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MULTIWALL CARBON NANOTUBES ALTER THE THERMAL PROFILE AND ANTIBIOTIC ELUTION OF ORTHOPAEDIC BONE CEMENT

Alison Carroll Tickle
University of Kentucky, alison.tickle@uky.edu

ABSTRACT OF THESIS

MULTIWALL CARBON NANOTUBES ALTER THE THERMAL PROFILE AND ANTIBIOTIC ELUTION OF ORTHOPAEDIC BONE CEMENT

Multiwall carbon nanotubes (MWNTs) have extraordinary mechanical and thermal transport properties. They significantly improve the static and dynamic mechanical properties of acrylic orthopaedic bone cement when added to the dry cement polymer powder. Understanding the role MWNTs play on bone cement polymerization temperatures will lead to improved mechanical integrity of the cement-bone interface in joint arthroplasties. It was determined through thermal testing that MWNTs increased the polymerization time of the methylmethacrylate by 45-460% and decreased the peak exothermic temperature of bone cement with and without antibiotics. The flow of heat produced during polymerizing cement was reduced 25-85% with the addition of MWNTs to the cement powder. This decreases the probability of thermal necrosis and "hot" spots caused by high exothermic polymerization temperatures that can destroy the bone adjacent to the cement. These high temperatures also affect the potency and range of antibiotics used in arthroplasty. Isothermal and elution studies determined that MWNTs altered the heat flow and amount of antibiotic release from bone cement during polymerization. Antibiotic elution from bone cement containing MWNTs could match the elution seen in pure cement. The alteration of the flow of heat from bone cement leads to new options for heat-labile antibiotics in total joint arthroplasty.

KEYWORDS: Multiwall Carbon Nanotubes, Orthopaedic Bone Cement, Bone Cement Polymerization Temperature, Bone Cement Isothermal Reactions, Antibiotic Elution

Alison C. Tickle

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By

Alison Carroll Tickle

David Pienkowski
Director of Thesis

Abhijit Patwardhan
Director of Graduate Studies

May 3, 2010

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Alison Carroll Tickle

The Graduate School

University of Kentucky

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THESIS

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By

Alison Carroll Tickle

Lexington, Kentucky

Director: Dr. David Pienkowski, Associate Professor of Biomedical Engineering

Lexington, Kentucky

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Section 1.1.1 History and Functions

The idea of knee replacement or resurfacing was first conceptualized in the late 1860s [1]. Modern arthroplasties have been performed around the world for over sixty years. This procedure can be performed as a result of trauma or injury, osteo-arthritis, rheumatoid arthritis, congenital disease, or other miscellaneous causes. Total hip replacement was first successfully performed in the United Kingdom in 1962, and approved for use in the United States in 1969 [2, 3]. Acrylic bone cement is used in the fixation of artificial joints and the anchoring of metallic prostheses to bone.

Otto Röhm was one of the first to discuss the polymerization of methacrylates more than 70 years ago [4]. The manufacture of polymethacrylates commercially led to a rapid development of new polymers. The polymer polymethylmethacrylate has been used in medical applications since the late 1930s [5]. In 1936, heat-curable dough was patented by the company Kulzer. The dough was made by mixing polymethylmethacrylate (PMMA) powder with liquid methylmethacrylate (MMA) and a heat-sensitive initiator. In 1943, Kulzer and the company, Deguss, developed the first cold-curing cement [4]. This type of cement does not require additional heating to cure. The cement materials were used to fill defects in the skeleton and to produce cranial plates. Sir John Charnley first succeeded in anchoring femoral head prostheses in the femur with self-curing cement in 1958 [6]. Prosthetic devices with polymethylmethacrylate (PMMA) permitted better range of motion while preserving the stability of the joint. Knee designs improved rapidly during the 1970s and 1980s as a result of the more reliable fixation that cement provided [1].

Bone cement fills the space between the prosthesis and bone. It acts as an elastic buffer and a load transfer system that moves the load from the implanted prosthesis to the host bone. This distribution of stresses is important for the long-term stability of the implant. Cement fracture can result if the external stresses are greater than the cement's ability to transfer the load [4]. Bone cement is also commonly used in resurfacing arthroplasty in addition to primary and revision total joint arthroplasty. The success or failure of a replacement joint is determined by the success of the bone cement fixation.

Section 1.1.2 Current Clinical Data

Joint replacement surgeries, most notably hip and knee, are some of the most frequently performed surgical procedures in the world. Total hip and knee joint replacements were the most common major orthopaedic surgical procedures in 2006, with approximately one million performed annually world-wide [7]. About 160,000 of those total hip and knee replacement procedures were carried out in England and Wales and about 500,000 in the United States [8, 9]. Total joint replacement is the most successful method of treating end-stage arthritis. It improves the quality of life and functional capability of arthritic disease patients. In Sweden, over 95% of hip arthroplasties and over 98% of knee arthroplasties use bone cement for the fixation of at least one component [7]. In the United Kingdom, bone cement is used to secure the metallic prosthesis to the bone in more than 90% of total joint replacements [7, 10]. In the United States, over the period 1979-2000, approximately 77% of primary total knee joint replacements were cemented [11]. The *in vivo* longevity of cemented total joint replacements is very good. The 10-year survival rate of cemented total hip joint replacements (THJRs) being between 94-96% [11].

In 2003, 202,500 primary total hip arthroplasties and 402,100 primary total knee arthroplasties were performed in the US according to Nationwide Inpatient Sample (NIS) and National Hospital Discharge Survey (NHDS) data [12]. The number of total hip revision arthroplasties that year was 36,000 and the number of total knee revisions was

32,700. Over the next two decades there is going to be a massive demand for primary and revisions surgeries. Overall, revision rates in the first three years after a hip or knee replacement in England since April of 2003 were low. Only about one in 75 patients needed a prosthesis revision within three years. The cemented hip and cemented knee prostheses had the lowest revision rates [13].

A methodology to project the prevalence of arthroplastic surgery in future years was developed [12]. Using NIS data, the demand for primary total hip arthroplasty was estimated to increase by 174% between 2005 and 2030. If the number of total knee arthroplasties performed yearly continues at the current rate, the demand for primary total knee arthroplasty is predicted to increase by 673% by 2030. The total number of revision arthroplasty procedures performed in 2005 is expected to double by 2026 for revision hip arthroplasty and by 2015 for revision knee arthroplasty. The total number of total hip arthroplasty revisions was estimated to increase by 137% by 2030 and the total number of total knee arthroplasty revisions was estimated to increase by 601% [12]. They projected a massive increase in demand for primary and revision total joint procedures over the next two decades that will need to be addressed by increasing economic resources, operative efficiency, number of surgeons, and implant longevity. The results are based on historical data which may not be an accurate prediction of the future depending on the quality of the available data, the sample size, and improvements to the implant technology or surgical procedure.

If the number of infection cases is increasing along with the number of procedures being completed, infection during arthroplasty is becoming a growing problem. As the number of infection cases increase, the number of revisions that must be completed also increases. Deep infection after total knee arthroplasty (TKA) or total hip arthroplasty (THA) frequently results in the need for multiple surgical interventions, the need for extended duration parenteral antibiotics, and possible compromised function of subsequent revision TKA or THA [14]. The health care costs of reconstructing previously infected TKAs and THAs have been estimated to be approximately 5 times

that of a primary TKA or THA [15]. Deep infection represents a tremendous economic burden [16].

The Swedish Knee Registry reported that deep infection occurs in 1.7% of total knee arthroplasty patients with osteoarthritis and 4.4% of patients with rheumatoid arthritis [17]. Other sources report infection rates of 1-3% in patients undergoing knee arthroplasty for osteoarthritis [18] and up to 8% for patients having a knee replacement for rheumatoid arthritis [19]. The overall incidence of deep infection has also substantially increased between 1990 and 2003 for both total hip arthroplasty and total knee arthroplasty [20]. In 2003, approximately 1.2% of the total hip and total knee arthroplasties performed in the United States were associated with deep infection. The risk of infection after revision arthroplasty is higher than after primary arthroplasty [21]. The number of infections seen in the United States currently reaches as high as 8,000 to 10,000 per year [22]. Because of the enormous number of surgical procedures completed each year, even with low infection rates, the impact on morbidity, mortality, and medical costs is huge [23]. Deep infections are extremely costly to treat and cause the patient pain and discomfort. The annual hospital charges for primary total hip arthroplasty were estimated at $5.1 billion and estimated at $9.1 billion for primary total knee arthroplasty in 2005 [24]. Hospital charges for revision total hip arthroplasty and revision total knee arthroplasty were estimated in 2005 as $1.3 billion and $0.91 billion, respectively [16].

The incidence of deep infection after primary TKA is rising and has been projected to reach 6.8% by 2030 [25]. This infection percentage may be increasing as a result of patient or infectious organism resistance to the commonly used antibiotics. Also, the infectious organisms may start working synergistically to increase the presence of infections. It was also projected that hip arthroplasty revisions done because of infection will increase from 8.4% in 2005 to 47.5% in 2030 [25]. Similarly, knee arthroplasty revisions as a result of infection were projected to increase from 16.8% in 2005 to 65.5% in 2030 [25]. The incidence of deep infection was projected to exceed 50% after 2030 for total hip arthroplasty and by 2022 for total knee arthroplasty [25]. The actual

percentages of total surgery infection numbers were estimated to increase from 1.4% in 2005 to 6.5% for total hip arthroplasty [25]. For total knee arthroplasty, the total infection burden is projected to increase from 1.4% in 2005 to 6.8% in 2030 [25]. Annual hospital charges were estimated to increase between 2005 and 2015 by 340% to $17.4 billion for primary total hip arthroplasty and by 450% to $40.8 billion for primary total knee arthroplasty [25]. Hospital charges for revision total hip arthroplasty and revision total knee arthroplasty were projected to increase by 290% to $3.8 billion and by 450% to $4.1 billion by 2015 [25]. This provides strong motivation for clinical and technological innovators to develop more effective and timely countermeasures for infection at the site of a joint arthroplasty.

Section 1.2 Bone Cement

Section 1.2.1 Composition and Structure

The biomaterial used in most arthroplasties for fixing components to bone cement. In 2008, there were over 30 commercially available plain acrylic bone cement brands approved by relevant regulatory authorities [11]. Each of the commercial bone cement manufacturers makes products with slight differences. The basic composition of the products is the same, typically called the two-component bone cement system. The manufacturers supply polymer powder in sterile packaging and monomer liquid in an ampule. MMA is the main ingredient of the monomer, 97-99 wt% [11]. It is a clear, colorless, intensely smelling, flammable liquid. MMA is an ester of methacrylic acid with a polymerizable double bond. The MMA also contains *N,N*-dimethyl-para-toluidine (DMPT) (0.4-2.8 wt%), a tertiary amine that acts as an activator [11] and enables cold curing of the polymer, eliminating the need to preheat the material prior to polymerization [5]. The liquid is also stabilized with small amounts of hydroquinone (15-75 ppm) to guarantee shelf-life and to prevent polymerization during storage of the product [4, 11]. Even small amounts of the monomer liquid are detectable by smell, because the odor threshold is only approximately 0.2 ppm [1].

The polymer powder is comprised of beads of pre-polymerized PMMA-based polymer, or MMA copolymers (83-99 wt%) and benzoyl peroxide (BPO) (0.75-2.6 wt%). The BPO is the polymerization reaction initiator and is required to initiate curing of the cement. It reacts with the DMPT to create free radicals that break the carbon double bonds and start the polymerization process [5]. The powder also contains a radiopacifier. The two most commonly added ones include barium sulfate ($BaSO_4$) or zirconium dioxide (ZrO_2), (9-15 wt%). The radiopacifiers are contrasting agents that confer radiopacity and aid in the radiographic assessment of implants. A few manufacturers also add chlorophyllin to their cements to tint the cement to a green color. This allows for better distinction from body tissues during surgery [5]. Additives like these, plus antibiotics, do not take part in the curing process or free-radical polymerization. There are considerable differences between the powder components of different commercialized cements that account for the variations in properties of cements, therefore influencing their performance and the success of the arthroplasty. The mechanical and elution properties of the different cements vary as a result of their different components or differences in cement preparation [1].

Section 1.2.2 Polymerization Process

There are two processes that occur when the two-component system is combined. First, the polymer powder takes up the monomer liquid, called the "wetting" stage. This mixture quickly forms a viscous fluid or dough, called the "dough" stage. During this phase, the monomer and polymer powders experience swelling and dissolution processes that are important for the characterization of bone cement. Second, a chemical process is initiated. The initiator, BPO, from the polymer powder and the DMPT, from the liquid, interact to produce free radicals in what is known as the "initiation reaction". These radicals are able to start the polymerization of MMA by adding to the polymerizable double-bond of the monomer molecule. A polymer chain begins to build up by adding monomer molecules [26]. Polymer chains from the PMMA become available for free radical polymerization and entanglements of these chains with newly formed chains leads

to a connection between the newly formed PMMA with what was already present [5]. The number of radicals generated is high, so many rapidly growing polymer chains are formed. This leads to the fast conversion of MMA to PMMA. The polymerization process takes only a few minutes. Radical polymerization of the MMA generally does not proceed to completion. The mobility of remaining monomer molecules is hindered at high conversion rates. There is approximately 2-6% residual monomer directly after curing [27]. Over the few weeks after curing, the amount of monomer remaining unpolymerized decreases.

The dough phase is important because is offers the possibility of moulding and being used to support a prosthesis, while allowing its insertion. The doughy phase is also susceptible to outside factors. The temperature of the monomer or polymer, ambient room temperature, and humidity impacts polymerization. Lower temperatures inhibit the monomer-polymer reaction and less monomer is allowed to evaporate. This leads to a higher concentration of free monomer, a prolongation of handling time, and a longer setup time from the liquid to the doughy state [1].

The polymerization of acrylic bone cement is exothermic, with the maximum polymerization temperature being high enough that thermal necrosis of the peri-prosthetic tissue may occur [11]. Cement also plays a role in chemical necrosis of the bone as a result of the release of unreacted monomer liquid before polymerization of the cement [28]. The high polymerization temperature was one of the believed reasons for aseptic loosening of prostheses. The high exothermic termperature of bone cement has also been identified as playing a role in impaired local blood circulation and the formation of a membrane at the cement-bone interface. There is 57kJ of heat formed per mole of MMA (molar mass of MMA = 100 g) [26]. This heat formation results in an increase in the temperature of the curing bone cement. The maximum temperature can be influenced by the chemical composition of the cement, by the powder to liquid ratio, and by the radiopacifier. The maximum *in vitro* temperature according to ISO and ASTM standards is approximately 140-176^{o}F. This maximum temperature is only held for a very short

time, and when measured *in vivo* it is lower. Clinical trials showed a maximum temperature of approximately 104-115^{o}F at the interface of bone and cement [4]. The lower *in vivo* temperatures are because of the thin layer of the bone cement used and heat dissipation to the prosthesis and to surrounding tissue [26].

Section 1.2.3 Properties

Each manufacturer's cement has a unique set of material properties that the surgeon must understand. Some of these differences include the viscosity of the initial liquid phase, the length of time for the liquid phase, the length of time for the doughy phase, and the time from doughy state to the solid state. Surgeons may prefer one characteristic or manufacturer according to the requirements of their arthroplasty technique. Most surgeons prefer one type of cement and then make adjustments as necessary for each surgery type [1]. There have been many types of cement and multiple manufacturers. These products have also been sold and passed from one manufacturer to another over time. Some of the major manufacturers of bone cement include Biomet (Warsaw, Indiana, US), Zimmer (Warsaw, Indiana, US), Smith & Nephew (Memphis, Tennessee, US), Stryker (Mahwah, New Jersey, US), and Depuy (Warsaw, Indiana, US). Their common current bone cement products include Refobacin, Palacos, VersaBond, Simplex, and CMW, respectively (Table 1).

There are four different handling phases. The first is the mixing phase (up to 1 minute). It is the period where the powder is thoroughly mixed into the liquid. The second phase is the waiting phase (up to several minutes) and is the period to reach the non-sticky state of the material. The working phase (2-4 minutes) is the period in which the cement is injectable and should be used by the surgeon to manipulate the cement and place it in the joint. The last phase is the hardening phase (1-2 minutes) and is the period of final setting and the development of the heat of polymerization [4]. Viscosity is the most important handling property for the surgeon.

The viscosity of bone cement is determined by the chemical composition and the powder to monomer ratio. There are typically two categories of bone cements: high and low viscosity. High viscosity bone cements have short wetting phases, lose their stickiness quickly, have a shorter waiting phase, are injectable almost directly after mixing, and have a longer handling time. Low viscosity bone cements have a long lasting liquid to low viscosity wetting phase and the material usually remains sticky for three minutes or longer. Common high and low viscosity cements are displayed in Table 1.1. Viscosity of the initial mixture should be low enough to allow material insertion in the bone cavities. When curing starts, monomer is rapidly consumed by the propagation reaction and the viscosity increases [29].

Table 1.1 Current Bone Cements

Brand	Viscosity	Manufacturer	Location
Palacos R	High	Zimmer	Warsaw, Indiana, US
Palamed		Heraeus	Wehrheim, Germany
Smartset HV		DePuy	Warsaw, Indiana, US
Cemfix 1		Teknimed	Vic en Bigorre, France
Osteopal	Low	Zimmer	Warsaw, Indiana, US
Palacos LV		Heraeus	Wehrheim, Germany
Simplex P		Stryker	Limerick, Ireland
Osteobond		Zimmer	Warsaw, Indiana, US
Versabond		Smith & Nephew	Memphis, Tennessee, US
Cemfix 3		Teknimed	Vic en Bigorre, France

All of these manufacturers produce slightly different products. Each bone cement formulation has generally the same composition but there are slight differences that impact the properties of the bone cement. These differences can include type and amount of copolymer, powder particle size, type and amount of radio pacifier, percentage of initiator (BPO), exact chemical formulations, sterilization method, polymerization

reactions, and setting times. In these studies we used two of the above listed cements, one of each viscosity. The two cements were chosen because of their differering viscosities, handling types, and market share.

Another way commercialized cements differ is by molecular weight. Molecular weight is the characteristic parameter for the length of the polymer chains incorporated in the polymer powder or resulting from the polymerization of the MMA. Molecular weight influences the swelling properties and the mechanical properties of the bone cement. The molecular weight of the polymer powder is affected by the type of sterilization procedure used. Sterilization by γ-irradiation or β-irradiation significantly lowers the molecular weight of the cement. Ethylene oxide sterilization has no influence on the molecular weight of the polymer or change any material properties, but it is more complex, time-consuming, and therefore more expensive [4].

Another variation in cement formulation is mixing method. This is an aspect that has been extensively studied. Hand mixing is generally defined as mixing in a ceramic bowl with a metal spatula at stirring frequency of 1-2 Hz. Mixing time is typically one minute. This type of manual mixing method has been shown to create cement mixtures that are porous and contain voids. These voids and pores decrease the mechanical integrity of the cement and can lead to fatigue fractures. Mixing methods that lead to a more efficacious bone cement mantle include mechanical mixing, vacuum mixing, or centrifugation. Vacuum mixing systems typically have a fixed central axis. The cement is mixed for one minute under a pressure of about 300 mmHg. Additional vacuum mixing setups can include a rotating-central axis. In this setup the same mixing procedure is followed. Manual mixing decreases the amount of unmixed powder but vacuum mixing decreases the number of voids in the mixture [30]. The variations caused by mixing must be taken into account in relation to how the cement is going to be used.

Acrylic bone cement is the only material used for anchoring a total joint prosthesis to the contiguous bone in a cemented arthroplasty. It has drawbacks including thermal necrosis,

chemical necrosis, cement shrinkage, and uneven peri-prosthetic cement distribution. Cement shrinkage can lead to loosening, and thereby reduce the clinical life of the arthroplasty. The mechanical properties of the cement will also be affected. The resistance to fracture at both the cement-prosthesis and cement-bone interface can be decreased. Despite the drawbacks, the success rate of arthroplasties is very high. At least ninety percent of implanted cemented hip and knee arthroplasties in patients over fifty years old were successful in maintaining their results over time without deterioration [31].

Section 1.2.4 Antibiotic Laden Bone Cement

Bacterial infections during orthopaedic surgery can come from the atmosphere in the operating room, surgical equipment, and resident bacteria on the patient's skin or already in the body. Device-associated infections are the result of bacterial adhesion or biofilm formation at an implantation site. One of the most critical steps in preventing implant-associated infection is the inhibition of bacterial adhesion [32]. The pathogenesis of post-implant infections differs from other post-surgical infections because of the presence of biomaterials. The interstitial milieu surrounding prosthetic implants is known to represent a region of local immune depression and is often referred to as immuno-incompetent fibro-inflammatory zone [33]. When strange materials are implanted into the human body an inflammatory response known as the foreign body reaction develops. Therefore, any material that is applied to the human body should be able to perform with an appropriate host response, or show a high degree of biocompatibility [5]. Bone cement is one of these materials.

A prosthesis-related infection is difficult to treat. With modern standards and improved sterility within the operating room environment and peri-operative antibiotic prophylaxis, the incidence of infections associated with orthopaedic implants has become very low. It leads to complex revision procedures, failure of the implant, and possibly the need for complete removal of the implant. Standard antibiotic protocols that are effective against

other infections fail in these cases. Typical treatment involves the removal of the infected prosthesis. Antibiotic treatment can then be successful once the foreign body materials have been removed. These antibiotics can be administered systemically or locally. Local administration of antibiotics can be achieved by implanting antibiotic-releasing carriers such as antibiotic bead chains or antibiotic-loaded bone cement [34, 35]. Thermostable antibiotics are added to PMMA and leached out into the blood stream. These methods aim to reach and maintain local antibiotic concentration at a level that cannot be attained using systemic administration without side effects [5].

The most extensively studied and earliest commercially available device for controlled release of antibiotics was developed in the 1970's by Buchholz and Engelbrecht [36]. They had the idea of releasing antibiotics from the newly introduced non-biodegradable polymethylmethacrylate (PMMA) bone cement. Antibiotic bone cement is still widely accepted as a way to reduce bone infection. Buchholz reported in 1981 that there was a success rate of 77% for exhange arthroplasty where the old prosthesis was replaced by a new one that was cemented with antibiotic-loaded bone cement [37]. Performing a second exchange arthroplasty increased the success rate to 90% [38]. There are some drawbacks to this device: PMMA enables only a small fraction of the antibiotic to diffuse through the polymer pores [39-41] and it may possibly house resistant bacteria. Also, since PMMA is not biodegradable, when clinical failure occurs, a secondary surgery may be necessary to remove the PMMA before bone can regenerate. In the bone cement systems with antibiotics, the soluble drug is slowly released from the polymerized bone cement surrounding the implant. Incorporation of antibiotics into bone cement is currently limited to antibacterial drugs that are able to withstand the heat generated by polymerization [32]. Loaded drugs are released through mechanisms of water pore penetration, soluble matrix dissolution, and outward diffusion of solubilized drug via matrix imperfections. The typical release pattern is characterized by an initial burst release followed by a long tail of low, ineffective, and largely incomplete release that continues for days or months. A number of studies have shown that less than 10% of the trapped drug is eventually released from the cement [39-41].

Studies of antibiotic-laden bone cement and the elution characteristics of specific antibiotics from bone cement have been ongoing for over thirty years. Using bone cement as a reservoir for antibiotics, allows for antibiotics to be delivered immediately and locally. It is fully accepted that antibiotic substances mixed in powder form with the prepolymerized polymer powder before mixing with liquid monomer elute after curing from the surface into an aqueous medium. The process is largely diffusion and the rate depends on the chemical composition of the cement, the concentration of the antibiotic, the surface area of the exposed cement, the rate at which the eluent is cleared from the surface, and the chemical stability of the antibiotic itself [2, 36, 38, 42]. Antibiotics are released from bone cement in a bi-phasic fashion. There is peak release initially, followed by a long low release that continues for days or months. As the amount of antibiotic included in the cement is increased, the relative amount released also increases. It is accepted that the local concentrations of antibiotic eluted during the first days of implantation are vastly greater than those available from systemic administration [43].

Gentamicin is one of the most common antibiotics included in commerical antibiotic-loaded bone cements. It has wide-spectrum antimicrobial activity, is water soluble, has thermal stability, and low allergenicity [35]. Gentamicin is a naturally occurring antibiotic produced by the bacterial strain *Micromonospora purpurea* and has been in clinical use for over 50 years [44]. It is an aminoglycoside that has concentration-dependent antibacterial activity. If the antibiotic concentration is high enough, all the bacteria will die within a short period of time. Another antibiotic that is commonly used in loaded bone cements is Tobramycin. Tobramycin is an aminoglycoside antibiotic that is used to treat gram-negative bacterial infections.

Bone cement with the addition of antibiotic has become a standard practice in Europe and Scandinavia, for both primary and revision knee and hip arthroplasties. In Norway, the use of antibiotic-containing bone cement increased from approximately 40% in 1987 to

90% in 1998 in total hip arthroplasties [18]. In 2004, the United States Food and Drug Administration approved antibiotic bone cement use in infected total joint arthroplasties. Prevention of infection would save the patient significant morbidity and the health care system significant costs [19]. The current average cost of treating an infection at the site of a total joint arthroplasty is approximately $60,000, with an average net loss to the hospital of $20,000 per patient [16]. Much has been done to improve operating procedures to minimize contaminiation and bacterial exposure. The use of antibiotic bone cement was found to be cost-effective in eliminating infection and reducing the costs of difficult revisions [18].

Section 1.2.5 Variations and Adaptations

Through the years, surgeons and researchers investigated the different ways bone cement is prepared and the possible additions to the bone cement material that could be made. The common components of cement have also been investigated individually to determine what happens to the cement's material properties when their amounts and ratios are changed. Simplex P cement reduced the *N,N*-dimethyl-para-toluidine (DMPT) content from 2.5 vol% to between 0.8-1.4 vol%. This resulted in a cement with approximately 54% higher setting time, 7% lower maximum exotherm temperature, 4% lower ultimate compressive strength, and the computed polymerization rate at 37^{o}C (k') was approximately 97% lower [11]. The increased setting time puts this type of cement at the maximum limit recommended per ISO 5833 standards (14 minutes). This may pose a problem during preparation and handing for use in a cemented total joint replacement. The increasing handling time may cause economic burden because of the cost per minute in the operating room, scheduling limitations, and additional employee wages.

It has been identified that the presence of radiopacifiers in the cement mixture influences different mechanical properties of bone cement including tensile strength, flexural strength, and fracture toughness. In tensile strength testing it was seen that the addition

of barium sulphate particles significantly decreased tensile strength. This was not seen in ZrO_2, possibly because of the different size and shape of these particles. The barium sulphate particles tended to form agglomerates and didn't seem to anchor themselves to the polymer matrix like the zirconium dioxide agglomerates. Zirconium dioxide cement and barium sulfate cement were found to improve fatigue crack propagation resistance [45].

One of the components of bone cement that is less studied is the effect of initiator benzoyl peroxide on curing parameters and mechanical properties. Samples with lower BPO concentrations had the highest amount of residual monomer present. This may act as an internal plasticer that allows the molecules to reach higher deformation before failure. As the concentration of BPO increased, the polymerization exotherm was increased, accompanied with a shorter dough time and less residual monomer. The mean ultimate tensile strength and the Young's modulus were found to increase with increasing BPO concentration until 2 wt%. During the initiation process of mixing, the DMPT is the active ingredient that induces reaction of the BPO which produces free radicals capable of initiation polymerization. The efficiency of these free radicals falls between 50 and 80%. BPO and DMPT concentrations are the variables that essentially control the rate of polymerization. Peak temperature is expected to increase and the setting time to decrease by increasing both the DMPT and BPO concentrations. The peak temperature decreased with decreasing BPO concentration. The difference in peak temperature for a formulation with a 2 wt% BPO concentration and a formulation with 0.75 wt% was $10^{o}C$. This decrease should not be neglected as any decrease in peak temperature is beneficial to the reduction of long-term necrosis. The setting time increased with decreasing initiator concentration, with a difference of about 5 minutes between the highest and lowest BPO concentrations. Also, as found in literature, the addition of barium sulphate did not affect the curing characteristics of the cement but did affect the mechanical properties. An optimum of 1.5% by weight of benzoyl peroxide was found to yield suitable handling characteristics along with good mechanical properties [46].

In more recent years, surgeons and engineers also began adding elements to bone cement to increase their mechanical strength, fatigue life, or ability to prevent infection. Gladius Lewis reviewed many of the different commercial cements, including reinforced cements [11]. In one sample set, PMMA matrix was reinforced with 0.5-10 wt% multi-walled carbon nanotubes. Two weight percent was found to be the optimum reinforcement mass percentage based on the values of mechanical properties [11]. With this loading, the nanotubes have their long axis oriented to the plane of the incipient crack, and there is an absence of inadequately dispersed nanotubes, as was seen at higher loading. For reinforced cements in general, fatigue life, fracture toughness, ultimate tensile strength, and ultimate compressive strength were improved over control cements. However, for flexural strength, there was little or no gain. There are some problems with reinforced cements, although literature contains very little or no discussion of these main challenges. Those challenges include being able to develop accurate methods of blending the reinforcing agent with the matrix to insure that the agent does not aggregate, obtaining perfect bonding between the reinforcing agent and the matrix so that there are no crack initiation sites at the agent-matrix interface, and limiting the viscosity of the curing cement to keep its handling easy. Also, it must be ensured that the interstices of the bone are completely free, to try to achieve a perfect cement-bone interface [11].

One of the reasons fibers are used to reinforce bone cement is that it is believed that they will increase the fracture toughness properties of bone cement which can prevent failure within the bone cement mantle. The fracture toughness of a polymer (PMMA) can also be increased by increasing the molecular weight of the polymer [47]. Fiber reinforcements of bone cement may be a more practical route to enhance the fracture resistance of bone cement along with increasing the actual strength of the cement. Using fibers with a higher heat capacity has the potential to decrease the peak temperatures observed in the surrounding bone tissue during cement curing [48]. One of the concerns with adding fiber reinforcements to the polymer matrix is that the viscosity of the cement becomes too high to work in a cement gun. Titanium fibers added at 5% to the polymerizing matrix of bone cement were shown to increase the fracture toughness of the

bone cement without significantly affecting the viscosity [49]. Steel has higher volumetric heat capacity than titanium so any temperature reduction would be larger for steel-reinforced bone cement. Steel also has high fracture toughness, a large energy absorption capacity, and it will undergo 30-60% plastic deformation before failure. Steel fibers ranging in diameter from 21.9-52.4 μm and in length from 1-3 mm have been used to augment cement [48]. Fracture toughness of the bone cement was increased by at least 78% and increased as the volume fraction of fibers increased. SEM results of the fractured ends revealed pull-out regions, which indicated that the bonding between the fibers and the matrix is not good. The peak temperatures obtained during curing were decreased in the steel-fiber-reinforced samples [48].

Section 1.3 Carbon Nanotubes

Section 1.3.1 History and Properties

Carbon nanotubes can be traced back to fullerene chemistry (buckyball, C_{60}) in 1985. Carbon nanotubes themselves were discovered by Iijima in 1991. Their discovery has lead to significant scientific, engineering, and medical research about these materials and in discovering ways to use them. Carbon nanotubes (CNTs) are long cylinders of covalently bonded carbon atoms. Every carbon atom is connected to three other carbon atoms in a perfect network. This perfect molecular network, allows nanotubes to maintain their identity up to temperatures around 212^{o}F [50]. There are two types of carbon nanotubes: multi-walled carbon nanotubes (MWNTs) and single-walled carbon nanotubes (SWNTs). MWNTs consist of two or more concentric cylindrical shells of graphene sheets coaxially arranged around a central hollow core with interlayer separations. Figure 1.1 shows a transmission electron micrograph (TEM) of the walls of a multiwall carbon nanotube. MWNTs are flexible and resilient [51] hollow tubular structures that have diameters from several nanometers to several hundred nanometers [52] and are 10-100 μm long [53]. SWNTs have a single graphene cylinder and have an

average diameter of 0.4 to 3 nm [52, 53]. MWNTs have physical characteristics of solids and are micro-crystals with high aspect ratios of 1000 or more [54].

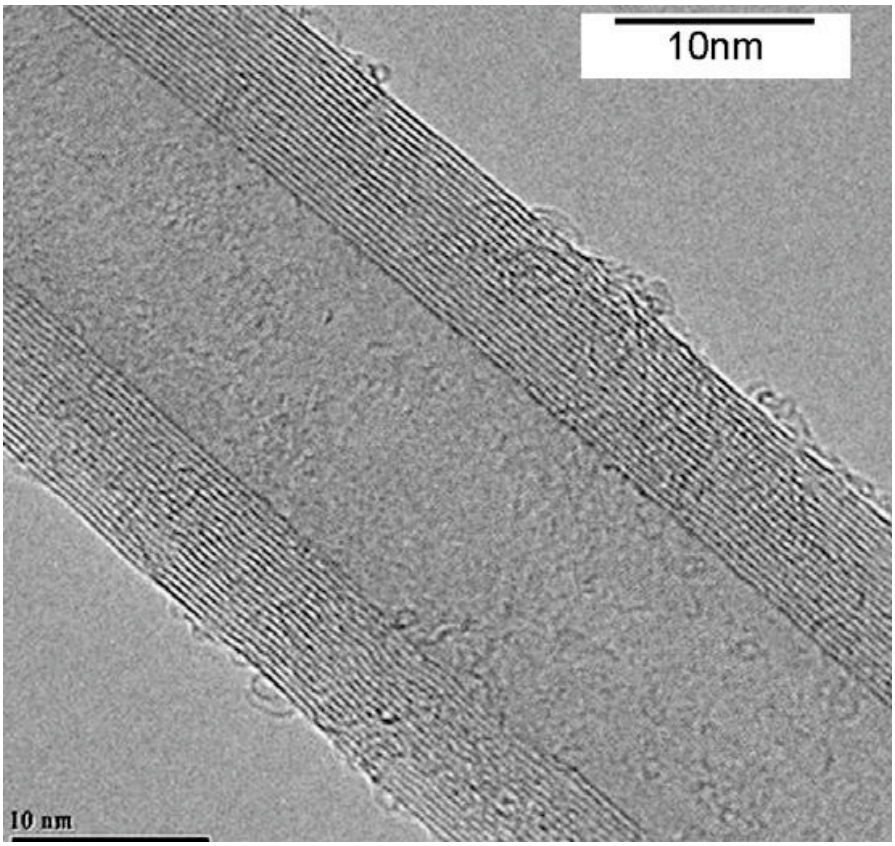


Figure 1.1 Transmission electron micrograph of the walls of a multiwall carbon nanotube with an outside diameter of ~20 nm [Reprinted from Carbon, 45, Brock Marrs, Rodney Andrews, and David Pienkowski, Multiwall carbon nanotubes enhance the fatigue performance of physiologically maintained methyl methacrylate-styrene copolymer, Page 2099, Copyright 2007, with permission from Elsevier].

The structure and properties of carbon nanotubes have been analyzed using direct measurement and modeling techniques. Their properties are remarkable and have led to their use in a wide variety of application. Carbon nanotubes have extraordinary mechanical properties, including exceptionally high tensile strength and stiffness. They have tremendously high strength, are 50 to 100 times stronger than steel at one-sixth the weight of steel [55], have an on-axis thermal conductivity that rivals that of diamond [50] and remarkable magnetic properties (due to encapsulated catalyst metals) [50]. Table 1.2 displays some of carbon nanotubes' theoretical and experimental properties. The

electronic properties of perfect MWNTs are similar to perfect SWNTs because the coupling between the cylinders is weak in MWNTs. Electronic transport basically occurs ballistically (no scattering) over long nanotube lengths, enabling them to carry high currents with essentially no heating [56]. The measured room temperature thermal conductivity for an individual MWNT (>3000 W/m K) is greater than that of natural diamond or the basal plane of graphite (2000 W/m K) [57].

Table 1.2 Properties of Carbon Nanotubes [54]

Property	SWNTs	MWNTs
Elastic modulus	1 TPa	0.3-1 TPa
Strength	50-500 GPa	10-60 GPa
Resistivity	5-50 μΩ cm	5-50 μΩ cm
Thermal conductivity	3000 W/mK	3000 W/mK

Carbon nanotubes have huge industrial application potential and are therefore being extensively researched for a variety of industries, products, and other uses. The global market for carbon nanotubes was estimated at $12 million for 2002 and expected to grow up to $700 million by 2005 [58]. Electronics.ca published a report stating that the global carbon nanotube market is projected to exceed $1.9 billion by 2010. This would be a compound annual growth rate of more than 80% during the analysis period. The MWNT market was estimated at $290 million for 2006. The revenues from these materials are higher due to their simple production and low cost when compared to SWNTs [58].

Section 1.3.2 Carbon Nanotube Composites

The fiber-like structure of carbon nanotubes, their low density, high aspect ratio, and extraordinary mechanical properties make them particularly attractive for reinforcement in composite materials [52]. The conductivity, strength, elasticity, toughness, and durability of a composite material can be substantially improved with the addition of carbon nanotubes [50]. The first realized major commercial application of MWNTs was in polymer composites. Polymer and carbon nanotube composites began as a NASA research project for a variety of applications. They found the composites had unique

mechanical, surface, and multi-functional properties; and strong interactions with the matrix because of the nano-scale microstructure and extremely large surface area [54]. The properties of nanotube/polymer composites vary significantly depending on the distribution, the type, diameter, and length of the nanotubes. Polymer nanocomposites also depend on several factors including but not limited to the process used to produce nanotubes, nanotube purification, and amount of impurities, nanotube size, aggregation, and nanotube orientation [59]. The combination of carbon nanotubes' superlative mechanical, thermal, and electronic properties makes them an ideal candidate as an advanced filler material in nanocomposites [60].

Carbon nanotube composites have higher shear stress values than traditional fiber composites, possibly due to good bonding between the nanotubes and polymer matrix. Incorporation of nanotubes into plastics can provide structural materials with dramatically increased modulus and strength [54]. Depending on the polymer matrix, conductivities of 0.01 to 0.1 S/cm can be obtained for 5% nanotube loading. The low loading levels and the nanofiber morphology of the MWNTs allow electronic conductivity to be achieved while avoiding or minimizing degradation of other performance aspects, including mechanical properties and viscosity. The critical challenges lie in uniformly dispersing the nanotubes and achieving nanotube-matrix adhesion to provide effective stress transfer [56]. The thermal transport properties of CNT polymer composites could also improve due to the excellent thermal conductivity of CNTs [54]. This offers an opportunity for CNT polymer composites in a variety of different uses.

The high modulus and low weight of carbon fibers make them ideal reinforcing agents in a variety of composite materials. To take advantage of the high Young's modulus and high strength of carbon nanotubes the load transfer efficiency is very important. If the adhesion between the matrix and the carbon nanotubes is not strong enough to sustain high loads, the benefits of the high tensile strength of carbon nanotubes are lost. One of the ways to improve adhesion and shear strength is to functionalize the nanotubes, or

increase the surface roughness or surface reactivity [61]. Functionalization may slightly lower the thermal conductivity of the MWNTs, but the property is so significant that it can still be exploited to make thermally conductive composites [62]. Effective reinforcement of a polymer with carbon nanotubes requires four things: large aspect ratio, good dispersion, alignment, and interfacial stress transfer. The aspect ratio must be large to maximize the load transfer to the nanotubes to optimize composite strength and stiffness. Carbon nanotubes must also be uniformly dispersed as isolated nanotubes individually coated with polymer. This is important to achieve efficient load transfer to the nanotube network. A more uniform stress distribution is achieved as a result, and the presence of stress-concentration centers is minimized [60].

The most important part of making a successful composite is the complete and effective dispersion of the nanotubes through the matrix without destroying the integrity of any of the involved materials. To maximize the advantage of CNTs as effective reinforcements in high strength composites they should not form aggregates and must be well dispersed to prevent slippage [63]. There are several techniques to improve the dispersion of CNTs in polymer matrices, such as by optimum physical blending, *in situ* polymerization, and chemical functionalization [54]. One effective method found for dispersion of MWNTs is shear mixing [59]. High shear mixing energy resulted in more uniform dispersion but the MWNTs were broken. The dispersion of nano-fillers in polymer matrices is more difficult than that of micro-fillers due to the strong tendency to agglomerate in the nano-fillers [54]. Dispersion of CNTs is crucial to the production of both a homogenous product and to composite performance. Dispersion of CNTs in composites was determined quantitatively by Andrews' group by optical microscopy of polished sections. The group investigated the uniformity of fiber distribution across the specimen and the frequency of agglomerates in the matrix using the microscope. At any given temperature, the dispersion efficiency can be related to the mechanical energy input into the mix. Increasing either the residence time and/or rotor speed can increase the energy input and therefore improve the dispersion. When low concentrations (<0.5 vol%) of MWNTs are dispersed into polymers, a remarkable reduction in surface electrical resistivity can be

produced. But there is generally only a small increase in elastic modulus and a decrease in tensile strength. The reduction in tensile strength can be attributed to an increase in the frequency distribution of defects associated with the nanotubes that initiate failure or attributed to the poor interfacial bonding between the nanotubes and the matrix and the absence of alignment of the fibers in a preferred direction [59]. At higher concentrations of MWNTs the stiffness and strength of the composites were significantly improved [64].

The quality of nanotube dispersion in polymer matrices can be determined using scanning electron microscopy (SEM), as well as optical microscopy, polarized Raman imaging, and transmission electron microscopy (TEM). Gaps in expected mechanical properties especially for lower percentages of CNTs in composites can be explained by imperfect dispersion of CNTs and poor load transfer. Agglomeration leads to defect sites in the composites and limits the efficiency of carbon nanotubes on the polymer matrices. Poor dispersion and rope-like entanglement of CNTs leads to drastic weakening of the composites. Alignment of CNTs in the polymer plays a role in the mechanical and functional properties of composites [54]. Even modest nanotube agglomeration impacts the diameter and length distributions of the filler and overall is likely to decrease the aspect ratio. Nanotube agglomeration reduces the modulus of the filler relative to that of the isolated nanotubes because there are only weak dispersive forces between nanotubes [52].

The toxicity of carbon nanotubes in terms of medical use is less studied. Past reports tended to report negative conclusions about carbon nanotubes and their potential use in the human body. Authors suggested that carbon nanotubes were toxic to humans and that strict industrial hygiene measures should be taken to limit exposure during production and handling. One study published in 2005 showed that multiwall carbon nanotubes that were administered intratracheally to rats induced inflammatory and fibrotic reactions in the animals, as well as pulmonary lesions and agglomerates of nanotubes in the airways [65]. Current research shows that if certain precautions are taken in carbon nanotube production they are less harmful to humans. One of those precautions is

functionalization. This involves chemical modification and solubilization of single-walled and/or multi-walled carbon nanotubes [66].

Many researchers have tried to improve the mechanical properties of bone cement by adding small amounts of other materials including metal, glass, polymer, or carbon fibers. These efforts typically had little success because of inadequate dispersion, poor fiber-matrix bonding, and filler-damage scale mismatch. Scale compatibility was one of the reasons that led to the discovery of MWNTs as a reinforcement material for bone cement. Their small diameters are far more comparable to the size of the polymer chains and the scale of fatigue damage. There is increased physical interface between the MWNTs and the polymer matrix because of the large surface area to volume ratio of the MWNTs [67]. Acrylic bone cement has been shown to have less than ideal resistance to mechanical fatigue and impact, but carbon nanotubes can substantially improve these mechanical properties because of their prodigious tensile strength and large surface area to volume ratio which confers outstanding nanotube-cement matrix bonding [55]. Although improved surgical technigue has increased the probability of prosthesis survival, reducing or eliminating bone cement fracture by improving the material would further enhance the longevity of cemented prostheses [68].

Previous work produced by members of our laboratory showed that bone cement can be successfully loaded with MWNTs. The MWNTs improved the mechanical properties and fatigue life of the bone cement material [67]. The methods and results of their experiments are described as follows. MWNTs were synthesized by the University of Kentucky's Center for Applied Energy Research using a chemical vapor deposition process where a mixture of ferrocene catalyst and xylene were injected into a multi-zone, heated furnace under a hydrogen-argon (10-90) atmosphere (675 cm^3/min) [67]. Iron from the decomposed ferrocene acted as a catalyst for the formation of the ordered lattice structure of the carbon nanotubes. The carbon nanotubes were then harvested in clusters that required disaggregation before and during dispersion into the polymer matrix [67].

Marrs *et al* dispersed MWNTs throughout the molten matrix of pre-polymerized methyl methacrylate and styrene (MMA-co-Sty) copolymer powder. The mixture was subjected to high-shear mixing with two heated (220^{o}C) stainless steel, counter-rotating sigma rotors in the mixing chamber of a Haake Rheomix (Haake, GMBH, Germany). The bone cement powder was added to the mixing chamber followed by the as-produced MWNTs. The materials were shear mixed by the sigma rotors to disentangle and thoroughly disperse the MWNTs throughout the molten polymer. Nanocomposites consisting of 0.5, 1, 2, 5, and 10 wt% MWNTs were produced using a dilution method. The molten material was collected and allowed to cool. The hard composite materials were then crushed and sieved to a particle size of equal to or less than 2 mm. A 12-ton laboratory press was used to hot-press the crushed particles into films of uniform thickness (1.6 mm) under vacuum [55]. The films were then machined into dog-bone-shaped specimens and bar shaped specimens, which were then annealed to remove any surface flaws and to alleviate any residual stresses that formed during machining.

After curing at room temperature for 24 hours, the bar specimens were tested to failure in 3-point bending using a Q Test^{TM} 10 Elite (MTS Inc., Minneapolis, MN) materials testing system. The flexural strength, flexural yield strength, bending modulus, and strain were recorded for each specimen. Fatigue testing in 4-point bending was then performed in air at room temperature on an Instron 1331 (Instron Corp., Canton, MA) servohydraulic materials testing system. A sinusoidal wave profile was applied with a minimum load of 4 N and a maximum load of 40 N at a frequency of 5 Hz. The number of cycles to failure was recorded for each specimen. The 2 wt% MWNT composite was found to be nearly optimal for 3-point bend mechanical properties. Flexural strength was enhanced by 12.8% and flexural yield strength was enhanced by 13.1%. The 2 wt% MWNT concentration also had a 3.1-fold increase in the mean actual fatigue life. Bone cement samples with small weight percentages of MWNTs were found to have enhanced flexural strength, yield stress, and fatigue performance [55].

SEM images of the fractured surface of fatigue-failed specimens showed the MWNTs protruding from the bone cement matrix as long finger-like projections. Visually the nanotubes appeared to be randomly spaced but aligned along the direction of loading. It is believed that the MWNTs reoriented so that they offered resistance to crack growth by spanning the crack in a direction perpendicular to the plane of crack growth [55]. This should occur because of the nanotubes being well-dispersed, the anticipated strong nanotube-matrix as a result of their high surface area to volume ratio, and their extremely strong tensile properties. The nanotubes would slow crack growth and enhance the longevity of the cement mantle [55]. It was suggested that decreases in material properties with larger MWNT concentrations is a result of agglomerated or clumped MWNTs in the composite. These agglomerations may act as fracture initiation sites. If there are enough of them, there may be enough detrimental effects that the beneficial effects of the MWNTs are eliminated [55].

The fatigue specimens were measured and aged in phosphate buffered saline (PBS) at 37^{o}C for 6-60 days [67]. Fully reversed tension-compression fatigue testing was performed in a heated (37^{o}C) PBS environment with an Instron 8521 servohydraulic materials testing system (Instron Corp., Canton, MA). Specimens were sinusoidally loaded at 5 Hz to peak tensile/compressive stress amplitudes of 20, 30, and 35 MPa until failure or 2 million cycles. SEM was used to examine individual MWNTs within the nanocomposite matrix after fatigue testing. Testing at the 20 MPa peak stress amplitude showed that the 2 wt% and 5 wt% MWNT samples had 565% and 592% greater fatigue lives, respectively, when compared to the control group. The 0.5 wt% had the smallest increase but the value was still 307% greater than the control samples. At large percentages of MWNTs, the results were less than ideal. The irregularities with those results are believed to be due to imperfectly disaggregating and dispersing the larger amounts of MWNTs into the MMA-co-Sty matrix. Examination of the SEM crack images showed the MWNTs protruding from the cracked faces in the normal direction to crack growth, shown in Figure 1.2. Some of the MWNTs were seen to bridge the growing crack. Multiwall carbon nanotubes were shown to clearly enhance the fatigue

performance of MMA-co-Sty. MWNTs retard the mechanism of fatigue failure by preventing or minimizing the initiation of catastrophic cracks and by slowing damage to the accumulation of existing or newly forming cracks [67]. The MWNTs were believed to bridge cracks and reduce the extent of plastic deformation experienced by the matrix. The effectiveness of MWNTs as reinforcement is dependent on the concentration of MWNTs, their dispersion, and the peak stress of the dynamic loading cycle.

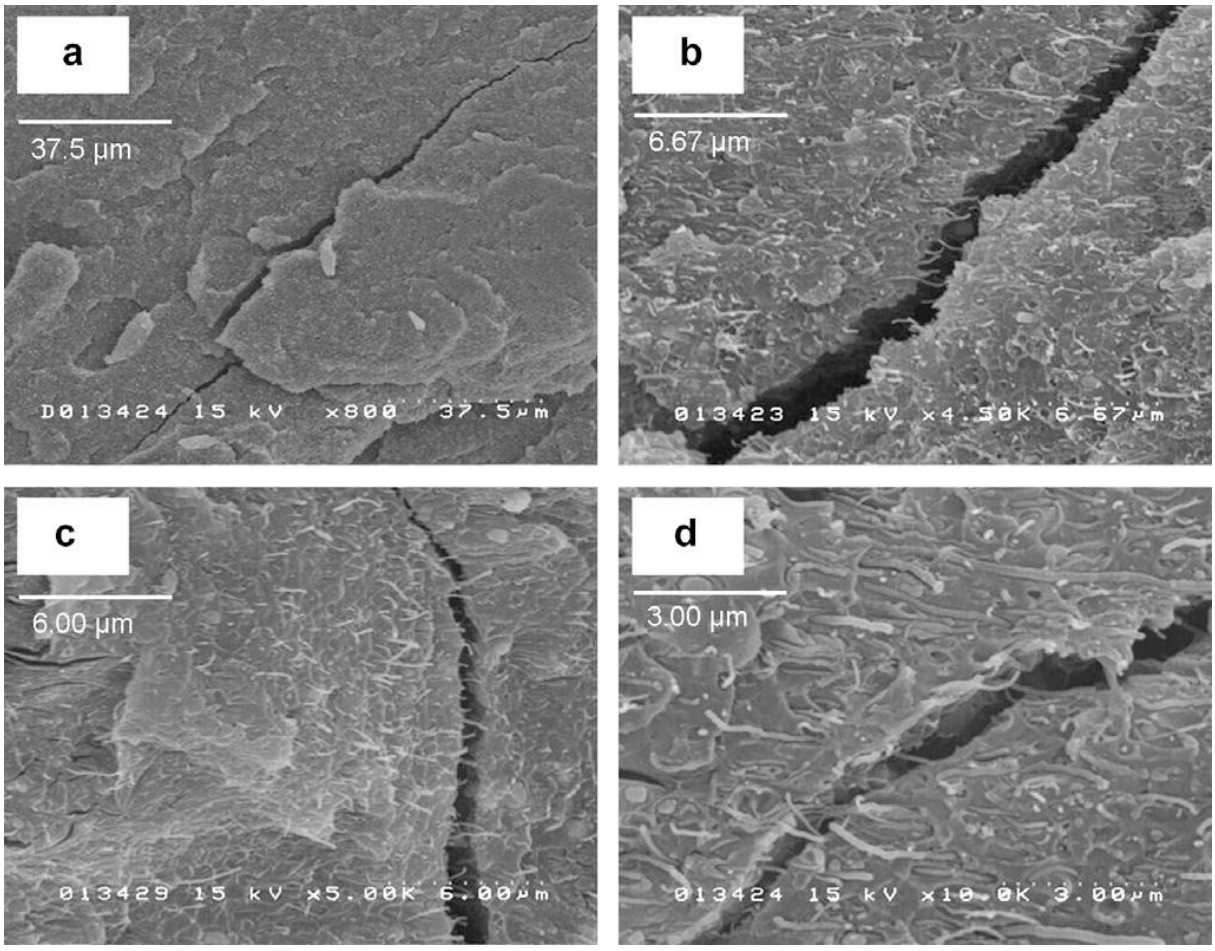


Figure 1.2 SEM images showing the growth of one micro-crack (a) on the surface of one of the 5 wt% MWNTs specimens that had been stressed at 20 MPa. Images (b), (c), and (d) showed the MWNT matrix-reinforcing behavior across the crack [Reprinted from Carbon, 45, Brock Marrs, Rodney Andrews, and David Pienkowski, Multiwall carbon nanotubes enhance the fatigue performance of physiologically maintained methyl methacrylate-styrene copolymer, Page 2101, Copyright 2007, with permission from Elsevier].

The addition of carbon nanotubes to bone cement may also offer thermal benefits to the cement to enhance implant longevity. The high temperatures seen at cement-bone interfaces during *in vivo* polymerization could be lowered as a result of the high axial thermal conductivity of MWNTs. The addition of steel fibers (5-15%) reduced the peak temperature of curing PMMA [48]. The addition of MWNTs to bone cement may help avoid polymerization induced "hot" spots and subsequent hyperthermia-based destruction of bone adjacent to the cement mantle. The mechanical integrity of the cement-bone interface may be improved and the implant performance enhanced [55].

Section 1.4 Objectives

The goals of this thesis are: 1) to determine if MWNTs change the flow of heat in polymerizing bone cement, and if so, what are the kinetics of this alteration and 2) to determine if MWNTs alter the flow of heat in antibiotic laden bone cement, and if so, to quantify the diffusion profile of antibiotics in Palacos and Simplex bone cement.

Chapter 2 - Polymerization Kinetics of Acrylic Bone Cement with Multiwall Carbon Nanotubes

Section 2.1 Introduction

The properties and performance of acrylic-based bone cements and the supporting bone are strongly dependent on the polymerization kinetics including the chemical reaction of the MMA monomer [69]. The polymerization kinetics of bone cement must be determined to study the thermal behavior of a bone-cement-prosthesis system. The progress of the polymerization reaction can be seen in the temperature rise in the bone cement mixture due to the exothermic polymerization of MMA [70]. Polymerization kinetics, and the amount of left over monomer, depend on the temperature of the polymerizing material. The temperature distribution during polymerization is one of the most important determinants for the success of the cementation procedure [71]. The exothermic polymerization reaction, coupled with the poor heat conductivity of PMMA, has been theorized to result in localized "hot spots" which nucleate small voids. These voids can then later become sites for the initiation of fatigue cracks which ultimately lead to mechanical failure of the cement and implant loosening. The hot spots are caused by poor heat dispersion across the polymer. Polymerization occurs almost sporadically in the bone cement material. Failure and loosening of an implant will force the patient to undergo a painful, costly, and difficult revision surgery.

The heat generated during polymerization causes a temperature increase in the whole system. The peak temperatures are first located at the cement/bone interface and then move into the middle of the cement over the course of the reaction. The temperatures are highest in this region because the temperature of the bone is higher than the temperature of the implant. The higher bone temperature aids in the polymerization kinetics of the bone cement. When a balance between heat generation and thermal conduction is obtained, the temperature reaches a maximum and is subsequently followed by cooling as the conduction dominates the reaction [70]. The temperature of the mixture drops after the polymerization reaction is completed. The heat is dissipated into the surrounding

environment. The temperature of bone tissue surrounding the bone cement increases due to the heat released by the curing bone cement [70].

Toksvig-Larsen completed a study to investigate the temperatures experienced at the cement interface in hip arthroplasties. Temperature measurements were performed with thermocouples with a range of 0-110^{o}C with a total accuracy of ±1 ^{o}C. The thermocouples were placed so that their tips laid flush with prepared bone surface. The points of measurement included the middle of the circumference of the acetabulum and the trochanteric region of the femur. The mean maximum cement-curing temperature in the acetabulum was 43^{o}C (109.4 ^{o}F) and 40^{o}C (104 ^{o}F) in the femur. In the acetabulum, 5 of 28 recordings were above 44^{o}C and 2 of 28 recordings were above 47 ^{o}C. In the femur, 4 of 41 recordings were above 47 ^{o}C. The longest duration above 44^{o}C was 7 minutes and above 47^{o}C was 2 minutes and 20 seconds [72]. In 1984, Eriksson found the threshold temperature for impaired bone regeneration to be in the range of 44-47^{o}C when measured at a distance of 0.5 mm and applied for 1 minute [73]. There are many factors that influence the rate of build-up and dissipation of the amount of heat generated during bone cement polymerization. These factors include the rate of setting, the size and shape of the bone cement mass, and the thermal properties of the surrounding materials – including bone, blood, and the plastic and metallic components of the prosthesis [72].

Thermal factors, such as thermal injury to the periprosthetic tissues and osseous necrosis of those tissues can lead to aseptic loosening of cemented arthroplasties. This is a complex phenomenon that is also believed to be affected by mechanical factors, such as fatigue failure that occurs due to crack growth from voids. Thermal injury is associated with the high heat produced during the *in situ* polymerizing of the cement [28, 74]. Osseous necrosis results from leakage of unreacted or residual monomer from the cured cement to the surrounding tissues [31, 75]. It is also believed that a lower polymerization reaction rate *(k)* and a higher degree of monomer conversion will lead to a smaller amount of residual monomer [29]. This will contribute to long-term *in vivo* stability of cemented arthroplasties.

One of Lewis' goals was to determine the influence of three variables on the polymerization reaction rate. Those three variables included the amount of copolymer as a proportion of the total powder weight (COP), the amount of DMPT as a proportion of the total volume of the liquid monomer (ACC), and the accelerator. The study was completed using differential scanning calorimetry (DSC) in the nonisothermal mode [13]. DSC measurements may be used for determining the progress of curing of bone cement by assuming that the heat evolved during the polymerization reaction is directly proportional to the overall extent of the reaction. For each of the cements, the correct ratio of powder to liquid according to manufacturer was manually mixed in a polyethylene bowl that was open to ambient air for 1 minute. A spatula was used to transfer approximately 3 mg of material very quickly to the center of a DSC aluminum sample pan. The sample pan was situated in a DuPont 910 DSC (Instrument Specialists, Spring Grove, IL) that was operating under a nitrogen purge, with a constant flow rate of 100 $cm^3\ min^{-1}$. The DSC test was conducted immediately to prevent complete curing of the cement prior to test start. The pan was heating in the chamber from the initial dough temperature, T_i, to a final temperature, T_f, of 150°C at a predetermined rate of heating. T_i was taken to be the temperature of the air in the room, 23°C±1°C. The exothermic heat flow from the polymerizing cement (H) was recorded as a function of its temperature (T_d) during the heating period. This is known as a thermogram. A variety of different heating rates were used, including 5, 10, 15, and 20 K min^{-1}. At each heating rate triplicate DSC runs were performed. If it is assumed that the heat generated during the polymerization reaction is directly proportional to the extent of the reaction (i.e. the reaction rate is proportional to the mole fraction of the unreacted liquid monomer), then the reaction rate constant (k) [in s^{-1}], at a specified value of T_d, can be calculated using:

$$k = (H) / (A - B) \qquad \text{Equation 2.1} \qquad [13]$$

where H is the heat flow at temperature T_d, A is the total area of the thermogram (the area of the thermogram between T_i and T_f), and B is the area of the thermogram between T_i

and T_d. The following Arrhenius equation was used with the fit of the k-T_d results to calculate Q and $\ln Z$:

Equation 2.2 $$k = Z\exp[-Q/(RT_d)]$$ [13]

where Z is the frequency factor (in s^{-1}), Q is the activation energy (in J mol^{-1}), and R is the molar gas constant (=8.314 J mol^{-1} K^{-1}). Six widely dispersed values of T_d were selected from each thermogram. For each cement, twelve values of Q and $\ln Z$ were obtained and means and standard deviations were calculated from each. Each result was also compared to a control polymerization reaction rate value, *k'*, that was calculated at a physiologically relevant temperature of 37°C. The *k'* estimates were statistically analyzed using one-way or 2x2 factorial ANOVA for each of the individual parameters (COP, ACC, and accelerator). For the interaction effect of COP and ACC, factorial ANOVA was used. In all cases, $p < 0.05$ was taken as significant. The maximum heat flow seen in the thermograms increased as the heating rates increases. Figure 2.1 below shows typical thermograms obtained from the DSC tests.

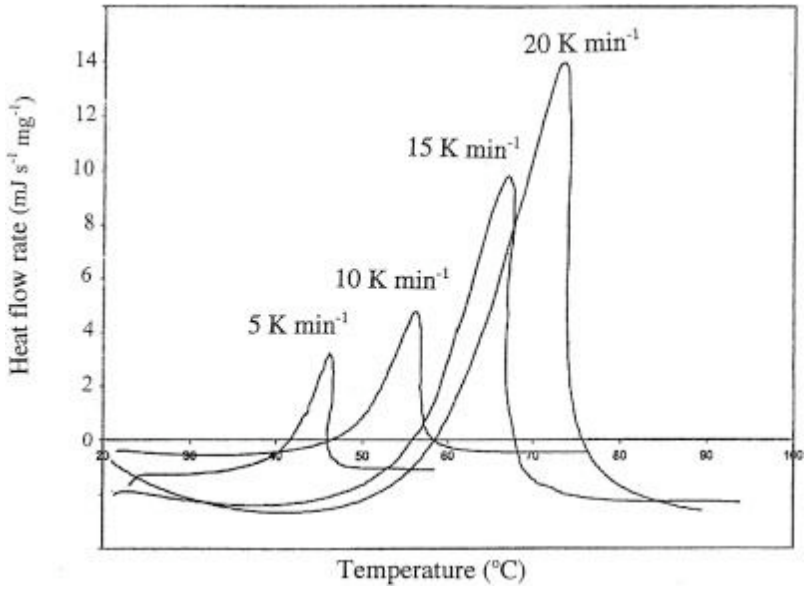


Figure 2.1 Typical thermograms obtained from the DSC tests [Reprinted from Journal of Biomedical Materials Research Part B: Applied Biomaterials, 81B, Gladius Lewis and Sanjay R. Mishra, Influence of Changes in the Composition of an Acrylic Bone Cement on its Polymerization Kinetics, Page 524, Copyright 2007, with permission from John Wiley and Sons].

Section 2.2 Methods

The following describes the study design for the first aim of this investigation. The variables included type of bone cement, percentage of MWNTs added, and heating rate. Calculations were made to identify the activation energy, frequency factor, control polymerization reaction rate value, peak heat flow, and width at half maximum of peak heat flow. MWNT loading ranged from 0.167wt% to 1.33wt%. There were eight samples used in each experimental group. These are the methods for the first aim of this research, to understand how MWNTs influence the polymerization kinetics of orthopaedic bone cement.

Differential Scanning Calorimetry (DSC) is a thermoanalytical technique that measures the difference in the amount of heat required to increase the temperature of a sample in relation to a reference, measured as a function of temperature. The sample and reference are placed in the DSC chamber where they then are subjected to the same temperature changes throughout the experiment. A computer program is connected to the DSC that is used to design the experiment; the user can choose the temperatures and methods to test the sample. The DSC can detect energy or heat capacity changes with great sensitivity. Typically the DSC is used to study phase transitions of a material, such as melting, glass transitions, or exothermic decompositions. When a material or sample undergoes a phase transition, the amount of heat flowing to that sample will be different than a reference sample to maintain both at the same temperature. For example, an exothermic process like bone cement polymerization requires less heat to raise the sample temperature. An endothermic process like a liquid becoming a solid requires more heat. While performing the experiment with the DSC, the computer program records the heat flow data and can output graphs of this information once the experiment is completed. Typically these graphs, thermograms, show a curve of heat flux versus temperature or time. A variety of measurements can be made from these curves.

Section 2.2.1 Polymerization Kinetics

The current investigation began because the thermal benefits of MWNTs in bone cement had not been explored. MWNTs (~25nm diameter; ~100μm length) were produced at the University of Kentucky Center for Applied Energy Research [55] and treated in a nitric acid bath to remove the residual catalyst particles [67]. Both a 0.25 and 1% mixture (by weight) of MWNTs were disaggregated and dispersed throughout dry pre-polymerized bone cement powder using a dual-blade shear mixer. Thirty gram batches of hand-mixed powder were passed through the mixer three times, one minute each time, to ensure complete dispersion of the MWNTs in the bone cement powder. The materials were mixed at room temperature and the MWNTs were not inserted into the polymer powder beads. A method of mixing that included heating, cooling, and breaking down was used in previous studies completed by our lab [55]. This method results in the MWNTs being actually in the polymer beads. The mixing method employed in the present investigation was simpler, quicker, and easier to make a variety of mixtures. This may lower the effects that the MWNTs have on the polymerization kinetics of bone cement.

Scanning electron micrographs (Figures 2.2 and 2.3) of a small representative sample of the resulting 1 wt% MWNT bone cement powder revealed the successful dispersion of MWNTs throughout the bone cement. The larger globular shapes in Figure 2.3 are the barium sulphate molecules. Visual observation can result in useful information regarding the spatial dispersion of multiwall carbon nanotubes in a polymer matrix [55]. Liquid monomer was prepared in the laboratory using 97.5% by volume methylmethacrylate (MMA) (ACROS Organics, Morris Plains, NJ) and 2.5% N,N-Dimethyl-p-toluidine (DMPT) (ACROS Organics, Morris Plains, NJ). The DMPT acts as an accelerator and the 10-20 ppm monomethyl ether of hydroquinone (MEHQ) in the MMA acts as a stabilizer.

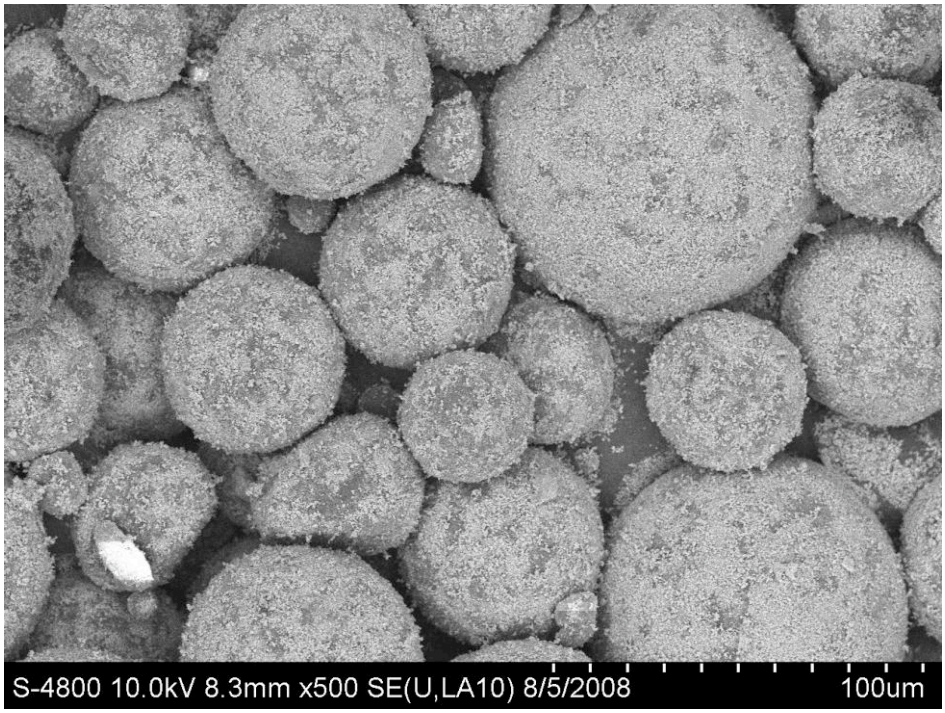


Figure 2.2 SEM image of the surfaces of Simplex bone cement polymer molecules with 1 wt% MWNTs dispersed on the polymer particles.

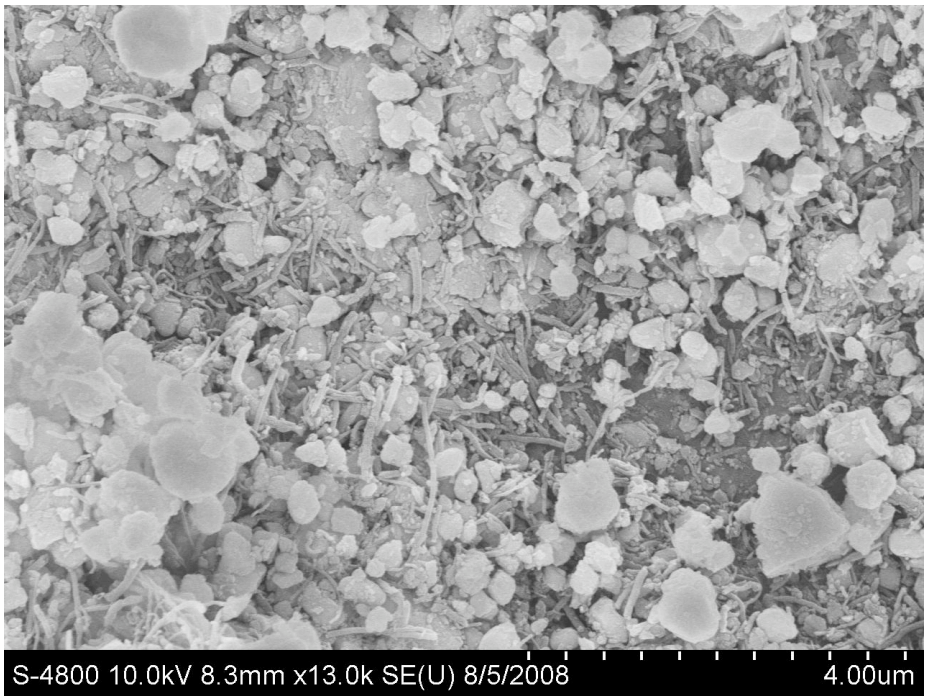


Figure 2.3 SEM Image of the MWNTs (finger-like shapes) covering the surface of one Simplex polymer molecule

Cement samples were manually mixed in a ratio of 2 g of powder to 1 mL of as-noted monomer for 1 minute. This diluted the concentration of MWNTs in the bone cement to 0.17 wt% and 0.67wt%. For each sample, approximately 26 mg of cement was quickly transferred to an aluminum sample pan, covered, pressed and then placed in a Q100 DSC (TA Instruments, New Castle, DE), operating under a nitrogen purge, with a constant flow rate of 100 cm^3 min^{-1}. The specimen was heated from room temperature (T_i) to 150°C (T_f) at one of three heating rates (5, 10, and 20°C min^{-1}). The exothermic heat flow (H) was recorded. Thermograms were obtained for each cement formulation at each heating rate. Triplicate experiments were conducted for combination of concentration and heating rate.

The data from the thermograms was analyzed to calculate the parameters reaction rate, activation energy, and frequency factor. First the heat flow data was shifted to start at zero and then the data was normalized with the mass of the corresponding sample. The area under each data point, corresponding to one recorded time point (1 second), was calculated using the trapezoid rule. The slope and y-intercept were then found for the normalized heat flow data versus time. These values were used in the equation of a line to form a line connecting the end points of the data, shown in red in Figure 2.8. The areas at each time point were better approximated using this line as the bottom limit instead of the horizontal axis. The areas under each time point along this new line were calculated using the trapezoid rule as before. This amount was then subtracted from the original area to have the best approximate area values. The total area under the curve, A, down to that line and not the horizontal axis, was then found by adding all the small area values together. This value was then compared with the original thermogram using the computer program Universal Analysis which is compatible with the original DSC data recorded. This served as a check for my analysis.

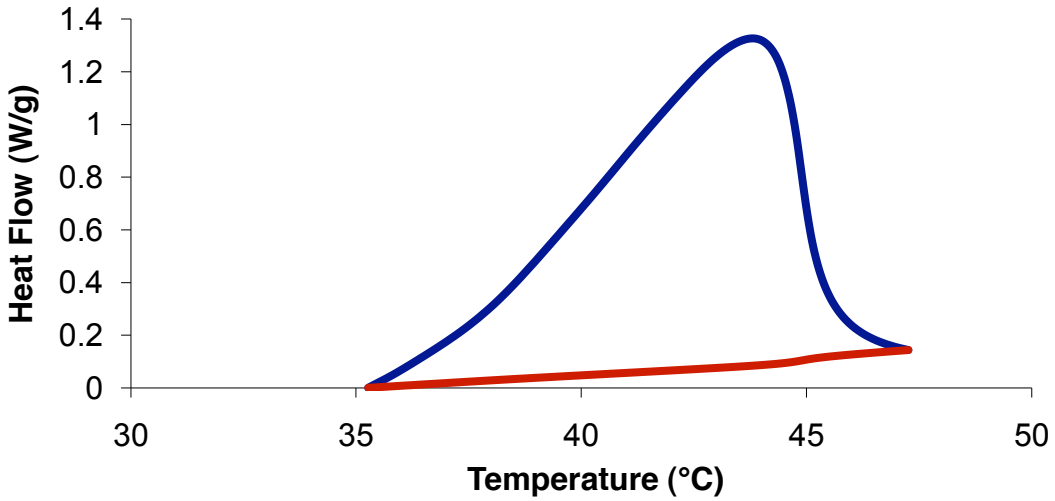


Figure 2.4 Example thermogram showing recorded heat flow data in blue and straight line used in accurately calculating area under the curve in red.

Assuming first order polymerization kinetics [31], the reaction rate constant (k) of each thermogram was calculated at a selected temperature (T_d) using Equation 2.1 from above. The activation energy, Q (J mol^{-1}), and the frequency factor, Z (in s^{-1}), were calculated by fitting the k and T_d results to the Arrhenius Equation 2.2. For each thermogram, several values of T_d below the temperature corresponding to the peak heat flow were selected for analysis. The heat flow value and corresponding values of A and B, were used to compute Q and ln Z. The area under the curve was also calculated. The area indicates the number of reactions that occurred during polymerization. A control polymerization reaction rate value, k', was also calculated for each sample. This value was calculated using the equation for k above, using 37°C as the temperature, and the average Q and ln Z values for that sample set. The Q, ln Z, area under curve, and k' values were analyzed using 2-way ANOVA, comparing both influence of heating rate and MWNTs.

Section 2.2.2 Isothermal Heating

Polymerization kinetics can also be studied using an isothermal setting on a DSC [29]. For these studies, instead of studying the polymerizing kinetics of bone cement while heating it at different rates, the DSC chamber will be heated to a physiologically relevant

37°C and held at that constant temperature for a specified amount of time. This experimental method was used on two types of cement, Simplex (Stryker) and Palacos (Zimmer). The measurements recorded by the DSC were used to further understand exactly how the cement polymerization occurs in the presence of both MWNTs and antibiotics. The heat flow recorded by the DSC should rival the heat flow experienced by the tissues surrounding bone cement in an arthroplasty. These experiments will be important in understanding heat flow in antibiotic loaded bone cement and if the addition of MWNTs can lower the peak temperatures experienced during polymerization without hindering the polymerization reactions.

Section 2.2.3.1 Simplex Bone Cement

Varying amounts, 0.25%, 1%, and 2% (by weight), of MWNTs produced at the Center for Applied Energy [55] were disaggregated and dispersed throughout dry pre-polymerized Simplex bone cement powder using a dual-blade shear mixer. Thirty-gram batches of hand-mixed powder were passed through this mixer three times to ensure complete dispersion of the MWNTs. Liquid monomer was prepared according to standard commercial formulations, same as above in polymerization kinetics studies. The first set of isothermal studies investigated the affect of MWNTs on the polymerization of bone cement containing the antibiotic tobramycin. For those samples, a clinically relevant (0.06 g, 3% by weight) dosage of tobramycin (X-Gen Pharmaceuticals, Big Flats, NY) was added to selected cement groups. The antibiotic was added in powder form to the bone cement prior to the addition of the monomer. It was hand mixed into the powder using a metal spatula.

The second antibiotic studied was cefazolin. This is a cephalosporin that has a lower denaturing temperature than some of the antibiotics more commonly used in arthroplasty. Completing isothermal DSC testing on bone cement samples with this antibiotic and MWNTs will help us to understand whether MWNTs lower peak polymerization temperatures enough to use a heat-labile antibiotic. These antibiotics tend to be less expensive than the more commonly used gentamicin and tobramycin. A clinically

relevant (0.05 g, 2.5% by weight) dosage of cefazolin (Novation, Irving, TX) was added to selected cement groups. The same amounts of MWNTs, 0.25%, 1%, and 2% (by weight), produced at the Center for Applied Energy [55] were disaggregated and dispersed throughout dry pre-polymerized bone cement powder using a dual-blade shear mixer. Thirty-gram batches of hand-mixed powder were passed through this mixer three times to ensure complete dispersion of the MWNTs. Liquid monomer was prepared according to standard commercial formulations, same as above in polymerization kinetics studies.

Components were manually mixed at room temperature for two minutes in the ratio of 2 g of powder to 1 mL of monomer. This diluted the concentration of MWNTs in the bone cement powders to 0.17 wt%, 0.67 wt%, and 1.34 wt%. Approximately 20 mg of material was quickly transferred to an aluminum sample pan, covered, pressed, and then placed in a specimen container in the chamber of a differential scanning calorimeter (DSC). The chamber was quickly heated to 37^{o}C and maintained for 15 minutes. The exothermic heat flow (thermogram) was recorded in triplicate for each MWNT and antibiotic loading. Maximum heat flow (HF_{max}) and the width at half maximum heat flow (D) parameters were measured from these thermograms. Mean values of HF_{max} and D were analyzed using a 2-way ANOVA.

Section 2.2.3.2 Palacos Bone Cement

Palacos R and Palacos bone cement that had been pre-mixed with the antibiotic gentamicin (Palacos+G) was studied using isothermal testing. The samples with antibiotic contained approximately 0.025 g (1.25% by weight) of gentamicin that had already been mixed into the bone cement powder by the manufacturer. MWNTs produced at the Center for Applied Energy [55] were disaggregated and dispersed throughout the dry pre-polymerized Palacos bone cement powder using a dual-blade shear mixer in varying amounts, first including 0.1%, 0.25%, 0.5%, and 1% (by weight). Mixtures were made from Palacos R and Palacos+G with each MWNT concentration, as well as control samples without MWNTs. Twenty-gram batches of hand-mixed powder

were passed through this mixer three times to ensure complete dispersion of the MWNTs. Figure 2.5 (a,b) shows two SEM images of the Palacos powder that had been shear mixed with 1 wt% MWNTs. Figure 2.6 (a,b,c) shows three SEM images of the Palacos+G powder that was shear mixed with 1 wt% MWNTs. The images of the cement powders show that our method of mixing does not damage the polymer beads.

(a)

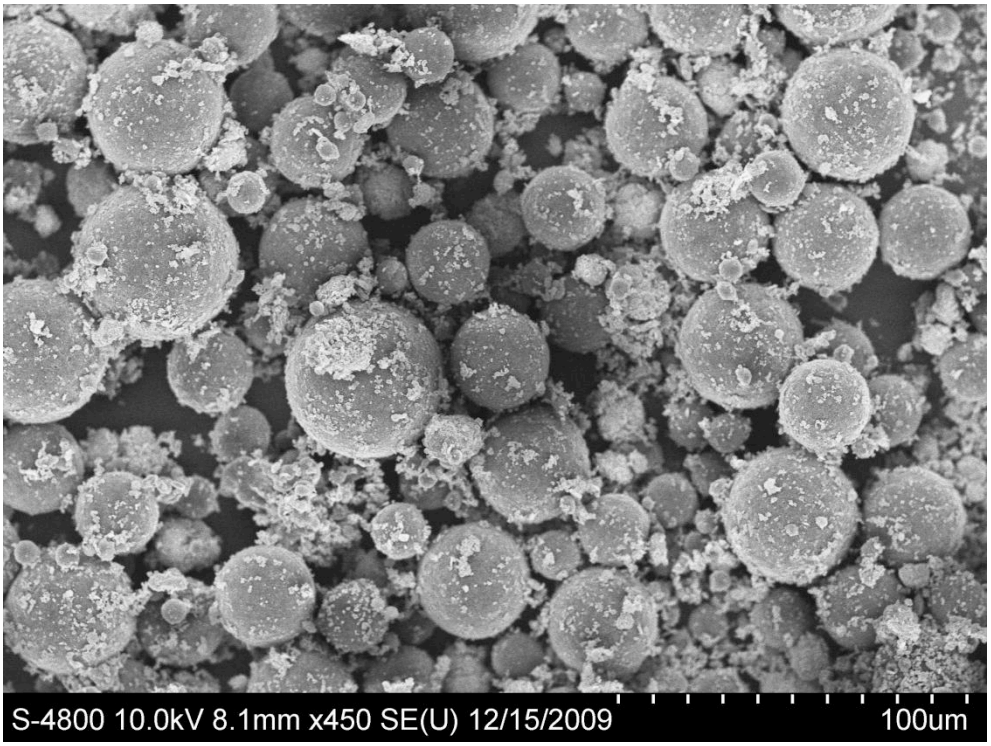


Figure 2.5 (a) Palacos R polymer beads with visible zirconium dioxide molecules and (b) Surface of one Palacos R polymer bead showing dispersion of MWNTs and zirconium dioxide.

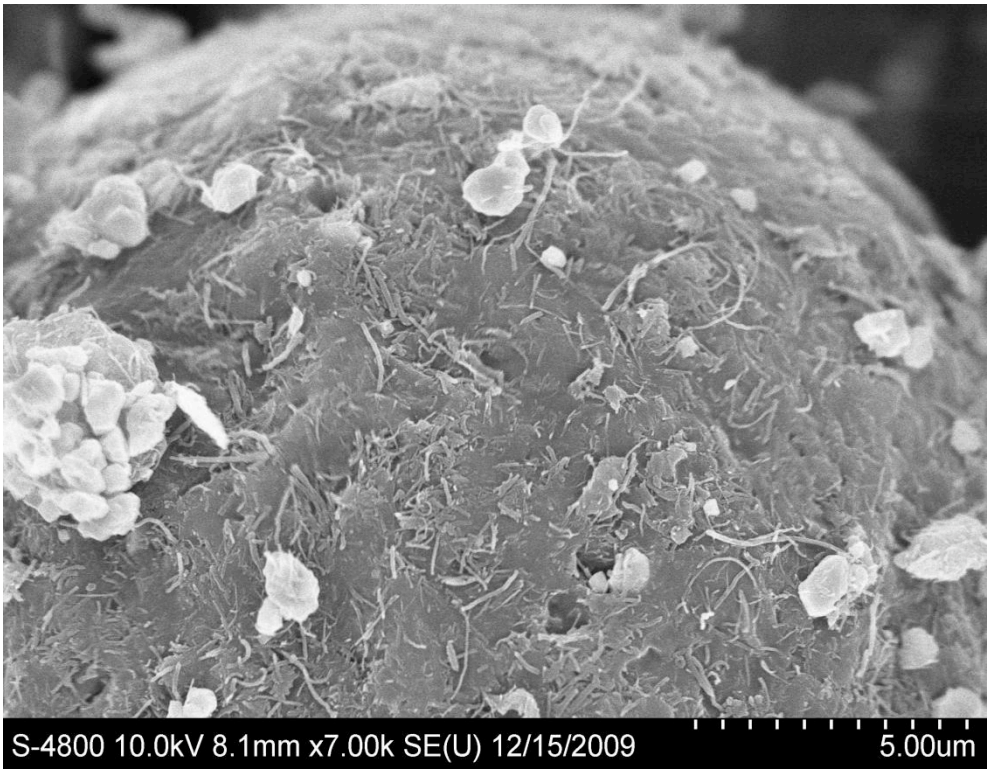
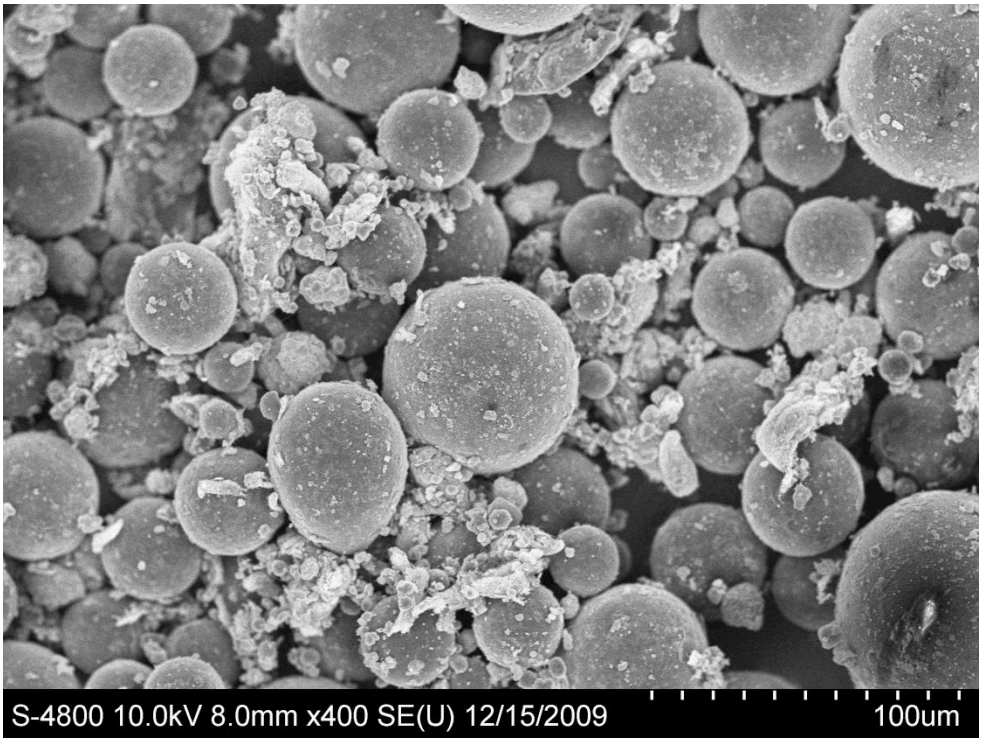
(b) 

Figure 2.5 Continued

(a)



(b)

Figure 2.6 (a) Palacos+G polymer beads with visible zirconium dioxide molecules, (b) Surface of one Palacos+G polymer bead showing dispersion of MWNTs and zirconium dioxide, and (c) Agglomeration of MWNTs in Palacos+G powder.

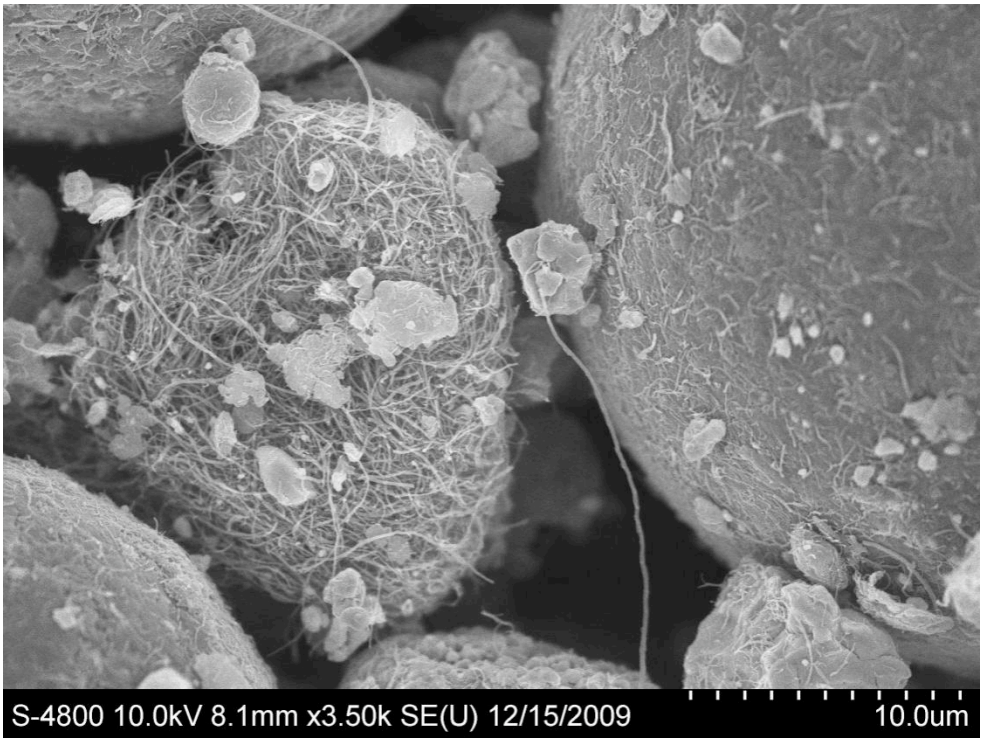
(c) 

Figure 2.6 Continued

The liquid monomer used was the commercially prepared ampule that came with the bone cement powder. Components were manually mixed at room temperature for two minutes in the ratio of 2 g of powder to 1 mL of monomer. This diluted the concentration of MWNTs to 0.067 wt%, 0.167 wt%, 0.33 wt%, and 0.67 wt% from 0.1%, 0.25%, 0.5%, and 1% (by weight), respectively. After the cement components were mixed together for one minute, approximately 20 mg of material was quickly transferred to an aluminum sample pan, covered, pressed, and then placed in a specimen container in the chamber of a differential scanning calorimeter (DSC). The chamber was quickly heated to 37^{o}C and maintained for 15 minutes. The exothermic heat flow (thermogram) was recorded in triplicate for each MWNT and antibiotic loading. Maximum heat flow (HF_{max}) and the width at half maximum heat flow (D) parameters were measured from these thermograms. Mean values of HF_{max} and D were analyzed using a 2-way ANOVA.

Figure 2.7 displays SEM images of the 0.33wt% Palacos R and G composites. Figure 2.7 (a) shows the surface characteristics of a fractured Palacos R composite. The porosity of

the material is evident. Figure 2.7 (b) shows the polymer beads and interstitial regions after polymerization of the Palacos R sample. Figure 2.7 (c) shows the surface of the Palacos G composite. Figure 2.7 (d) shows the polymerization of the polymer beads with antibiotic and nanotubes in the Palacos+G composite. It is evident that complete organized polymerization seen in bone cement with MWNTs does not occur. Figure 2.8 displays SEM images of the 0.67 wt% Palacos R composite.

(a)

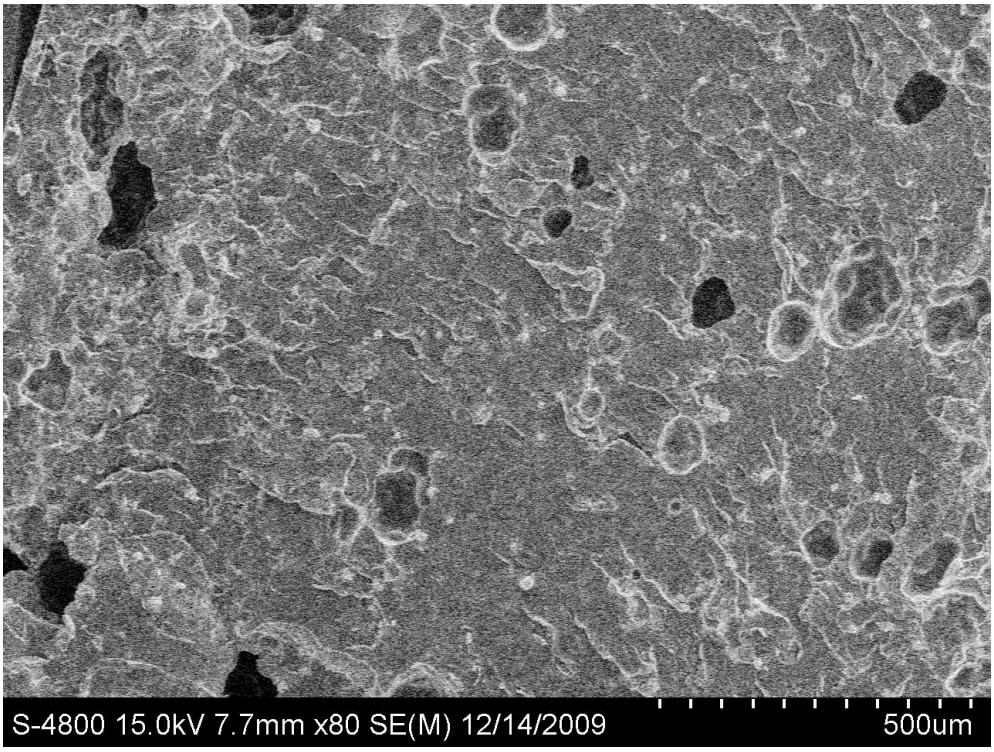
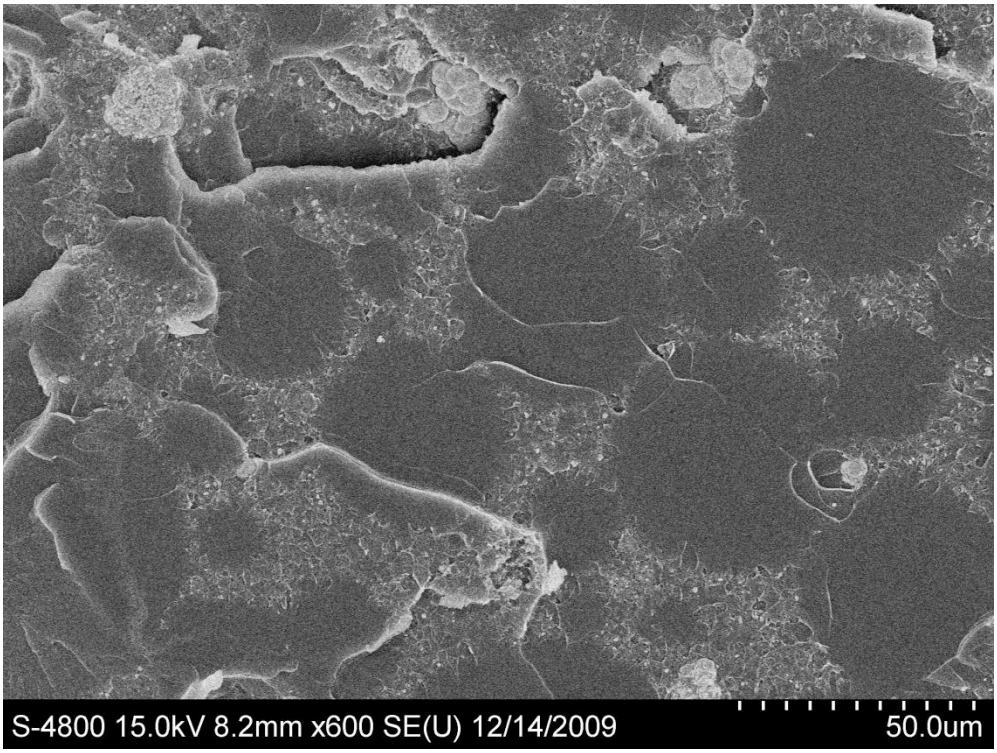


Figure 2.7 (a) 0.33 wt% MWNT Palacos R composite surface, (b) 0.33 wt% MWNT Palacos R composite polymerization, (c) 0.33wt% MWNT Palacos+G composite surface, and (d) 0.33wt% MWNT Palacos+G composite polymerization.

(b)



(c)

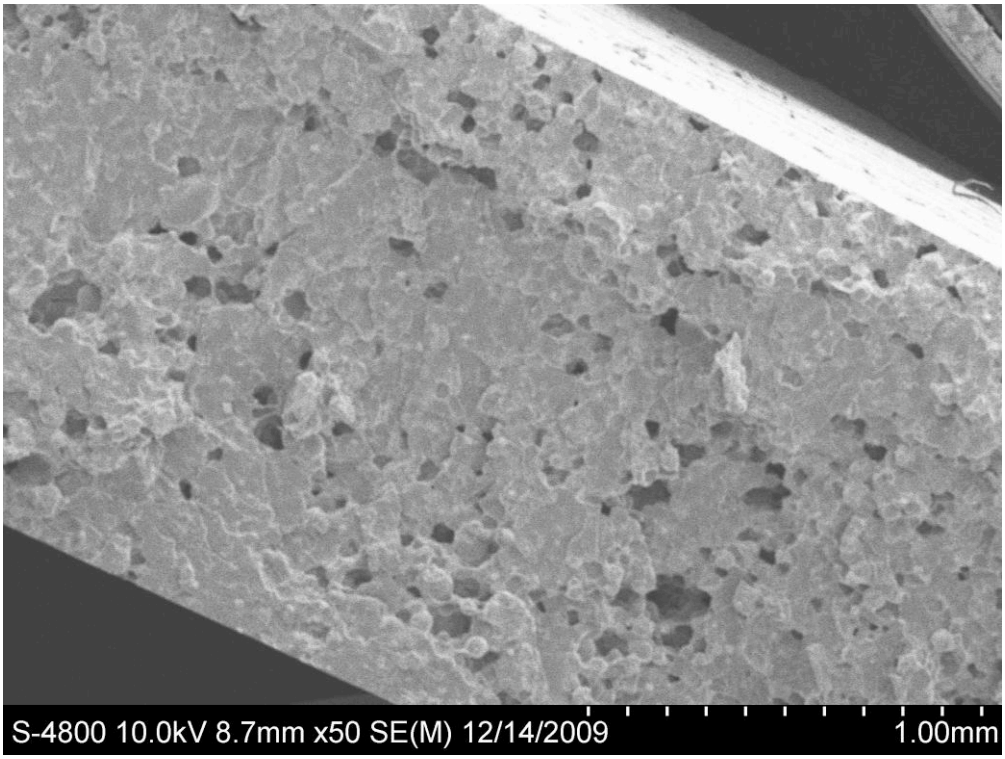


Figure 2.7 Continued

(d)

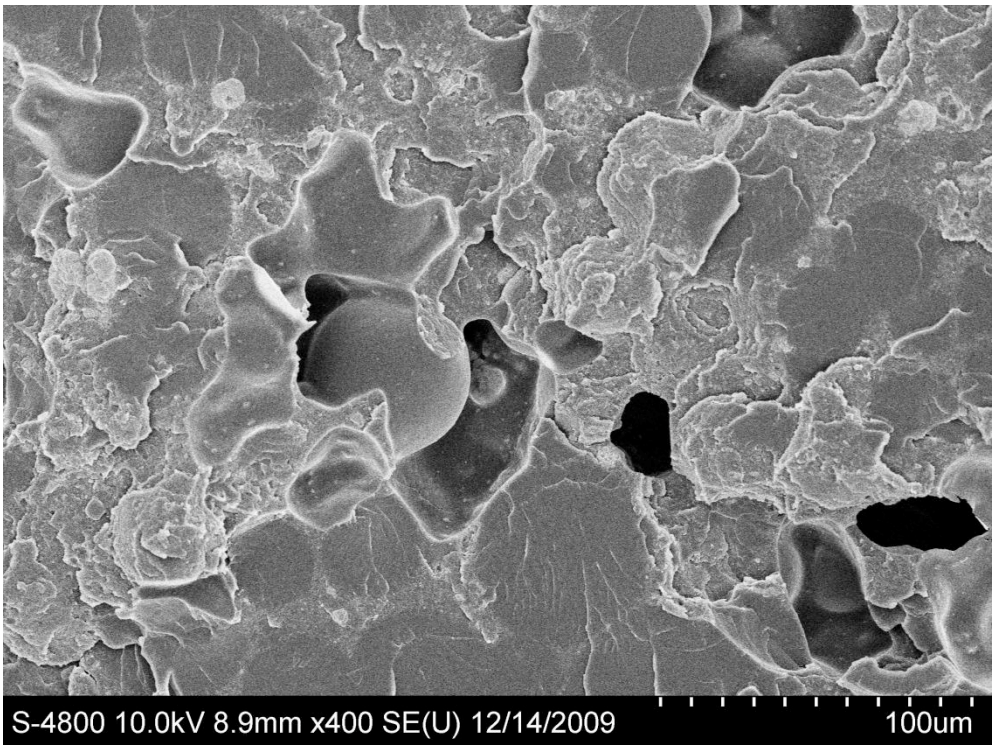


Figure 2.7 Continued

(a)

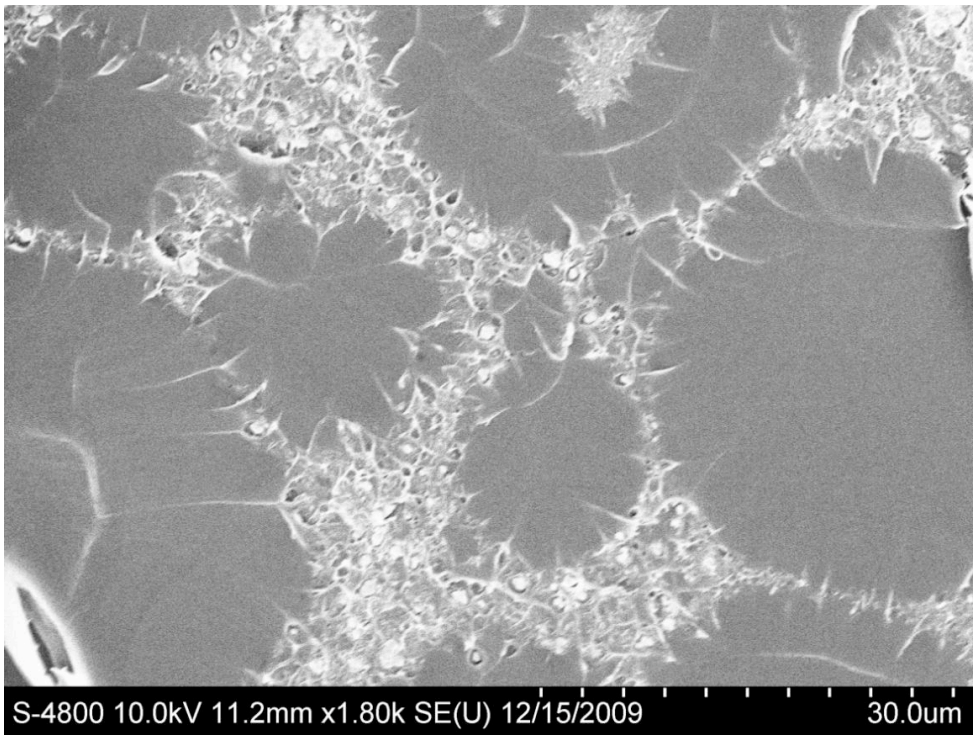


Figure 2.8 (a),(b) 0.67 wt% MWNT Palacos R composite polymerization showing polymer beads and interstitial space

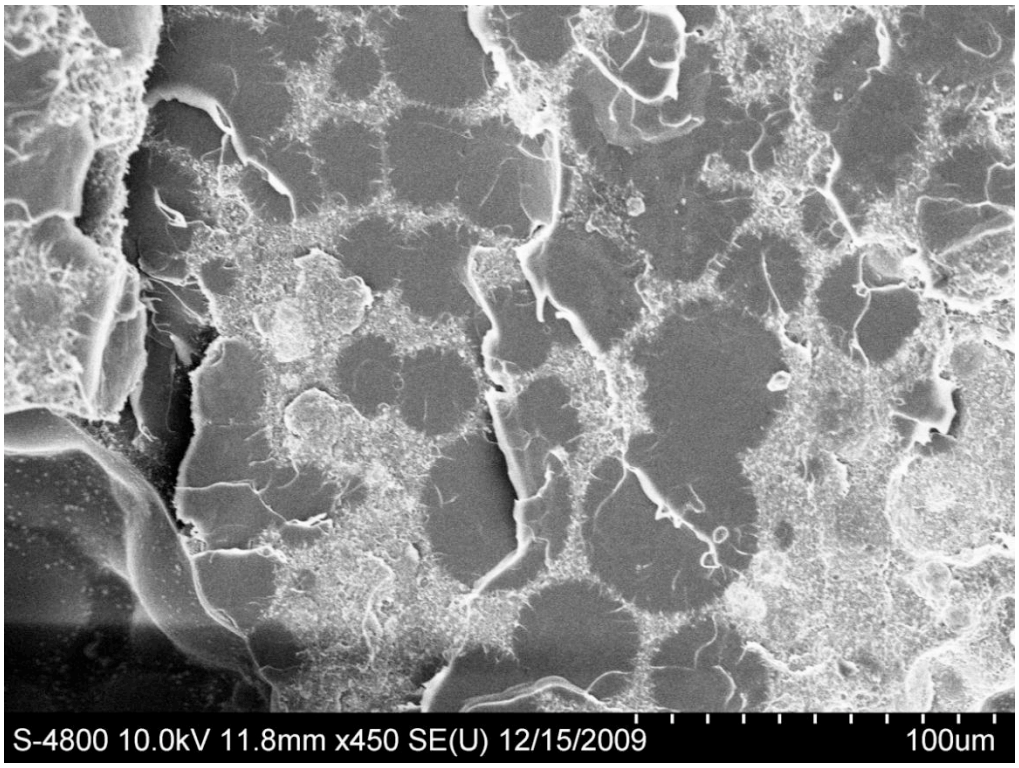
(b) 

Figure 2.8 Continued

The porosity of bone cement affects the mechanical properties of the material and therefore the replacement joint. Pores prevent even dispersion of heat during polymer polymerization. This indicates that the pores limit the complete result of adding MWNTs to bone cement powder to help systemize the polymerization process and reduce "hot spots". The amount of MWNTs added to the cement powder must be optimized to prevent too much porosity or the method of mixing the MWNTs into the powder needs to be improved. Improving the mixing method, or possible using the more elaborate heating and cooling method, would limit the amount of porosity and therefore limit its negative effects on complete and constant polymerization.

Section 2.3 Results

Section 2.3.1 Polymerization Kinetics Results

These are results for the first aim of this research. They explain how MWNTs influence the polymerization kinetics of orthopaedic bone cement when heated at a specific rate during the curing process. Incorporation of MWNTs into Simplex bone cement powder was found to significantly affect the polymerization kinetics of the bone cement. The thermograms collected from DSC showed that the MWNTs caused a dramatic drop in the peak heat flow and broadened the range of temperatures for all heating rates with both MWNT percentages (Figure 2.9).

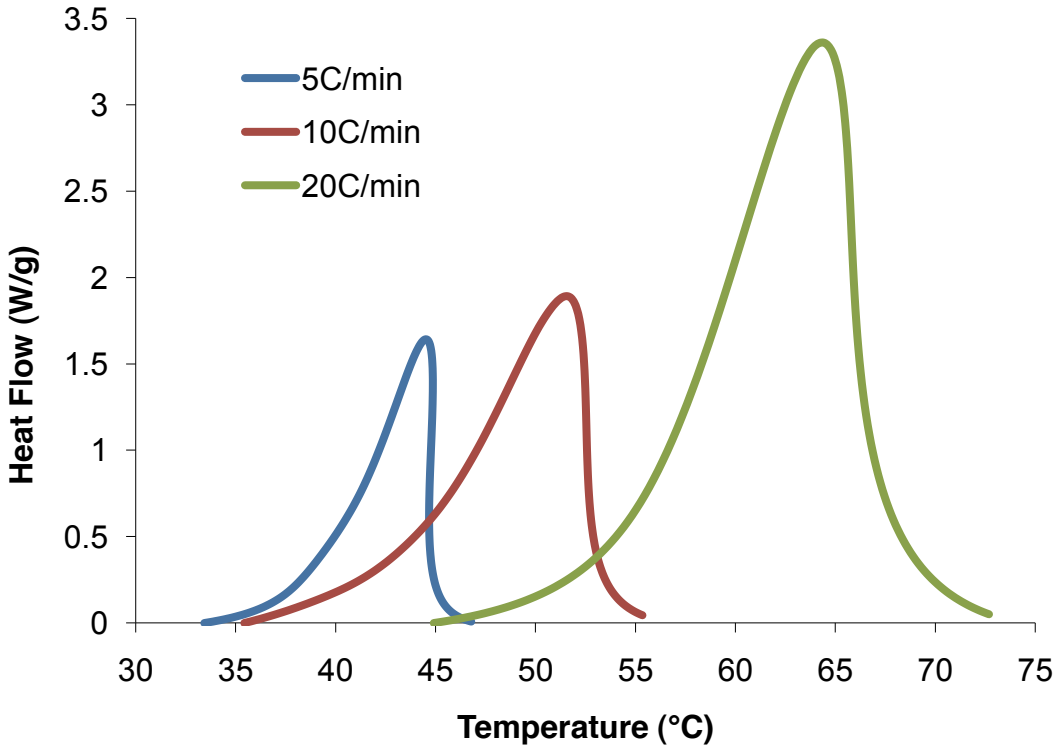


Figure 2.9 Thermogram trend for curing bone cement samples without MWNTs at the corresponding heating rates

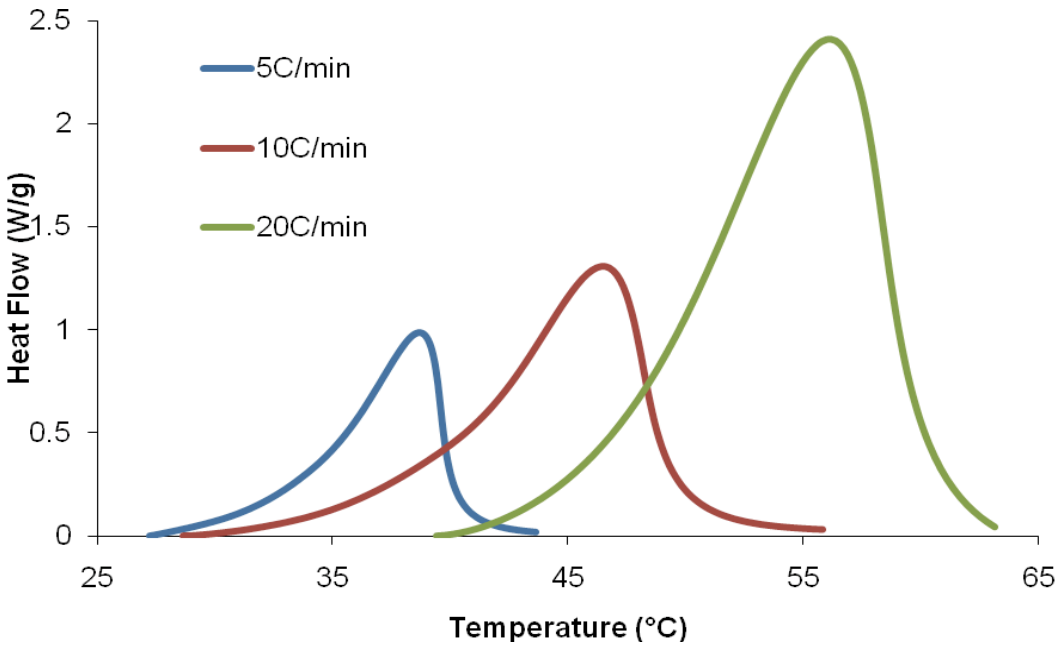


Figure 2.10 Thermogram trend for curing bone cement samples with 0.17wt% MWNTs at the corresponding heating rates

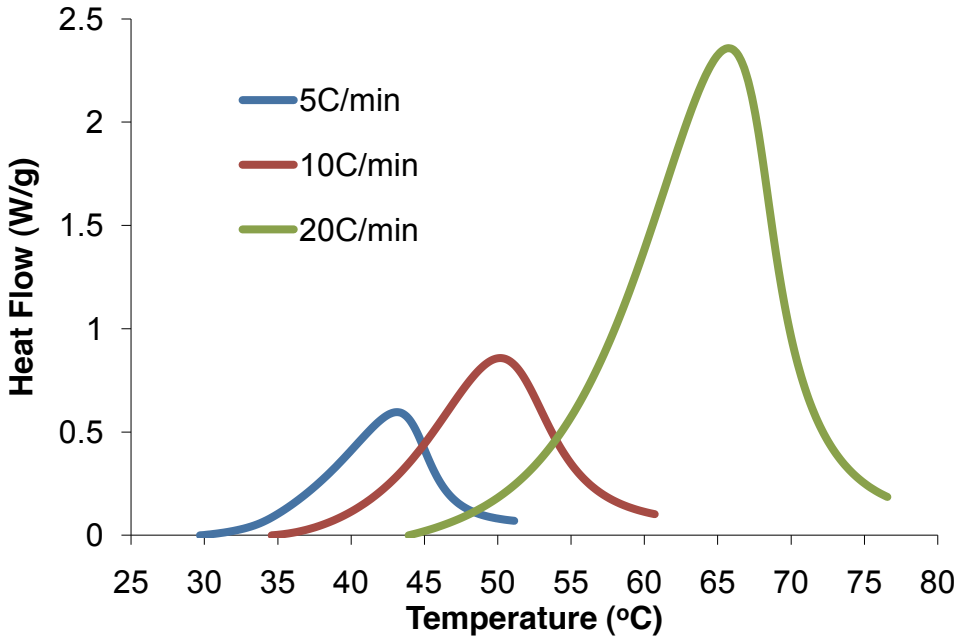


Figure 2.11 Thermogram trend for curing bone cement samples with 0.67wt% MWNTs at the corresponding heating rate

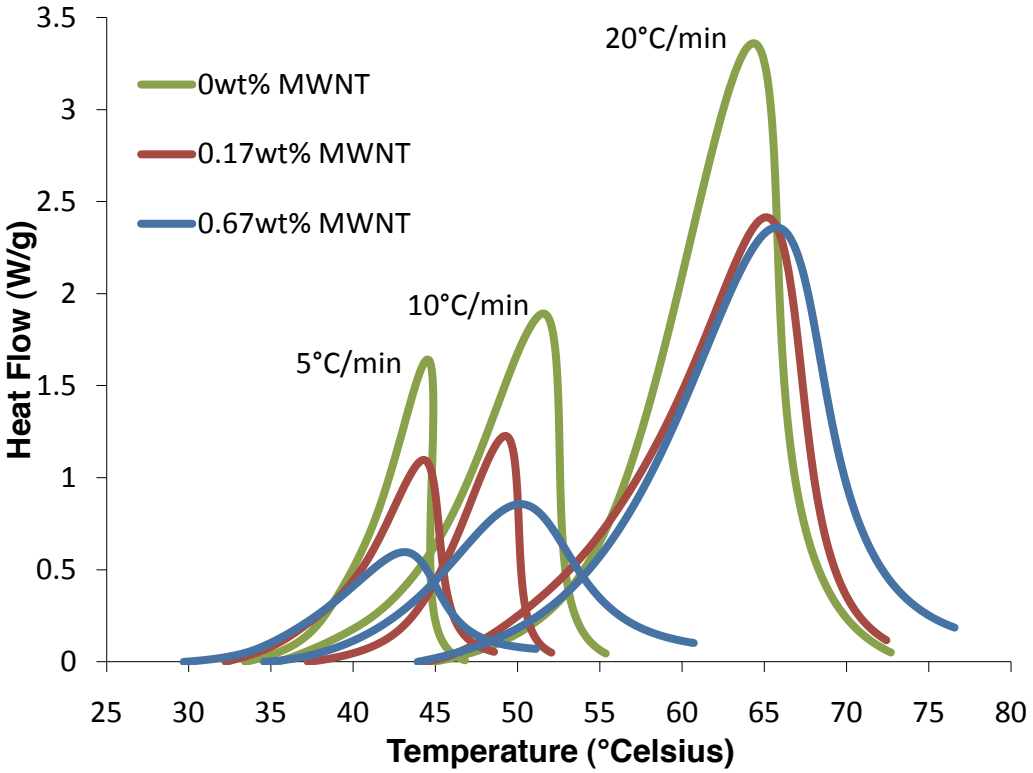


Figure 2.12 Average heat flow curves for curing pure and MWNT bone cements at the different heating rates

A 2-way Anova analysis showed the frequency factor and control polymerization values were significant for the 0.17wt% samples. The not significant combinations included the activation energy (Q) and the area under under the curve. At heating rates of 5, 10, and 20°C/min, the presence of MWNTs decreased the frequency factor of bone cement by 10.8%, 3.1%, and 9.4%, respectively (Table 2.1). The control polymerization values, k', increased by 140.8%, 140.4%, and 55.4% at each of the 5, 10, and 20°C heating rates. The change in heating rate significantly affected the activation energy and frequency factor (both p values being less than 0.001).

The 2-way Anova analysis for the 0.67wt% samples, at heating rates of 5, 10, and 20°C/min, showed significance for the activation energy, frequency factor, area under the curve, and control polymerization values. The presence of MWNTs decreased Q by 32.8%, 28.7%, and 14.9% respectively (p<0.001) (Table 2.1). Similarly, the 0.67wt% MWNTs decreased the frequency factor of bone cement at 5, 10, and 20°C/min by

34.4%, 30.8%, and 17.2%, respectively (p<0.001). The area under the curves decreased by 28.8%, 31.7%, and 12.0%, respectively (p<0.001). The control polymerization values, *k'*, increased by 21.4% and 19.9% at the 5 and 10°C heating rates. The value decreased by 37% at the 20°C heating rate. The *k'* value is very sensitive to changes and could easily become an increased value if the sample size was increased. A 1% change in one of the eight activation energy sample values resulted in a 12% change in k'. The change in heating rate significantly affected the activation energy and frequency factor (both p values being less than 0.001).

When both MWNT concentrations and the three heating rates were compared together, the influence of MWNT significantly affected the frequency factor (p=0.006) and the activation energy (p=0.008). The increase in heating rate had a greater impact on the frequency factor (p<<0.001) and activation energy (p<<0.001) than the influence of MWNTs though.

Table 2.1 Activation Energies, Frequency Factors, Areas, and Control Polymerization Values

MWNT (by wt)	H (°C/min)	Q (kJ mol^{-1})	ln Z (sec^{-1})	Area (°C*W/g)	k' (sec^{-1})	p value
0%	5	358.6 ± 80.6	133.1 ± 30.6	72.1 ± 3.6	0.002	---
	10	253.3 ± 29.5	91.8 ± 12.0	61.2 ± 11.7	0.002	---
	20	207.9 ± 11.3	73.0 ± 4.5	76.7 ± 10.5	0.000	---
0.17%	5	319.0 ± 26.2	119.5 ± 9.3	60.2 ± 16.1	0.014	NS
	10	241.5 ± 71.2	89.0 ± 28.1	58.9 ± 12.7	0.009	NS
	20	189.5 ± 78.6	66.4 ± 28.9	69.7 ± 8.4	0.001	NS
0.67%	5	257.5 ± 9.4	94.1 ± 3.2	53.9 ± 8.2	0.003	< 0.001
	10	189.6 ± 22.8	67.3 ± 8.5	44.5 ± 13.7	0.002	< 0.001
	20	179.1 ± 11.8	61.4 ± 4.2	68.0 ± 3.1	0	< 0.001

Section 2.3.3 Isothermal Heating Results

Section 2.3.3.1 Simplex Results

These are results for the first aim of this research. They explain how MWNTs influence the polymerization kinetics of orthopaedic bone cement during curing at a constant temperature. Thermograms collected from DSC showed that the MWNTs and the antibiotic tobramycin decreased the maximum heat flow (HF_{max}) and increased the full width at half maximum heat flow values (D) (Table 2.2). Figure 2.13 shows the influence of MWNT addition alone on the thermograms collected during isothermal testing. Figure 2.14 shows the influence of both MWNT and tobramycin addition.

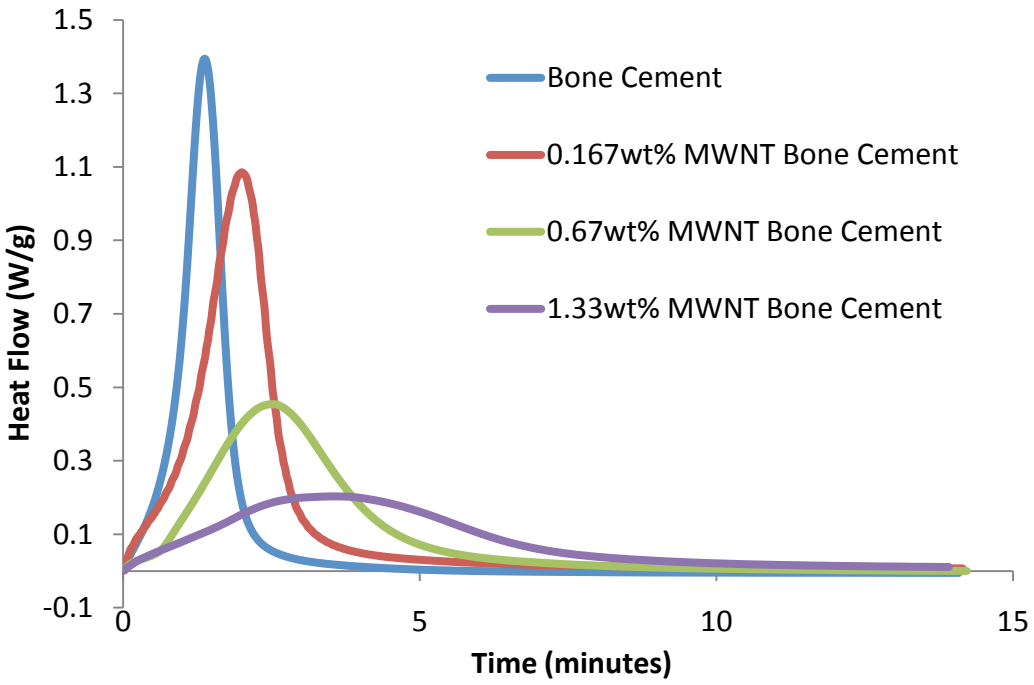


Figure 2.13 Selected heat flow curves for pure and MWNT loaded bone cement

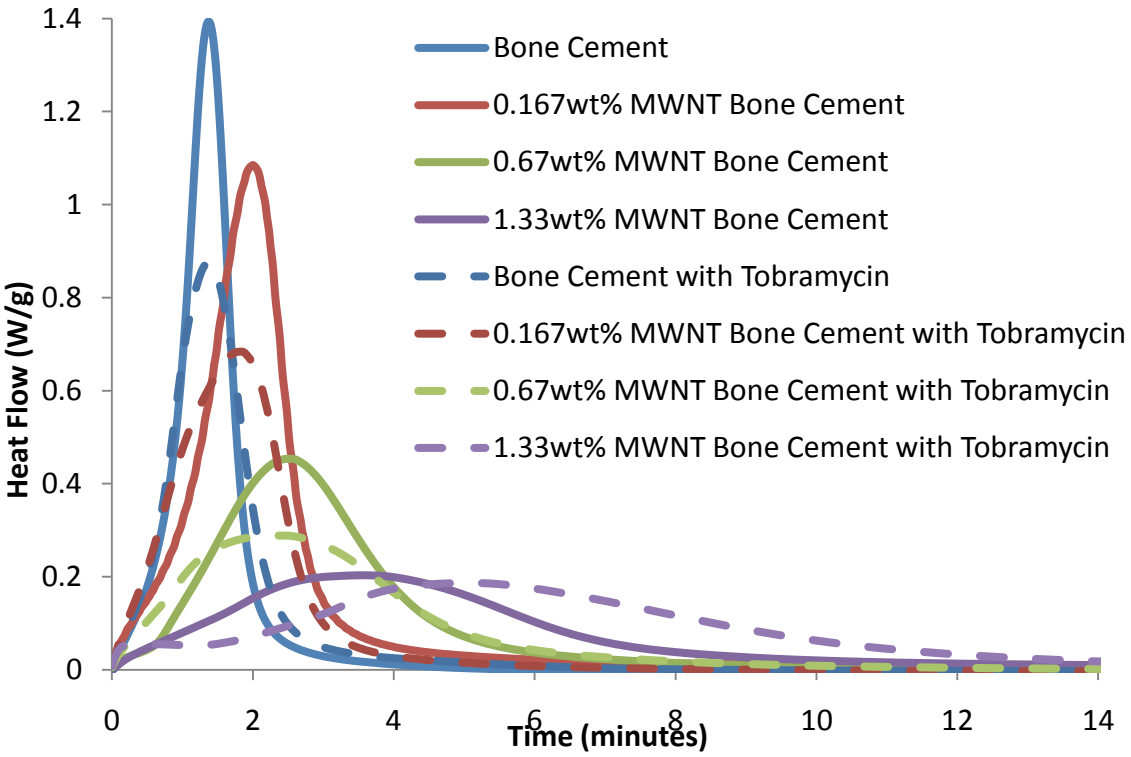


Figure 2.14 Selected heat flow curves for pure, MWNT loaded, and antibiotic loaded bone cement

Table 2.2 Maximum Heat Flow and Duration for MWNT and Tobramycin Loaded Simplex Bone Cement

MWNT (by wt)	Tobramycin (grams)	HF_{max} (W/g)	D (min)
0%	0	1.30±0.11	0.87±0.22
	0.06	0.85±0.05	1.45±0.31
0.17%	0	0.99±0.22	1.27±0.18
	0.06	0.63±0.14	1.84±0.42
0.67%	0	0.48±0.08	2.17±0.20
	0.06	0.35±0.08	3.10±0.75
1.34%	0	0.20±0.02	4.90±0.56
	0.06	0.20±0.04	5.55±1.21

MWNTs were associated with a 25-85% reduction in HF_{max} and a 45-460% increase in D (p<0.001). Tobramycin addition alone was associated with a 35% reduction in HF_{max} and a 70% increase in D (p<0.001). The interaction between the heat flow reduction caused by both MWNTs and antibiotic was significant with a 50-85% reduction in HF_{max} and a 110-535% increase in D (p<0.001).

To ensure that the decrease in peak heat flow and increase of width at half maximum in the samples with both MWNTs and antibiotic was not from antibiotic alone, the tobramycin was tested in the DSC alone. The antibiotic and monomer were mixed in the same ratio as in the cement samples and placed in the DSC at 37^{o}C for 15 minutes. The experiment was run in triplicate and the thermograms averaged. These thermograms were then subtracted from the original cement sample data with MWNTs but without antibiotic to see if the results would be the same. Those quantitative results showed that the antibiotic alone would decrease the peak heat flow by 3-25% (p<0.001) and increase the width at half maximum by up to 50% (p<0.001). The differences seen were still significant numerically but the combination of the antibiotic into the MWNT cement mixture prior to heating in the DSC shows a greater drop in peak heat flow and increase in width at half maximum. The combination of the MWNTs and the antibiotic must work together to impact the thermal properties of the polymerizing bone cement. Figure 2.15 compares the applications of antibiotic. Visually it is easy to identify that the samples with the tobramycin mixed in prior to heating reduced the peak heat flow more substantially than when the antibiotic effect was just subtracted off mathematically.

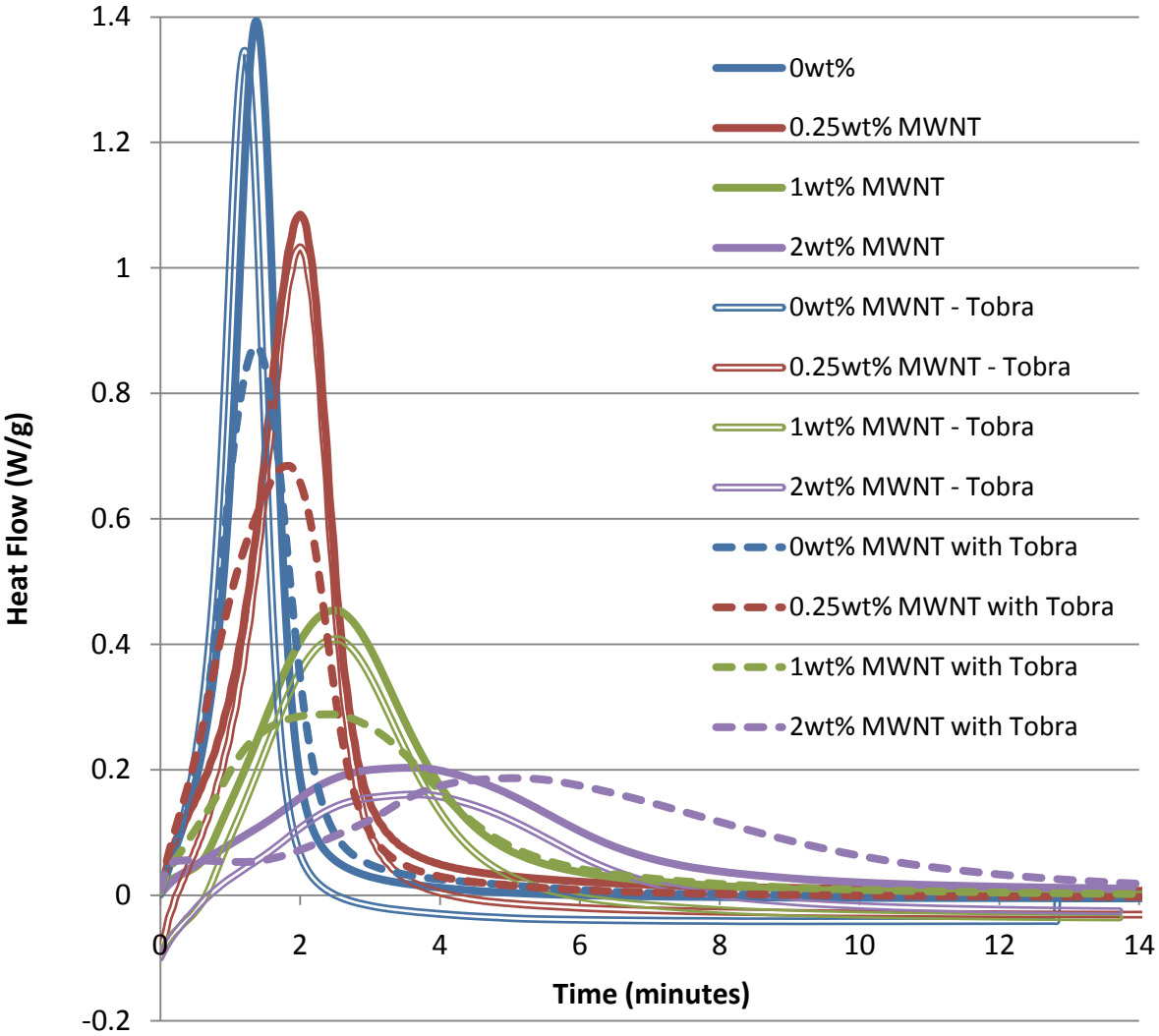


Figure 2.15 Comparison of cement samples with tobramycin actually in samples (MWNT with tobra) and the quantitative effects found by combining the tobramycin and MWNT cement results (MWNT –tobra).

Thermograms collected from DSC showed that the MWNTs and the antibiotic cefazolin decreased the maximum heat flow (HF_{max}) and decreased the full width at half maximum heat flow values (D) (Table 2.3). Figure 2.16 shows the influence of MWNT and the cefazolin antibiotic addition on the thermograms collected during isothermal testing.

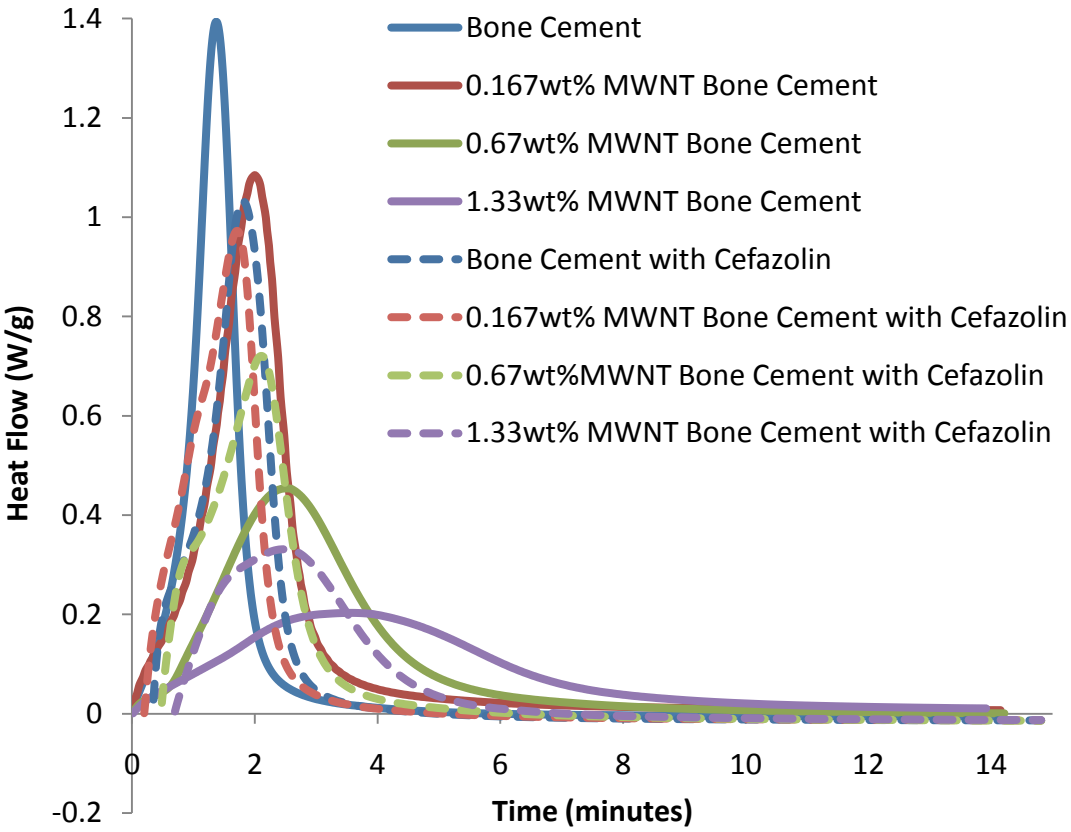


Figure 2.16 Selected heat flow curves for pure, MWNT loaded, and cefazolin loaded bone cement

Cefazolin addition without MWNTs was associated with a 32% reduction in HF_{max} and a 85% decrease in D (p<0.001) (Table 2.3). The interaction between the heat flow reduction caused by both MWNTs and antibiotic was significant with a 20-75% reduction in HF_{max} and a 85-90% decrease in D (p<0.001) (Table 2.3). MWNTs in bone cement augmented with cefazolin were associated with decreases in peak height and width at half peak values that were significant (p<<0.001) .

Table 2.3 Maximum Heat Flow and Duration for MWNT and Cefazolin Loaded Simplex Bone Cement

MWNT (by wt)	Cefazolin (grams)	HF_{max} (W/g)	D (min)
0%	0	1.30±0.11	0.87±0.22
	0.05	0.88±0.14	0.13±0.08
0.17%	0	0.99±0.22	1.27±0.18
	0.05	1.04±0.08	0.10±0.02
0.67%	0	0.48±0.08	2.17±0.20
	0.05	0.80±0.11	0.13±0.01
1.34%	0	0.20±0.02	4.90±0.56
	0.06	0.34±0.02	0.11±0.02

In the experiments using tobramycin as the antibiotic, the width at half maximum values increased as the percentage of MWNTs included increased. The opposite effect was seen with the cefazolin antibiotic. In those samples, the width at half maximum values decreased as the percentage of MWNTs included increased. The widths at half maximum values were used as a way to help quantify polymerization of the material. It was hypothesized that the presence of MWNTs would decrease the peak polymerization temperatures reached and increase the time of polymerization to achieve complete polymerization. This result was seen in the individual isothermal studies just looking at the addition of MWNTs as well as in the studies with the presence of the antibiotic tobramycin. The peak heat flow values recorded decreased with the addition of MWNTs but in order for complete polymerization of the monomer, the duration of polymerization increased. Graphically this can be examined by looking at the area under the thermograms. In the cefazolin cases, the polymerization duration appears to be decreasing. This would indicate that complete polymerization is not occurring in these samples. Figure 2.17 below compares the influence of MWNT and both antibiotic additions. In this figure you can examine the thermogram peaks and widths among all isotherm sample types.

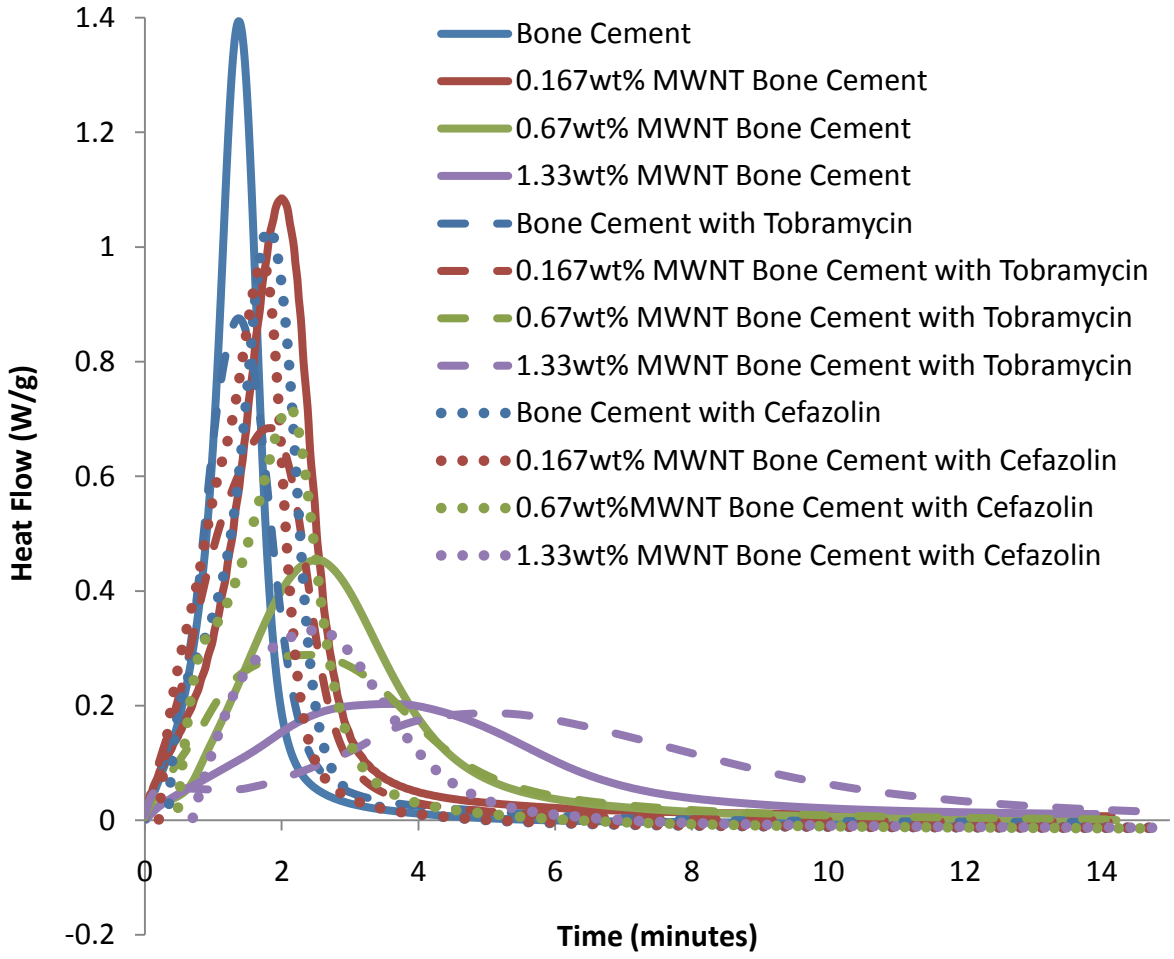


Figure 2.17 Selected heat flow curves for pure, MWNT loaded, tobramycin, and cefazolin loaded bone cement

Section 2.3.3.2 Palacos Results

Thermograms collected from DSC showed that the MWNTs decreased the maximum heat flow (HF_{max}) and increased the full width at half maximum heat flow values (D) but in a different way than in the Simplex cement (Table 2.4). Figure 2.18 shows the influence of MWNT addition on the Palacos R and Palacos+G thermograms collected during isothermal testing.

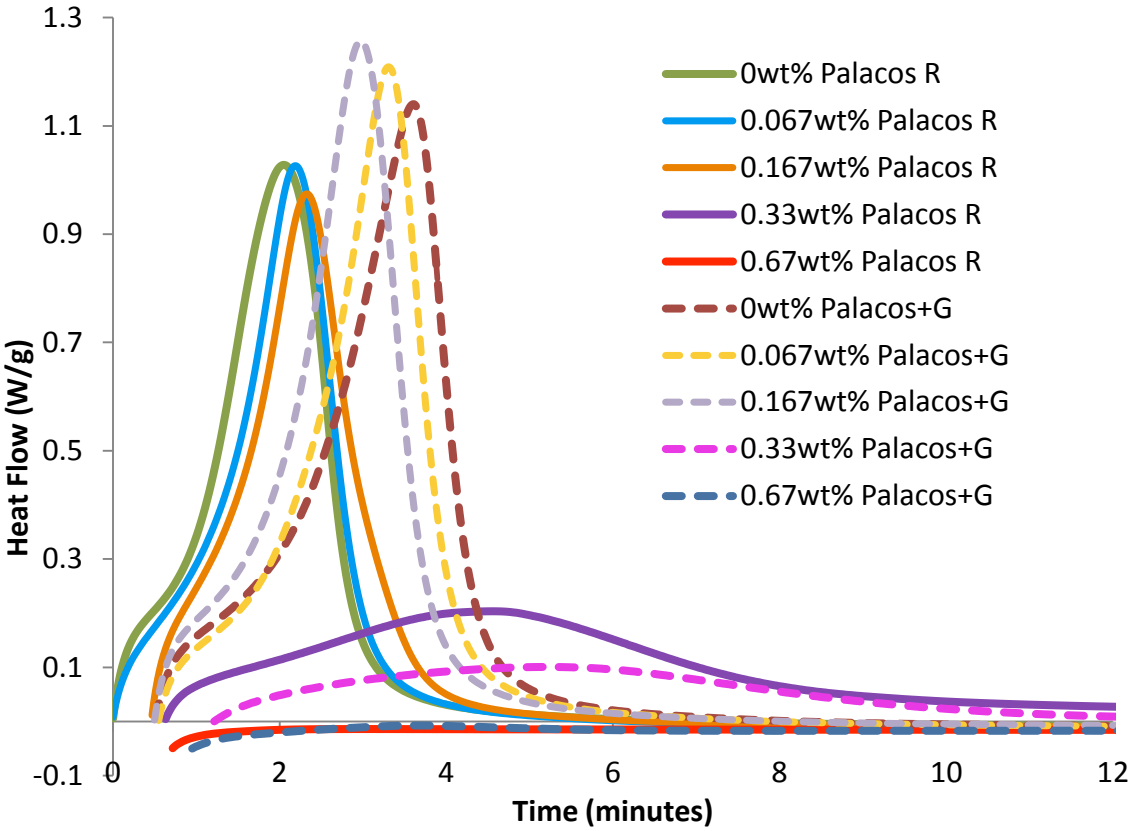


Figure 2.18 Selected heat flow curves for pure, MWNT loaded, and gentamicin loaded Palacos bone cement

MWNTs were only associated with one significant reduction in HF_{max} and increase in D, and that was in the 0.33 wt% samples (75% decrease and 235% increase, respectfully, $p<<0.001$). The 0.067 wt% and 0.167 wt% samples had no significant reduction in peak heat flow or width at half maximum as a result of MWNT addition. There was no measurable peak in the 0.67 wt% samples. The pure Palacos+G samples with no MWNTs had an average reduction in peak height reduction of 25% and an average increase in width at half peak of 15% ($p<<0.001$). Similar curves were seen in the Palacos+G samples with the addition of MWNTs. Only the 0.33 wt% samples had significant changes in measured variables. HF_{max} decreased by 88% and D increased by 300% in those samples ($p<<0.001$). The antibiotic alone only significantly affected the width at half peak in the samples 0.67 wt% samples ($p=0.01$). The heat flow reduction caused by both MWNTs and antibiotic was significant with a p value $<<0.001$).

Table 2.4 Maximum Heat Flow and Duration for MWNT and Gentamicin Loaded Palacos Bone Cement

MWNT (by wt)	Gentamicin (grams)	HF_{max} (W/g)	D (min)
0%	0	0.91±0.17	1.52±0.20
	0.025	1.15±0.01	1.28±0.06
0.067%	0	1.13±0.09	1.24±0.14
	0.025	1.09±0.29	0.83±0.66
0.167%	0	1.01±0.03	1.36±0.17
	0.025	1.36±0.09	1.06±0.12
0.33%	0	0.22±0.07	5.09±0.42
	0.025	0.11±0.04	6.21±0.50

Since there was no measurable peak in the 0.67 wt% samples, it was assumed that polymerization of the bone cement did not occur in those samples. The temperature of the samples did not rise like the other samples. The MWNTs prevented the polymerization reaction from occur in both the samples with and without antibiotic. From this point forward, only percentages of MWNTs less than 0.67 wt% were used in testing with Palacos R and Palacos+G cement.

After completing the isotherm polymerization studies with Palacos R and Palacos+G, it was recognized that the polymerization kinetics of Simplex and Palacos cements were different. When comparing the effects of different percentages of nanotubes, significant differences between the two types of cement were found. The isotherm study of Palacos was expanded to include other weight percentages of MWNTs to try to identify where the cut-off point for complete polymerization was. It was already known that 0.67 wt% MWNTs did not allow polymerization to occur. Intermediate percentages including 0.302 wt%, 0.268 wt%, and 0.201 wt% were investigated. Figure 2.19 shows the isotherm thermograms for these percentages and the ones included in the previous study.

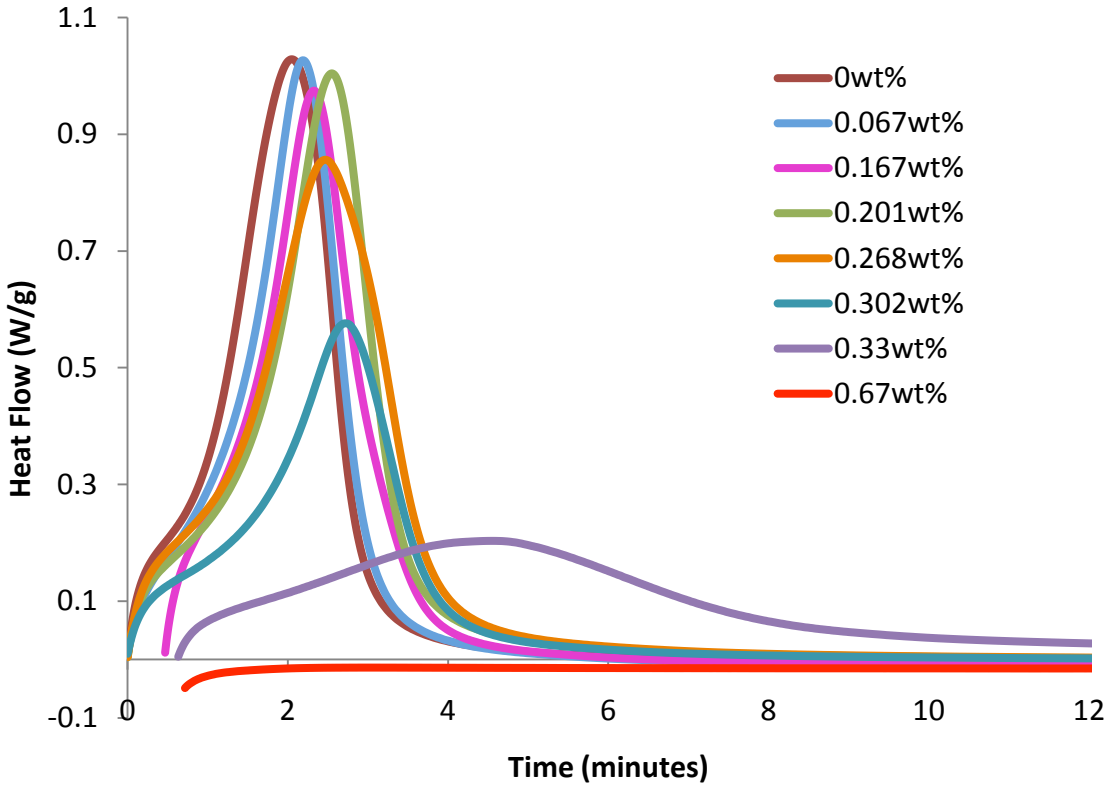


Figure 2.19 Selected heat flow curves for a variety of MWNT loadings in Palacos bone cement

MWNTs were only associated with significant reductions in HF_{max} and increases in D in the samples with percentages of MWNTs greater than 0.201 wt% (25-75% decrease and 1-235% increase, $p<<0.001$). Table 2.5 shows the average heat flow maximum and width at half maximum values for all the MWNT Palacos samples.

Table 2.5 Maximum Heat Flow and Duration for all MWNT Palacos Bone Cement Samples

MWNT (by wt)	HF_{max} (W/g)	D (min)
0%	0.91±0.17	1.52±0.19
0.067%	1.13±0.09	1.24±0.14
0.167%	1.01±0.03	1.36±0.17
0.201%	0.98±0.18	1.37±0.30
0.268%	0.94±0.07	1.53±0.23
0.302%	0.69±0.10	1.65±0.04
0.33%	0.22±0.07	5.09±0.42

The first MWNT percentage that significantly decreased peak heat flow was 0.268 wt%. Only increasing the MWNT percentage by 0.05 wt% decreased the average heat flow by 25%. This indicates that the range of 0.4-0.45 wt% MWNTs is where MWNTs decrease peak heat flow but still allows polymerization to occur. Once the percentage of MWNTs is again raised by 0.05 wt%, the average peak heat flow values decrease an additional 50%. The width at half maximum at this point is also significantly increased (235%) but complete polymerization of the material may not be occurring. The Palacos cement appears to be much more susceptible to the MWNTs changing its polymerization than the Simplex cement.

Section 2.3.3.3 Comparison of Simplex and Palacos Bone Cement

MWNTs influenced the polymerization kinetics of the two cement brands differently. Graphical analysis was completed on the average thermograms for both types of cements shown in Figure 2.20. The differences between the peaks of the thermograms with different MWNT percentages are very distinct.

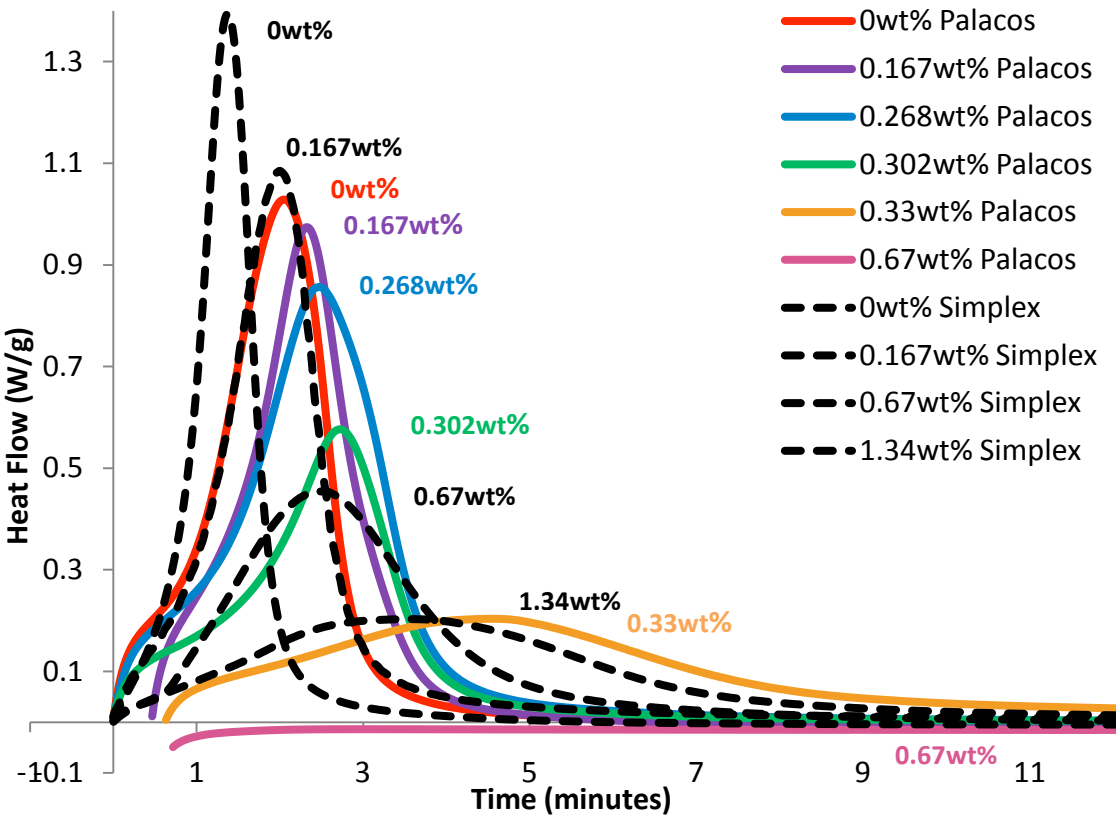


Figure 2.20 Selected thermograms for Palacos and Simplex bone cements

Comparing, HF_{max} and D from the previous isothermal experiments shows the peak heat flow values between the two pure cements with MWNTs has an average difference of 26% (p=0.03). The Palacos cement had lower heat flow values when compared to the Simplex cement. The pure Palacos samples also had an average width at half maximum that was 46% larger (p=0.03) than the Simplex samples. The other samples that could be directly compared were the 0.167 wt% MWNT samples. The variable differences in these samples were smaller. Palacos had a peak heat flow that was on average 5% lower than Simplex, and a width at half peak that was on average 6% wider (p>0.05). As the percentages of added MWNTs increased, the differences in peak height also increased. In the 0.67wt% case, the Simplex samples still have a visible positive peak heat flow while the Palacos samples have an almost horizontal line below the positive horizontal axis. The difference in average HF_{max} was that the Palacos values were 230% lower than the Simplex values (p<0.01). The widths at half maximum of the Palacos samples were

not calculated due to the lack of a real peak. Table 2.6 displays the peak heat flow values and width at half maximum values for all the samples in the figure.

Table 2.6 Heat Flow Maximum and Duration Comparison for Palacos and Simplex Bone Cement
(N/D means No Data and N/M means Not Measured)

MWNT (by wt)	Cement Type	HF_{max} (W/g)	D (min)
0%	Palacos	0.99±0.12	1.41±0.16
	Simplex	1.30±0.11	0.87±0.22
0.167%	Palacos	1.01±0.03	1.36±0.17
	Simplex	1.06±0.10	1.27±0.18
0.268%	Palacos	0.94±0.07	1.53±0.23
	Simplex	N/D	N/D
0.302%	Palacos	0.69±0.10	1.65±0.04
	Simplex	N/D	N/D
0.33%	Palacos	0.22±0.07	5.09±0.42
	Simplex	N/D	N/D
0.67%	Palacos	-0.01±0.01	N/M
	Simplex	0.48±0.08	2.17±0.20
1.34%	Palacos	N/M	N/M
	Simplex	0.20±0.02	4.90±0.56

Visually and numerically it can be seen that the Palacos 0.33 wt% MWNT samples had very similar thermograms to the Simplex 1.34 wt% MWNT samples. The polymerization of the Palacos samples began about 45 seconds after the Simplex samples. This is one of the differences between the MWNT effects on the polymerization of the different commercial bone cement powders. It is interesting to see how a small percentage of MWNTs inhibits polymerization in the Palacos cement. The polymerization in these samples may occur over a longer period of time but all of our experiments were limited to 15 minutes.

Section 2.4 Discussion

Section 2.4.1 Polymerization Kinetics Conclusions

Multiwall carbon nanotubes influence the polymerization characteristics of bone cement. MWNTs significantly and positively affected the thermal properties of Simplex bone cement. They increased the time required to completely polymerize the methylmethacrylate monomer which adds time for the surgeon to position the implant. More importantly, they decreased the rate of heat release, shown by decreases in activation energy and frequency factor, and this will likely contribute to a decrease in the peak exothermic temperature of the bone cement. Reducing the temperature of the *in situ* polymerizing cement means that there is less likelihood of thermal necrosis and, therefore, a more biologically viable bone cement-bone interface will result. This will positively affect the mechanical integrity of the interface and improve implant longevity. The addition of MWNTs to bone cement may help avoid polymerization induced "hot" spots. MWNTs can also be used to distribute heat from the cement mantle that can be the cause of hyperthermia-based destruction of adjacent bone by conducting the heat to the metal in the implant.

The carbon nanotubes must be inhibiting some of the polymerization reaction. The areas under the curve were similar in all samples with the same MWNT percentage, even with the different heating rates. But the areas under the curve did decrease with the addition of MWNTs. The areas relate to the total energy in the system in relation to the heating rate, or the number of reactions that occurred during polymerization. If the areas are roughly the same, the same degree of polymerization has occurred. The addition of carbon nanotubes caused a signficant decrease in thermogram area. The polymer part of the reaction cannot be controlled as easily as the kinetics reaction.

It has been suggested that a low *k'* indicates a higher degree of conversion of the liquid monomer, which may lead to a smaller amount of residual monomer, contributing to

long-term *in vivo* stability of the replacement [11]. The *k'* values of the samples with MWNTs significantly increased when compared to the control samples. This indicates that the amount of residual monomer is actually increasing, there is a lower degree of monomer conversion in the MWNT samples. The addition of MWNTs to the bone cement powder is likely preventing all of the polymerization reactions from occurring. The MWNTs may be getting in the way of some of the free radical reactions by either physically being in the way or actually chemically binding to some of the free radicals themselves. To have more complete polymerization of the bone cement material, the percentage of MWNTs added to the mixture must be minimal. If the percentage of MWNTs is too large, where near complete polymerization does not occur, not only will the thermal properties be affected but the mechanical properties as well. The strength of bone cement is dependent on the polymerization reactions. If enough polymerization reactions do not occur, the material does not harden, and could not be used in orthopaedic surgery like it is today. An optimal balance of MWNT weight percent must be found through future studies. This optimal range should have the increase in mechanical properties due to the MWNTs presence accounting for the subsequent decrease in polymerization reactions which decreases the mechanical properties. A limitation of these studies is the inability to know how many polymerization reactions are occurring. If this was better understood it would be easier to identify the optimal percentages of MWNTs to ensure the best performance material.

The high exothermic temperatures of bone cement polymerization also limit the therapeutic potency of the antibiotics used in arthroplasty. Antibiotics that are not heat labile denature at temperatures above 100^{o}F. Despite contemporary efforts to minimize contamination, infection remains a significant concern in all arthroplasty procedures. Antibiotics are denatured at certain temperatures, a temperature which can be reached during cement polymerization. Presently there are less expensive antibiotics that would positively prevent orthopaedic infections, but these antibiotics denature at temperatures exceeded during polymerization. Lowering the polymerization temperature to below 100^{o}F would increase the choice of antibiotics available to the patient and the surgeon. If

the lowered temperature could be achieved without diminishing antibiotic potency, it would also positively affect the mechanical integrity of the interface and would greatly improve implant longevity.

Section 2.4.2 Isothermal Heating Conclusions

MWNTs were shown to substantially alter the flow of heat liberated during the polymerization of both Simplex and Palacos bone cement. The benefits of this include: 1) new options for use of heat-labile antibiotics in TJA, 2) improved antibiotic potency, 3) fewer "hot spots" that can nucleate fatigue cracks, 4) greater biological viability of the bone-bone cement interface, and 5) mechanical strengthening of the matrix otherwise weakened by antibiotic incorporation. These benefits support our belief that MWNTs in antibiotic laden bone cement can result in enhanced clinical performance of cemented total joint implants.

The polymerization of Palacos cement is more affected by the presence of MWNTs than Simplex cement because of its viscosity and molecular weight differences. Palacos is sterilized by ethylene oxide and is a high molecular weight cement. Simplex is sterilized by γ-irradiation and is a low molecular weight cement [4]. Palacos powder has a measured molecular weight of approximately 950 kDa and Simplex powder has molecular weight estimated at 100 kDa [76]. Palacos being a high viscosity cement plus the addition of MWNTs may make the mixture too viscous. If the viscosity is too high, the free radicals cannot find the double bonds and polymerization will not occur. The lower viscosity, Simplex cement, cures to a much greater extent with MWNTs. Questions arise as a way to possibly alter this result and allow the higher viscosity cements like Palacos to polymerize better with the presence of MWNTs. Adding more BPO could limit the negative effects of MWNT presence, but this raises the issue of how would this change the mechanical properties of the material. Concerns exist that MWNTs are getting in the way or attaching to the free-radicals themselves; thus preventing the joining with the double bonds and polymerization. Currently the reactions

occur in a more localized way, sporadically throughout the sample. This leads to hotter regions in the material that cause the polymerization heat spike. If the reactions could be more systemic, the polymerization reactions would be more gradual and the maximum temperatures reached could be minimized. Also, we want to see optimization among the maximum temperature reached during polymerization, mechanical properties, and elution properties. The MWNT percentage that provides the best result in each of those cases needs to be identified. This percentage should decrease peak heat flow, increase polymerization time, and increase mechanical stability. These studies could also have been expanded by finding a direct calculation to go from the measured heat flow to temperature in the polymerizing material. It would be easier to understand the affects of MWNTs on bone cement polymerization and the nature of what would occur when the material is implanted into the body.

Chapter 3 - Elution Properties of Antibiotic Bone Cement with Multiwall Carbon Nanotubes

Section 3.1 Background

The use of bone cement as a source of antibiotics has been around for almost forty years. Methylmethacrylate is clearly capable of serving as a route of administration for antibiotics. PMMA appears to be an adaptable material that can be manipulated to modify the antibiotic elution profile to match a given clinical situation [77]. Controversies do exist over the best use of different antibiotics, cements, and their combinations. It is not yet known how to provide the best antibiotic to patients who have different requirements with the antibiotic-cement combination to meet their needs. Antibiotics in bone cement leach out of the hardened plastic material by diffusion. The idea is that the antibiotics are released from the cement gradually over time in such a way that the local levels of antibiotics exceed the minimal inhibitory concentration of the pathogen [78]. The amount has been found to be proportional to the surface area of the cement [35]. It is quite obvious from elution kinetics that not every bone cement or antibiotic is suitable for use in an antibiotic bone cement mixture for arthroplasty. The potential benefits of antibiotic-loaded cement outweigh its potential risks [79].

Antibiotic-loaded cement is used in two ways. One way is to treat infection, using 1- 3.6 g of antibiotic per 40 g of acrylic cement to have effective elution kinetics and sustained therapeutic levels of antibiotic [80]. Higher doses, between 6 and 8 g of antibiotic per 40 g of bone cement, can be used in cement beads or spacers [81]. Not all the bone cement is used in every case, but the whole 40 g of bone cement is mixed with the antibiotic to maintain the correct ratio. The packets of bone cement come premeasured from the cement manufacture. The second way antibiotic-loaded cement is used is as prophylaxis. This requires low doses of antibiotics in the bone cement to avoid adverse mechanical effects because of the use of this cement as mechanical fixation for an implant [82]. Low-dose antibiotic-loaded bone cement (ALBC) is typically defined as ≤ 1 g of

powdered antibiotic per 40 g of bone cement. Figure 3.1 is a flowchart created by Jiranek *et al* that outlines the different uses of antibiotic-loaded bone cement [82]. Continuous use of the same antibiotics develops the potential for creating drug resistant bacteria that are resistant to this type of treatment.

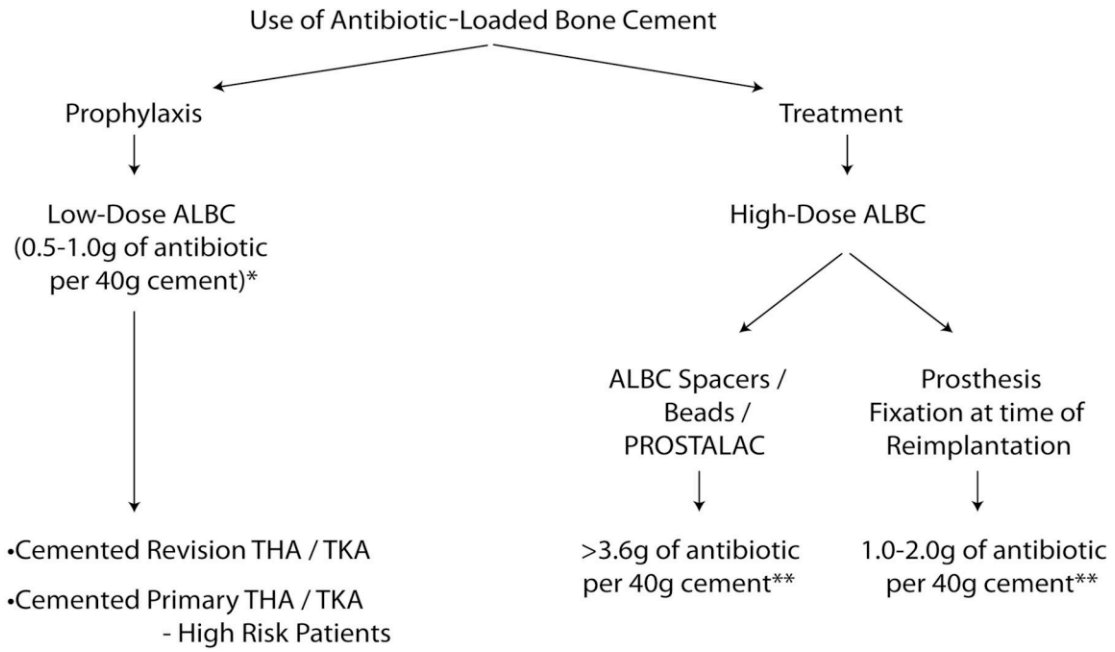


Figure 3.1 Guidelines for clinical use of antibiotic-loaded bone cement. *Antibiotics recommended for prophylaxis include gentamicin or tobramycin. **The antibiotic(s) used depends on the susceptibility of the microorganisms identified or suspected [82].

The United States Food and Drug Administration (FDA) approved the use of antibiotic-impregnated bone cement products for prosthetic fixation after eradication of a previous infection. The commercially available materials only contain a low-dose of antibiotics. Bone cement beads and spacers used to treat established infections typically use a higher dose of antibiotics to achieve desired elution and sustained therapeutic levels. Using high-dose antibiotic loaded cement requires the orthopaedic surgeon to hand-mix the appropriate agents as needed [83]. Table 3.1 displays the current FDA approved premixed antibiotic bone cement products.

Table 3.1 Food and Drug Administration-Approved Premixed Antibiotic Bone Cements Used in Study [14]

Product	Distributor	Viscosity	Antibiotic (Amount)
Cemex Genta	Exactech	Low	Gentamicin (1.0 g)
Cobalt G-HV	Biomet	High	Gentamicin (0.5 g)
Palacos R + G	Zimmer	High	Gentamicin (0.5 g)
Simplex P	Stryker	Medium	Tobramycin (1.0 g)
Smartset GHV	DePuy	High	Gentamicin (1.0 g)

There are conflicting reports discussing the mechanism by which antibiotics are released from bone cement, including looking at whether they can diffuse through the cement or are removed only from its surface [42]. It is known that there are many factors that affect elution profiles, including the type of cement used, the choice of antibiotic, and the preparation method. Antibiotic release must be dependent on factors including its molecular weight, the molecular weight and degree of cross-linking of the polymer , the solubility of the drug in the polymer, and the relative solubility of the drug in the polymer and in the medium outside the matrix [42]. The mechanical properties of the cement are also important and choices about antibiotic loading must be made based on the need for the cement. Porosity is also important in elution. When porosity is created by materials in the cement, elution is improved, but too much porosity can compromise desired mechanical properties as much as 30-50% [22]. But the compressive strength of PMMA is many times greater than required in the clinical setting and even a 45% decrease would not compromise its function as a spacer [83].

It was also proposed that antibiotic elution may change if more than one antibiotic is introduced into the cement. The idea that the addition of a second antibiotic improves antibiotic elution has been termed the synergistic effect. Initially it seems that the elution is strongly affected by the amount of antibiotic that is on the surface of the test sample. Once that antibiotic is eluted, the presence of the second antibiotic may facilitate the elution of the first antibiotic from open cavities within the cement. It is believed that the synergistic effect is due to the increased porosity of the cement caused by the elution of antibiotic molecules, therefore improving the overall elution rate [84]. Masri *et al* termed

the phenomenon the process of "passive opportunism of antibiotic elution from bone-cement" [84].

It has been generally accepted that Palacos R (Smith & Nephew Orthopaedics, Memphis, TN) has superior elution characteristics compared with other cement types [79]. Maximizing the efficiency of antibiotic release may improve therapeutic efficacy and cost efficiency [85]. Maximizing antibiotic release from cement may offer clinical benefit and at least may offer theoretical advantages, such as decreased development of antibiotic resistance, improved treatment efficacy, and increased cost efficiency. If these benefits can be obtained without an increase in cost or risk to the patient, choosing the cement with the maximal elution rate seems warranted.

Being able to find a way to use liquid gentamicin instead of the costlier powdered antibiotics would be a significant way to decrease related costs. Currently, tobramycin is widely used in the US but it is very expensive ($120 per 1.2 g dose [83]) and has been in short supply. Gentamicin has been used to treat musculoskeletal infections for years but is as expensive as tobramycin when in its powdered form, and is unavailable in some countries. Liquid gentamicin is much cheaper ($4 per 480 mg dose [83]), is readily available, and is one of the most commonly used agents in clinical settings. If tobramycin in bone cement spacers could be replaced with liquid gentamicin, it was estimated that an annual antibiotic cost savings of $7,400,000 could be achieved in the US for total joint implant infections treatment [22].

Hsieh investigated the use of liquid gentamicin in combination with vancomycin [83]. It is known that using liquid antibiotic in bone cement for the implantation of prosthesis is unsuccessful because the cement has inferior mechanical properties. But because antibiotic-impregnated beads or spacers are only temporary, the mechanical properties are not as important. During a five week study period, vancomycin elution was enhanced by 146% with the addition of gentamicin liquid and gentamicin elution was enhanced by 45% with the addition of vancomycin. The liquid gentamicin also significantly increased

the porosity of the specimens, both with and without vancomycin. Ultimate compressive strength was reduced by 13%, 37%, and 45% in samples with vancomycin alone, gentamicin liquid alone, and vancomycin with liquid gentamicin, respectively [83].

One of problems with antibiotic loaded bone cement is that the antibiotics elute very quickly out of the cement, into the blood stream, and away from the intended site of action. If the elution time could be extended, the "dwell time" of the antibiotic could be extended, thereby rendering the antibiotic more effective against existing or potential infection. Most of the antibiotic that is added to the cement also becomes trapped in the polymer matrix and is never able to elute into the surrounding system. The antibiotic that is eluted typically comes from the exterior of the bone cement.

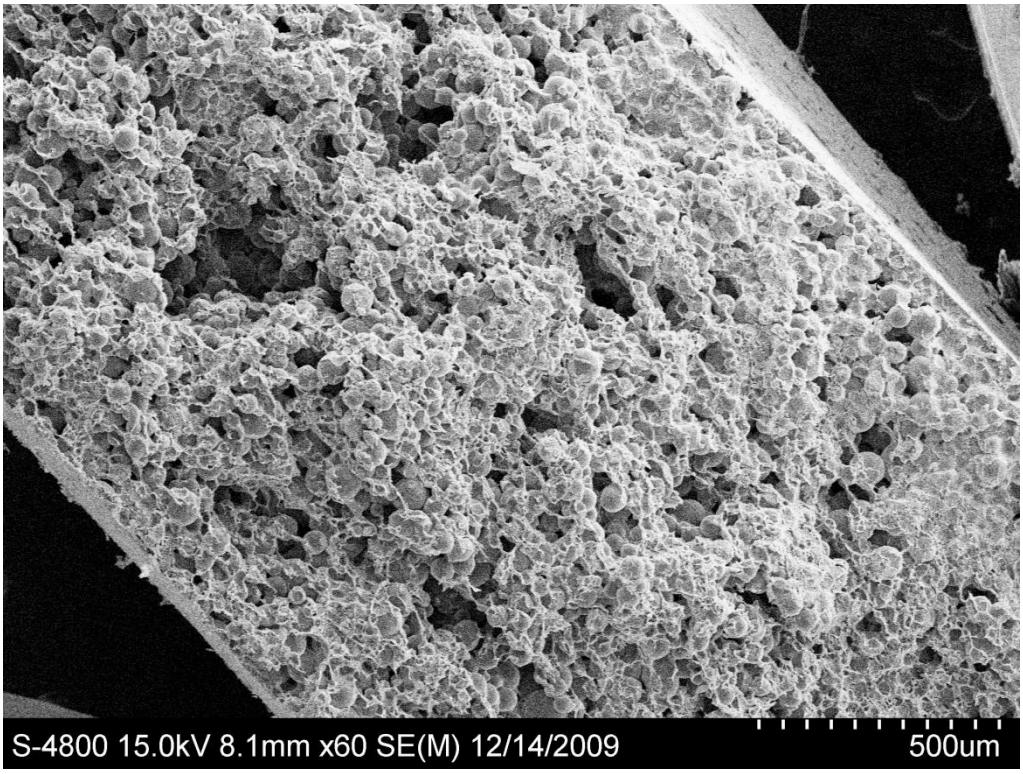
Carbon nanotubes are now being considered for drug delivery. They can be implanted at sites where a drug is needed without trauma, and slowly release the drug over time [86]. Carbon nanotubes could also potentially be used as part of the antibiotic bone cement drug delivering system. Their presence in the bone cement matrix could assist the antibiotic in getting out of the center of the bone cement. The purpose of this specific study was to determine if MWNTs affect the elution rate of antibiotics or improve the amount of antibiotic eluted out of bone cement.

Section 3.2 Methods

The following describes the study design for the second aim of this investigation. The variables included percentage of MWNTs added and elution sampling time. Calculations were made to identify the total antibiotic elution and the elution rate throughout the study. MWNT loading ranged from 0.33wt% to 1.34wt%. There were eight samples used in each experimental group. These are the methods for the second aim of this research, understanding how MWNTs influence the elution of antibiotics from orthopaedic bone cement.

After performing an extensive literature search, a pilot elution study was developed. Palacos bone cement (Zimmer, Warsaw, IN) both with and without the premixed antibiotic gentamicin (Palacos+G) was used to create MWNT mixtures. MWNTs produced at the Center for Applied Energy [55] were disaggregated and dispersed throughout the dry pre-polymerized Palacos bone cement powder using a dual-blade shear mixer. Mixtures were made with Palacos and Palacos+G for each MWNT concentration, as well as control samples without MWNTs. This resulted in 4 mixtures with antibiotic and 4 mixtures without antibiotic. Three of the mixtures in each group contained MWNTs. Commercial formulations with 40 g of sterile bone cement powder were used to create the mixtures. The Palacos+G formulation contained approximately 0.5 grams of gentamicin (1.25 wt%) per box. Forty-gram batches of hand-mixed powder were passed through the shear mixer three times to ensure complete dispersion of the MWNTs. The liquid monomer used was the commercially prepared ampule that came with the bone cement powder. Components were manually mixed at room temperature for two minutes in the ratio of 40 g of powder to 20 mL of monomer. The MWNT mixtures had percentages of 0.33%, 0.67%, and 1.34% (by weight). Figure 3.2 (a,b) displays SEM images of one of the 0.33 wt% MWNT Palacos R composites with liquid gentamicin. Figure 3.2 (a) shows the immense porosity in the cement sample with liquid antibiotic and Figure 3.2 (b) shows the alteration of polymer molecules during polymerization.

(a)



(b)

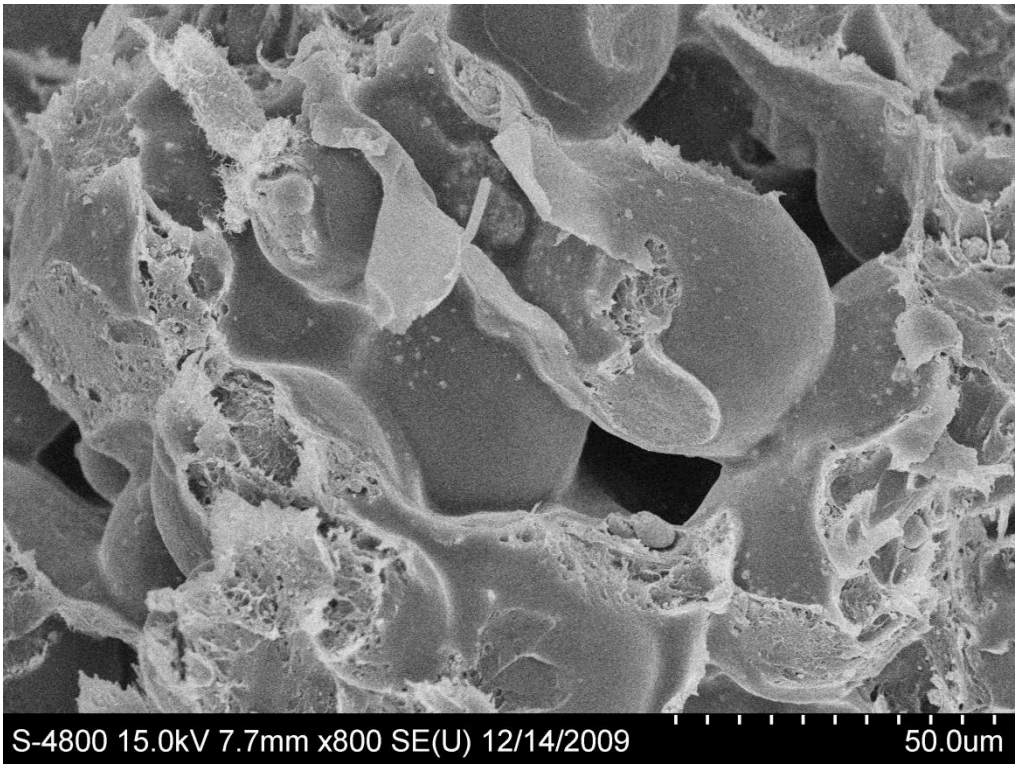


Figure 3.2 (a) Surface characteristics of 0.33 wt% MWNT Palacos R and liquid gentamicin composite and (b) Altered polymerization of 0.33 wt% MWNT Palacos R and liquid gentamicin composite.

After mixing the components manually until the cement had reached the doughy phase, the material was manually pressed into an acrylic mold that produced 6 mm diameter spherical shaped specimens. Five specimens were formed for each of the test groups. They were allowed to cure for one hour at room temperature. The specimens were then weighed individually. Each specimen was immersed in a plastic test tube containing 30 mL of PBS and kept at room temperature until the designated sampling times. At each sampling time, the tubes were slowly shaken three times and then 0.5 mL of the PBS solution was removed and stored at -20^{o}C until analysis. No new PBS was added to the tubes. Samples were taken at 1, 3, 6, and 24 hours. This method was adapted from an extensive literature search that covered current methods used in antibiotic bone cement studies. Most current literature measures antibiotic concentration in the samples using fluorescence polarization immunoassay (FPIA). We did not have the capability to use this technique but were able to use particle-enhanced turbidimetric inhibition immunoassay (PETINIA) instead. This method is a precise and accurate alternative to FPIA [87]. The assay has a range of 0.5-12.0 μg/mL. The antibiotic concentration results were multiplied by the total concentration of PBS in the test tube to calculate total antibiotic release per bead at each sample interval. This value was then divided by the elution time for the given interval to get an elution rate in μg/hour for each bead.

Section 3.3 Results

These are the results for the second aim of this research. They explain how MWNTs influence the elution of the antibiotic gentamicin from spherical samples of orthopaedic bone cement. Gentamicin was released from bone cement with and without MWNTs (Figure 3.3 and 3.4). The elution occurred mainly in the first few hours, 66-86% of total antibiotic was eluted during the first three hours. The addition of MWNTs did increase the amount of antibiotic released and the elution rate in all of the cases except for the 0.67wt%. The initial gentamicin elution after 1 hour was increased by 9% with the addition of 0.33 wt% MWNTs and increased by 25% with the addition of 1.34 wt% MWNTs. The concentration with 0.67% MWNTs by weight decreased the amount of initial antibiotic elution by 27%. The total gentamicin elution after 24 hours was

increased by 32% with the addition of 0.33 wt% MWNTs and increased by 23% with the addition of 1.34 wt% MWNTs. The concentration with 0.67% MWNTs by weight decreased the amount of initial antibiotic elution by 35%. The initial elution rate was also increased in the 0.33 wt% and 1.34 wt% samples (by 9 and 25%, respectively). The initial elution rate decreased by 27% in the 0.67 wt% MWNT samples. The final elution rate increased in the 0.33 wt% and 1.34 wt% samples (by 32 and 23%, respectively). The final elution rate decreased by 35% in the 0.67 wt% MWNT samples. Table 3.2 shows the average elution amounts and elution rates for each sample type and time. Figure 3.3 displays the total accumulated gentamicin elution amount per bead per sampling time and Figure 3.4 displays the elution rate per sample type and time. Due to time and budget constraints, this study was only completed one time. Only one batch of 40 mg cement was mixed and used in this run. A replicate study and modified studies are outlined as future work for this project.

Table 3.2 Elution Amounts and Rates for Each Sample

MWNT Percentage (wt%)	Sampling Time (hr)	Total Elution Amount (μg)	Elution Rate (μg/hr)
0	1	61.95	61.95
	3	75.4	25.13
	6	79.8	13.3
	24	95.2	3.97
0.33	1	67.85	67.85
	3	87	29
	6	102.6	17.1
	24	131.6	5.48
0.67	1	47.2	47.2
	3	58	19.33
	6	62.7	10.45
	24	67.2	2.8
1.34	1	79.65	79.65
	3	95.7	31.9
	6	102.6	17.1
	24	120.4	5.02

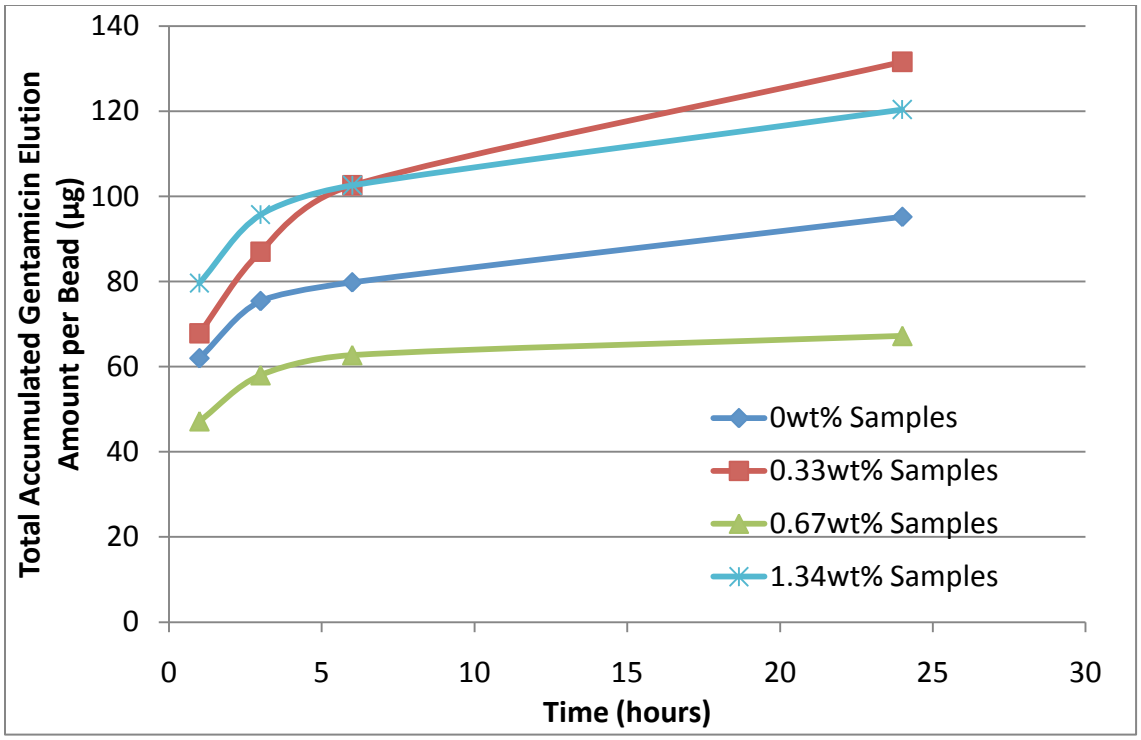


Figure 3.3 Accumulated gentamicin elution per sampling time for each MWNT concentration.

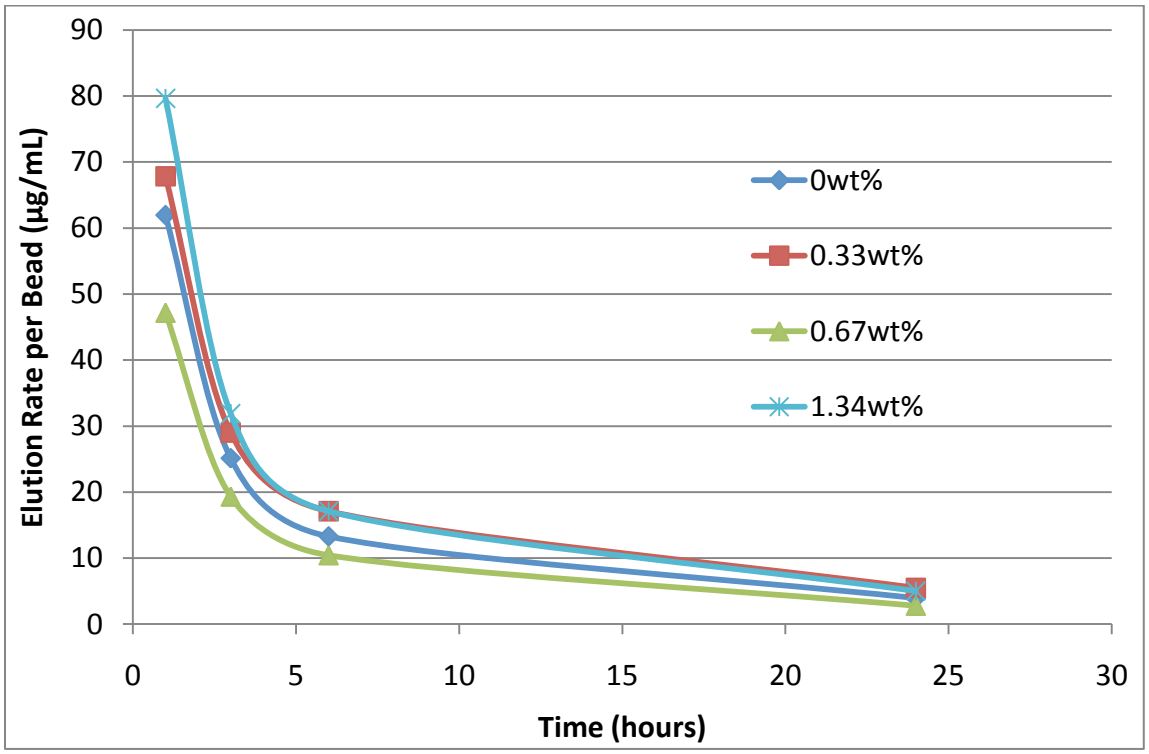


Figure 3.4 Elution rate per sample type.

After completing the pilot study it was determined that the optimal MWNT percentage to use in the expanded elution study was 0.33 wt%. This concentration had the highest amount of gentamicin eluted from the sample after 24 hours. The hypothesis of the complete elution study is that the addition of MWNTs to bone cement will increase the total antibiotic elution amount and decrease the elution rate so that antibiotic is eluted for a longer period of time when compared to pure bone cement without MWNTs. The complete elution study protocol included using a MWNT concentration of 0.33 wt% and two forms of gentamicin, the powdered, pre-mixed form in Palacos+G and liquid gentamicin that would be added to Palacos. In both cases controls were made without nanotubes. MWNTs produced at the Center for Applied Energy [55] were disaggregated and dispersed throughout the dry pre-polymerized Palacos bone cement powder using a dual-blade shear mixer. This resulted in 6 mixtures, a) pure Palacos, b) pure Palacos with 0.33 wt% MWNTs, c) pure Palacos+G, d) pure Palacos+G with 0.33 wt% MWNTs, e) pure Palacos with liquid gentamicin, and f) pure Palacos with liquid gentamicin and 0.33 wt% MWNTs. One commercial formulation of 40 g of sterile bone cement powder was used in each of the six mixtures. The Palacos+G formulation contains approximately 0.5 grams of gentamicin (1.25 wt%) per box. The equivalent amount of liquid gentamicin was used in the Palacos samples. The small batch size was one of the biggest limitations of this study.

Forty-gram batches of hand-mixed powder were passed through the shear mixer three times to ensure complete dispersion of the MWNTs. The liquid monomer used was the commercially prepared ampule that came with the bone cement powder. Components were manually mixed at room temperature for two minutes in the ratio of 40 g of powder to 20 mL of monomer. Once the cement had reached the doughy phase, it was manually pressed into an acrylic mold that produced 6 mm diameter spherical shaped specimens. Six specimens were formed for each of the test groups. They were allowed to cure for one hour at room temperature. The specimens were then weighed individually. Each specimen was immersed in a plastic test tube containing 40 mL of PBS and kept at room temperature until the designated sampling times. At each sampling time, the tubes were

slowly shaken three times and then 0.5 mL of the PBS solution was removed and stored at -20^{o}C until analysis. No new PBS was added to the tubes. Samples were taken at 1, 3, 6, 24, 48, 96 (4 days), 168 (7 days), 336 (14 days), and 576 (24 days) hours.

The antibiotic concentration in each of the samples was measured using particle-enhanced turbidimetric inhibition immunoassay (PETINIA). The antibiotic concentration results were multiplied by the total concentration of PBS in the test tube to calculate total antibiotic release per bead at each sample interval. This value was then divided by the elution time for the given interval to get an elution rate in µg/hour for each bead. The initial antibiotic elution concentration, total antibiotic elution amount, and elution rate from each sample type was compared using a 2-way ANOVA. Table 3.3 displays the average gentamicin elution amounts for the sample beads in each category and the initial and final elution rates. Initial elution rate corresponds to the elution rate after 1 hour and the final elution rate corresponds to the elution rate after 24 days.

Table 3.3 Total Elution Amount and Elution Rates for Each Sample Type (Average ± Standard Deviation)

Antibiotic Type	MWNT Percentage (wt%)	Total Elution Amount (µg)	Initial Elution Rate (µg/hr)	Final Elution Rate (µg/hr)
Powder	0	264.96±20.93	104.0±12.65	0.46±0.04
Liquid		87.0±19.38	62.67±15.73	0.15±0.03
Powder	0.33	60.0±7.78	36.67±8.91	0.10±0.01
Liquid		187.2±64.52	118.67±38.67	0.33±0.11

Initial elution amount was 50% lower in the samples with liquid antibiotic than powder antibiotic. The addition of MWNTs decreased the initial elution amount by 95% in the powder antibiotic samples and increased 14% in the liquid antibiotic samples. The MWNT loaded samples with liquid antibiotic had an increase of 62% elution. The ANOVA results for the initial elution amounts had the forms of antibiotic being significant (p=0.03) and the interaction between presence of MWNTs and antibiotic form being significant (p<<0.001).

Total elution amount was 100% lower in the samples with liquid antibiotic than powder antibiotic. The addition of MWNTs decreased the final elution amount by 126% in the powder antibiotic samples and decreased 34% in the liquid antibiotic samples when compared to the Palacos+G. The MWNT loaded samples with liquid antibiotic had an increase of 100% elution from the Palacos and liquid gentamicin samples. The ANOVA results for the total elution amounts had the presence of MWNTs being significant (p=0.002) and the interaction between presence of MWNTs and antibiotic form being significant (p<<0.001).

Initial elution rate was 50% lower in the samples with liquid antibiotic than powder antibiotic. The addition of MWNTs decreased the initial elution rate by 96% in the powder antibiotic samples and increased 13% in the liquid antibiotic samples. The MWNT loaded samples with liquid antibiotic had an increase of 62% elution. The ANOVA results for the initial elution rate had the forms of antibiotic being significant (p=0.04) and the interaction between presence of MWNTs and antibiotic form being significant (p<<0.001).

Final elution rate was 100% lower in the samples with liquid antibiotic than powder antibiotic. The addition of MWNTs decreased the final elution rate by 126% in the powder antibiotic samples and decreased 33% in the liquid antibiotic samples. The MWNT loaded samples with liquid antibiotic had an increase of 73% elution. The ANOVA results for the initial elution rate had the presence of MWNTs being significant (p=0.002) and the interaction between presence of MWNTs and antibiotic form being significant (p<<0.001).

Figure 3.5 and Figure 3.6 show the total accumulated gentamicin amount per bead and the elution rates for each sample type. Figure 3.5 and Figure 3.6 do not display elution amounts or rates for the control samples without antibiotic. These samples tested using PETINIA came back with elution rates of zero.

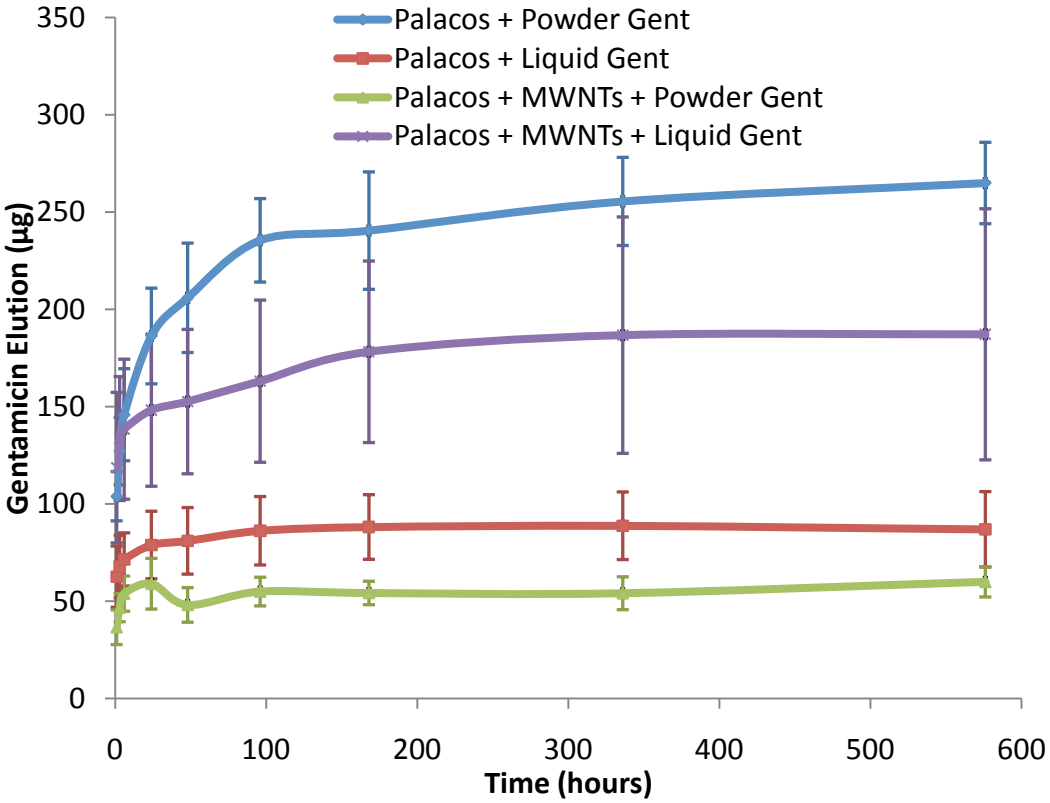


Figure 3.5 Total accumulated gentamicin release per bead.

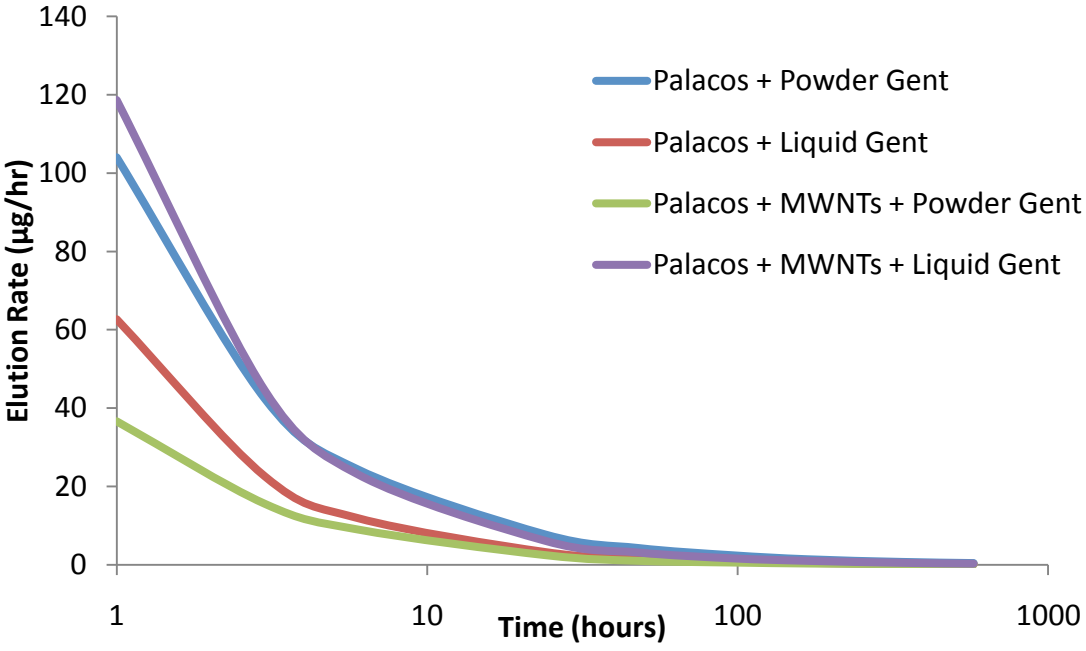


Figure 3.6 Elution rate per bead for each sample type.

Section 3.4 Discussion

The pure Palacos+G cement had the largest amount of total gentamicin elution after 24 days. If the standard deviation of the Palacos cement with liquid gentamicin and 0.5 wt% MWNTs is taken into account, those samples had total elution amounts in the same range as the Palacos+G samples. The elution rates of these two samples were also the most similar. This is an important finding because of the price of liquid gentamicin. It has a much lower cost than the pre-mixed powdered gentamicin. If bone cement could be made with the cheaper liquid antibiotic and a small percentage of MWNTs, the elution of antibiotic could be the same as the Palacos+G used presently.

When comparing the samples with the same form of antibiotic and the presence of MWNTs, the MWNTs significantly decreased the total elution amount, initial elution rate, and final elution rate in the powdered antibiotic case. While in the liquid antibiotic case, the total elution amount, initial elution rate, and final elution rate were increased. The increased elution rates initially indicate that more of the antibiotic is being released initially. To have a more prolonged antibiotic release, the elution rates should be decreased enough to prolong the release of the antibiotic but not too low in that not enough antibiotic is being released. This may be the reason for the decrease in elution in the Palacos+G samples with MWNTs added. The MWNTs may be decreasing the elution rates too much, both initially and over time that not enough antibiotic is getting out. The presence of 0.5wt% MWNTs has already been found to negatively impact polymerization. Complete polymer polymerization does not occur in Palacos samples with 0.5 wt% MWNTs. If the MWNT percentage was decreased to between 0.4-0.5 wt%, more complete polymerization would occur as a result of increased conversion of the monomer to polymer, as well as better elution. Elution rates and amounts must be impacted by the polymerization kinetics of the material.

The study was also completed at room temperature and not a more physiologically relevant temperature. The MWNTs and the antibiotic will not change chemically at 37^{o}C, but the study should be completed in the most accurate situation for better

implantation understanding. At a higher surrounding temperature, the rate of diffusion of the antibiotic out of the bone cement polymer will increase. It has also been shown that polymerization occurs better in the Simplex cement, so an elution study should also be run with Simplex cement, MWNTs, and antibiotic to see if the elution properties could be improved by just using a different brand of cement. This brand of cement does have initially higher heat flow values during polymerization, even in samples without MWNTs, than Palacos cement. This may indicate that the polymerization reaction can negate some of the MWNT influence to more completely polymerize, also allowing for further proper elution of the antibiotic out of the system.

Chapter 4 - Conclusions and Future Work

Section 4.1 Conclusions

Multiwall carbon nanotubes alter the polymerization kinetics of bone cement. MWNTs increased the time required to completely polymerize the methylmethacrylate monomer and decreased the peak exothermic temperature of the bone cement. The likelihood of thermal necrosis is reduced and the mechanical integrity of the cement-bone interface will be greatly improved. The addition of MWNTs to bone cement may also help avoid polymerization induced "hot" spots and subsequent hyperthermia-based destruction of bone adjacent to the cement mantle.

The strength of bone cement is dependent on polymerization reactions. If enough polymerization reactions do not occur, the material does not harden, and bone cement could not be used for orthopaedic surgery. To have more complete polymerization of the bone cement material, the percentage of MWNTs added to the mixture must be optimized. This optimal range should have the increase in mechanical properties due to the MWNTs presence make up for the subsequent decrease in polymerization reactions.

The high exothermic temperatures of bone cement polymerization also limit the therapeutic potency of the antibiotics used in arthroplasty. Presently there are less expensive antibiotics that would positively prevent orthopaedic infections, but these antibiotics denature at temperatures exceeded during polymerization. Lowering the polymerization temperature would increase the choice of antibiotics available to the patient and the surgeon. If the lowered temperature could be achieved without diminishing antibiotic potency, it would also positively affect the mechanical integrity of the interface and would greatly improve implant longevity.

MWNTs were shown to substantially alter the flow of heat liberated during the polymerization of bone cement. The benefits of this include: 1) new options for use of heat-labile antibiotics in TJA, 2) improved antibiotic potency, 3) fewer "hot spots" that

can nucleate fatigue cracks, 4) greater biological viability of the bone-bone cement interface, and 5) mechanical strengthening of the matrix otherwise weakened by antibiotic incorporation. These benefits support the claim that MWNTs in antibiotic laden bone cement can result in enhanced clinical performance of cemented total joint implants.

Section 4.2 Future Work

There are many directions for future research involving bone cement augmented with multiwall carbon nanotubes. One important thing to know would be the potency of the antibiotic that is released from the augmented bone cement. This is typically investigated using a bioassay. The results will show whether or not the MWNTs denature or change the antibiotic that is released and if the antibiotic has killing power against the bacteria. This knowledge is even more important in understanding the potential of the MWNT bone cement that the antibiotic elution alone.

There are a variety of other studies typically performed with bone cement including fatigue and mechanical testing studies. It is already known that MWNTs improve the mechanical and fatigue properties of bone cement [67], but additional testing on other types of cement should be completed. The different commercial bone cements are affected by the MWNTs differently during polymerization. When testing low viscosity cements like Simplex a higher percentage of MWNTs by weight percent is needed to alter polymerization. In higher viscosity cements like Palacos, a lower percentage of MWNTs must be used in cement mixtures. The higher viscosity cement is greater affected by the presence of MWNTs and polymerization does not occur at high weight percents of MWNTs. Mechanical testing should include tension and compression testing, in either 3 or 4-point bend tests.

Ultrasound has been found to be effective in enhancing the efficacy of antibiotics [88]. It has been hypothesized that gentamicin elution could be accelerated with the use of

ultrasound. Another future work study could be to establish the effect of the application of low-intensity low-frequency ultrasound on the release of antibiotics from bone cement [89]. The hope is to be able to access antibiotics that remain isolated within the bone cement and allow them to be released in the surrounding environment. Application of ultrasound during the early postoperative days combined with usage of antibiotic-loaded bone cement or systemic antibiotics may contribute to the prevention of implant infection in future clinical practice [90].

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VITA

Alison Carroll Tickle was born on July 28, 1985 in Livingston, New Jersey. She received a Bachelor of Science in Engineering Science and Mechanics from Virginia Polytechnic Institute and State University in Blacksburg, Virginia in May 2007.