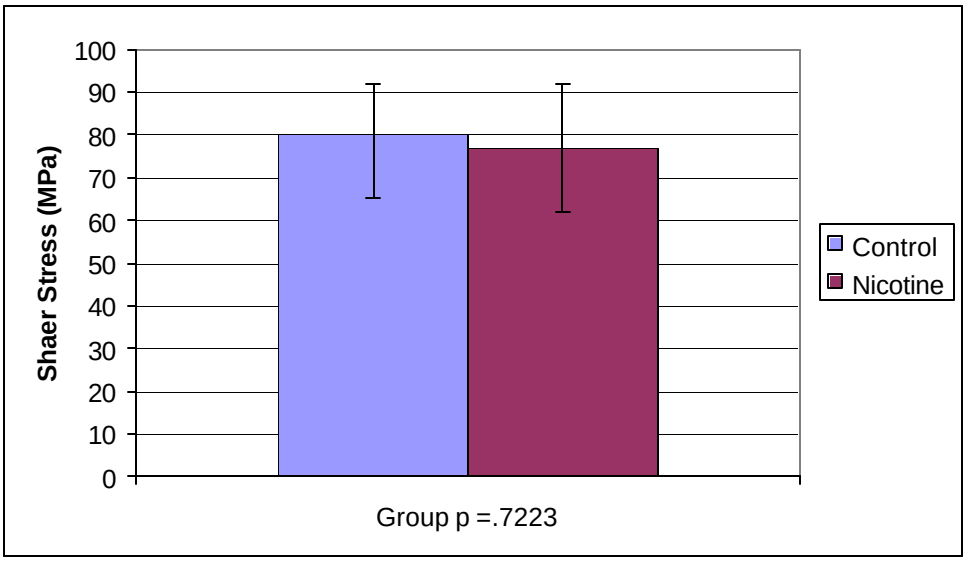


Figure 5.5 – 2: Shear stress for nicotine and control group

Table 5.5 – 2: Values for figure 5.5 – 2

Group	Shear Stress	Standard Deviation
Control	80.28573	11.85253
Nicotine	76.92219	15.06744

5.6 Histomorphometry for Experiment 1

Figures 5.6 – 1 & 2 and table 5.6 – 1 & 2 is the porosity measurements. Figures 5.6 3 & 4, and table 5.6 3 & 4 is the average pore radius measurements. Figures 5.6 – 5 & 6 and table 5.6 – 5 & 6 is the % Dry Density measurements. Figures 5.6 – 7 & 8 and table 5.6 – 7 & 8 is the % Wet Density measurements. Figures 5.6 – 9 & 10 and table 5.9 – 5 & 10 is the % Mineralization measurements. Figures 5.6 – 11 & 12 and table 5.11 – 5 & 12 is the % Organic Wt. Dry measurements. Figures 5.6 – 13 & 14 and table 5.6 – 13 & 14 is the % Ash measurements. Figures 5.6 – 15 & 16 and table 5.6 – 15 & 16 is the % Organic Wt. Wet measurements. Figures 5.6 – 17 & 18 and table 5.6 – 17 & 18 is the % Water measurements.

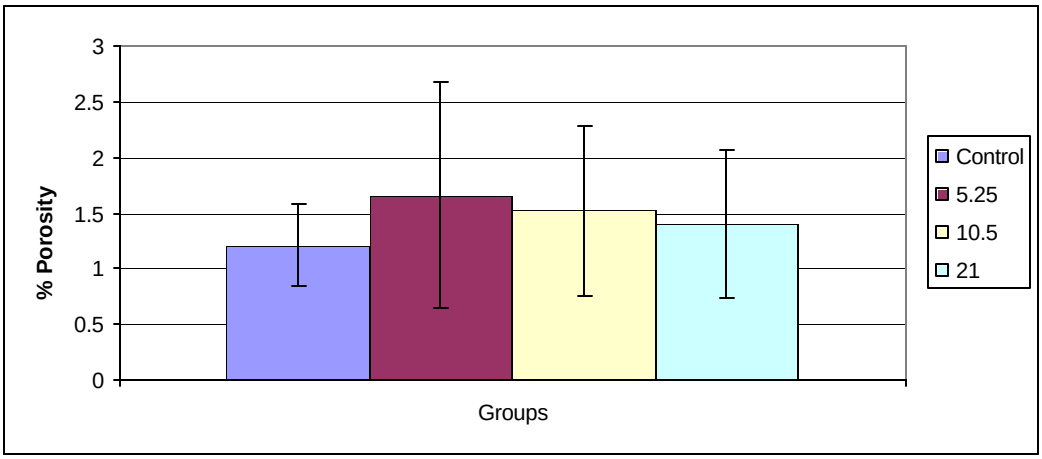


Figure 5.6 –1: % porosity for each group

Table 5.6 – 1: Values for figures 5.6 – 1 and p values for each group

Groups	% Porosity	STD		Groups	Prob>F
Control	1.2082	0.368		all	0.8729
5.25	1.6614	1.0105		5.25 & C	0.5
10.5	1.5186	0.7644		10.5 & C	0.543
21	1.3991	0.668		21 & C	0.6652
				5.25 & 10.5	0.8153
				5.25 & 21	0.6308
				10.5 & 21	0.788

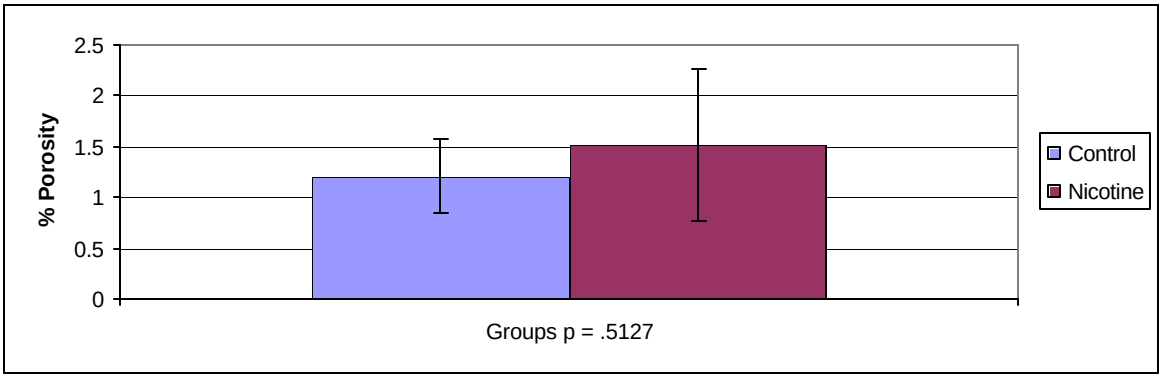


Figure 5.6 – 2: % Porosity for the nicotine and control groups

Table 5.6 – 2:Values for figure 5.6 – 2

Groups	% Porosity	Standard Deviation
Control	0.012082	0.00368
Nicotine	0.015089	0.007463

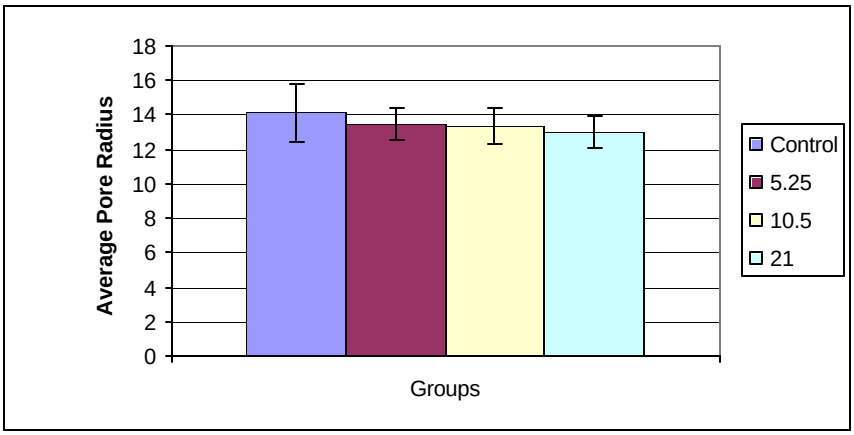


Figure 5.6 – 3: Average Pore Radius for each group

Table 5.6 – 3: Values for figures 5.6 – 3 and p values for each group

Groups	Average Pore Radius	Standard Deviation
Control	14.11342	1.699251
5.25	13.48146	0.972664
10.5	13.34851	1.049135
21	13.01278	0.911264

Groups	Prob>F
all	0.5843
5.25 & C	0.5561
10.5 & C	0.452
21 & C	0.2325
5.25 & 10.5	0.851
5.25 & 21	0.4596
10.5 & 21	0.5835

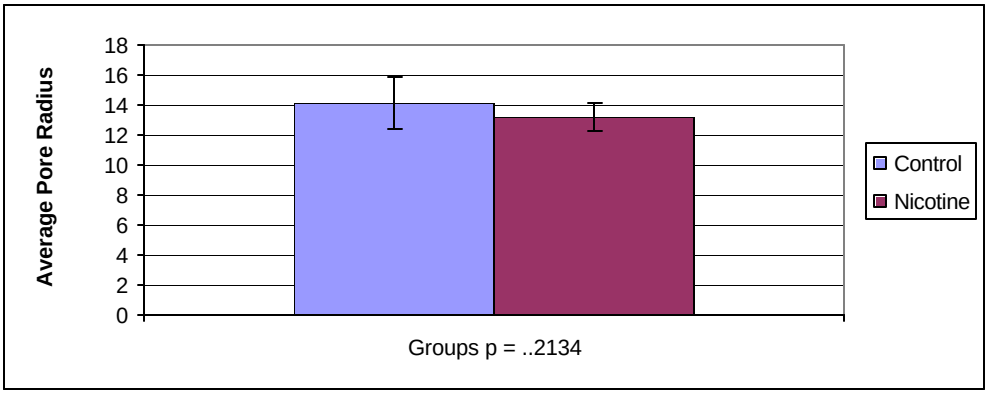


Figure 5.6 – 4: Average Pore Radius for the nicotine and control group

Table 5.6 – 4: Values for Figure 5.6 - 4

Groups	Average Pore Radius	Standard Deviation
Control	14.11342	1.699251
Nicotine	13.24967	0.925567

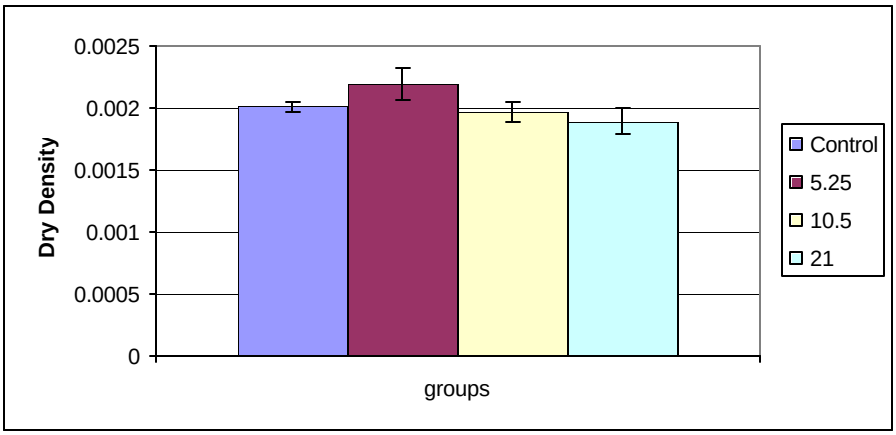


Figure 5.6 – 5: Dry Density % for the groups

Table 5.6 – 5: Values for figures 5.6 – 5 and p values for each group

groups	%Dry Density	Standard Deviation
Control	0.002009	0.000046
5.25	0.002193	0.000126
10.5	0.001971	0.000083
21	0.001893	0.0001

Groups	Prob>F
all	0.0025
5.25 & C	0.0643
10.5 & C	0.5042
21 & C	0.107
5.25 & 10.5	0.0154
5.25 & 21	0.003
10.5 & 21	0.2026

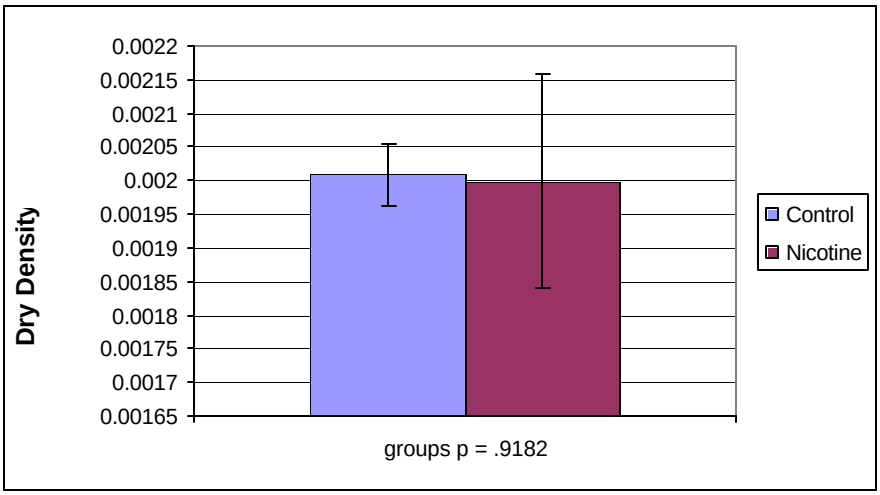


Figure 5.6 – 6: % Dry Density for nicotine and control groups

Table 5.6 – 6: Values for figure 5.6 – 6

groups	% Dry Density	Standard Deviation
Control	0.002009	0.000046
Nicotine	0.001999	0.000158

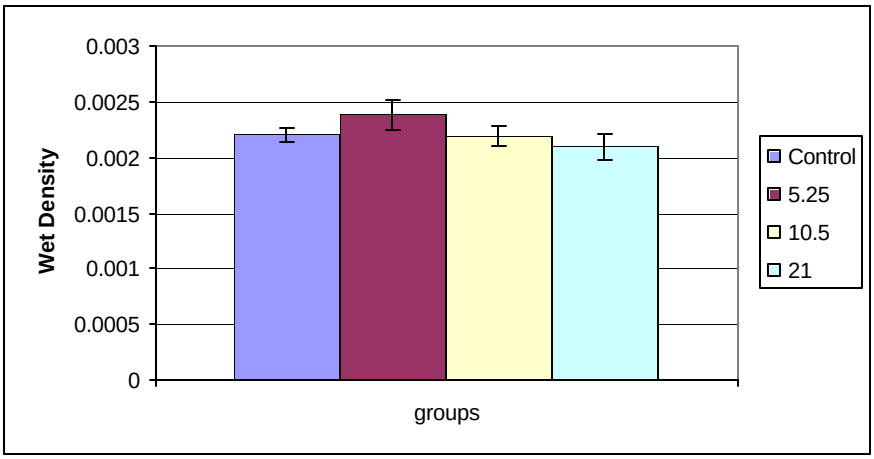


Figure 5.6 – 7: % Wet Density for all groups

Table 5.6 – 7: Values for figures 5.6 – 7 and p values for each group

groups	% Wet Density	Standard Deviation
Control	0.002204	0.000062
5.25	0.002387	0.000135
10.5	0.002199	0.00009
21	0.002093	0.000109

Groups	Prob>F
all	0.0062
5.25 & C	0.0836
10.5 & C	0.9404
21 & C	0.1532
5.25 & 10.5	0.9856
5.25 & 21	0.0051
10.5 & 21	0.1161

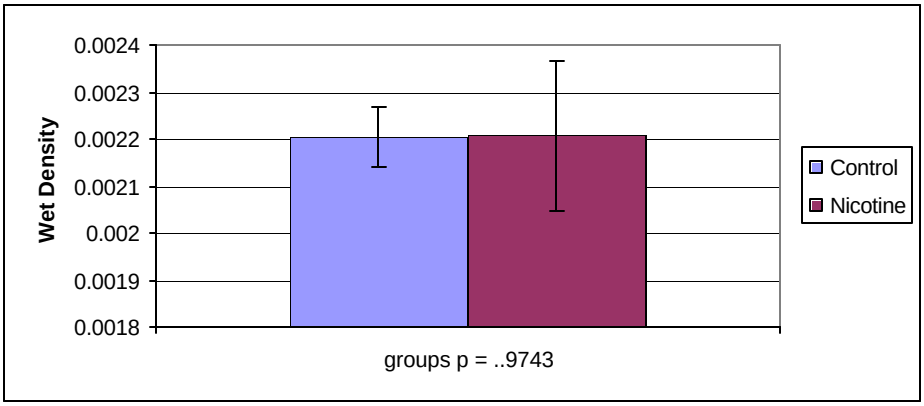


Figure 5.6 – 8: % Wet Density for nicotine and control groups

Table 5.6 – 8: Values for figures 5.6 - 8

groups	% Wet Density	Standard Deviation
Control	0.002204	0.000062
Nicotine	0.002207	0.000159

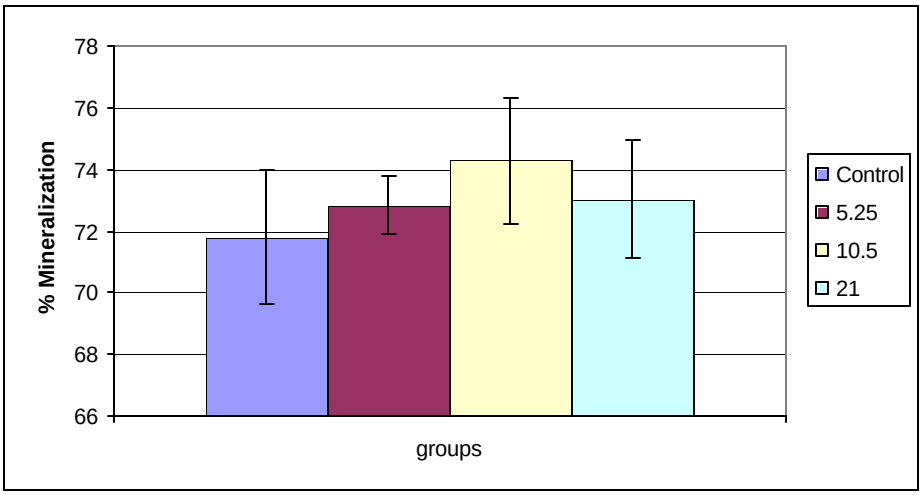


Figure 5.6 – 9: % Mineralization for all groups

Table 5.6 – 9: Values for figures 5.6 – 9 and p values for each group

groups	% Mineralization	Standard Deviation
Control	71.79066	2.161804
5.25	72.84097	0.914438
10.5	74.29161	2.044408
21	73.03156	1.938047

Groups	Prob>F
all	0.3345
5.25 & C	0.4127
10.5 & C	0.1515
21 & C	0.4104
5.25 & 10.5	0.2332
5.25 & 21	0.8609
10.5 & 21	0.3221

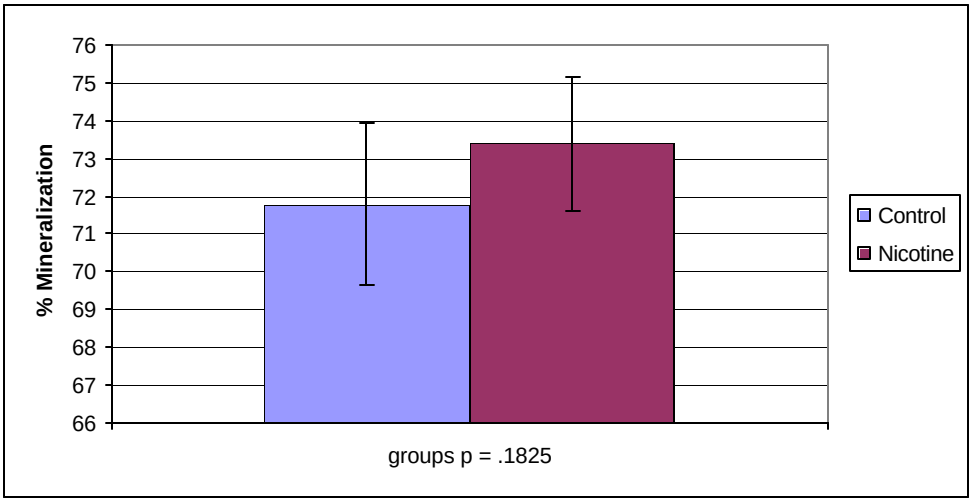


Figure 5.6 – 10: % Mineralization for nicotine and control groups

Table 5.6 – 10: Values for figures 5.6 - 10

groups	% Mineralization	Standard Deviation
Control	71.79066	2.161804
Nicotine	73.40075	1.773747

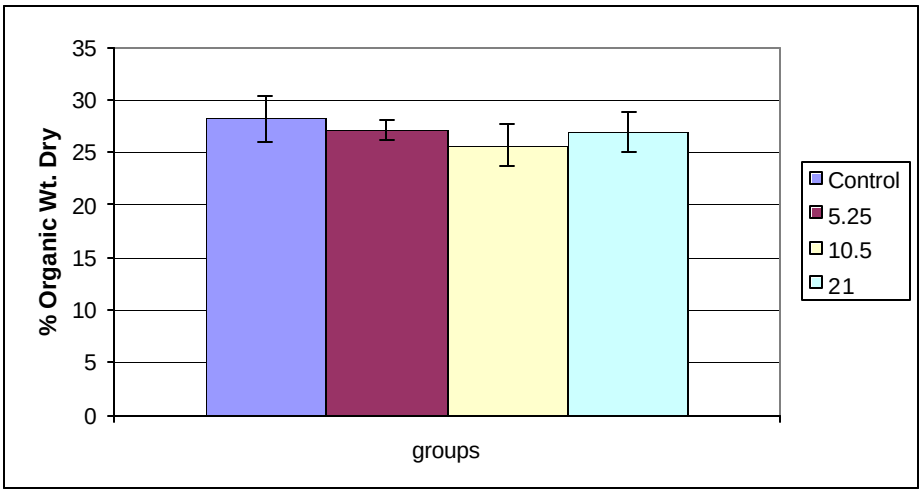


Figure 5.6 – 11: % Organic Wt. Dry for all groups

Table 5.6 – 11: Values for figures 5.6 – 11 and p values for each group

groups	% Organic wt. Dry	Standard Deviation
Control	28.20934	2.161804
5.25	27.15903	0.914438
10.5	25.70839	2.044408
21	26.96844	1.938047

Groups	Prob>F
all	0.3345
5.25 & C	0.4127
10.5 & C	0.1515
21 & C	0.4104
5.25 & 10.5	0.2332
5.25 & 21	0.8609
10.5 & 21	0.3221

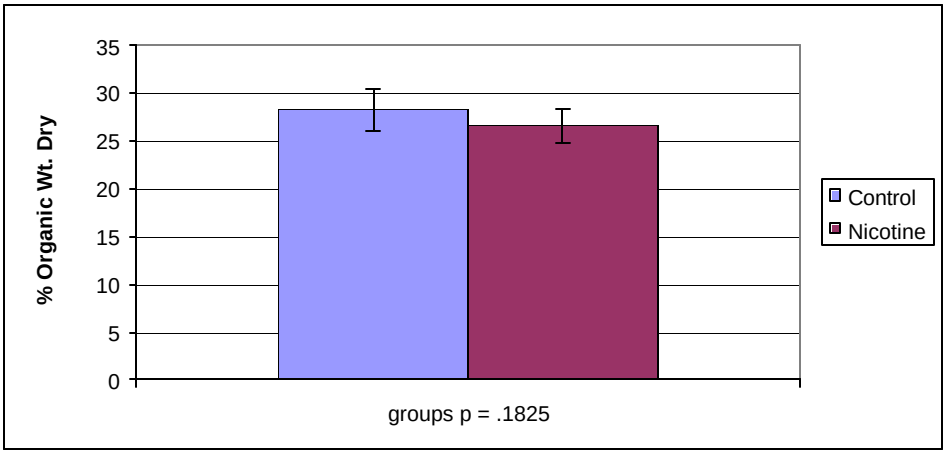


Figure 5.6 – 12: % Organic Dry for nicotine and control groups

Table 5.6 – 12: Values for figures 5.6 - 12

groups	%Organic Wt. Dry	Standard Deviation
Control	28.20934	2.161804
Nicotine	26.59925	1.773747

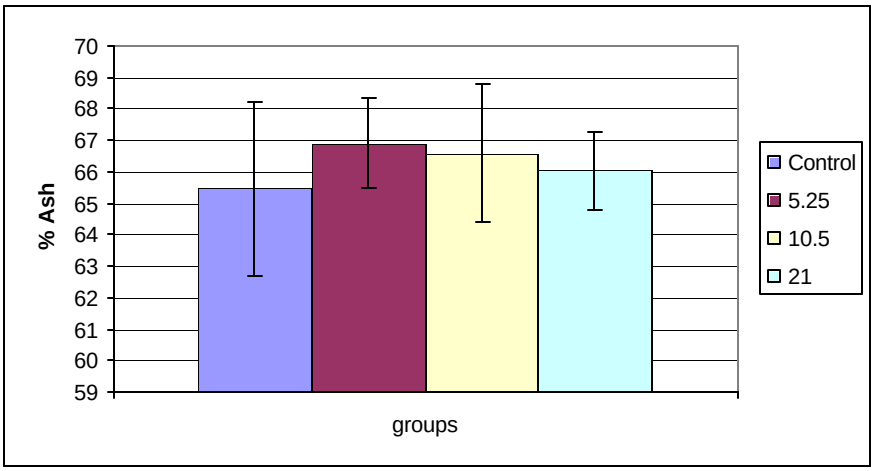


Figure 5.6 – 13: % Ash for all groups

Table 5.6 – 13: Values for figures 5.6 – 13 and p values for each group

groups	% Ash	Standard Deviation
Control	65.45607	2.736882
5.25	66.90258	1.431898
10.5	66.57826	2.191925
21	66.04006	1.250519

Groups	Prob>F
all	0.7388
5.25 & C	0.3992
10.5 & C	0.5436
21 & C	0.6611
5.25 & 10.5	0.8068
5.25 & 21	0.3415
10.5 & 21	0.6204

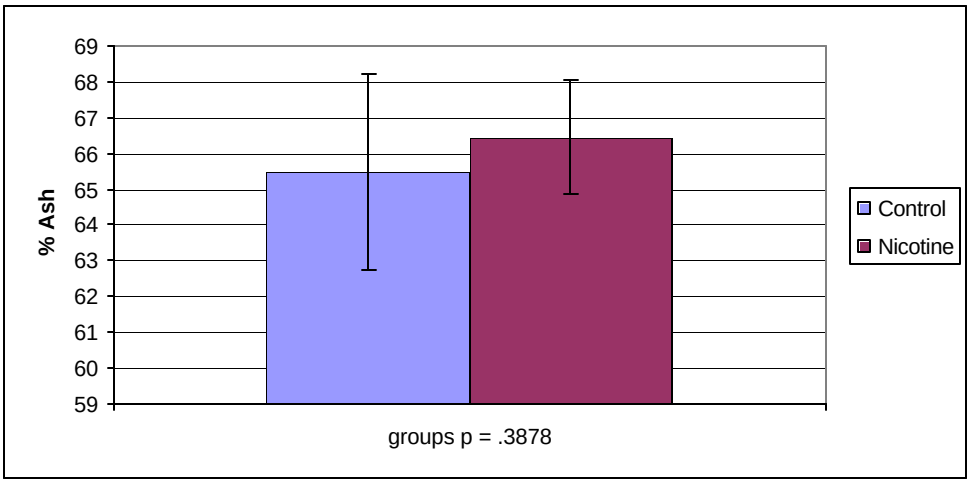


Figure 5.6 – 14: % Ash for nicotine and control groups

Table 5.6 – 14: Values for figures 5.6 - 14

groups	%Ash	Standard Deviation
Control	65.45607	2.736882
Nicotine	66.44947	1.583351

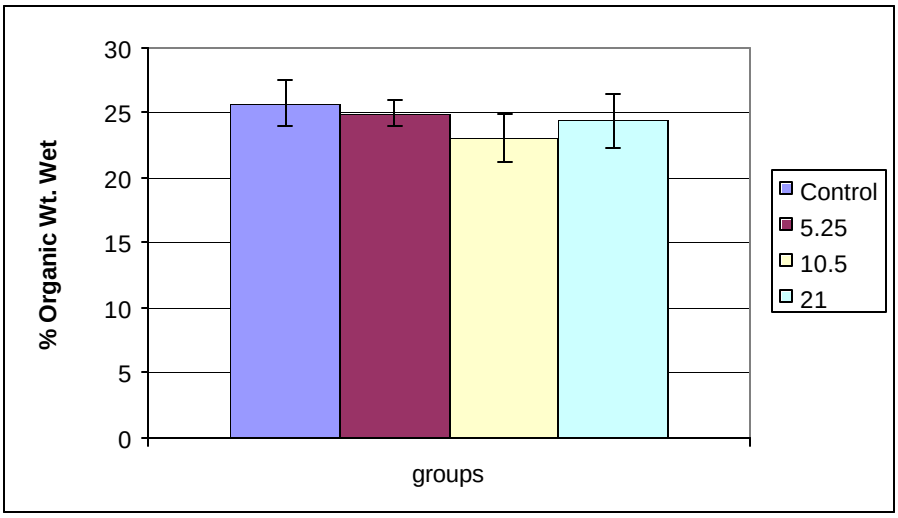


Figure 5.6 – 15: % Organic Wt. Wet for all groups

Table 5.6 – 15: Values for figures 5.6 – 15 and p values for each group

groups	%Organic wt. Wet	Standard Deviation
Control	25.70161	1.737238
5.25	24.94712	1.021928
10.5	23.03476	1.824483
21	24.41216	2.096796

Groups	Prob>F
all	0.2264
5.25 & C	0.4984
10.5 & C	0.0882
21 & C	0.3924
5.25 & 10.5	0.1052
5.25 & 21	0.6525
10.5 & 21	0.2803

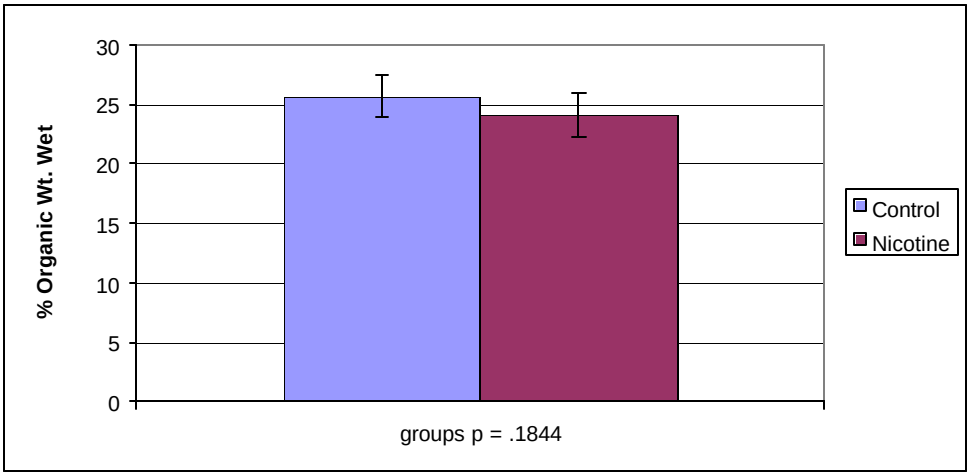


Figure 5.6 – 16: % Organic Wt. Wet for nicotine and control groups

Table 5.6 – 16: Values for figures 5.6 – 16

groups	%Organic Wt. Wet	Standard Deviation
Control	25.70161	1.737238
Nicotine	24.09568	1.84312

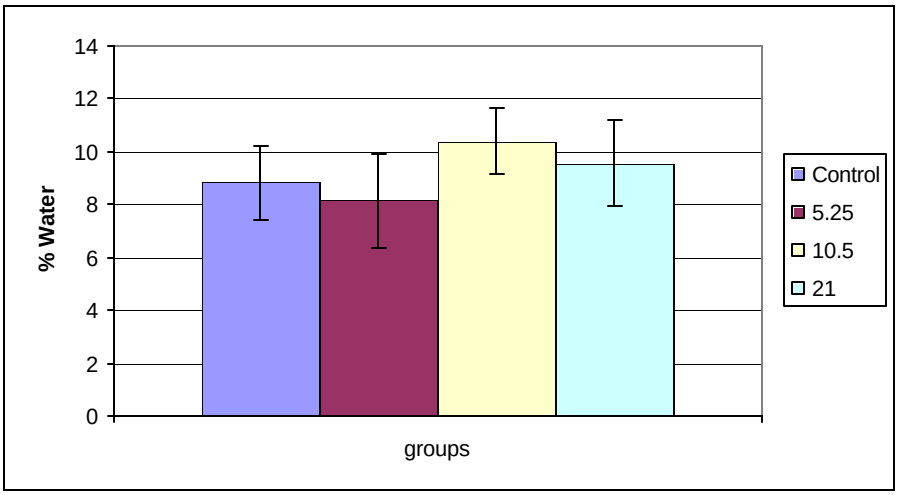


Figure 5.6 – 17: % Water for all groups

Table 5.6 – 17: Values for figures 5.6 – 17 and p values for each group

groups	% Water	Standard Deviation
Control	8.842324	1.398437
5.25	8.150296	1.773523
10.5	10.38698	1.258453
21	9.547779	1.634604

Groups	Prob>F
all	0.2097
5.25 & C	0.6031
10.5 & C	0.1567
21 & C	0.5455
5.25 & 10.5	0.0618
5.25 & 21	0.2356
10.5 & 21	0.3733

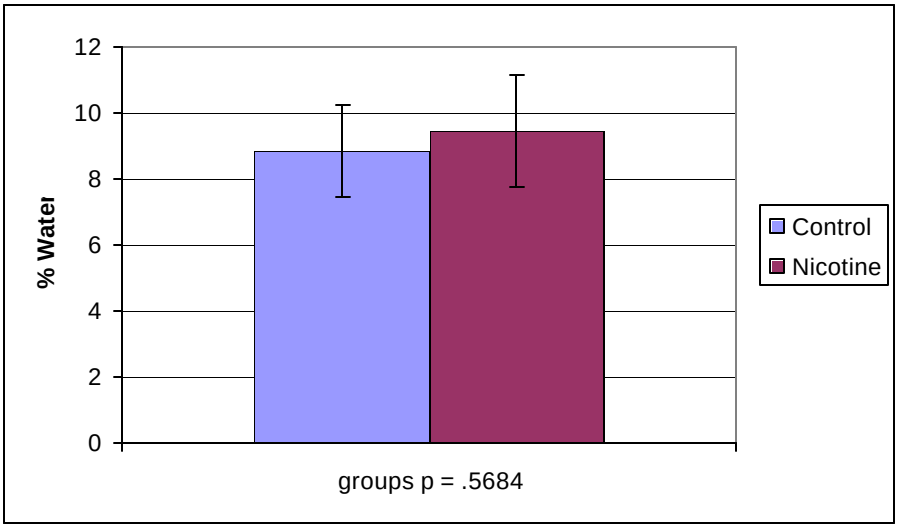


Figure 5.6 – 18: % Water for nicotine and control groups

Table 5.6 – 18: Values for figures 5.6 - 18

groups	% Water	Standard Deviation
Control	8.842324	1.398437
Nicotine	9.454851	1.697356

5.7 Weight and Nicotine Levels for Experiment 2

All individual test data for each rabbit is in Appendix B for group 2. For week one blood draw for Rabbit F8 no blood could be obtained from the rabbit. This affected the average nicotine level seen for the 5 week smoking group. Figure 5.7 – 1 and table 5.7 – 1 is the average nicotine level seen during the 5 weeks. Figure 5.7 – 2 and table 5.7 – 2 is the nicotine level for each measurement seen during the 5 weeks. Figure 5.7 – 3 and table 5.7 – 3 is the average weight difference seen during the 5 weeks. Figure 5.7 – 4 and table 5.7 – 4 is the weight for each measurement seen during the 5 weeks.

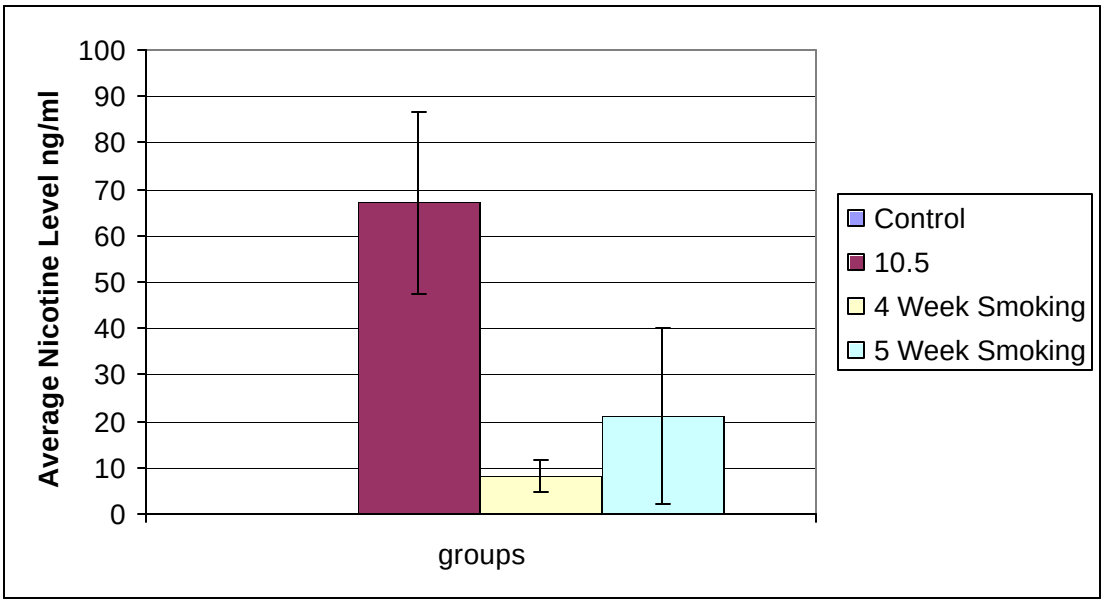


Figure 5.7 - 1: Average nicotine level seen in each group during the 5 week period

Table 5.7 - 1: Value for figure 5.7 – 1 and p values for each group

groups	Nicotine Level (ng/ml)	Standard Devation
Control	0	0
10.5	67.11111	19.55974
4 Week Smoking	8.161111	3.580777
5 Week Smoking	21.26111	18.95643

Groups	P values
ALL	0.0001
Control vs 10.5	0.0001
Control vs 4 weeks smoking	0.0007
Control vs 5 weeks smoking	0.0347
10.5 vs 4 weeks smoking	0.0001
10.5 vs 5 weeks smoking	0.0006
4 vs 5 weeks smoking	0.1272

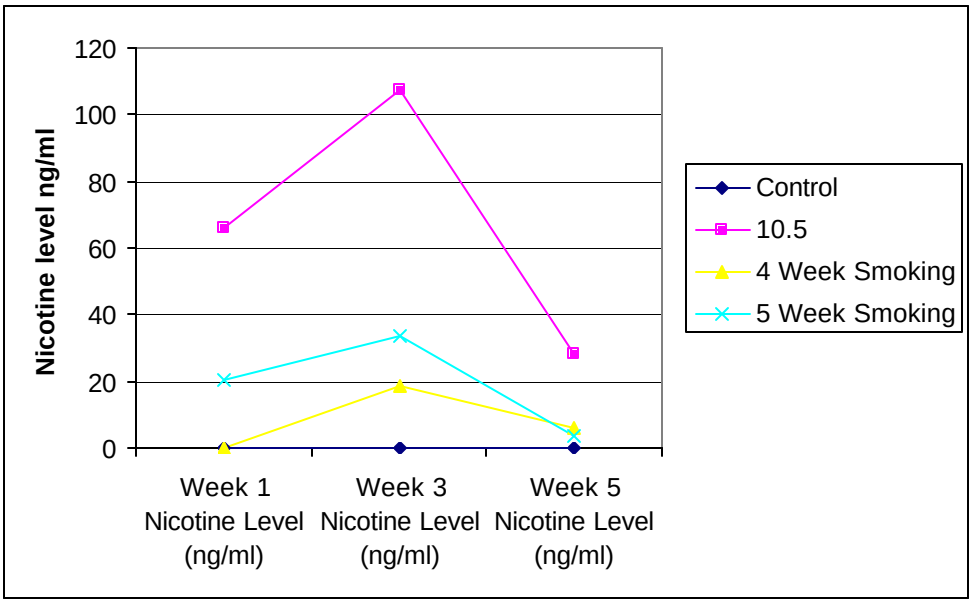


Figure 5.7 - 2: Nicotine levels during the 5 weeks period

Table 5.7 – 2: Values for figure 5.7 – 2

groups	Week 1 Nicotine Level (ng/ml)	Week 3 Nicotine Level (ng/ml)	Week 5 Nicotine Level (ng/ml)
Control	0	0	0
10.5	66.22222	107.1111	28
4 Week Smoking	0	18.66667	5.816667
5 Week Smoking	20.14	33.78333	3.483333

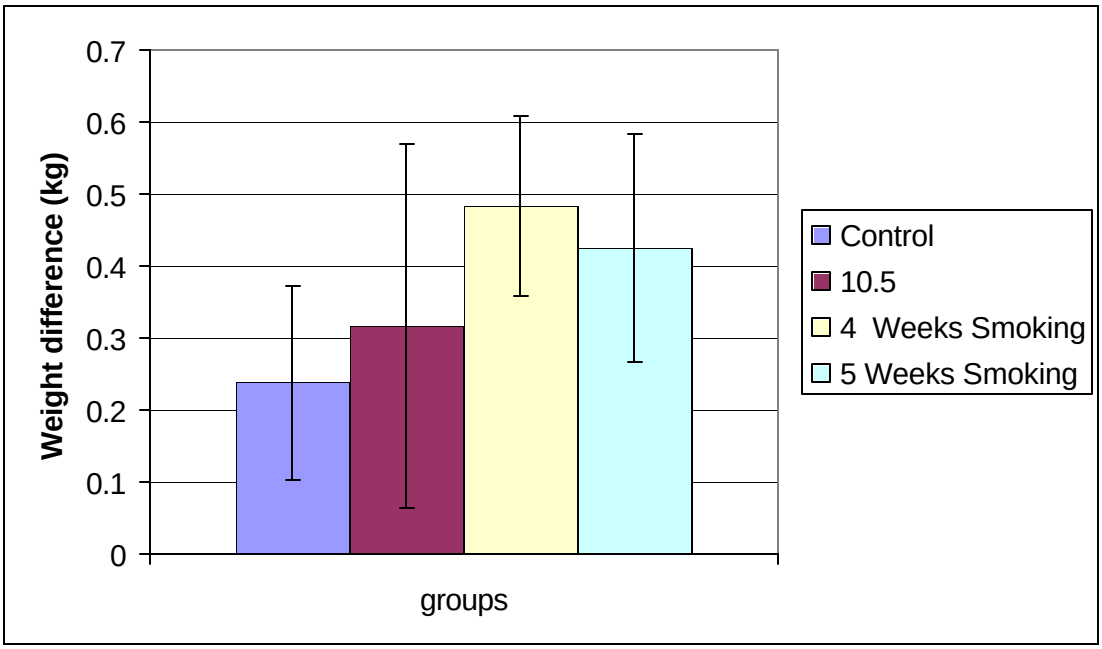


Figure 5.7 – 3: Average Weight of the rabbits during the 5-week period

Table 5.7 – 3: Values for figure 5.7 – 3 and p values for each group

groups	Weight Differenece	Stanrdard Devation
Control	0.238	0.134052
10.5	0.315556	0.252938
4 Weeks Smoking	0.483333	0.125645
5 Weeks Smoking	0.425	0.159217

Groups	P values
ALL	0.1535
Control vs 10.5	0.5402
Control vs 4 weeks smoking	0.0121
Control vs 5 weeks smoking	0.0674
10.5 vs 4 weeks smoking	0.1592
10.5 vs 5 weeks smoking	0.3659
4 vs 5 weeks smoking	0.4972

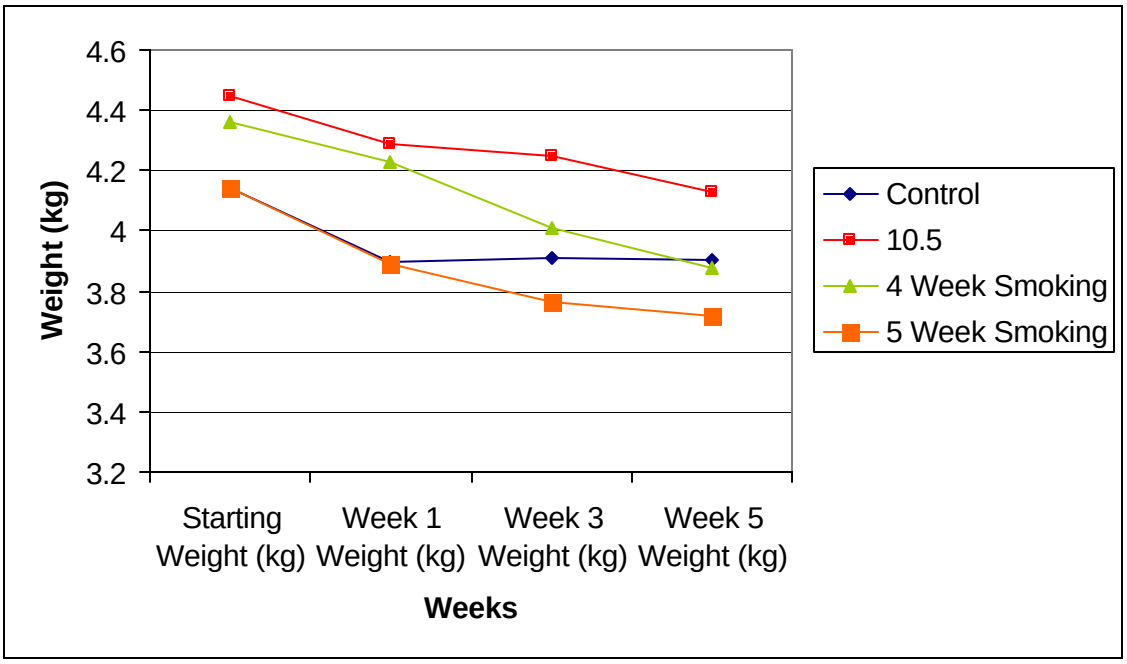


Figure 5.7 – 4: Average Weight of the rabbits during the 5-week period

Table 5.7 – 4: Values for figure 5.7 – 4

Groups	**Starting Weight (kg)**	**Week 1 Weight (kg)**	**Week 3 Weight (kg)**	**Week 5 Weight (kg)**
Control	4.14	3.8975	3.908	3.902
10.5	4.444444	4.286667	4.248889	4.128889
4 Week Smoking	4.36	4.228333	4.011667	3.876667
5 Week Smoking	4.141667	3.891667	3.763333	3.716667
Groups	**Standard Deviation**	**Standard Deviation**	**Standard Deviation**	**Standard Deviation**
Control	0.409878	0.383003	0.371981	0.309467
10.5	0.433333	0.402119	0.412839	0.443352
4 Week Smoking	0.424311	0.281668	0.3629	0.398129
5 Week Smoking	0.252936	0.250553	0.185436	0.168008

5.8 Fracture Toughness Values for Experiment 2

Figure 5.8 –1 and table 5.8 – 1 is the fracture toughness values for each group.

Table 5.8 – 2 is the dimension for fracture toughness for each group.

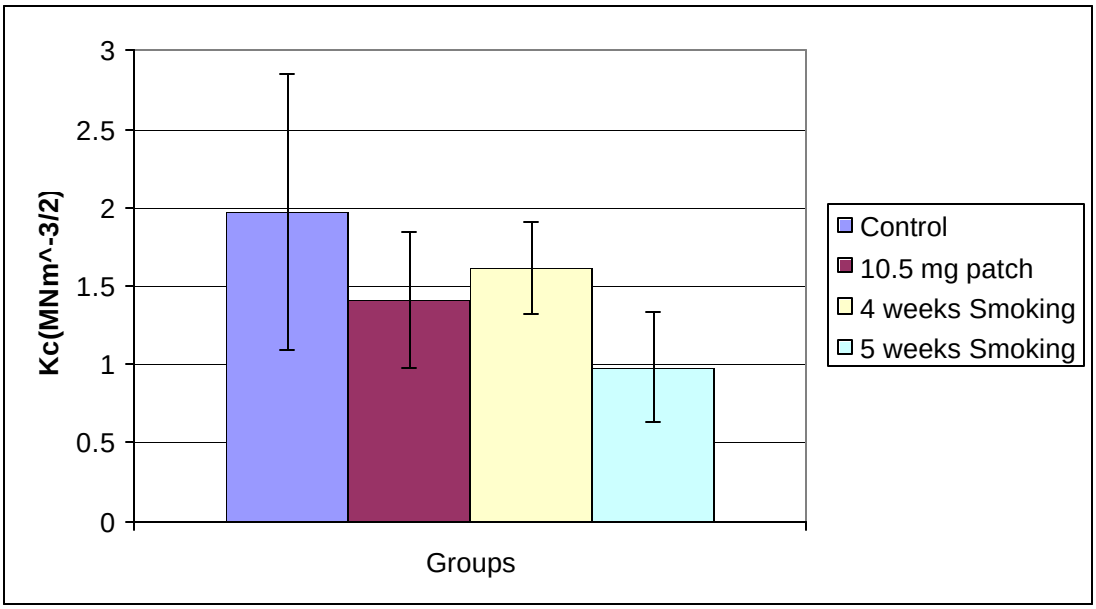


Figure 5.8 – 1: Fracture toughness values for each group

Table 5.8 –1: Values for figure 5.8 – 1 and p values for each group

group	Kc (MNm^-3/2)	Standard Deviation
Control	1.97201	0.883856
10.5	1.413139	0.43044
4 weeks Smoking	1.610427	0.29439
5 weeks Smoking	0.982683	0.3458

Groups	P values
ALL	**0.0274**
Control vs 10.5	0.1318
Control vs 4 weeks smoking	0.3671
Control vs 5 weeks smoking	**0.0317**
10.5 vs 4 weeks smoking	0.3473
10.5 vs 5 weeks smoking	**0.062**
4 vs 5 weeks smoking	**0.0069**

Table 5.8 – 2: Dimension for fracture toughness specimen for each group

group	thickness	a	W	a/W	Load
Control	0.000596	0.003144	0.005514	0.570547	6.77494
10.5	0.000572	0.002753	0.005374	0.512767	5.928689
4 week Smoking	0.000547	0.002928	0.005452	0.537505	5.94065
5 week Smoking	0.000512	0.002507	0.005323	0.470753	3.992683
group	**Standard Deviation**	**Standard Deviation**	**Standard Deviation**	**Standard Deviation**	**Standard Deviation**
Control	0.000087	0.000357	0.000307	0.05861	2.220938
10.5	0.000081	0.000367	0.000222	0.068955	2.236776
4 week Smoking	0.000066	0.00019	0.000137	0.039237	1.079493
5 week Smoking	0.000057	0.000256	0.00023	0.043073	0.945486

5.9 Porosity for Experiment 2

Figure 5.9 – 1 and Table 5.9 – 1 is the porosity for values for each group. Figure 5.9 – 1 and Table 5.9 – 1 is the average pore radius for values for each group. Figure 5.9 – 2 and table 5.9 – 2 is for the average pore radius of each group.

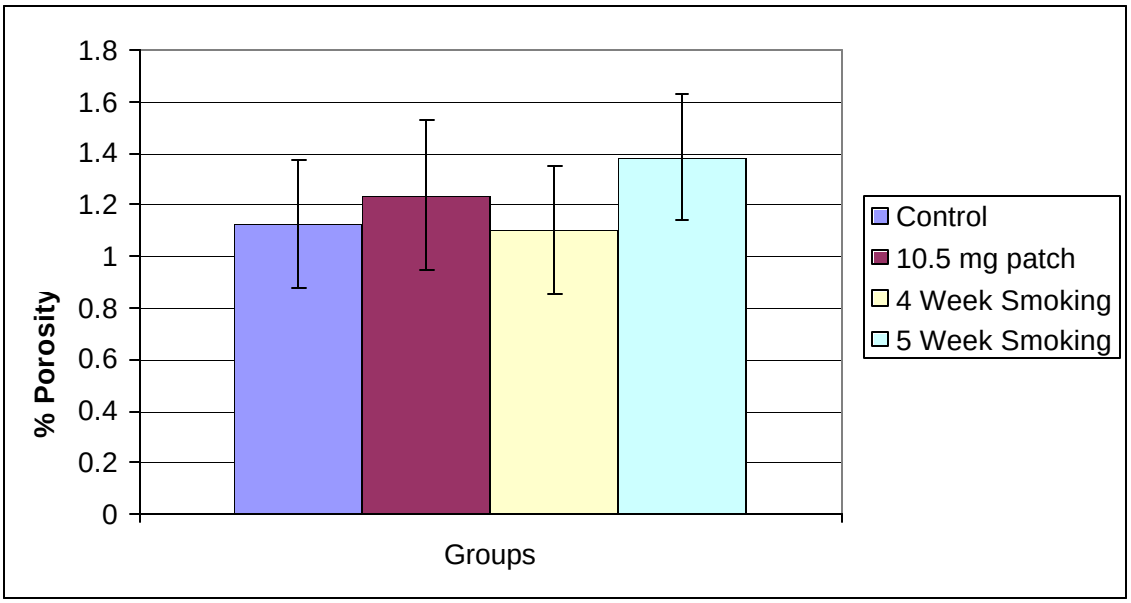


Figure 5.9 – 1: Average % porosity for each group

Table 5.9 – 1: Values for figure 5.9 – 1 and p values for each group

Groups	% Porosity	Standard Deviation
Control	1.126	0.2464
10.5	1.2378	0.2931
4 Week Smoking	1.1033	0.2483
5 Week Smoking	1.3867	0.242

Groups	P values
ALL	0.2652
Control vs 10.5	0.4854
Control vs 4 weeks smoking	0.8831
Control vs 5 weeks smoking	0.1115
10.5 vs 4 weeks smoking	0.3734
10.5 vs 5 weeks smoking	0.3223
4 vs 5 weeks smoking	0.0732

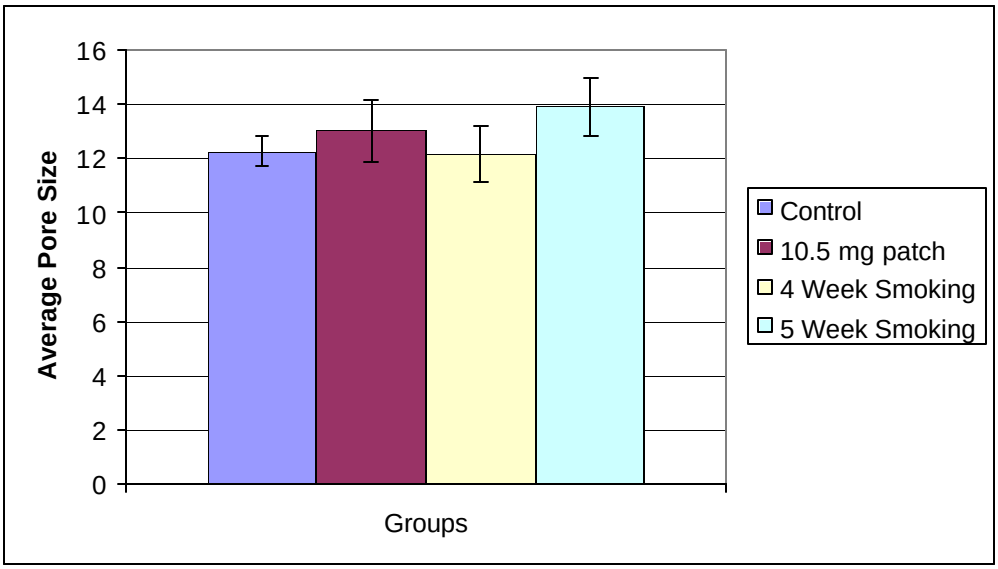


Figure 5.9 – 2: Average pore size for each group

Table 5.9 – 2: Values for figure 5.9 – 2 and p values for each group

Groups	Average Pore Size	Standard Deviation
Control	12.2667	0.570189
10.5	13.01503	1.156733
4 Week Smoking	12.16335	0.992366
5 Week Smoking	13.90523	1.070683

Groups	P values
ALL	**0.027**
Control vs 10.5	0.2046
Control vs 4 weeks smoking	0.842
Control vs 5 weeks smoking	**0.0135**
10.5 vs 4 weeks smoking	0.1643
10.5 vs 5 weeks smoking	0.157
4 vs 5 weeks smoking	**0.0152**

Chapter 6 Discussion

6.1 Discussion of Results of experiment 1.

There was significant difference seen in the nicotine levels between each group of experiment 1. There was a close to a significance difference seen in the weight loss difference between the nicotine groups as a whole versus the control group with $p = .0530$; a power analysis indicated a power of .5013 and the number of rabbits needed to determine a significant difference is 18. A number of studies show similar results in that there was significant difference in weight loss between the nicotine groups, and control(Syversen, Nordsletten et al. 1999), but there was just as many papers showing weight gain or no significant difference between the groups (Daftari, Whitesides et al. 1994; Silcox, Daftari et al. 1995; Fung, Mendlik et al. 1998; Raikin, Landsman et al. 1998; Silcox, Boden et al. 1998; Fung, Iwaniec et al. 1999; Iwaniec, Fung et al. 2000; Iwaniec, Fung et al. 2001; Akhter, Iwaniec et al. 2003). It has been shown that people who smoke weigh less then non-smokers, and after cessation of smoking the average person gains 8 lbs and a about 10% gains close to 30lbs (Williamson and Madans 1991). People who weigh less have lower BMD & increased chance of fractures(Piet 2003). Nicotine could play a part in increasing the risk of fractures by lowering the body weight and in time causing the BMD to be lower.

Rabbits administered nicotine for five weeks had no significant difference measured in any of the tests done for experiment 1. Nicotine has been shown in other studies to have no effect on bone strength in rats for varied amounts of time, dose, age of the rat and ovariectomy or not (Fung, Mendlik et al. 1998; Fung, Iwaniec et al. 1999; Syversen, Nordsletten et al. 1999; Iwaniec, Fung et al. 2000; Iwaniec, Fung et al. 2001;

Akhter, Iwaniec et al. 2003). In another series of tests conducted using the rabbit spine from this study, higher spine fusion results showed that 5.25 ng/ml patch had a higher fusion rate than the other groups, although the difference was not significant. The fracture toughness study showed similar, i.e. 5.25 ng/ml group had a higher, but no significant, (p= .0384) values compared to the control group. A power analysis indicated a power of .1226 and the number of rabbits needed to find a difference is 32.

6.2 Discussion of Results of Experiment 2

The nicotine levels in experiment 2 were significantly different for each group. In the fracture toughness study, a significant difference was seen in the fracture toughness values for the 5 week smoking chamber group compared to all other groups. However, no significant difference could be found in the other groups compared to each other. There was also a close to significant difference in porosity between the control group and 5 week smoking group (p= .1115) and a power analysis was done on these groups and found that the power was .3514 and the number of rabbits needed in each group to determine if there is significant difference was 16. The average pore radius was significantly different between the different groups. From this study it suggests that the nicotine has no effect on weakening bones' fracture toughness, however, cigarette smoke does. Therefore something else other than nicotine may be responsible for the effect of nicotine on bone. A cigarette contains over 4,000 chemicals that maybe responsible for the smoking effect above. For example, a study done on ovariectomized rats estrogen replacement, by Lee using Polycyclic Aromatic Hydrocarbons (PAH) 7,12-dimethylbenz(a)anthracene (DMBA), and Benzo-(a)pyrene (BaP) showed a decrease in both vertebral compression and three point bending test (Lee, Lee et al. 2002).

Therefore, it is possible that PAH might be the chemical in cigarette that weakens bone. As noted previously, the 5 week smoking groups had significantly different fracture toughness value than the 4 week smoking group. The group that was exposed for 4 weeks started receiving cigarette smoke a week after the surgery and only received first and second hand smoke on the last week before sacrifice. This mean that either there is a difference between the first and second hand smoke that caused the weakening in bones or that the body recovering during the first week after the surgery absorbs more of the chemicals from cigarette smoke.

The spine fusion results have not been completed at this time. If this study shows that the 5 week smoking group has a lower fusion rate than the control and nicotine groups, then it may suggest that after a spine fusion giving the patient a nicotine patch during the first weeks of recovery may aid in the healing of the fusion and reduce the need for cigarettes at this critical time.

6.3 Limitations and Recommendations

There were several limitations to the project. The most significant limitation was the failure to control nicotine levels in the smoking rabbits to match those in the patch rabbits. The blood serum levels indicated significantly lower nicotine levels in the smoking group making it difficult to compare strictly on the basis of smoking versus nicotine. No measurements were made of BMD to see if the bone BMD changed in any of the nicotine or smoking groups. No histology measurements of trabecular bone were made. Also no dynamics histology was done to see if the bone-remodeling rate was affected. No other serum levels were measured in the rabbits, ie: 25-hydroxyvitiamin D

serum, to see if nicotine or smoking changes any of these chemical levels. In addition, no other chemical was measured for the cigarette smoking groups beside nicotine levels.

Some recommendations for future studies in this field are to find out what chemical does weaken bone and investigate if there are better ways to machine fracture toughness specimens to help reduce the error due to machining. If the current spine fusion study shows that the 5 week smoking group has lower fusions rate than the controls and nicotine groups then maybe a clinical study could be completed to see if people who are given a nicotine patch after spine fusion have better fusions rate than those who are allowed to smoke right after the surgery.

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Appendix A

Table A1-a: Weight and Nicotine measurements for every Experiment 1

Rabbit #	Nicotine Dose (mg/ml)	Surugy Date	Sacurefic Date	notes:
R179	10.5	5/6/2003	6/10/2003	paraplegia
R181	10.5	5/23/2003	6/27/2003	
R187	10.5	5/8/2003	6/12/2003	
R188	10.5	5/23/2003	6/27/2003	rear right leg
R191	10.5	5/9/2003	6/13/2003	
R168	21	5/8/2003	6/12/2003	
R176	21	5/5/2003	6/10/2003	
R180	21	5/6/2003	6/10/2003	
R184	21	5/7/2003	6/11/2003	
R192	21	5/9/2003	6/13/2003	
R196	21	5/7/2003	6/11/2003	left rear leg
R166	5	5/22/2003	6/26/2003	
R174	5	5/22/2003	6/26/2003	
R178	5	5/22/2003	6/26/2003	
R186	5	5/8/2003	6/12/2003	
R185	Control	5/7/2003	6/11/2003	
R189	Control	5/9/2003	6/13/2003	
R195	Control	5/6/2003	6/10/2003	

Table A1-b: Weight and Nicotine measurements for every rabbit in Experiment 1

Rabbit #	Nicotine Dose (mg/ml)	Starting weight (kg)	Week 1 weight (kg)	Week 3 weight (kg)	Week 5 Weight (kg)	Weight Difference (kg)
R179	10.5	4.53	4.08	3.4	3.56	0.97
R181	10.5	4.25	4.08	3.99	3.85	0.4
R187	10.5	4.15	3.98	3.92	3.84	0.31
R188	10.5	4.51	4.38	3.91	3.79	0.72
R191	10.5	4.55	4.47	4.5	4.52	0.03
R168	21	4.04	3.65	3.4	3.36	0.68
R176	21	4.35	4.1	3.71	3.98	0.37
R180	21	4.52	4.49	4.36	4.26	0.26
R184	21	4.63	4.29	4.06	3.97	0.66
R192	21	4.24	4.22	4.1	3.9	0.34
R196	21	4.8	4.6	4.15	3.92	0.88
R166	5	3.94	3.9	3.9	3.77	0.17
R174	5	4.77	4.83	4.83	4.75	0.02
R178	5	4.37	4.3	4.12	4.07	0.3
R186	5	4.33	4.14	4.05	3.86	0.47
R185	Control	4.89			4.93	-0.04
R189	Control	4.67			4.44	0.23
R195	Control	4.67	4.63	4.63	4.63	0.04

Table A1-c: Weight and Nicotine measurements for every rabbit in Experiment 1

Rabbit #	Nicotine Dose (mg/ml)	Nicotine Full (Days)	Nicotine 1/2 one patch on of the 2 (Days)	Nicotine patch off (days)	Nicotine Serum level 1 week	Nicotine Serum level 3 weeks	Nicotine Serum level 5 weeks
R179	10.5	32	1	0	170	130	33
R181	10.5	34	1	0	94	140	57
R187	10.5	26	9	0	240	140	100
R188	10.5	31	3	1	65	86	23
R191	10.5	5	20	10	8.2	50	24
R168	21	26	7	2	170	160	70
R176	21	22	2	12	260	7.7	76
R180	21	28	3	0	190	290	110
R184	21	26	4	5	380	19	160
R192	21	20	9	4	290	59	200
R196	21	26	1	7	72	13	65
R166	5	6	x	27	0	16	0
R174	5	15	x	19	7	9.2	6.5
R178	5	16	x	18	0	21	12
R186	5	3	x	30	0	7.7	10
R185	Control	x	x	x	0	0	0
R189	Control	x	x	x	0	0	0
R195	Control	x	x	x	0	0	0

Table A2-a: Fracture toughness measurement and values for each rabbit in Experiment 1

Rabbit #	Nicotine Dose (mg/ml)	Nicotine Dose (mg/ml)	thickness (m)	a (m)	W (m)	a/W
R179	10.5	10.5	0.00054	0.00189	0.00476	0.397059
R181	10.5	10.5	0.00055	0.00268	0.0053	0.50566
R187	10.5	10.5	0.00058	0.0029	0.0048	0.604167
R188	10.5	10.5	0.00059	0.00252	0.005425	0.464516
R191	10.5	10.5	0.00076	0.00255	0.00545	0.46789
R168	21	21	0.00062	0.00242	0.0049	0.493878
R176	21	21	0.00054	0.0027	0.0055	0.490909
R180	21	21	0.00069	0.00263	0.00554	0.474729
R184	21	21	0.00042	0.00226	0.00496	0.455645
R192	21	21	0.00059	0.00266	0.005645	0.471213
R196	21	21	0.00067	0.00288	0.00565	0.509735
R166	5	5	0.00057	0.0025	0.0055	0.454545
R174	5	5	0.00047	0.0028	0.005	0.56
R178	5	5	0.00055	0.00265	0.005415	0.489381
R186	5	5	0.00054	0.00244	0.00528	0.462121
R185	Control	Control	0.00052	0.0026	0.005	0.52
R189	Control	Control	0.00069	0.00304	0.00538	0.565056
R195	Control	Control	0.00064	0.00246	0.00523	0.470363

Table A2-b: Fracture toughness measurement and values for each rabbit in Experiment 1

Rabbit #	Nicotine Dose (mg/ml)	f(a/W)	Max Load (N)	Kc (MNm^-3/2)	Complinace Slope Inverse
R179	10.5	7.270231	6.3468	1.238528	2.247696
R181	10.5	9.767915	6.5204	1.590653	2.51067
R187	10.5	13.76891	2.41	0.825787	4.2123
R188	10.5	8.674538	6.4935	1.296204	2.392344
R191	10.5	8.75609	6.9306	1.081606	2.423655
R168	21	9.430979	5.9247	1.287459	3.101737
R176	21	9.34934	7.54673	1.761833	2.797985
R180	21	8.925424	10.0232	1.741932	1.948938
R184	21	8.466053	4.4038	1.260429	3.462604
R192	21	8.837679	6.4785	1.291602	3.128911
R196	21	9.889565	6.1702	1.211651	2.3912
R166	5	8.440778	10.6249	2.121539	2.065262
R174	5	11.65799	3.2752	1.14889	3.648304
R178	5	9.307811	8.9603	2.060669	2.49501
R186	5	8.617418	5.4756	1.202536	2.514458
R185	Control	10.20866	4.5296	1.257593	3.749531
R189	Control	11.86883	7.0713	1.658315	2.464876
R195	Control	8.816687	5.0804	0.967771	2.62743

Table A3-a: Bending Stress measurements for each rabbit in Experiment 1

Rabbit #	Nicotine Dose (mg/ml)	max load (N)	max deflections (mm)	bo diameter short outside mm	bo/2 radius mm	bi diameter short inside mm
R179	10.5	370.67	-1.3798	7.182	3.591	5.164
R181	10.5	305.91	-0.9302	7.036	3.518	4.855
R187	10.5	310.41	-0.8713	7.2	3.6	5.455
R188	10.5	343.22	-1.1992	6.727	3.3635	4.709
R191	10.5	297	-1.0108	6.764	3.382	4.873
R168	21	320.42	-1.1914	6.964	3.482	4.8
R176	21	340.38	-1.0116	6.527	3.2635	4.345
R180	21	333.98	-1.5604	7.945	3.9725	6.327
R184	21	337.53	-1.1759	7.291	3.6455	5.491
R192	21	300.46	-1.179	7.073	3.5365	5.291
R196	21	503.93	-1.5968	7.218	3.609	5
R166	5	377.73	-1.3061	7.236	3.618	5.236
R174	5	429.31	-1.2333	7.491	3.7455	5.436
R178	5	367.63	-1.0844	6.745	3.3725	4.655
R186	5	222.24	-0.217	7.327	3.6635	4.873
R185	Control	299.32	-0.9333	7.509	3.7545	5.473
R189	Control	284.1	-1.0961	7.618	3.809	5.855
R195	Control	413.24	-1.0658	7.382	3.691	5.091

Table A3-b: Bending Stress measurements for each rabbit in Experiment 1

Rabbit #	Nicotine Dose (mg/ml)	thickness 1 mm	thickness 2 mm	thickness 3 mm	thickness 4 mm	Average mm
R179	10.5	1.255	1.091	1.255	0.891	1.123
R181	10.5	1.238	1.164	1.309	1.018	1.18225
R187	10.5	0.891	0.855	1.055	0.855	0.914
R188	10.5	1.491	1.164	1.091	0.855	1.15025
R191	10.5	1.218	1	1.073	0.873	1.041
R168	21	1.418	1.018	1.345	1.145	1.2315
R176	21	1.073	1	1.364	1.145	1.1455
R180	21	1.073	0.636	1.091	0.964	0.941
R184	21	1.018	0.891	1.236	0.945	1.0225
R192	21	1.127	0.945	1.127	0.836	1.00875
R196	21	1.327	1.109	1.2	1.091	1.18175
R166	5	1.036	1	1.273	0.982	1.07275
R174	5	1.164	0.982	1.218	1.055	1.10475
R178	5	1.182	1.109	1.055	1	1.0865
R186	5	1.364	1.291	1.127	1.164	1.2365
R185	Control	1.091	0.982	1.473	0.982	1.132
R189	Control	1.091	0.764	1.109	0.927	0.97275
R195	Control	1.291	1.273	0.909	1.018	1.12275

Table A3-c: Bending Stress measurements for each rabbit in Experiment 1

Rabbit #	Nicotine Dose (mg/ml)	ao diameter long outside mm	ao/2 radius mm	ai diameter long inside mm	Moment N*m	Moment of Inerta hollow ellipsce m^4	Bending Stress pa	bending Stress in Mpa
R179	10.5	10.255	5.1275	7.764	11.49077	5.57E-10	2.96E+08	296.4226
R181	10.5	9.012	4.506	6.555	9.48321	4.83E-10	2.76E+08	276.1243
R187	10.5	9.564	4.782	7.6	9.62271	4.65E-10	2.98E+08	297.7136
R188	10.5	9.673	4.8365	7.164	10.63982	4.53E-10	3.16E+08	316.2677
R191	10.5	9.127	4.5635	6.855	9.207	4.13E-10	3.02E+08	301.8623
R168	21	9.455	4.7275	6.673	9.93302	5.02E-10	2.76E+08	275.6943
R176	21	10.091	5.0455	7.636	10.55178	4.35E-10	3.17E+08	316.9927
R180	21	10.855	5.4275	8.673	10.35338	6.76E-10	2.43E+08	243.2799
R184	21	9.509	4.7545	7.309	10.46343	5.12E-10	2.98E+08	297.9684
R192	21	9.818	4.909	7.564	9.31426	4.84E-10	2.72E+08	272.1037
R196	21	9.982	4.991	7.527	15.62183	5.66E-10	3.98E+08	398.4963
R166	5	9.164	4.582	6.818	11.70963	5E-10	3.39E+08	338.962
R174	5	10.055	5.0275	7.746	13.30861	6.03E-10	3.31E+08	330.682
R178	5	8.727	4.3635	6.509	11.39653	4.03E-10	3.82E+08	381.6397
R186	5	9.436	4.718	6.982	6.88944	5.72E-10	1.76E+08	176.3703
R185	Control	10.073	5.0365	7.6	9.27892	6.16E-10	2.26E+08	226.1583
R189	Control	9.746	4.873	7.618	8.8071	5.66E-10	2.37E+08	236.8848
R195	Control	9.782	4.891	7.6	12.81044	5.72E-10	3.31E+08	330.5925

Table A4-a: Femoral Neck Load for each measurement in Experiment 1

Rabbit #	Nicotine Dose (mg/ml)	Ultimate Load (N) Fx neck
R179	10.5	762.2787
R181	10.5	1053.348
R187	10.5	1167.121
R188	10.5	1226.378
R191	10.5	1097.909
R168	21	1032.964
R176	21	1173.284
R180	21	1251.265
R184	21	1053.111
R192	21	1100.99
R196	21	1379.497
R166	5	1059.037
R174	5	1007.365
R178	5	1169.254
R186	5	938.3898
R185	Control	1046
R189	Control	989.3506
R195	Control	986.5063

Table A5-a: Shear Stress Measurements for each rabbit in Experiment 1

Rabbit #	Nicotine Dose (mg/ml)	bo diameter short outside mm	bo radius	bi diameter short inside mm	ao diameter long outside mm	ao radius	ai diameter long inside mm
R179	10.5	5.203	2.6015	3.035	7.355	3.6775	4.802
R181	10.5	5.018	2.509	2.982	6.75	3.375	4.5
R187	10.5	4.875	2.4375	2.786	7.304	3.652	4.964
R188	10.5	5.571	2.7855	3.161	6.786	3.393	4.304
R191	10.5	5.036	2.518	3.321	6.911	3.4555	4.964
R168	21	5.018	2.509	2.607	6.982	3.491	4.5
R176	21	5.25	2.625	3.232	7.339	3.6695	5.179
R180	21	5.696	2.848	3.339	7.804	3.902	5.446
R184	21	5.161	2.5805	3.268	7.768	3.884	5.929
R192	21	5.054	2.527	3.018	7.196	3.598	4.946
R196	21	5.411	2.7055	2.946	7.143	3.5715	4.536
R166	5	5.63	2.815	3.256	7.912	3.956	4.804
R174	5	5.224	2.612	3.172	7.724	3.862	5.259
R178	5	5.138	2.569	3.121	6.983	3.4915	4.5
R186	5	5.086	2.543	3.564	7.103	3.5515	5.259
R185	Control	5.138	2.569	3.069	8.052	4.026	5.586
R189	Control	5.672	2.836	3.793	7.397	3.6985	5.293
R195	Control	5.224	2.612	3.172	7.207	3.6035	4.845

Table A5-b: Shear Stress Measurements for each rabbit in Experiment 1

Rabbit #	Nicotine Dose (mg/ml)	thickness 1 mm	thickness 2 mm	thickness 3 mm	thickness 4 mm	Average mm
R179	10.5	1.297	1.131	1.231	0.995	1.1635
R181	10.5	1.054	1	1.214	1.018	1.0715
R187	10.5	1.036	1.018	1.286	1	1.085
R188	10.5	1.214	1.089	1.304	1.25	1.21425
R191	10.5	1.036	0.839	0.964	0.911	0.9375
R168	21	1.232	1.161	1.286	1.286	1.24125
R176	21	1.268	1.018	0.929	0.982	1.04925
R180	21	1.161	1.214	1.25	1.107	1.183
R184	21	0.893	0.946	1	0.947	0.9465
R192	21	1.179	0.982	1.107	1.036	1.076
R196	21	1.304	1.286	1.357	1.143	1.2725
R166	5	1.643	1.05	1.488	1.382	1.39075
R174	5	1.172	1.017	1.31	1.017	1.129
R178	5	1.276	1.017	1.224	1.052	1.14225
R186	5	1	0.759	0.879	0.828	0.8665
R185	Control	1.224	1	1.207	1.103	1.1335
R189	Control	1.069	0.948	1.052	0.948	1.00425
R195	Control	1.14	0.864	1.053	0.985	1.0105

Table A5-c: Shear Stress Measurements for each rabbit in Experiment 1

Rabbit #	Nicotine Dose (mg/ml)	Torsion N*m	Polar Moment of Inerta hollow ellipsce (m^4)	Shear Stress (pa)	Shear Stress (Mpa)
R179	10.5	2.8476	5.15E-10	57580751	57.58075
R181	10.5	3.2201	3.94E-10	82065987	82.06599
R187	10.5	4.4264	4.47E-10	96481733	96.48173
R188	10.5	3.8562	4.95E-10	86869665	86.86966
R191	10.5	3.8331	3.89E-10	99195465	99.19547
R168	21	3.7639	4.49E-10	84163816	84.16382
R176	21	4.2154	4.95E-10	89466604	89.4666
R180	21	3.8859	6.7E-10	66055913	66.05591
R184	21	3.4343	5.14E-10	68927559	68.92756
R192	21	3.2332	4.55E-10	71850566	71.85057
R196	21	4.3729	5.33E-10	88711294	88.71129
R166	5	3.0783	7.26E-10	47747727	47.74773
R174	5	3.609	5.66E-10	66646347	66.64635
R178	5	3.7507	4.5E-10	85660025	85.66003
R186	5	2.4521	4E-10	62409400	62.4094
R185	Control	4.2814	6.05E-10	72708872	72.70887
R189	Control	4.5648	5.51E-10	93944608	93.94461
R195	Control	3.2992	4.65E-10	74203707	74.20371

Table A6-a: Volume 1 Measurements for each rabbit in Experiment 1

		Volume 1			
Rabbit #	Nicotine Dose (mg/ml)	W	L	H	V1
R179	10.5	0.54	2.53	6.53	7.224826
R181	10.5	0.5	2.542	6.79	7.059294
R187	10.5	0.5	2.41	6.68	6.478604
R188	10.5	0.58	2.44	6.75	7.730476
R191	10.5	0.83	2.13	6.6	9.060618
R168	21	0.5	2.52	6.32	6.392404
R176	21	0.49	2.38	6.81	6.402442
R180	21	0.64	2.55	6.86	9.184901
R184	21	0.58	2.9	6.75	9.531376
R192	21	0.5	2.46	6.83	6.830104
R196	21	0.64	2.35	6.66	8.006021
R166	5	0.57	2.62	6.72	8.24494
R174	5	0.51	2.44	6.23	6.1504
R178	5	0.52	2.35	6.93	6.834832
R186	5	0.6	2.5	6.59	8.000044
R185	Control	0.34	2.65	6.7	4.968558
R189	Control	0.64	2.44	6.65	8.374021
R195	Control	0.61	2.49	6.72	8.290636

Table A6-b: Volume 2 Measurements and Average Measurement for each rabbit in Experiment 1

	Volume 2					
Rabbit #	Nicotine Dose (mg/ml)	W2	L2	H2	V2	Vaverage
R179	10.5	0.54	2.63	6.51	7.549042	7.386934
R181	10.5	0.53	2.59	6.79	7.655589	7.357441
R187	10.5	0.54	2.56	6.68	7.537972	7.008288
R188	10.5	0.59	2.44	6.64	7.705404	7.71794
R191	10.5	0.85	2.33	6.57	10.34153	9.701075
R168	21	0.48	2.5	6.25	5.992036	6.19222
R176	21	0.49	2.58	6.78	7.031896	6.717169
R180	21	0.68	2.3	6.86	8.592757	8.888829
R184	21	0.43	2.87	6.8	7.040995	8.286186
R192	21	0.52	2.62	6.86	7.712436	7.27127
R196	21	0.68	2.28	6.61	8.111861	8.058941
R166	5	0.55	2.76	6.69	8.427544	8.336242
R174	5	0.42	2.51	6.21	5.227113	5.688756
R178	5	0.55	2.21	6.94	6.707694	6.771263
R186	5	0.62	2.75	6.57	9.254063	8.627053
R185	Control	0.44	2.54	6.69	6.094443	5.531501
R189	Control	0.65	2.28	6.67	7.842905	8.108463
R195	Control	0.62	2.52	6.73	8.567165	8.428901

Table A6-c: Wet Wt. Measurements for each rabbit in Experiment 1

		Wet Wt			
Rabbit #	Nicotine Dose (mg/ml)	1	2	3	average
R179	10.5	0.016	0.0161	0.0161	0.016067
R181	10.5	0.017	0.0167	0.0168	0.016833
R187	10.5	0.0145	0.0146	0.0145	0.014533
R188	10.5	0.0178	0.0178	0.0173	0.017633
R191	10.5	0.021	0.0211	0.0212	0.0211
R168	21	0.0138	0.014	0.0142	0.014
R176	21	0.0142	0.0144	0.0141	0.014233
R180	21	0.0179	0.0179	0.0179	0.0179
R184	21	0.0168	0.017	0.0168	0.016867
R192	21	0.0145	0.0143	0.0141	0.0143
R196	21	0.0174	0.0175	0.0174	0.017433
R166	5	0.0196	0.0193	0.0193	0.0194
R174	5	0.014	0.014	0.0138	0.013933
R178	5	0.0174	0.0172	0.017	0.0172
R186	5	0.0192	0.0196	0.019	0.019267
R185	Control	0.0125	0.0122	0.0126	0.012433
R189	Control	0.0171	0.0173	0.0175	0.0173
R195	Control	0.0188	0.0188	0.0188	0.0188

Table A6-d: Defatted Wt. Measurements for each rabbit in Experiment 1

		Defatted Wt			
Rabbit #	Nicotine Dose (mg/ml)	1	2	3	average
R179	10.5	0.0158	0.0165	0.0161	0.016133
R181	10.5	0.0164	0.016	0.0162	0.0162
R187	10.5	0.0148	0.0144	0.014	0.0144
R188	10.5	0.0167	0.0163	0.0164	0.016467
R191	10.5	0.0205	0.0205	0.0205	0.0205
R168	21	0.0135	0.0134	0.0138	0.013567
R176	21	0.0133	0.0132	0.0139	0.013467
R180	21	0.0179	0.0178	0.0173	0.017667
R184	21	0.0163	0.0165	0.0167	0.0165
R192	21	0.0139	0.0137	0.0139	0.013833
R196	21	0.017	0.0168	0.0165	0.016767
R166	5	0.0193	0.019	0.0192	0.019167
R174	5	0.0128	0.0131	0.0131	0.013
R178	5	0.0163	0.0167	0.0168	0.0166
R186	5	0.0191	0.0188	0.0185	0.0188
R185	Control	0.0121	0.0127	0.0121	0.0123
R189	Control	0.0163	0.0171	0.0171	0.016833
R195	Control	0.0185	0.0185	0.0188	0.0186

Table A6-e: Dry Wt. 1 & 2 Measurements for each rabbit in Experiment 1

		Dry Wt 1				Dry Wt day 2
Rabbit #	Nicotine Dose (mg/ml)	1	2	3	average	1
R179	10.5	0.0142	0.0148	0.0145	0.0145	0.0145
R181	10.5	0.0148	0.0154	0.0158	0.015333	0.0151
R187	10.5	0.0131	0.013	0.0132	0.0131	0.013
R188	10.5	0.0155	0.0157	0.0155	0.015567	0.0158
R191	10.5	0.0182	0.0189	0.0188	0.018633	0.0189
R168	21	0.0125	0.0126	0.0132	0.012767	0.0125
R176	21	0.0125	0.0124	0.0128	0.012567	0.0124
R180	21	0.0163	0.016	0.0164	0.016233	0.0166
R184	21	0.0157	0.0155	0.0159	0.0157	0.0158
R192	21	0.0126	0.013	0.013	0.012867	0.0134
R196	21	0.0154	0.0156	0.0158	0.0156	0.0156
R166	5	0.0181	0.0184	0.0184	0.0183	0.0181
R174	5	0.0129	0.0124	0.0131	0.0128	0.0127
R178	5	0.0159	0.0151	0.0159	0.015633	0.0156
R186	5	0.0171	0.0174	0.0177	0.0174	0.0172
R185	Control	0.0108	0.0115	0.0111	0.011133	0.0111
R189	Control	0.0158	0.0161	0.0158	0.0159	0.0157
R195	Control	0.0175	0.0174	0.017	0.0173	0.017

Table A6-f: Ash Wt. Measurements for each rabbit in Experiment 1

		Ash Wt			
Rabbit #	Nicotine Dose (mg/ml)	1	2	3	average
R179	10.5	0.011	0.0113	0.011	0.0111
R181	10.5	0.0108	0.0111	0.0112	0.011033
R187	10.5	0.0098	0.0101	0.0101	0.01
R188	10.5	0.0111	0.0117	0.0115	0.011433
R191	10.5	0.0137	0.0135	0.0137	0.013633
R168	21	0.0088	0.0091	0.009	0.008967
R176	21	0.0096	0.0093	0.0093	0.0094
R180	21	0.0124	0.0121	0.012	0.012167
R184	21	0.0111	0.0111	0.0113	0.011167
R192	21	0.0095	0.0093	0.0096	0.009467
R196	21	0.0116	0.0114	0.0114	0.011467
R166	5	0.0131	0.0134	0.0132	0.013233
R174	5	0.0094	0.0094	0.0094	0.0094
R178	5	0.0116	0.0115	0.0115	0.011533
R186	5	0.0124	0.0127	0.0124	0.0125
R185	Control	0.0078	0.0078	0.0078	0.0078
R189	Control	0.0117	0.0118	0.0119	0.0118
R195	Control	0.0123	0.0122	0.0124	0.0123

Table A6-g: Composition. Measurements for each rabbit in Experiment 1

Rabbit Number	Nicotine Dose (mg/ml)	Wet Density	Dry Density	% Min	%Org (Dry)	%Ash	%Org (wet)	% Water
R179	10.5	0.00218	0.00196	76.552	23.448	69.087	21.162	9.751037
R181	10.5	0.00229	0.00208	71.957	28.043	65.545	25.545	8.910891
R187	10.5	0.00207	0.00187	76.336	23.664	68.807	21.33	9.862385
R188	10.5	0.00228	0.00202	73.448	26.552	64.839	23.44	11.72023
R191	10.5	0.00218	0.00192	73.166	26.834	64.613	23.697	11.69036
R168	21	0.00226	0.00206	70.235	29.765	64.048	27.143	8.809524
R176	21	0.00212	0.00187	74.801	25.199	66.042	22.248	11.7096
R180	21	0.00201	0.00183	74.949	25.051	67.97	22.719	9.310987
R184	21	0.00204	0.00189	71.125	28.875	66.206	26.877	6.916996
R192	21	0.00197	0.00177	73.575	26.425	66.2	23.776	10.02331
R196	21	0.00216	0.00194	73.504	26.496	65.774	23.709	10.51625
R166	5	0.00233	0.0022	72.313	27.687	68.213	26.117	5.670103
R174	5	0.00245	0.00225	73.438	26.563	67.464	24.402	8.133971
R178	5	0.00254	0.00231	73.774	26.226	67.054	23.837	9.108527
R186	5	0.00223	0.00202	71.839	28.161	64.879	25.433	9.688581
R185	Control	0.00225	0.00201	70.06	29.94	62.735	26.81	10.45576
R189	Control	0.00213	0.00196	74.214	25.786	68.208	23.699	8.092486
R195	Control	0.00223	0.00205	71.098	28.902	65.426	26.596	7.978723

Table A7-a: Porosity and radius size measurement for each rabbit in Experiment 1

	Nicotine Dose (mg/ml)	% Porosity	average area pore size um
R179	10.5	0.02183724	14.84612
R181	10.5	0.02341927	13.9448
R187	10.5	0.01548242	12.87641
R188	10.5	0.0093724	12.9025
R191	10.5	0.00581641	12.1727
R168	21	0.01834635	11.32048
R176	21	0.02533464	13.65804
R180	21	0.01304427	12.70747
R184	21	0.00818555	13.25417
R192	21	0.01014583	13.34112
R196	21	0.00888737	13.7954
R166	5	0.00777865	12.71318
R174	5	0.03040625	13.01157
R178	5	0.01054622	14.89447
R186	5	0.01772656	13.30664
R185	Control	0.01293359	14.04367
R189	Control	0.00805013	12.45012
R195	Control	0.01526172	15.84647

Appendix B

Table B1-a: Weight and Nicotine measurements for every rabbit in Experiment 2

Rabbit #	Group	Surgery Date	Sacurfice Date	Starting Weight (kg)	Week 1 Weight	Week 3 Weight	Week 5 Weight	Weight Difference (kg)
289	Control	2/16/2004	3/22/2004	3.6	3.5	3.52	3.57	0.03
290	Control	2/16/2004	3/22/2004	4.4	4.3	4.27	4.18	0.22
291	Nicotine	2/17/2004	3/23/2004	4.6	4.5	4.53	4.57	0.03
292	Nicotine	2/17/2004	3/23/2004	3.7	3.54	3.65	3.54	0.16
293	Control	2/9/2004	3/15/2004	4.5		4.2	4.11	0.39
294	Nicotine	2/26/2004	4/1/2004	4.2	4.13	4.32	4.33	-0.13
295	Nicotine	2/17/2004	3/23/2004	5.2	4.96	4.92	4.76	0.44
296	Nicotine	2/18/2004	3/24/2004	4.5	4.33	4.13	4.03	0.47
297	Nicotine	2/18/2004	3/24/2004	4.3	4.28	4.04	3.9	0.4
298	Nicotine	2/19/2004	3/25/2004	4.9	4.69	4.73	4.58	0.32
300	Nicotine	2/19/2004	3/25/2004	4.2	4.01	3.97	3.74	0.46
301	Control	2/20/2004	3/26/2004	4.4	4.14	4.05	4.09	0.31
303	Control	2/20/2004	3/26/2004	3.8	3.65	3.5	3.56	0.24
304	4 Week S	2/24/2004	3/30/2004	4.5	4.23	3.99	3.84	0.66
305	4 Week S	2/24/2004	3/30/2004	4.4	4.36	4.12	3.98	0.42
306	4 Week S	2/24/2004	3/30/2004	5.1	4.7	4.64	4.58	0.52
307	4 Week S	2/25/2004	3/31/2004	3.93	3.91	3.56	3.38	0.55
308	4 Week S	2/25/2004	3/31/2004	4.23	4.17	3.96	3.77	0.46
309	4 Week S	2/25/2004	3/31/2004	4	4	3.8	3.71	0.29
310	Nicotine	2/26/2004	4/1/2004	4.4	4.19	3.9	3.71	0.69
F1	5 Week S	4/12/2004	5/17/2004	3.68	3.44	3.44	3.45	0.23
F3	5 Week S	4/12/2004	5/17/2004	4.13	3.87	3.83	3.85	0.28
F4	5 Week S	4/12/2004	5/17/2004	4.29	4.13	3.89	3.83	0.46
F6	5 Week S	4/14/2004	5/19/2004	4.32	4.12	3.97	3.88	0.44
F7	5 Week S	4/14/2004	5/19/2004	4.07	3.88	3.75	3.61	0.46
F8	5 Week S	4/14/2004	5/19/2004	4.36	3.91	3.7	3.68	0.68

Table B1-b: Weight and Nicotine measurements for every rabbit in Experiment 2

Rabbit #	Group	Nicotine Full (Days)	Nicotine 1/2 one patch on of the 2 (Days)	Nicotine patch off (days)	Nicotine Serum level 1 week	Nicotine Serum level 3 weeks	Nicotine Serum level 5 weeks
289	Control	x	x	x	0	0	0
290	Control	x	x	x	0	0	0
291	Nicotine	14	8	13	200	92	0
292	Nicotine	13	11	11	47	180	0
293	Control	x	x	x	0	0	0
294	Nicotine	24	8	2	40	96	24
295	Nicotine	16	14	3	140	120	11
296	Nicotine	28	7	0	44	75	30
297	Nicotine	29	6	0	18	99	82
298	Nicotine	28	6	1	70	100	40
300	Nicotine	15	19	1	?	72	33
301	Control	x	x	x	0	0	0
303	Control	x	x	x	0	0	0
304	4 Week Smoking	x	x	x	0	17	0
305	4 Week Smoking	x	x	x	0	20	5.5
306	4 Week Smoking	x	x	x	0	21	5.8
307	4 Week Smoking	x	x	x	0	35	6.9
308	4 Week Smoking	x	x	x	0	19	6.7
309	4 Week Smoking	x	x	x	?	?	10
310	Nicotine	22	13	0	37	130	32
F1	5 Week Smoking	x	x	x	26	26	8.2
F3	5 Week Smoking	x	x	x	32	22	5.9
F4	5 Week Smoking	x	x	x	8.7	24	0
F6	5 Week Smoking	x	x	x	9	11	0
F7	5 Week Smoking	x	x	x	25	9.7	0
F8	5 Week Smoking	x	x	x	?	110	6.8

Table B1-c: Weight and Nicotine measurements for every rabbit in Experiment 2

Rabbit #	Group	Average nicotine level	Max nicotine level	Min Nicotine Level
289	Control	0	0	0
290	Control	0	0	0
291	Nicotine	97.33333	200	0
292	Nicotine	75.66667	180	0
293	Control	0	0	0
294	Nicotine	53.33333	96	24
295	Nicotine	90.33333	140	11
296	Nicotine	49.66667	75	30
297	Nicotine	66.33333	99	18
298	Nicotine	70	100	40
300	Nicotine	35	72	0
301	Control	0	0	0
303	Control	0	0	0
304	4 Week Smoking	5.666667	17	0
305	4 Week Smoking	8.5	20	0
306	4 Week Smoking	8.933333	21	0
307	4 Week Smoking	13.96667	35	0
308	4 Week Smoking	8.566667	19	0
309	4 Week Smoking	3.333333	10	0
310	Nicotine	66.33333	130	32
F1	5 Week Smoking	20.06667	26	8.2
F3	5 Week Smoking	19.96667	32	5.9
F4	5 Week Smoking	10.9	24	0
F6	5 Week Smoking	6.666667	11	0
F7	5 Week Smoking	11.56667	25	0
F8	5 Week Smoking	58.4	110	6.8

Table B2-a: Fracture toughness measurement and values for each rabbit in Experiment 2

291	Nicotine	0.00057	0.0028	0.00538	0.51115
292	Nicotine	0.00062	0.003	0.00543	0.54696
293	Control	0.00068	0.0029	0.00585	0.48889
294	Nicotine	0.00054	0.0029	0.005	0.57
295	Nicotine	0.00062	0.0021	0.00541	0.38078
296	Nicotine	0.00053	0.0028	0.0052	0.52885
297	Nicotine	0.0005	0.0031	0.0056	0.55714
298	Nicotine	0.00053	0.003	0.00576	0.51736
300	Nicotine	0.00049	0.0022	0.00535	0.41869
301	Control	0.0007	0.0037	0.0057	0.65439
303	Control	0.00053	0.0032	0.00558	0.56989
304	4 Week Smoking	0.00061	0.003	0.00569	0.53427
305	4 Week Smoking	0.00058	0.0027	0.00539	0.50278
306	4 Week Smoking	0.00045	0.0029	0.00539	0.53061
307	4 Week Smoking	0.00061	0.0028	0.00553	0.49729
308	4 Week Smoking	0.00049	0.003	0.0054	0.55556
309	4 Week Smoking	0.00054	0.0032	0.00531	0.60452
310	Nicotine	0.00075	0.0031	0.00524	0.58397
F1	5 Week Smoking	0.00046	0.0022	0.00507	0.42406
F3	5 Week Smoking	0.0006	0.0026	0.00566	0.45406
F4	5 Week Smoking	0.00052	0.0028	0.00546	0.50916
F6	5 Week Smoking	0.00044	0.0026	0.00542	0.4834
F7	5 Week Smoking	0.00053	0.0027	0.0051	0.52745
F8	5 Week Smoking	0.00052	0.0022	0.00523	0.42639

Table B2-b: Fracture toughness measurement and values for each rabbit in Experiment 2

Rabbit #	Group	f(a/W)	Max Load (N)	Kc (Nm^-3/2)	Kc (MNm^-3/2)	Complinace Slope Inverse
289	Control	11.8757	6.6192	2009213	2.009213	2.6645
290	Control	12.2754	4.2931	1381692	1.381692	3.037
291	Nicotine	9.93253	5.8828	1397584	1.397584	2.4437
292	Nicotine	11.1452	8.9424	2181481	2.181481	1.937
293	Control	9.29451	5.787	1034172	1.034172	3.357
294	Nicotine	12.082	3.3201	1050533	1.050533	5.12
295	Nicotine	6.98519	9.0861	1391764	1.391764	2.219
296	Nicotine	10.4993	4.0446	1111111	1.111111	3.11
297	Nicotine	11.5419	5.0236	1549631	1.549631	3.408
298	Nicotine	10.1248	6.0624	1525967	1.525967	2.92
300	Nicotine	7.67883	3.3291	713262.1	0.713262	4.117
301	Control	17.1233	10.3195	3343561	3.343561	1.85
303	Control	12.0772	6.8559	2091411	2.091411	2.467
304	4 Week Smoking	10.6852	6.733	1563531	1.563531	2.212
305	4 Week Smoking	9.68365	5.281	1200973	1.200973	2.789
306	4 Week Smoking	10.5592	4.6673	1491722	1.491722	3.322
307	4 Week Smoking	9.52631	7.6132	1598820	1.59882	2.78
308	4 Week Smoking	11.4783	5.3559	1707334	1.707334	3.201
309	4 Week Smoking	13.7885	5.9935	2100181	2.100181	2.256
310	Nicotine	12.724	7.6671	1796917	1.796917	2.18
F1	5 Week Smoking	7.7859	3.4668	824092	0.824092	4.31
F3	5 Week Smoking	8.42976	3.347	625044.9	0.625045	3.606
F4	5 Week Smoking	9.87216	5.1463	1322232	1.322232	2.653
F6	5 Week Smoking	9.1482	3.6524	1031482	1.031482	3.824
F7	5 Week Smoking	10.4524	5.2361	1445986	1.445986	2.426
F8	5 Week Smoking	7.83293	3.1075	647263.5	0.647263	3.388

Table B3-a: Porosity for Experiment 2

Rabbit #	Porosity	Radius
289	0.010210	12.689917
290	0.007767	12.085333
291	0.012204	14.102859
292	0.015241	14.804247
293	0.014126	12.576312
294	0.010340	11.195685
295	0.013753	13.860507
296	0.009671	11.969880
297	0.010907	12.712015
298	0.009641	13.212694
300	0.018429	13.266483
301	0.011564	11.342799
303	0.012883	12.639387
304	0.013507	12.679905
305	0.010660	12.966466
306	0.012391	11.698264
307	0.013550	12.869522
308	0.007828	10.363446
309	0.008495	12.402669
310	0.011549	12.011041
F1	0.014653	13.81530727
F3	0.012558	13.64026948
F4	0.017566	15.84608063
F6	0.010317	14.20458625
F7	0.013654	12.86125989
F8	0.014527	13.0642382