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ABSTRACT

THE *IN VITRO* EVALUATION OF VARIOUS BIODEGRADABLE COMPOSITES USED IN INTERNAL FIXATION DEVICES

by
Hui-chen Hsieh

In vitro degradation kinetics and mechanical properties of various composites, comprising a polycarbonate (DTE polymer) reinforced with CaP glass fiber, synthetic ceramic and non-ceramic hydroxyapatite (HA-500,OsteoGen HA) were investigated.

They were soaked in the SBF solution with a constant pH of 7.4 at 37°C for 5 days. The DTE/CaP composite degraded in an acid manner such that a large amount of NaOH was required but with a small decrease in calcium ion concentration. By contrast, the DTE/OsteoGen HA composite required comparable amounts of NaOH, but with a concomitantly large decrease in calcium ion concentration. This showed that the OsteoGen HA acted as a good nucleating substrate for HA formation on the composites. The DTE/HA-500 composite did not require the addition of as much NaOH, nor did it cause a significant decrease in calcium ion concentration, reflecting its inactive properties.

The moduli of the HA-500 and the OsteoGen HA composites obtained at room temperature increased the modulus of the DTE polymer by more than 33% and 56%, respectively. Plasma surface modification of OsteoGen HA particles provided a moderate improvement in the modulus of the modified OsteoGen HA composites by 90%. However, the moduli of these composites decreased sharply after the materials were soaked in the SBF solution for 5 days or tested in the 37°C water environment. It is believed that the moduli decreases are due to poor fabrication processes, not the actual degradation of the materials. It is concluded that CaP glass fiber and HA-500 composites are unacceptable and the modified OsteoGen HA composite shows the most promise as a biodegradable material for use in internal fixation.

THE *IN VITRO* EVALUATION OF VARIOUS BIODEGRADABLE COMPOSITES USED IN INTERNAL FIXATION DEVICES

by
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This thesis is dedicated to my lovely parents, Chou Chen and Tsung-hsien Hsieh, and my best friend, Matthew T. Ebling.

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CHAPTER 1

INTRODUCTION

1.1 Anatomy and Physiology of Bone

Bone maintains the shape of the body and provides a system of levers upon which muscles act to produce body movements, so the basic functions of bone are to carry a load and protect organs.[1] Therefore the strength and rigidity of bone are its primary qualities. Single or repeated mechanical overload will produce fracture. Before considering the mechanisms of fracture and its repair, it is important to understand the structure of bone.

1.1.1 The Structure of Bone

Bone is a complex material characterized by four levels of structure. At its fundamental level, hydroxyapatite (HA) crystals ($Ca_{10}(PO_4)_6(OH)_2$) are embedded between the ends of adjoining collagen fibrils.[2] This composite of rigid HA and flexible collagen provides the synergistic effect for bone so that it can absorb a lot of energy before failure and bears higher loads, yet retains its stiffness.

At the second level, the collagen/HA fibrils are formed into *lamellae* (sheets) with a preferred direction. The orientations of the fibers define directions of maximum and minimum strength for a primary loading direction.[2]

The third level of structure consists of the arrangement of lamellae. A circular concentric structure produces a tubular *haversian osteon* with a maximum strength along its longitudinal axis.[2]

The fourth level of structure is on the macroscopic level. Bone is divided into two types, a dense and compact *cortical* bone and a spongy trabecular *cancellous* bone. In long bone, for example, cortical bone forms the outer shell of the bone and it has

concentric layers (*Haversian system*), which consists of a central (*Haversian*) canal surrounded by rings of lamellae. Between the lamellae are small spaces called *lacunae* which contain bone cells called *osteocytes*. Minute canals (*canaliculi*) connect the osteocytes with one another and with the Haversian canals. Blood vessels contained in the Haversian canals and canaliculi supply the osteocytes with oxygen and nutrients and remove waste products.[3,4] Spongy bone is located in the intramedullary zone of the long bone and consists of an irregular latticework of thin plates of bone called *trabeculae*. The spaces between the trabeculae of some bones are filled with red marrow. The cells of red marrow are responsible for producing blood cells.[4]

1.1.2 Types of Bone Tissue

Bone cells play an important role in fracture healing. There are three types of bone cells : *osteoblasts*, *osteocytes*, and *osteoclasts*. Osteoblasts secrete some of the organic components and mineral salts involved in bone formation. Osteocytes, or mature bone cells, are the principal cells of bone tissue. Osteoclasts develop from circulating monocytes and their function is resorption or degradation.[4]

Bone tissue is a combination of cells and intercellular substances that form entire organs. Bone tissue can be classified as: (1) *Woven bone* that exists in fracture calluses (2) *Primary bone* (lamellar) forms trabecular bone (3) *Secondary bone* is cortical bone.

1.1.3 Behavior and Mechanical Properties of Bone

Bone exhibits both elastic and viscoelastic behavior. The elastic modulus of human bone varies between 6 and 24 GPa, the maximum strength varying between 50 and 190 MPa, with the greatest occurring in longitudinal compression.[5] The Poisson ratio is approximately 0.33 and 0.42.[5] Beyond the elastic region of its stress-strain curve, bone is viscoelastic. Viscoelastic behavior describes the non-linear load response of bone to an

applied displacement. Because cortical bone is stiffer and denser, it exhibits less viscoelastic behavior than cancellous bone.[5]

The effects of structural parameters and external factors affect the mechanical properties of bone. Structural parameters include the mineral/collagen ratio, bone porosity or density, and trabecular orientation.[2] So, the mechanical behavior varies from bone to bone. External effects such as bone age, the rate of load application, the presence of holes or defects, and the extent of use, will change the mechanical properties of bone.[2]

1.1.4 Fracture Behavior & Mechanisms of Fracture Repair

Fracture of bone is caused by two mechanisms : (1) Impact Load - In impact fracture, bone will be subjected to large deformations such that, when the failure load of the material is exceeded, cracks will generate at weak interfaces and lead to failure as a load bearing structure.[2] (2) Cumulative Fatigue Damage - Fatigue proceeds by the advancement of a crack, usually initiated at concentrators in bone, at a stress below failure magnitude. Cumulative fatigue fracture occurs as this crack continues to grow with repetitive load faster than the ability of bone to heal itself.[2]

As a bone is fractured, three biological stages of fracture healing occurs: inflammatory, reparatation, and remodeling.[6] In the inflammatory stage, a hematoma accumulates within the medullary canal in the endosteum and beneath the periosteum (a fibrous membrane covering the bone). The bone in the fracture region becomes necrotic due to a lack of blood supply, and granulocytes, macrophages, and lymphocytes invade the region to digest the debris.[6] The latter two stimulate repair by releasing angiogensis factors and other cell growth factors. The inflammatory response not only activates the subsequent repair but also protects the healing tissue fron infection.

The reparative stage begins within two or three days after injury, as the hematoma becomes organized. This is the formation of fibrous tissue, fibrocartilage and hyaline cartilage.[6] These materials seal the fragment ends together. New bone is formed

underneath the periosteum around the ends of the fracture and grows toward the initial fracture site. The cartilage tissue is then replaced by bone in a timely fashion.

The final stage, bone remodeling, occurs over a long period of time. Osteoclasts remove the superfluous tissue around the fracture site until the bone returns to its original shape through bone resorption. Osteoblasts lay down new Haversian systems later in bone formation. Bone remodeling is a phenomenon in which bone adjusts its shape to optimize the amount of material for the loads it must bear.

1.2 Fracture Fixation Devices

By using fracture fixation devices, the reparative healing process is accelerated.[1] Meanwhile, macromotion must be prevented due to a non-union of the fractured bone, or else loosening of the prosthesis will occur. These points illustrate the significance of fracture fixation devices, by demonstrating how surgical intervention can align and stabilize the bone fragments with fracture fixation devices.

1.2.1 Requirements for Fracture Fixation Devices

The general requirements for fracture fixation devices include tissue compatibility, sufficient strength, wear resistance, the ability to transfer loads from devices to bone, and the ability to promote bone remodeling.

Tissue incompatibility, or tissue reaction to implants, includes an inflammatory immune, and healing response. The presence of a fluid-filled capsule, macrophages, and bone resorption adjacent to the device exist immediately after surgical intervention. Over time, the healing response to the foreign body reaction will effect bone formation. Good biomaterials should not hinder the natural healing process.

As a rule, fracture fixation devices are required to buttress the loads applied to it and be at least as strong as bone. In addition, fracture fixation devices are not immune to

wear because they are exposed to motion and a very hostile extracellular environment, where the release of particles, especially metals of less than 5 μm, should be controlled.

Stress shielding occurs when the device carries a significant portion of the normally encountered physiologic load on bone. Therefore, stress shielding impedes the normal bone remolding and results in bone atrophy. The stiffer and larger devices result in greater stress shielding. The size of the device is a mechanical design constraint, and is easily controlled. However, stiffness is a material constraint and is not easily controlled. In order for the ideal fracture fixation device to provide adequate stability, it should be initially stiff and then have a graded decrease in stiffness with time as the bone heals and stiffens. It is also important that the strength of the device should not cause shielding and, that the stronger the device, the better the protection against bone re-fracture.

To promote bone remodeling, the geometry of the device should not interfere with the cellular transport processes and responses needed for repair.

1.2.2 Geometry and Materials Used for Fracture Fixation Devices

Orthopedic surgeons use internal or external fixation methods to immobilize fracture sites. The devices include plates, screws, pins, K-wires, and intra-medullary (IM) nails.

Materials used for fracture devices are classified as metals, adhesives, ceramics and biodegradable polymers.[2] Metals and other non-biodegradable materials generally require removal after successful bone healing. Metals, principally 316-L stainless steel, cobalt-chromium alloys, titanium and its alloys (6Al-4V), are used for hardware and governed by national standards for maximum content of alloys and impurities and mechanical properties. Stainless steels can be produced with high elastic moduli and ductilities. The advantage of titanium alloy is its corrosion resistance.

Adhesives, cyanoacrylates and fibrin, used for repair of small nonload bearing fragments, are required to have sufficient bond strength, and be biocompatable, sterilizable, and able to adhere to moist surfaces.[2]

Ceramics such as tricalcium phosphates, calcium phosphate (CaP) fiber and hydroxyapatites (HA), and allograft bone are used as defect fillers and buttressing devices in fracture fixation situations. Ceramics, structurally come in varying degrees of porosity. Porosity is the most important property, since the higher the porosity, the greater the potential for bone ingrowth and the rate of dissolution, but the lesser in mechanical strength.[2]

Biodegradable polymers, on the other hand, can either be absorbed and excreted by the body. The significant advantage in employing these materials for fracture fixation is the elimination of a secondary surgery. Currently, there are numerous research activities that explore the behavior and properties of biodegradable materials.

1.2.3 Problems with Metallic Fixation Devices

Conventional fracture fixation methods have for many years used metallic materials. While offering many advantageous properties, like buttressing, they are not the ideal materials for fracture fixation, due to the much higher stiffness of the steel compared to the underlying bone.[7] Compare the E_{bone} = 6-20 GPa, with the E_{metal} = 100-200 GPa. The rigidity of steel is good during the early healing period, but it can have strong disadvantages later. Stress concentration might occur at the edge of the metallic implant and cause additional fracture. Also, completion of healing is prevented by a highly rigid fixation, since much of the load that is normally carried by the bone is transferred across the fracture site by the implant (stress shielding).[8] Furthermore, bone atrophy may occur with the possibility of refracture after removal of the fracture device.

Other problems associated with metals are corrosion and wear due to the aqueous environment from body fluid and motion between the plate and screws. Corrosion leads to the release of ions that may cause local infection or tumors.[7] It can also produce premature cracking due to stress and fatigue of the implant.

1.3 Biodegradable Materials for Internal Fixation Devices

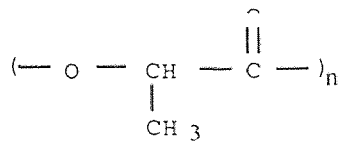
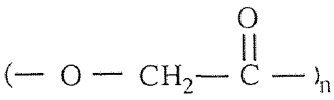
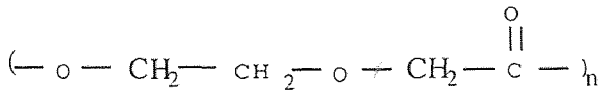
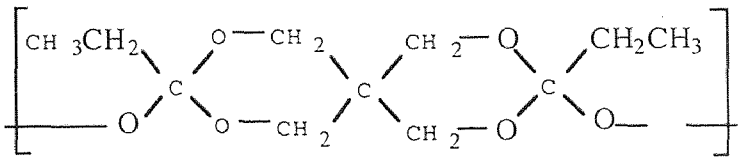
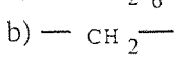
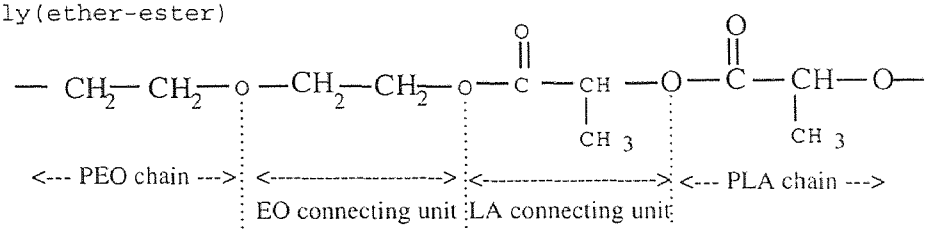
Since metallic devices pose certain disadvantages, biodegradable polymers have been utilized to replace traditional materials for bone fixation. Some of the criteria involved in selecting degradable materials include: (1) a sufficient initial strength and stiffness comparable to bone, (2) a degradation rate is similar to the remodelling rate and slow enough for healing, and (3) a high biocompatibility. There are three major advantages over conventional metallic implants: (a) Gradual load transfer to the healing bone and then minimize bone atrophy, (b) no corrosion, and (c) no need for surgical removal.[8]

1.3.1 Currently Used Biodegradable Polymers

Several investigators have demonstrated that the implants of poly-lactic acid (PLA), poly-glycolic acid (PGA) , and poly-dioxanone (PDS), which were initially developed as absorbent sutures, are completely absorbed within the body.[7,9,10,11] They are the most widely used materials for absorbent orthopedic implants clinically. Some of the first uses of these materials as fracture fixation devices, conducted in 1971, were PLA rods, screw and plates to treat mandibular fractures in dogs.[12,13] Recently, PLA was used in maxillofacial fractures, because the implant in these cases does not need to be very strong.[11] Further research has developed the self-reinforced (SR) PGA rods (Biofix®) and oriented materials such as PDS pins (Orthosorb®) for cancellous bone fixation.[10] There are other biodegradable materials available, including: poly-β-hydroxybutyrate (PHB), poly-β-hydroxyvalerate (PHV), polyorthoester (POE), polycaprolactone (PCL), PLA co-polymerized with PGA, POE and a new tyrosine-derived polycarbonate.[7,14] Table 1 shows the structural formulas of some biodegradable polymers.[8]

Currently polymeric materials for fracture fixation devices satisfy corrosion, wear, strength and remodelling requirements. Yet, they fail the requirements for stiffness since the elastic moduli of biodegradable polymers (1-10 GPa) are low relative to bone (20GPa).

Table 1 Structural formulas of some biodegradable polymers

Name	Structural Formula
Poly(lactic acid)	
Poly(glycolic acid)	
Polydioxanone	
Poly(ortho ester)	 Where R can be a) — $(CH_2)_6$— b) — CH_2—  —CH_2—
Copoly(ether-ester)	

Figures. 1 and 2 provide comparisons of tensile yield strength and tensile moduli of some polymers and composites, stainless steel, and cortical bone (initial mechanical properties).[8] The degradable polymers are not as stiff as cortical bone (tensile modulus : cortical bone > PGA > PLA > POE).[8]

In addition, there are some biocompatability problems with these biodegradable polymers. The results of a study of 516 patients who were treated with SR-PGA rodshad complication rates of 1.2% failure during fixation reoperation, 1.7% bacterial infection from the wound, and 7.9% late noninfectious inflammatory tissue response.[15]

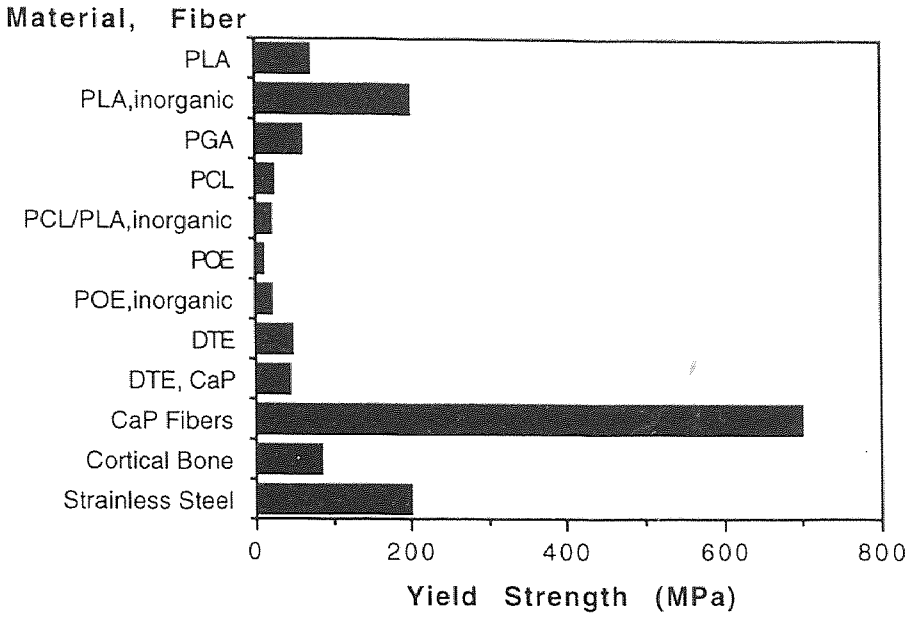


Figure 1 Representative tensile yield strengths

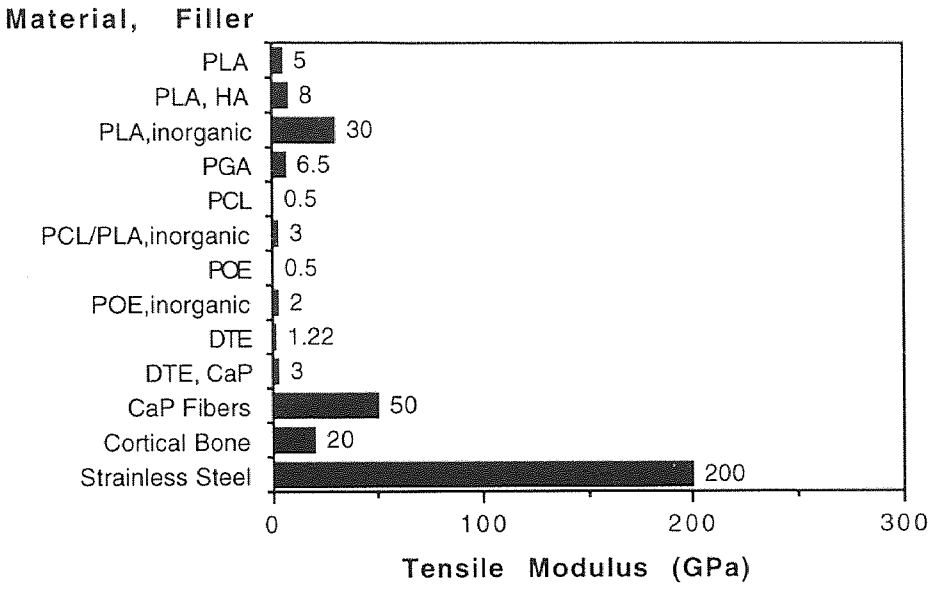


Figure 2 Representative tensile moduli

Some studies of PLLA materials indicate small particles released from the PLLA cause late foreign-body inflammatory reactions and bone resorption.[16] The lactic-acid-rich degradation products lower the value of the local extracellular fluid (ECF) pH, that is surrounded by bone. It is hypothesized that this acidity tends to cause abnormal bone resorption and/or demineralization which could lead to a cytotoxic environment.[17]

1.3.2 Biodegradation Mechanism

The degradation mechanism of these polymers used in internal fixation devices is mainly by hydrolysis and, to a large extent, through non-specific enzymatic action.[11] Water first diffuses into the material and causes swelling due to the disruption of intramolecular bonding within the material. In PLA, PGA, and PDS, water is believed to cleave covalent bonds of the polyester groups within the polymer chains leading to chain breakdown. As a result, molecular weight and mass decreases, with a concomitant loss of mechanical strength. A quantitative relationship between polymer molecular weight and tensile strength has been determined.[18] Table 2 summarizes the mechanical degradation studies.[8]

Briefly, the modes of degradation of polymers are classified as either bulk degradation or surface erosion. As the rate of water permeation into the polymers increases above the rate of polymer hydrolysis, *bulk degradation* occurs. However, if polymer hydrolysis exceeds water permeation, it is called *surface erosion*.[20]

The differences in the final metabolism of these polymers are relatively slight, but the rates of degradation do vary.[11] There are several factors that influence the degradation rate such as molecular weight, crystallinity, thermal history, mechanism of hydrolysis, glass transition temperature, and geometry of the implant. For example, a porous thin sheet depolymerizes much more rapidly than a dense block.[11] Another example is PLA, which degrades quickly by bulk degradation, since PLA is a strong semi-crystalline polymer with a relatively simple chemical structure.[21]

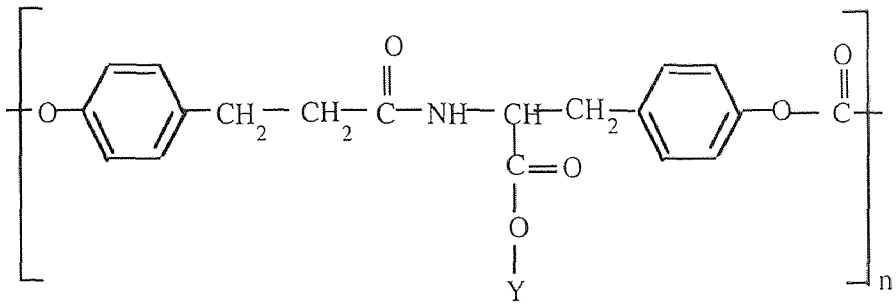
Table 2 Summary of Mechanical Degradation Studies.[8]

Materials	*Fiber*	*Notes*	*Author*	*Strength*	*% **	*Modulus*	*% **	*Time (days)*	*Condition*
PLLA	None	Low mol.wt.	Tunc	Tensile	12	Flex.	70	84	Buffer-7, 37°C
PLLA	None	High mol.wt.	Tunc	Tensile	16	Flex.	76	84	Buffer-7, 37°C
PLLA	CaP	Uncirectional	Lin	Tensile	35	Tensile	45	23	Saline-7.4, 37°C
PLLA	CaP	45° layup	Lin	Flex.	52	Flex.	60	9	Saline-7.4, 37°C
PLA	HA		Verheyen	Flex.		Flex.	50	21	Phosphate Buffer
PLLA	PLLA		Vainionpaa	Flex.	43			336	Water-37°C
PGA	PGA	2.0 mm	Pellinen	Flex	8			42	Water-37°C
PGA	PGA	3.4 mm	Pellinen	Flex.	39			42	Water-37°C
PGA	PGA	4.5 mm	Pellinen	Flex.	54			42	Water-37°C
PGA/PLA	None		Tormala	Tensile	4			28	Water-37°C
PGA/PLA	None		Tormala	Flex	7			28	Water-37°C
PGA/PLA	Carbon		Tormala	Tensile	9			28	Water-37°C
PGA/PLA	Carbon		Tormala	Flex	11			28	Water-37°C
PDO	None		Markela	Shear	57			21	Water-37°C
PDO	None		Markela	Shear	5			42	Water-37°C
90/10 POE	CSM		Andriano	Tensile	60	Tensile	39	84	Water-37°C

* Percentage of property remaining

1.3.3 Tyrosine Polycarbonates

Some studies indicate the primary problem with biodegradable polymers used in fixation devices such as PLA, PGA, and PDS is massive acidic degradation products that cause late inflammatory reactions and increased osteoclastic activity (bone resorption). [15,16,23] Thus, a new type of bioabsorbent materials, tyrosine-derived polycarbonates, was developed by Dr. J. Kohn, et. al.[14] The synthesis of these new materials was based on derivatives of the amino acid L-tyrosine. Tyrosine-derived dipeptides replaced the diphenols employed in the synthesis of commercial polycarbonates.[19] The length of the pendant chain can be modified by these dipeptides (ethyl, butyl, hexyl, and octyl esters of desamino-tyrosyl) to influence important polymer engineering properties(Fig. 3).[14]



Pendant Chain: Y = ethyl -> poly (DTE carbonate)
Y = butyl -> poly (DTB carbonate)
Y = hexyl -> poly (DTH carbonate)
Y = octyl -> poly (DTO carbonate)

Figure 3 Tyrosine-derived polycarbonates[14]

The initial tensile moduli of these polycarbonates were found to be in a range of 1.1-1.6 GPa.[19] In a recent comparative study with other degradable polymers, tyrosine-derived polycarbonates were found to be stiffer than PDS and POE, which have elastic moduli of less than 1 GPa. They were not as stiff as PLA and PGA which have moduli of 5 GPa and 6.5 GPa, respectively.[8,10] The DTE and DTB polycarbonates had a tensile failure at breakage of 67 and 60 MPa and failed without yielding after 4%

elongation, while the DTH and DTO were ductile, yielding at 5% elongation with a yield point of 62 and 51 MPa, respectively.[19]

The hypothesis of the degradation mechanism of this polycarbonate is that the pendant ester bonds will be cleaved first, and followed by a slower hydrolysis of the amide/carbonate bonds (Fig. 4).[19] Since the DTE polymer has a long and complex chemical structure but is completely amorphous, it appears to degrade slowly by surface erosion.

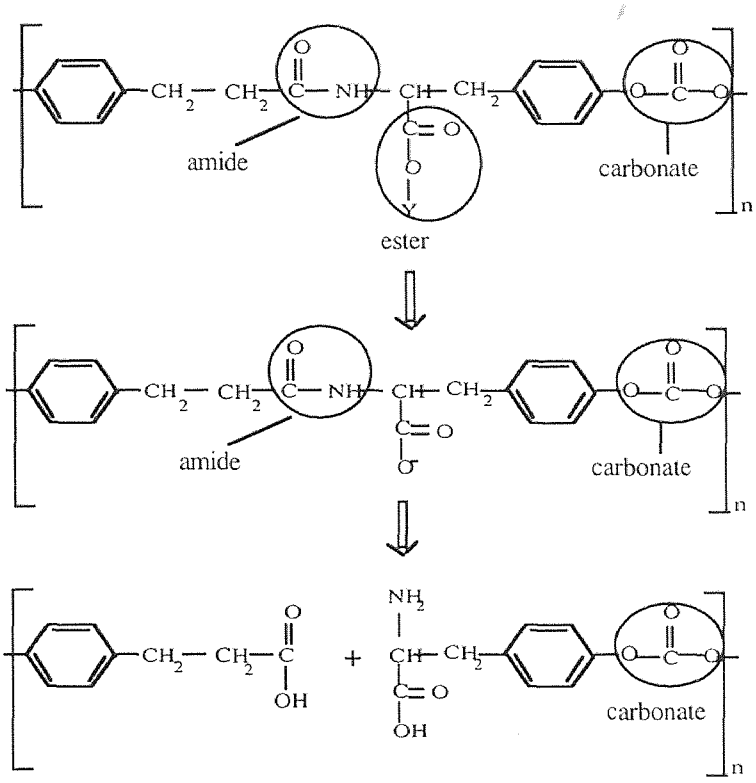


Figure 4 Possible degradation mechanism of polycarbonates.

Tyrosine-derived polycarbonates appear to be promising materials for orthopedic application. This is because they can degrade slowly to almost neutral metabolic products, and have been found to evoke only a mild foreign body response in animal studies. In addition, they elicit a bone growth response superior to that of PDS and

PLLA.[22,23] A recent *in vivo* study, bone ingrowth into DTE polymer was without surrounding inflammatory tissue or osteoclastic activity.[23]

In vitro cytotoxicity studies have been conducted too. The tyrosine-derived polycarbonates did not elicit any noticeable cytotoxic effect on fibroblast cells, except for the more hydrophobic poly-DTO carbonate which caused patchy cell death.[14] Recently, a study indicated that DTE and its degradation products showed no evidence of cytotoxicity and cells adhered and grew normally.[24] Cell proliferation was modulated by the pendant chain length; the least hydrophobic polycarbonate, poly-(desamino-tyrosyl-tyrosine ethylester) (DTE), was a more stimulating substrate for cell growth than the more hydrophobic polymers.

From these early studies, it can be concluded that DTE shows promise for use as a biodegradable implant material and will be the focus of this thesis. Different molecular weights are shown to have different effects with respect to the degradation and mechanical properties. The molecular weight of DTE polymer for this study had to be selected. More recently, the mechanical properties of poly(DTE carbonates) of two molecular weights was investigated after subcutaneous implantation in rats. The results from this study indicated that the failure strength of the 71.8-kDa DTE was significantly higher than the failure strength of the 44.2-kDa DTE at 2 and 6 weeks of post-implantation.[25] Based on this research, the higher molecular weight (>71 kDa) of poly(DTE carbonates) was chosen for this research.

1.4 Composites

Some researchers consider the reinforcement of polymers, such as making composites necessary to obtain mechanical properties sufficient for fracture fixation devices. Composites are the combination of a reinforcement material (a particle or fiber) in a matrix or binder material (a polymer or metal).[26] The advantage of composites is the ability to design the material's stiffness. This flexibility is governed by the amount and

the relative direction of the reinforcement material. This and the mechanical properties of the matrix itself are selected by the designer. Other major advantages of composites over other materials are high specific tensile strength, high specific modulus, improved fatigue life, and corrosion resistance.[26] Composites made of polymer, glass or ceramic do not corrode. Despite all these advantages, composites also have disadvantages, such as a high cost of manufacturing, and complexity of material behavior.[26]

1.4.1 Classification of Composites

Composites can be classified as particulate, laminated, and fibrous.[27] Fibrous composites can be further classified as either continuous or discontinuous. This study will be limited to discontinuous fibrous composites and particulate composites. Discontinuous fibrous-reinforced-composites are those that consist of a polymer matrix with short fibers. In most cases the short fibers are assumed to be randomly oriented in the composite. Depending on its critical length and direction, discontinous fibers can enhance both the stiffness and the strength of the composite. The function of the matrix is to bind the fibers together, transfer loads to the fibers, and protect them against environmental attack and damage. In particular, fibers are very effective in resisting fracture because a reinforcement having a long dimension discourages the growth of incipient cracks normal to the reinforcement that would lead to failure.

A particulate composite contains reinforcing materials which are macroscopically nondimensional. Since the distribution of the additive particle is usually random rather than controlled, particulate composites are therefore usually isotropic.[28] In a dispersion-hardening composite, the particles must resist the stress caused by dislocation pileups against it.[28] Thus, the particles are effective in enhancing the stiffness of the composites. Strength of a dispersion-hardened composite is directly proportional to the hardness of the dispersed particle.[28] Also, coherency strains between the particle phase and matrix affect the strength of the composite. The particles need to act as barriers to dislocation

flow, so that good coupling is necessary, which results in low energy at the particle-matrix interface.[28]

Mechanical properties of composites depend on the fillers' chemical composition, structure, orientation, dimension (aspect ratio), volume percentage, strength of bonds with the polymer, the characteristics of the polymer matrix, and the fabrication (e.g. injection molding, compression molding). For example, DTE/treated CaP fiber composites increased the tensile modulus of the DTE polymer by 74-116% as the fiber volume fraction was increased from 20% to 30%.[29] Also, injection molding was found to result in better mechanical properties due to the strong binding of polymer microspheres in the "Mechanical Evaluation of an HA/PDS Composite Material" study.[30]

1.4.2 Calcium Phosphate Ceramics as Reinforcement

Calcium phosphate (CaP) glass fiber, carbon fiber, and hydroxyapatite (HA) are widely used to be reinforcement fillers for making stiff composites. Carbon and inorganic fiber composites tend to increase the initial strength and modulus, but they often lose strength rapidly during environmental exposure (see Table 2, p.11).[8]

CaP glass fiber can be degraded by water. *In vivo* hydrolysis can occur at the P-O-P bonds producing P-OH end groups which are susceptible to redox reactions.[31] Water can also hydrate the entire chain, called the "wicking effect". The fairly rapid degradation rate could be lessened by using a hydrophobic polymer matrix to make a composite to protect the fiber. Also, the CaP glass fiber composites improve the mechanical properties of polymers.

Hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] is the major mineral component of bone. Synthesized HA is highly biocompatible and osteoconductive.[32,33] It acts as a trellis for the ingrowth of vessels and the subsequent deposition of new bone.[32] Some investigators suggest that there may be a relatively strong direct bonding of HA with host bone, creating better bone ingrowth.[32,33] In a review, Verheyen[33] observed that

HA/PLLA composite has better compressive and tensile strength, higher stiffness and a higher Vickers hardness number than unfilled PLLA.[33]

In order to improve the mechanical properties of the DTE polymer, calcium phosphate (CaP) glass fiber and hydroxyapatite (HA) were chosen as reinforcement agents in this study.

1.4.3 Composites Manufacturing

One of the major effects on the mechanical properties of composites materials are the fabrication parameters. Thus, the manufacturing processes have to be decided carefully at the start of any new design project. Along with choosing the proper processing parameters such as temperature, pressure, and the cooling rate, and manufacturing costs must be minimized.

Injection molding is the most widely used process for high-volume production of thermoplastic resin parts, reinforced or unreinforced composites. Pellets of resin with or without additive particles/fibers are fed into a hopper and then into a heated barrel containing a rotating screw that heats and mixes the material well. The heated resin is then forced at high pressure through sprues and runners into a matched-metal mold. Molding of this type is rapid, and parts can be very precise and complex.[34] The main factors which generally influence the resultant properties of the material are the thermodynamic and rheological factors, and the processing parameters. Good flow characteristics of materials under operating conditions can produce good composites by using injection molding. However, this process requires a large amount of polymer, making it inappropriate to investigate a new material like DTE.

Compression molding is one of the least expensive plastic forming processes. It offers more control over the dimensional accuracy of the product because the entire part surface is in contact with the mold. Machining can be eliminated since holes and slots can be molded into the part. However, compression molding has disadvantages as well.

Flow patterns within the cavity can result in weaknesses at knit (weld) lines, where different streams of compounds flow together in the mold and certain shapes can result in voids or incomplete mold filling.

There are other fabrication techniques which are also employed such as filament winding, braiding, hand lay-up and pultrusion. Due to the limited availability of materials and funding for this study, compression molding was chosen as the manufacturing process used.

1.5 Objectives

The use of biodegradable materials in fixation devices could eliminate a second surgery to retrieve the implant, eliminate the corrosion problems with metallic devices and provide load transfer to the healing bone, minimizing stress protecting atrophy. However, biodegradable fixation devices that have been developed previously have a number of problems, including low stiffness, rapid degradation, and acidic degradation products. The aim of this study was to investigate new composites that show the most promise as biodegradable materials for internal fixation. These were made of desaminotyrosyl-tyrosine polycarbonates with an ethyl pendant chain (DTE polymer) as a matrix, and calcium phosphate glass fibers and two types of hydroxyapatite (HA) as reinforcement fillers. The parameters to evaluate these composites in this study were : (1) the kinetics of *in vitro* degradation - the response of the sample to simulated body fluid (SBF) which contains only inorganic compounds that exist inside the body, and (2) mechanical properties - the tensile modulus and tensile strength were obtained at room temperature and in a 37°C distilled water system. This information can then be extrapolated to possible *in vivo* interactions in a biological setting, which provides an understanding of the chemical reactions and mechanical properties when the composites are implanted.

CHAPTER 2

MATERIALS AND METHODS

2.1 Fabrication Processes

There were a number of materials that were used in this study. These included DTE polymer, calcium phosphate (CaP) glass fiber and synthetic ceramic and synthetic non-ceramic hydroxyapatite (HA). The compression molding was used to make the polymer film and composites. In addition, plasma surface modification of HA particles was done to get better composites.

2.1.1 Desamino-tyrosyl-tyrosine-ethyl-ester Polycarbonate (DTE)

The polymer, polycarbonate (desamino-tyrosyl-tyrosine ethyl ester) (DTE) was synthesized in the Chemistry Department of Rutgers University, Piscataway, NJ. The monomer synthesis of the DTE was subdivided into three steps : 1) formation of the ethyl ester salt, 2) formation and extraction of the free base, and 3) coupling with desaminotyrosine (DAT). The HCl salt of tyrosine ethyl ester was prepared using a thionyl chloride technique. The method of polymerization used was a phosgenation/capping reaction followed by direct isopropyl alcohol precipitation of the DTE ester. The high molecular weight of DTE polymer was end-capped with acetic anhydride and had a white fiber appearance. Table 3 lists the physical properties of DTE.

Table 3 Poly (DTE carbonate) properties of Batch #DBII68

Weight Average Molecular Weight, M_W (da)	98,970
Number Average Molecular Weight, M_n (da)	58,266
Glass Transition Temperature (^oC)	95.80
Decomposition Temperature[a] (^oC)	290
Density[b] (g/cm^3)	~1.2

a - Obtained from reference [19]
b - Obtained from reference [29]

Molecular weights were measured by gel permeation chromatography (GPC) on a system consisting of a Perkin Elmer pump (Model 410) and a water differential refractometer (Model 410) at the Chemistry Department of Rutgers University, Piscataway, NJ. Two PL-gel columns (Polymer Laboratories) with pore sizes, 10^3 and 10^5 Å were operated at a rate of 1 ml/min, using tetrahydrofuran (THF) as the solvent medium. Molecular weights were reported as a weight average relative to polystyrene standards.

2.1.2 Calcium Phosphate Fibers (CaP) & Hydroxyapatite (HA)

In addition to the glass fibers used in this experiment, synthetic ceramic and synthetic non-ceramic hydroxyapatite were used as a possible replacement for the acidic glass fibers. So, three types of fillers were used :

(1) The calcium phosphate glass (CaP) fibers were fabricated at the New York State College of Ceramics, Alfred University, Alfred, NY. The fibers were composed of 54% P_2O_5, 27% Ca, 12% ZnO, 4.5% Fe_2O_3, and 2.5% Na. The fabrication required two processes called "glass preparation" and "fiber spinning". In the glass preparation phase, glasses containing phosphates with iron oxide were prepared by heating equal stoichiometric amounts of reagent grade Fe_2O_3, $CaCO_3$, and $NH_4(H_2PO_4)$ in a silica crucible at 1300°C in a furnace for 1 hour.[34] The glass was allowed to cool gradually. The glass was annealed at a temperature near its glass transition temperature of 300-400°C for about 1 hour in order to increase the stability of the chemical bond conformations. In fiber spinning, the glass was remelted and conditioned at 800°C until all the bubbles were removed. The glass was then in the form of a viscoelastic fluid which was configured into a fiber by extruding it through a platinum bushing. The fibers were wound onto a cylindrical drum spinning at a rate of 1200 r.p.m.[34] The wound fibers were cut into 300 mm long strands. Some of the physical and mechanical properties of the CaP fiber are listed in Table 4.

Table 4 The properties of calcium phosphate fiber.[34]

Nominal Fiber Diameter (μm)	20
Ultimate Tensile Strength (MPa)	700
Modulus of Elasticity (GPa)	50
Melting Temperature (°C)	759
Density (g/cm^3)	2.86

(2) Synthetic ceramic hydroxyapatite (Spherical HA) particles, obtained from Orthomatrix, Inc. (Dublin, California), have been used to enhance the stabilization of orthopedic implants and to promote bone ingrowth[35]. The average size of Spherical HA is 500 μm, and so it is called "HA-500" in this study. In general, HA ceramics that are formed at high temperature, like HA-500, are very inert and stable.

(3) Synthetic non-ceramic hydroxyapatite (Low-Temperature HA) particles were provided from Impladent Ltd. (Holliswood, New York) This kind of HA is named OsteoGen HA. OsteoGen HA particles were produced as nearly perfectly-formed clusters of relatively hexagonal-shaped crystals bound to a single nucleus, which were approximately 300-400 μm in size. A previous study concluded that the OsteoGen HA material is a biocompatible, osteoconductive material that conducts bone ingrowth.[36] This material has the additional property of being slowly resorbable, which is a beneficial characteristic for biodegradable implants.[36] In addition, OsteoGen-HA particles are highly hydrophilic, allowing the material to readily absorb water, such that the potential for migration or material loss is greatly reduced.

2.1.3 Plasma Surface Modification of Particles

To improve the coupling of the particle and polymer matrix interface, the OsteoGen HA particles were sent to Advanced Surface Technology, Inc. (AST), (Billerica, Massachusetts) for surface modification by using methane (CH_4) gas plasma treatment. This coupling technology uses a quartz reactor chamber, a radio frequency generator, a gas valve and vacuum pump, and a control system.

First, the particles were mounted on a glass rack positioned at the center of the plasma chamber. The pressure of this chamber was reduced to below 0.1 mmHg. The reacting methane gas monomer was then introduced into the chamber through the gas valve for 10 minutes. Second, the plasma was initiated by a radio frequency generator operating at 13.56 Hz with a reflecting power of between 50 and 100 W. During the reaction period, the pressure within the chamber was maintained at 50 mmHg. The thickness and surface energy of the substrates and the concentration of gas monomers in the reacting vapor determined the reaction time.[37] After that, the plasma was turned off. To prevent oxidation, the particles were treated with helium, an inert gas, which brought the system back to atmospheric pressure while still in the chamber. Finally, the particles were removed and vacuum packed until further use. OsteoGen HA particles are called "Modified OsteoGen HA" after plasma suface modification.

2.1.4 Fabrication of Polymer and Composite Sheets

The polymer alone and fiber/particle composites were fabricated via the prepreg method. The literature indicates that 30% volume fraction of DTE composites exhibited better mechanical properties[29], so the filler volume fraction that was used in this experiment was 30% by volume. The components that yielded a single 40 x 40 x 0.6 mm polymer and composite sheets were : (1) For the polymer alone, 1.2g of poly(DTE carbonates) and 8.0 mL of methylene chloride were prepared. (2) For a 30% fiber/particle by volume, 0.82 g of CaP fibers and HA particles, and 0.85 g of polymer, and 6.5 mL of methylene chloride were used. (CaP fibers were cut to their optimal packing lengths of 2-3 mm using an electronic cutting device. The HA particles and modified-HA particles were used unchanged from the manufacture.)

After weighing, the fibers/particles were arranged neatly and randomly into a 40-mm^2 aluminum foil cavity. The DTE polymer was dissolved in methylene chloride by using a Vortex Shaker, and then was poured into the cavity containing the

fibers/particles. The prepreg was dried for at least 24 hours in a vacuum dessicator prior to further processing.

Once dry, the prepreg was compression molded using a Carver Laboratory Press (Model C) with top and bottom heated plates and water heat-exchangers. The stainless steel mold used was a frame-type (42.0 x 40.0 x 0.8 mm) (see Fig. 5), with a special hole drilled at its center for accurate temperature monitoring. A thermocouple was inserted into this hole to provide a temperature reading. The prepreg was cut into four equal parts, stacked, and placed in the center of the mold for a more even fibers/particles distribution. Due to the polymer sticking on the mold surface, Teflon sheets (0.01 mm thick) were used between the mold surface and the material to ease the removal and prevent damage of the processed composite. The mold was then closed without pressure and was introduced to the heated plates. The composite was processed at approximately 120°C, and was held for 5 minutes at a constant pressure of 20.3 MPa . The temperature at the time of compression was not allowed to exceed 127°C. The mold was cooled to room temperature at a rate of 30°C/min under pressure. After processing, the Teflon sheets were removed from the polymer/composite sheets. Finally, the polymer/composite sheets were stored in a vacuum.

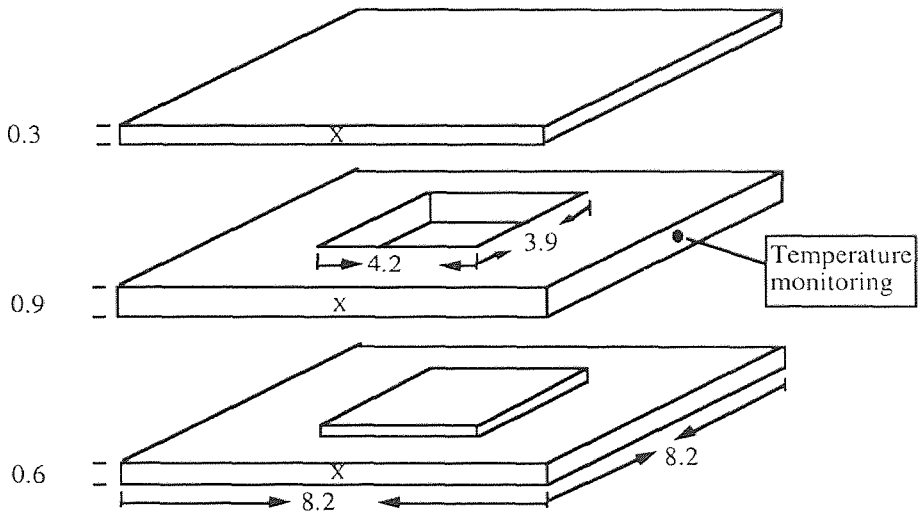


Figure 5 The frame-type mold used for compression molding

2.2 Evaluation of *In Vitro* Degradation

This study explored the *in vitro* response of materials listed in Table 5 to the simulated body fluid (SBF). To mimic an *in vivo* environment, the experiment was conducted in a closed, isothermal environment of 37°C with the SBF solution, which did not include Tris buffer, but instead utilized a highly sensitive titroprocessor system to maintain a constant pH of 7.4. The phosphate group is one of the building blocks of HA, the component of CaP fibers, and in the SBF solution, and phosphate ions have a strong affinity toward calcium ions in SBF or dissolved from the materials.[37] So, the amount of added acid or base to maintain a constant pH and the change of calcium concentration in SBF were measured to evaluate the degradation of materials or the formation of calcium and phosphate precipitates.

Table 5 Combinations of materials used in this study

Components Alone	*Composite*
DTE polymer	DTE/Regular CaP fiber
Regular CaP fibers	DTE/HA-500
HA-500	DTE/OsteoGen HA
OsteoGen HA	DTE/ Modified OsteoGen HA*

* Modified : Plasma Surface Modification of Particles

2.2.1 Preparation Of Simulated Body Fluid (SBF)

A SBF with ion concentrations (Na^+ 138.0 mM, K^+ 5.0 mM, Ca^{2+} 2.5 mM, Mg^{2+} 1.5 mM, Cl^- 148.0 mM, HPO_4^{2-} 1.0 mM, and SO_4^{2-} 0.5 mM) nearly equal to those of human blood plasma was prepared by dissolving NaCl, KCl, K_2HPO_4, $MgCl_2 \cdot 6H_2O$, $CaCl_2 \cdot 2H_2O$, and Na_2SO_4 in distilled water (see Table 6). The preparation of SBF is according to S. Yamada et al.[38], but the $NaHCO_3$ was not included because of the equilibrium problems which presented in maintaining a constant pH environment. In addition, Tris-Base and HCl were excluded and instead 0.1 M NaOH was added to each SBF trial solution to bring the pH to 7.4.

Table 6 The preparation used for 1000 ml solution of SBF

Components	Formula Weight (g/mol)	Approx. Concen. (mM)	Amount (g)
NaCl	58.44	137	8.00628
KCl	74.56	3.0	0.22337
K_2HPO_4	174.18	1.0	0.17418
Na_2SO_4	142.04	0.5	0.07102
$MgCl_2 \cdot 6H_2O$	203.13	1.5	0.30469
$CaCl_2 \cdot 2H_2O$	147.02	2.5	0.36755

The bicarbonate (H_2CO_3/HCO_3^-) and phosphate ($H_2PO_4^-/HPO_4^{2-}$) buffer systems in the human body provide only minor buffering capacity relative to the protein buffer system. The SBF solution in this study contains only inorganic compounds; that is, there are no proteins in SBF. Thus, it should be noted that the SBF is a relatively weak buffering system.

2.2.2 Experiment Set-up : Titration In Isothermal Environment

This experimental system included a titroprocessor system to maintain the SBF and sample solutions at the physiological pH of 7.4, and a pump system to create an isothermal environment of 37°C.

As chemical interactions occur in the SBF solution, the pH can fluctuate about the present value. The pH was maintained at 7.4 by adding acid (0.1 M HCl) or base (0.1 M NaOH) as necessary with a Brinkmann 682 Titroprocessor system (See Figure 5). The amount of added acid or base was recorded using a Brinkmann BR-110 dual channel recorder. Because the titroprocessor has only one channel, one experiment was conducted at a time. Also, the stirrer was needed to mix the solution competely.

A pump system was set up to ensure a constant circulation of deionized water that served to mimic an isothermal environment (37°C) that occurs *in vivo*. This system was comprised of a 100 ml double-jacket glass container filled with SBF solution in the

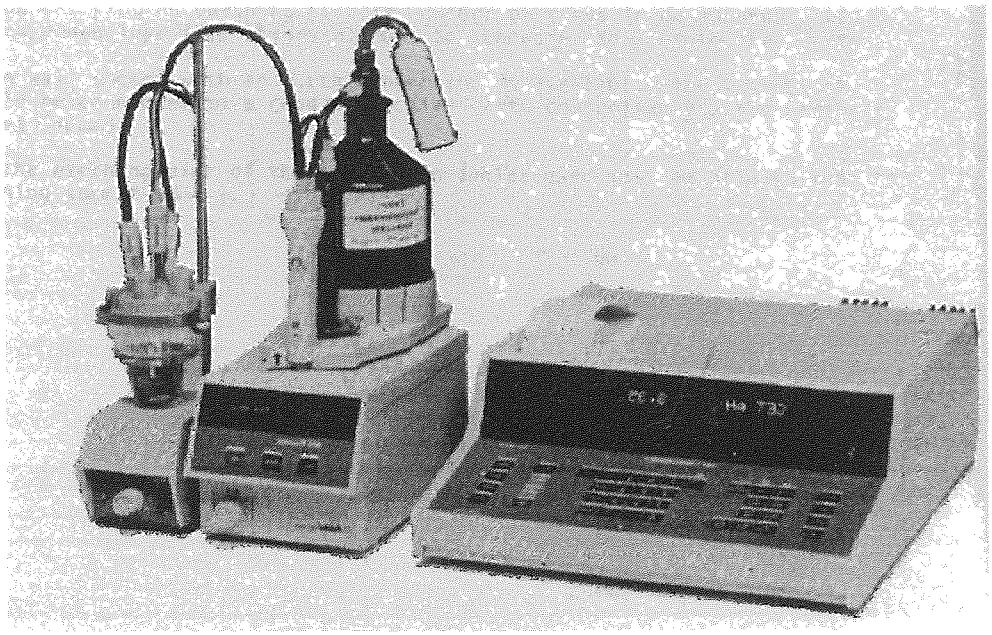
interior, and two side ports that served as inflow and outflow openings for a flexible tygon hose connecting the outer sleeve to a peristaltic pump and water bath held constant at 37°C (Fig. 6).

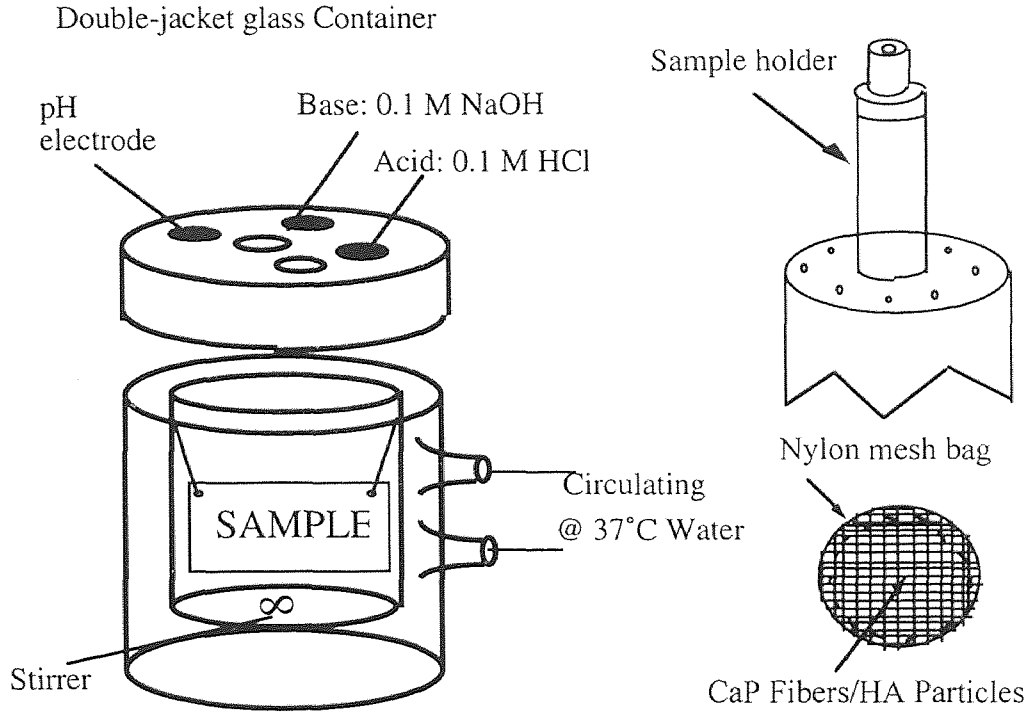
Materials were weighed and cleaned before experiments. The composites and the polymer film were suspended in the SBF solution in the container using polypropylene sutures from Polene. (Fig. 6) A polyethylene stand was constructed to hold a porous nylon mesh jacket that contained the desired amount of fillers (around 0.35 g) to be tested. The mass of materials to SBF solution volume ratio remained constant throughout the experiment at 0.0125 g/mL. The opening of the container was sealed with a five gated lid. Two gates were used for the acid and base inflow tubes, and a third gate was used to hold the pH electrode (Fig. 6). Before the container was closed, vacuum grease was put on the edge circumferentially to prevent evaporation. Next, the parameters of the titroprocessor system was programmed and then the experiment system ran up to 120 hours (5 days). Only early short time intervals were observed. This is because almost all the calcium ions were depleted from the SBF solution after 5 days in the OsteoGen HA composites experiment.

2.2.3 Measurement of Calcium Concentration

The dissolution of the sample was characterized by the concentration of calcium. Aliquots of 1 ml were taken daily from the SBF/Sample solutions. A Perkin Elmer Atomic Absorption Spectrophotometer was used to measure the atomic absorption spectrum of calcium in solution. Five known concentrations of calcium were used for calibration and to develop a linear relationship between absorption and concentration. Then the changes of calcium ion concentration in the experimental solution were calculated.

Figure 6 Experimental Set-up (titration in isothermal environment) : system and cells





2.3 Mechanical Test

Due to the materials used in the internal fixation, the mechanical properties of materials were important factors to evaluate. The tensile test was used to obtain the mechanical information. The original samples (control group) and the samples soaked in the SBF solution for 5 days (experimental group) were tested at room temperature, and at 37°C in distilled water to mimic *in vivo* conditions. During the preparation of specimens for mechanical testing and the short period of testing, the samples in experimental group were maintained wet by the SBF solution. Table 7 lists the summary of test groups. CaP fiber composites was excluded, because the results of the biodegradable testing (*in vitro* and *in vivo*) indicated the material could not be accepted.

Table 7 Summary of the mechanical testing groups for DTE polymer and composites

	25°C	25°C	37°C	37°C
DTE alone	Dry	Wet*	Water	Wet*
DTE/HA-500	Control	Experiment	Control	Experiment
DTE/OsteoGen	(Original)	(5 days)	(Original)	(5 days)
DTE/ModifiedOsteoGen				

Wet* : The sample was wet due to soaking in the SBF solution for 5 days

2.3.1 Preparation of Specimens

The specimens were based on ASTM D638 and ASTM D3039-76 for the polymer film and composites respectively, with slight modification in size due to high material cost and the smaller mold that was available. The polymer and composites sheets were cut into strips about 40 x 5 mm using a heated knife. All samples were measured with digital capilers. The mean width and thickness of each sample were obtained from three measurements along the samples' length.

To protect the tensile test specimens from grip damage and to avoid stress concentrations at the grip ends, cellulose triacetate tabs were glued to both ends of the specimen. The tabs were sanded using #240 grit carborundum paper to create a rough

surface to prevent grip slippage. The tabs were positioned so that the gauge length of the sample was about 20 mm. (Fig.7)

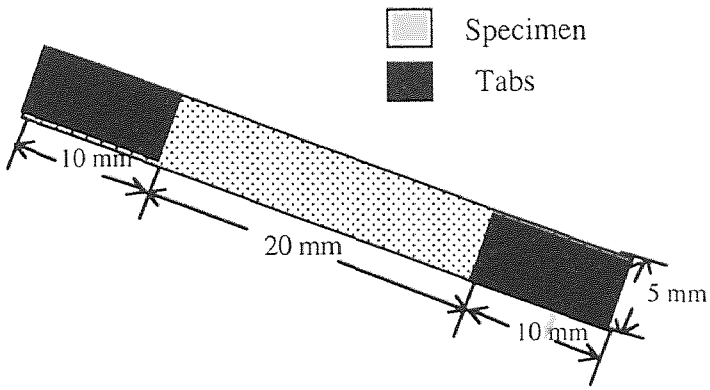


Figure 7 The tensile test specimen

2.3.2 The 37°C Environment Set-up

Different mechanical test parameters such as temperature, strain rate and load cell affected the results. For example, there were significant differences between the room temperature and 37°C environment.[25] To understand the mechanical properties of materials inside the body, the 37°C distilled water environment was constructed.

This system included two parts : (1) specific grips to hold the specimen, and (2) a plastic container filled with 37°C distilled water (Fig. 8). The system was designed by Dr. Frederick Kummer and Dr. John Ricci (Hospital for Joint Diseases, New York, NY). Two holes were drilled in the bottom of the container. One pore was to used for a bolt to hold the grip. Another opening was for a flexible tygon hose that reached to the water bath held constant at 37°C to channel the water out which was cooled down during the mechanical testing.

Before the specimen was tested, three steps needed to be prepared. First, the sample was held in place by the specific grips. Second, the tygon hose was closed by the tubbing clamp. Next, the 37°C distilled water was poured into the container. After

completion of these steps, the mechanical testing was tested in a 37°C distilled water environment to mimic *in vivo* conditions.

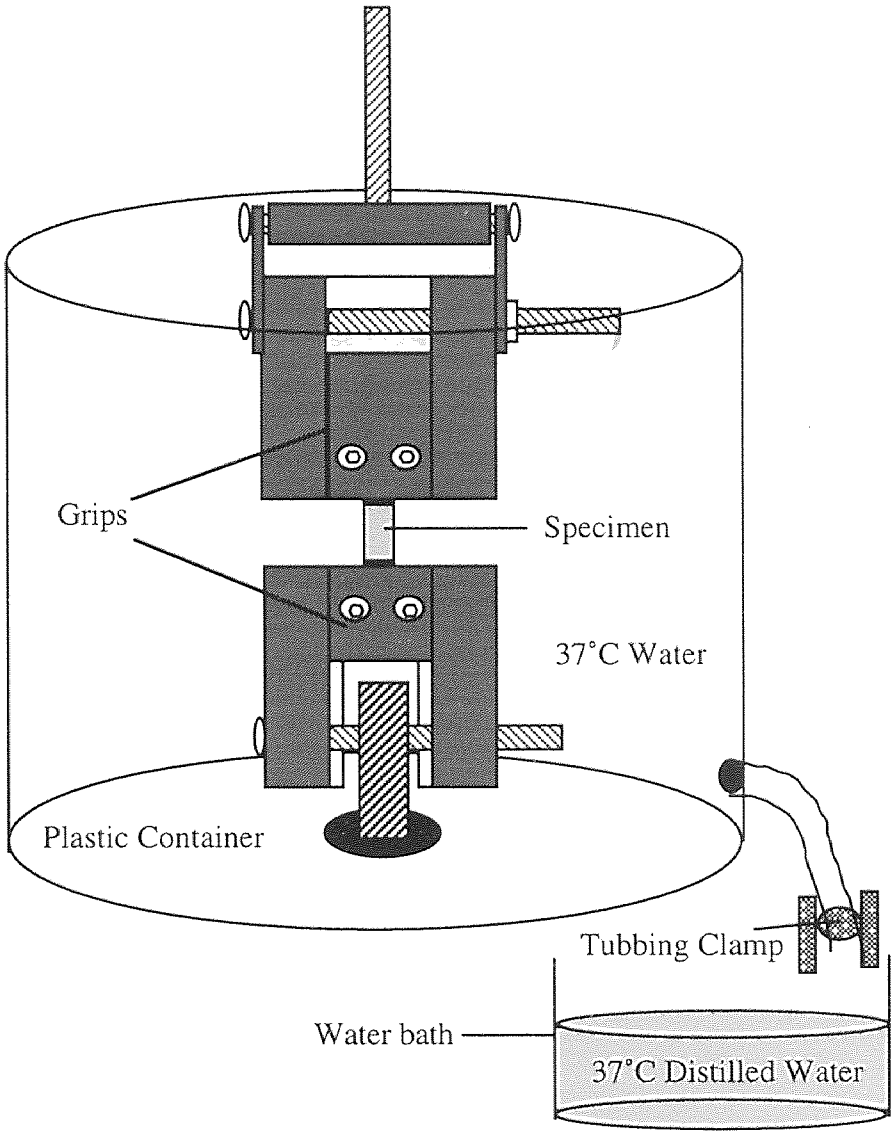


Figure 8 The 37°C distilled water system for tensile testing

2.3.3 Tensile Test

The tensile test was based on ASTM D638 and ASTM D3039-76 and was carried out using the Instron Uniaxial Testing Apparatus (Model 1321). There were two different test environments. (1) At room temperature, the specimens were held with pneumatic

grips. A 10 kN load cell was used to measure the load. The specimens were pulled at a constant cross-head speed of 0.2 mm/min until failure. (2) In 37°C distilled water system, the specimens were held by specific grips (see Fig. 8). A 500 N load cell was used. The cross-head speed was the same as at room temperature.

Load and displacement data were acquired at a sampling rate of 1 Hz using the LabTech data collection software, which showed the real-time force/deformation curve. An Omega X-Y-T chart recorder (Model Omegaline 1321) was performed as a backup recording system. These data, including load (F) and stroke (l-l_O) data, were then transferred to a spreadsheet, containing specimen information such as gauge length (l_O), width (w) and thickness (t) ($A = W*T$), to calculate the stress and strain for each specimen. The samples' moduli were obtained from the 20 points, which displayed the higher slope in the elastic region of stress-strain curve, by linear regression.

σ : stress ε : strain E : modulus

$$\sigma = \frac{F}{A} \qquad \varepsilon = \frac{l - l_O}{l_O} \qquad E = \frac{\sigma}{\varepsilon}$$

2.4 Scanning Electron Microscopy (SEM)

After the samples were soaked in the SBF solution for 5 days and the specimens were fractured due to the tensile testing, two specimens from each kind of material were randomly chosen for electron microscopic analysis. The cross-section area of failure site and surface of samples were gold coated at 40 millitorr using a Denton Vacuum Sputter System (Desk 1 Model). The coated specimens were examined via a Jeol model JSM-T300 scanning electron microscope, and specific sites were photographed using a Polaroid 545 camera coupled to the electon microscope.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Evaluation of *In Vitro* Degradation

First, to maintain the SBF and sample solutions at pH of 7.4, the kinetics of acid or base addition yielded important information about chemical processes occurring in the first five days. The graphs of NaOH addition vs. time for different samples (Fig.9, 11) showed a combination of acidic dissolution products, calcium and phosphate precipitates, or hydroxyapatite (HA) formation on the samples. The figures presented the addition of base (the positive direction on the Y-axis) or acid (the negative direction on the Y-axis), which depended on the pH changes in the SBF and sample solution occuring over time.

Second, the changes in calcium ion (Ca^{2+}) concentration in the experimental solution were measured (Fig. 10, 12). A decrease in calcium ions concentration indicated that a significant amount of HA nucleation and growth, or calcium and phosphate precipitates was occurring. By contrast, an increase in calcium ions concentration was possibly due to the degradation of materials.

In addition, the SEM micrographs, which were photographed after the samples were soaked in the SBF solution for 5 days, helped to understand the chemical reactions of the materials in the SBF solution (Fig. 13 - 16).

3.1.1 DTE Polymer Sheet

As expected, the DTE polymer sheet behaved in a neutral fashion requiring only minute amounts of both acid and base to maintain a constant pH (Fig.9) and there was only a slight change in calcium ion concentration (Fig.10). The result proved that the DTE polymer was a hydrophobic material, and degraded slowly and without acid products.

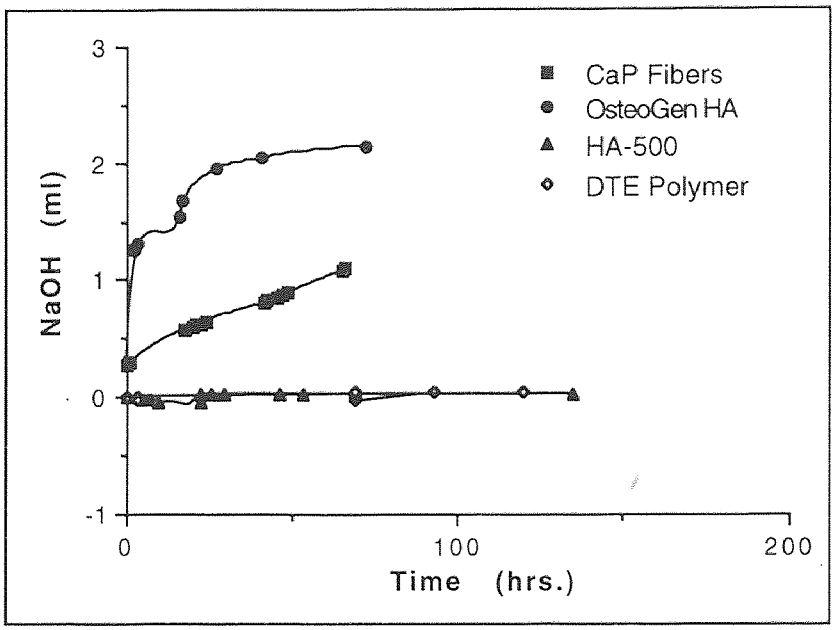


Figure 9 NaOH addition vs. Time of Components

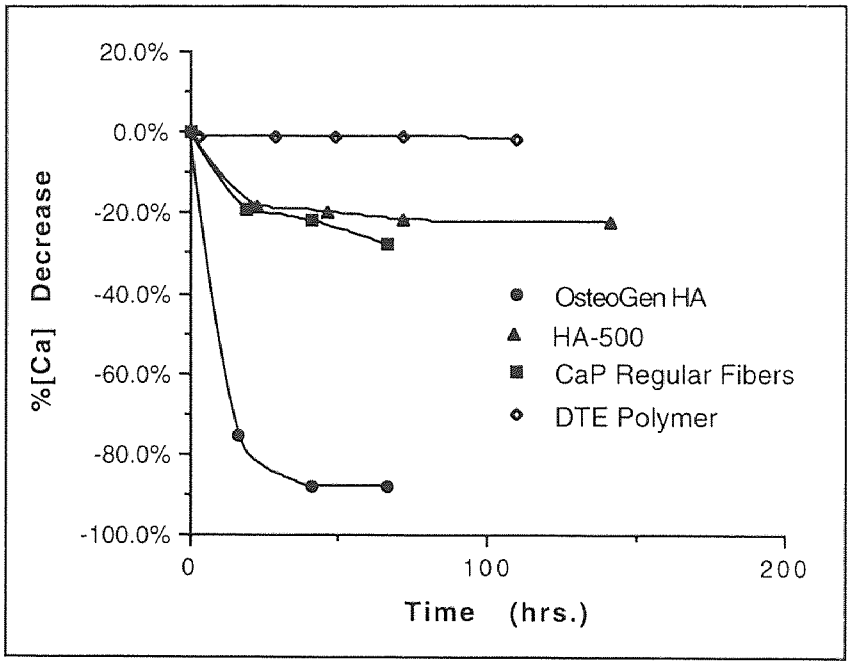


Figure 10 %[Ca] Decrease in SBF solution - Components

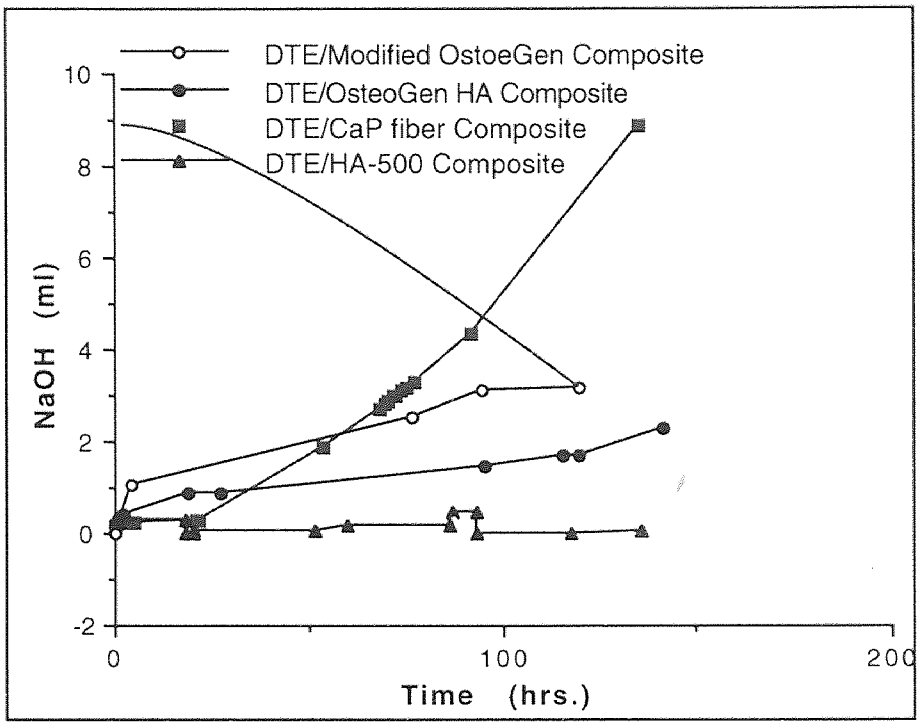


Figure 11 NaOH addition vs. Time of Composites

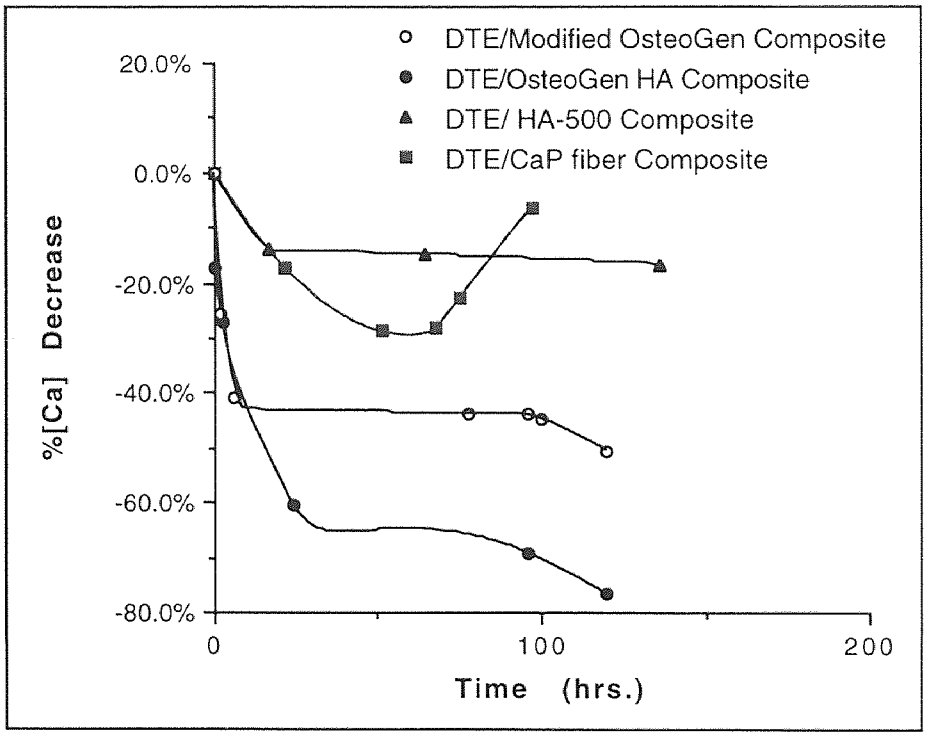


Figure 12 %[Ca] Decrease in SBF solution - Components

3.1.2 CaP Fibers and DTE/CaP Composites

In the short time period investigated, the regular calcium phosphate (CaP) glass fibers required small amounts of NaOH to be added in the titroprocessor system (Fig. 9). This may be due to the decrease in calcium ion concentration (Fig.10) in the SBF solution. Also, SEM analysis indicated that calcium phosphate precipitates formed on the surface of these fibers (Fig.14-a), leading to the necessary base addition.

DTE/CaP fiber composites were degraded to a large extent in SBF solution in an acidic manner, as observed by the large amount of base addition (8.5-9.5 ml) (Fig. 11) , mass loss (6.2%), and the changes in calcium ion concentration (Fig.12). The calcium ion concentration decreases initially which is probably due to the calcium phosphate precipitates formed in the CaP fiber composites (Fig.13) and then fiber breakdown might occur leading to an increase in calcium ion concentration. SEM analysis of the cross-section of the composite showed internal degradation of the fiber (Fig.14). This suggests an unknown reaction between the polymer and the fiber. Two hypotheses arise: (1) Rapid degradation of the composite was attributed to the process of solvent casting with methylene chloride and the subsequent compression molding of the system in aqueous condition might be the reason for the breakdown of the system in the SBF solution. (2) An unknown interaction between the polymer and fiber during solvent casting took place or poor bonding existed which caused an acidic release from the composite.

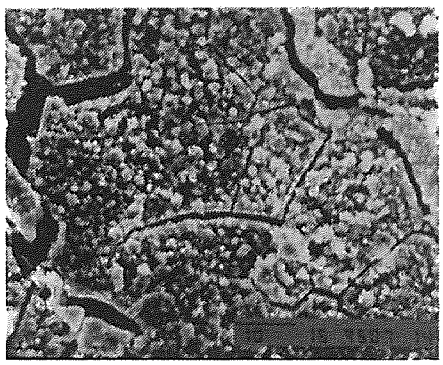


Figure 13 Surface View of DTE/CaP Fiber Composite,1500x.

In addition, calcium phosphate precipitates found in this study (Fig.13) is similar to precipitates found in the *in vivo* study[39]. The *in vivo* study also pointed out that the CaP fiber composites decrease bone ingrowth compared to the DTE polymer.[39]

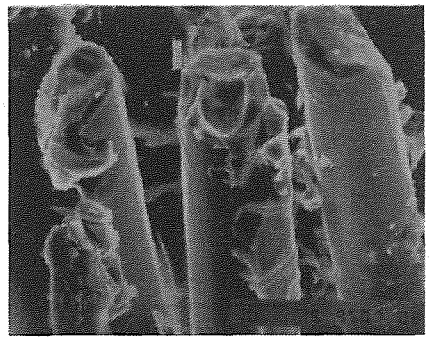


Figure 14 Cross Sectional View of DTE/CaP Fiber Composite,1500x.

3.1.3 HA Particles and DTE / HA Composites

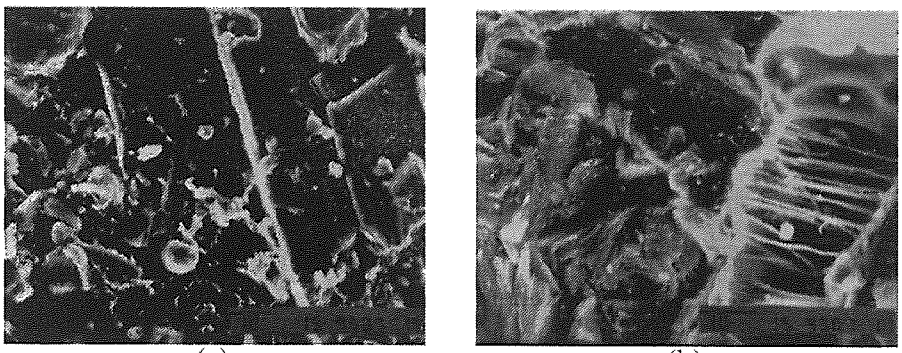
The OsteoGen hydroxyapatite had the highest initial rate of base addition compared to each material tested (Fig.9). It was believed the surface of these particles behaved like a nucleating substrate for the formation of HA. This hypothesis, supported by the curve of the NaOH addition, is mirrored in the rate of calcium ion depleted from the SBF solution (Fig.9, 10). The calcium ion concentration decreased by 76% during the first twenty hours of the experiment. After five days, 90% of all the calcium ions were depleted from the SBF solution. This value is significantly higher than the CaP fibers alone, 24.2%, and the fiber composites, 10% (Fig.10).

The DTE/OsteoGen HA composite required comparable amounts of NaOH (2.5-4.5 ml) (Fig.11) but with a concomitant large decrease in calcium ion concentration (~80%) (Fig.12). This showed the OsteoGen HA acted as a good nucleating substrate for HA formation on the composites. SEM analysis on this composite indicated scattered groups of precipitates unlike the continuous coating seen on the fiber composite (Fig.15). Also, the fact that a 6.2% mass loss was seen in the CaP fiber composites, while only a 0.2% mass loss was found in the OsteoGen HA composites, supported the hypothesis that the

base addition was possibly due to the surface nucleating effect of this composite, and not the actual degradation of the composites. The formation of an apatite surface *in vitro* in the SBF solution has been suggested to be indicative of bone bonding ability.[40] Another study concluded that the *in vivo* response to DTE/OsteoGen composites after six weeks in dogs was good and the material appeared to be osteoconductive [41].

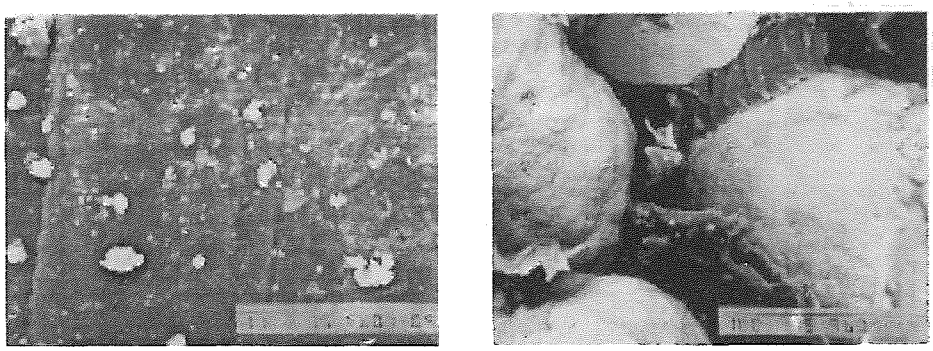
The amounts of NaOH added in the modified OsteoGen HA composites was similar to those in the unmodified OsteoGen HA composites (Fig.11), but the decrease of calcium ion concentration in the modified HA composites is not as much as those in the unmodifed OsteoGen HA composites (Fig.12). In both NaOH addition vs. time and calcium ion concentration decrease vs time curves had a high initial rate, which indicated that the modified OsteoGen HA composites still presented the ability of nucleation for HA formation but were less active than unmodified OsteoGen composites.

By contrast, the high temperature HA-500 ceramic particles and DTE/HA-500 composites did not require the addition of as much NaOH (Fig.11) nor did they cause a significant decrease in calcium ion concentration (Fig.12), reflecting the poor HA nucleating ability. This was supported by the SEM micrograph (Fig.16). As a result, the HA-500 composite was determined to be an inert and neutral material.



(a) (b)

Figure 15 DTE/OsteoGen HA Composite (a) Surface View ,1000x (b) Cross Sectional View, 1500x.



(a) (b)

Figure 16 DTE/HA-500 Composite (a) Surface View , 2000x (b) Cross Sectional View, 500x.

3.2 Mechanical Test

The tensile testing was used to evaluate the mechanical properties of the materials listed in Table 7 before and after soaking in the SBF solution. There were two different systems used for analysis: (a) Samples tested at room temperature ; (b) Samples tested in a 37°C water environment.

The tensile modulus of the material was obtained from the maximum slope in the elastic region of the stress-strain curve. Failure was defined by plateau and decrease of the force component during deformation. The maximum stress was in regards to the tensile strength of materials. The elongation at failure was the corresponding strain at the maximum stress.

Some specimens failed in the area too close to the grips, indicating a failure due to stress concentrations. These data points were removed from the calculations. The tensile test results for each specimen of each materials are included in appendix I (Tables 8-23).

3.2.1 Tensile Test at Room Temperature

Specimens were tested at room temperature and classified as a "control group" (materials not soaked in the SBF solution) and an "experimental group" (materials tested after soaked in the SBF solution for 5 days).

3.2.1.1 Control Group : The tensile modulus of the DTE polymer sheet in this study (1.1±0.089 GPa) was lower than that of the DTE polymer previously reported (1.46 GPa)[29]. The tensile strength fell in 31.735±6.139 MPa which was lower than a previous study (67±23 MPa) [29]. These differences are attributed to the fact that the polymer was made from the different DTE tyrosine monomer batch that was prepared at the Chemistry Department of Rutgers University at a different time. The polymer used in this study does not have the contaminates found in Perez's polymer.[29] Figure 17 illustrates the typical tensile stress-strain plot of the DTE polymer. Owing to a combination of strain softening and localized necking, the DTE polymer showed a load drop immediately after plastic deformation.

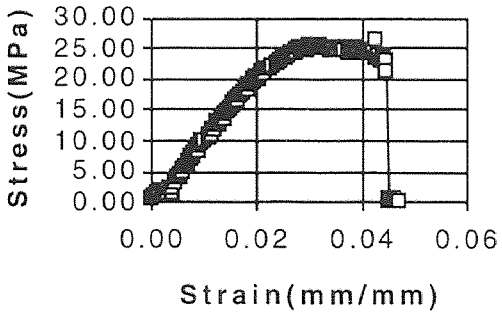


Figure 17 The DTE Polymer

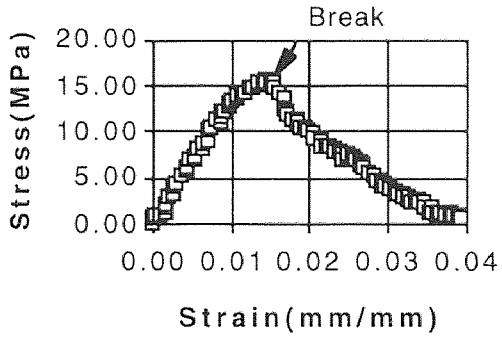


Figure 18 The HA-500 Composite

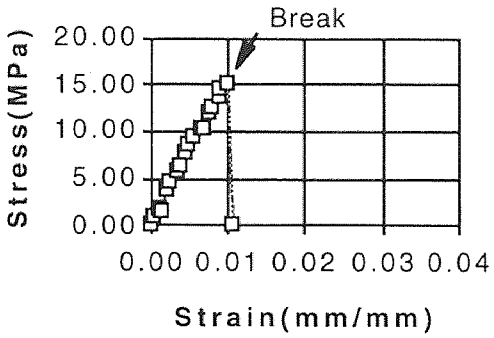


Figure 19 The OsteoGen Composite

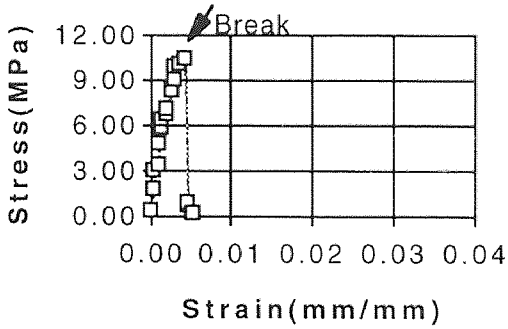


Figure 20 The Modified OsteoGen Composite

As expected, all of the DTE/HA particle composites had higher tensile moduli than the DTE polymer sheet by 33-90% (Fig.21). An unpaired t-test with unequal variances

and a 95% confidence level, showed that there was statistical significance ($p<0.0001$ respectively).

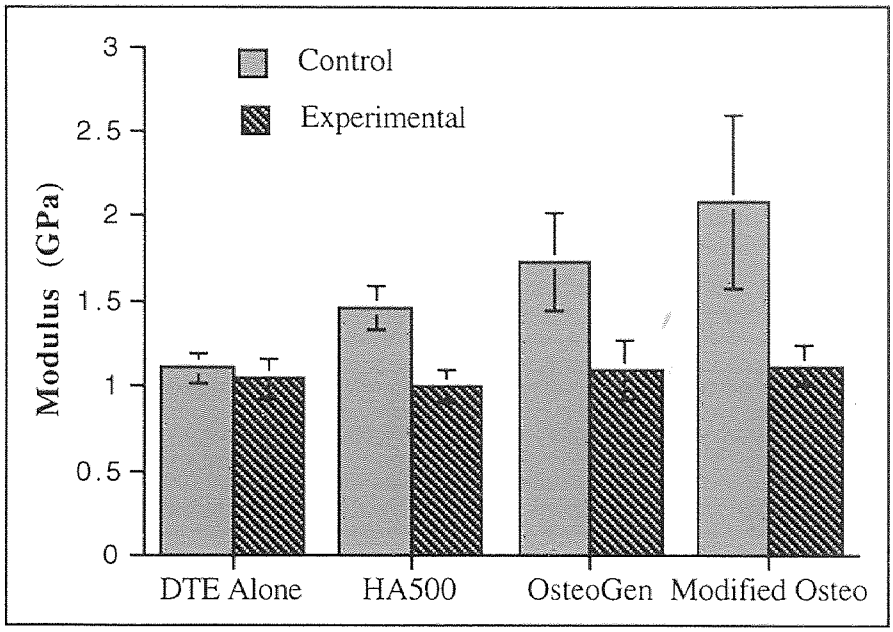


Figure 21 The tensile moduli of composites at room temperature.

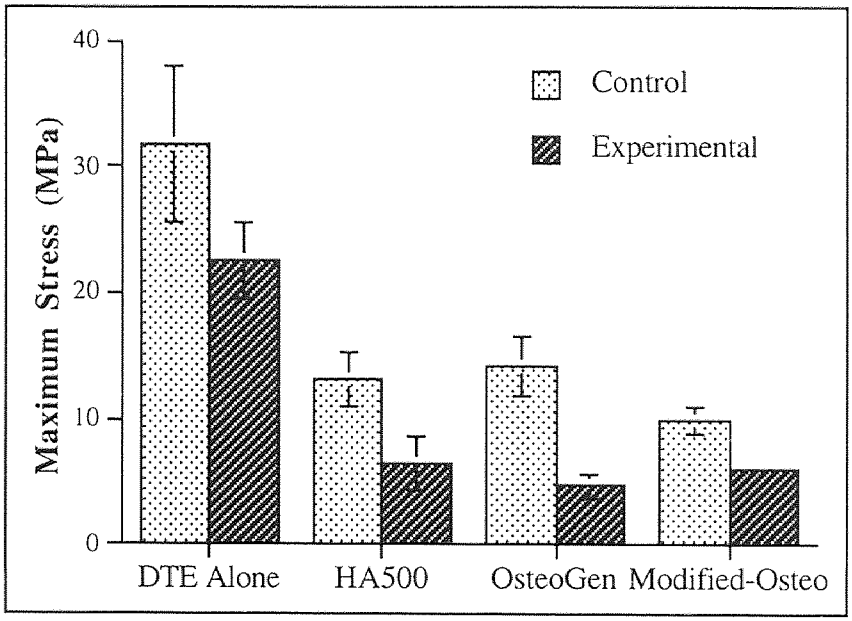


Figure 22 The tensile strength of composites at room temperature.

However, the tensile strength of all the composites decreased by 55-69% (Fig.22). These decreases can be accounted for by the fact that composites have more voids and defects inherent in the manufacturing process than the polymer have. The yield strains of all the composites are shorter than that of the DTE polymer alone. Figures 18, 19, and 20 depict the stress-strain curves for typical HA-500, OsteoGen, and modified OsteoGen composites, respectively. The composites are more brittle than the polymer alone.

OsteoGen HA composites had a tensile modulus that was 17.8% higher than HA-500 composites (Fig.21). There was considerable statistical significance ($p<0.058$). An explanation could be that the structure of OsteoGen HA particles consist of hexagonal-shaped crystals bound to a single nucleus, unlike spherical HA-500 that are larger than the OsteoGen particles. The tensile strengths of these two composites are close. OsteoGen HA particles do not increase the strength of the composite much relative to HA-500. OsteoGen HA composites exhibit brittle fracture (Fig.19), while HA-500 composites exhibit additional elongation after reaching maximum stress (Fig.18).

The modulus of the modified OsteoGen HA composites increased more than that of unmodified OsteoGen HA composites by 20.6% (Fig.21). Modified OsteoGen HA particles seem to improve the coupling of the particle and polymer matrix interface. Load is transferred to the modified HA particles better, thus they carry more load and result into a stiffer material. However, there was no statistical significance between the modulus of modified HA composites and unmodified HA composites ($p<0.15$). This is probably because the sample number of modified OsteoGen HA composites was too few (only three samples were tested). Also, the strength of modified OsteoGen HA composites is not higher than that of unmodified OsteoGen HA composites (Fig.22). The elongation of modified composites is smaller than that of unmodified composites (Fig.19, 20). It may be due to poor fabrication technique: more voids and defects were introduced into the modified OsteoGen HA composites probably by poor packing, poor mixing, or a flow problem during compression molding.

3.2.1.2 Experimental Group (soaked in the SBF solution for 5 days) : There is no difference between the modulus of the DTE polymer in the control group (dry) and the DTE polymer in the experimental group (wet) (Fig.21). Also, the stress-strain curve of the wet DTE polymer is similar to that of the dry DTE polymer (Fig. 17, 23). This proves that there is no degradation and water diffusion does not effect the stiffness of the DTE polymer after being soaked in the SBF solution for 5 days. However, water diffusion probably influence the tensile strength. The tensile strength of the wet DTE polymer is lower than that of the dry DTE (Fig.22).

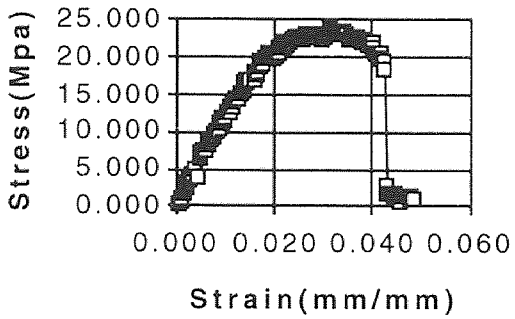


Figure 23 The DTE Polymer

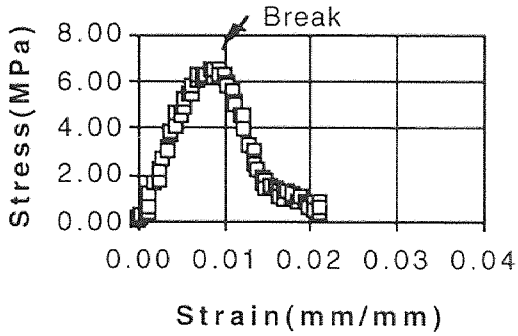


Figure 24 The HA-500 Composite

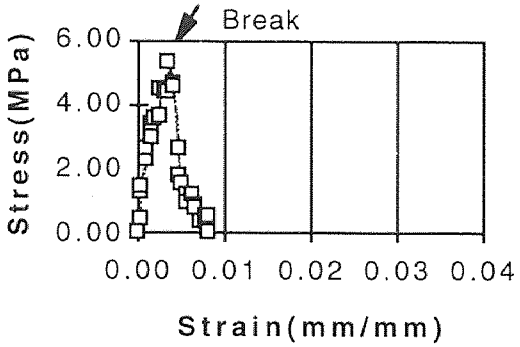


Figure 25 The OsteoGen Composite

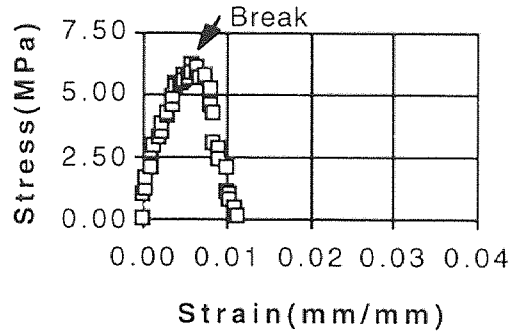


Figure 26 The Modified OsteoGen Composite

The moduli and tensile strengths of all the composites in the experimental group decreased sharply compared to the control group (Fig.21, 22). The moduli of all the composites in experimental group were almost the same as that of the DTE polymer.

Even the modified OsteoGen HA particles only increase the modulus of the composite slightly after the material was soaked in the SBF solution for 5 days. This suggests that in the experimental group load is not ransferred to particles well, thus polymer carries most load. As a result, the stiffness of composites is similar to that the DTE polymer alone.

Figures 24, 25, and 26 depict the stress-strain curves for typical HA-500, OsteoGen, and modified OsteoGen composites in the experimental group, respectively. The strain-stress curve of HA-500 composite in the experimental group (Fig.24) is similar to that in the control group (Fig.18), but the curves of the modified and unmodified OsteoGen composites are different. The load drop immediately after reaching the maximum load was not seen in the experimental group (Fig.25, 26).

3.2.2 Tensile Test in 37°C Environment

There were also two groups tested in the 37°C environment : "Control Group" (materials not soaked in the SBF solution), and "Experimental Group" (materials tested after soaked in the SBF solution for 5 days).

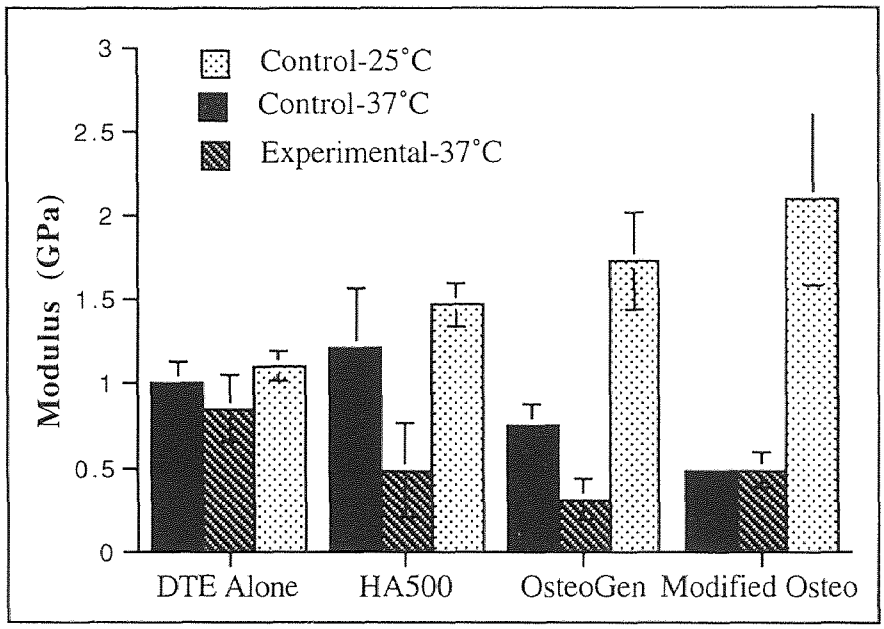


Figure 27 The moduli of composites in a 37°C water environment.

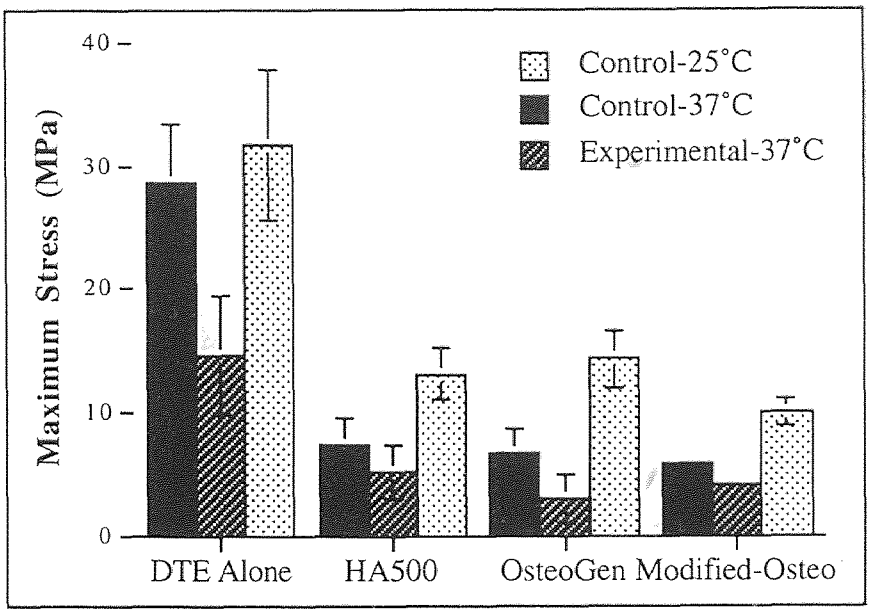


Figure 28 The tensile strengths of composites in a 37°C water environment.

3.2.2.1 Control Group : There were only minor differences between the DTE polymer tested in the 37°C environment and at room temperature. The modulus and tensile strength of the DTE polymer tested in the 37°C environment decreased slightly (~10%) (Fig. 27, 28). While the mode of the fracture was different from the material tested at room temperature, the DTE polymer tested in the 37°C environment failed by developing crazing cracks not a load drop seen in the sample tested at room temperature. The stress-strain curve is represented in figure 29. Also, figure 44 in appendix II shows the failure mechanism through SEM.

The moduli and tensile strengths of all of the composites decreased sharply when they were tested in the 37°C environment compared to the materials tested at room temperature (Fig.27, 28). Figures 30, 31, and 32 illustrate the stress-strain curves for typical HA-500, OsteoGen, and modified OsteoGen composites, respectively. These curves of all the composites are similar to that of the DTE polymer when the materials are tested in the 37°C environment. In addition, the failure mechanism of these composites through SEM are shown in appendix II (Fig. 45, 46, 47).

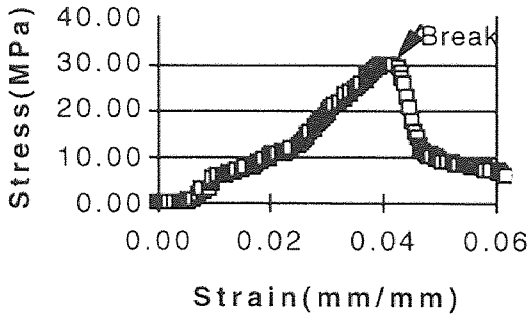


Figure 29 The DTE Polymer

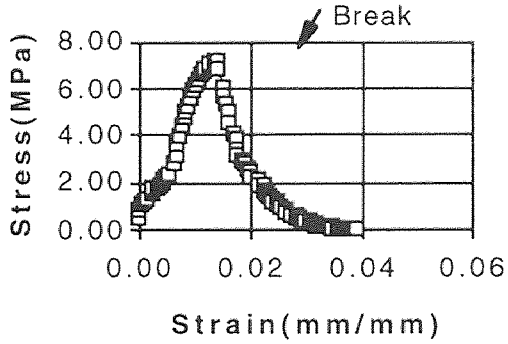


Figure 30 The HA-500 Composite

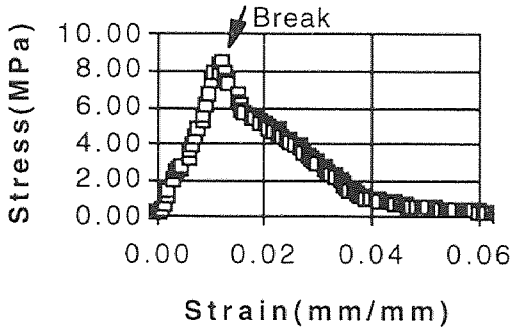


Figure 31 The OsteoGen Composite

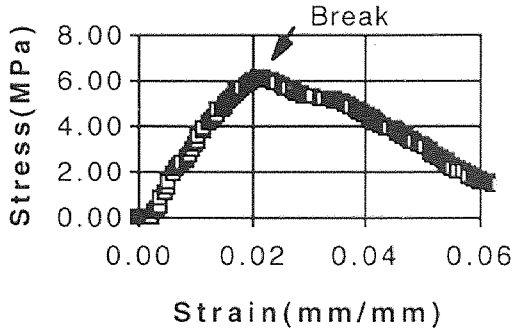


Figure 32 The Modified OsteoGen Composite

The modulus of the HA-500 composites tested in the 37°C environment is slightly lower than that of materials tested at room temperature. There was no statistical significance ($p<0.13$). However, the tensile strength was considerably lower by 47%.

At room temperature, the modulus of modified and unmodified OsteoGen composites increased by 57-90% compared to the DTE polymer. However, the modulus of modified and unmodified OsteoGen composites decreased sharply when they were tested in 37°C water (from 1.7 to 0.75 GPa and from 2.09 to 0.48 GPa, respectively) (Fig.27). They were even less than the moduli of the DTE polymer and HA-500 composites. The tensile strength of these materials decreased sharply too, compared to the materials tested at room temperature (Fig. 28). Due to a lack of modified OsteoGen HA particles, only three samples of the modified OsteoGen HA composites were tested.

3.2.2.2 Experimental Group (soaked in the SBF solution for 5 days) : When the testing environment was created in 37°C water, the moduli of both the DTE/OsteoGen and the DTE/HA-500 composites decreased by approximately 50%, the DTE polymer decreased by 23%, and the modified HA composites just decreased slightly by 0.4 %, which is comparable to the control group in the 37°C environment (Fig.27). The modified OsteoGen HA composites have a 21% higher modulus than the OsteoGen HA composites after materials were tested in the SBF solution for 5 days. There was statistical significance between the modified and unmodified OsteoGen composites tested in the 37°C environment after being soaked in the SBF solution for 5 days ($p<0.034$).

The tensile strength of these materials decreased comparably to the control group in the 37°C environment (Fig.28). The mode of fracture of the DTE polymer and HA-500 was similar to the control group in the 37°C environment. However, in the control group, the OsteoGen and modified OsteGen composites failed without yielding. While in the experimental group, they failed after yielding point, indicating higher ductility. This information was supported by the stress-strain curves of these materials (Fig.33, 34, 35, 36). The SEM micrographs are shown in appendix II (Fig.44-47).

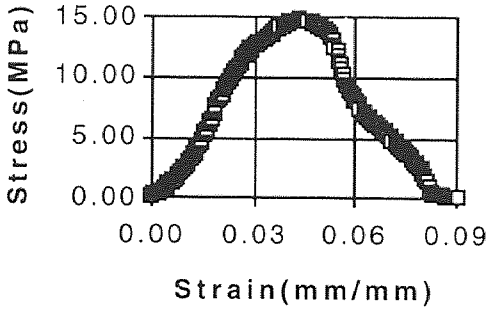


Figure 33 The DTE Polymer

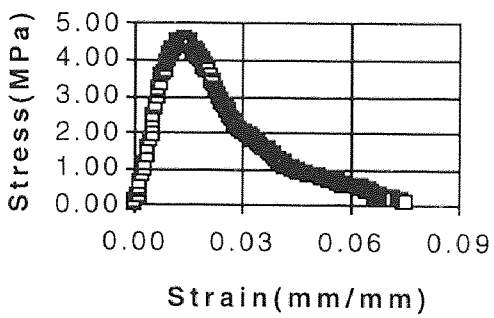
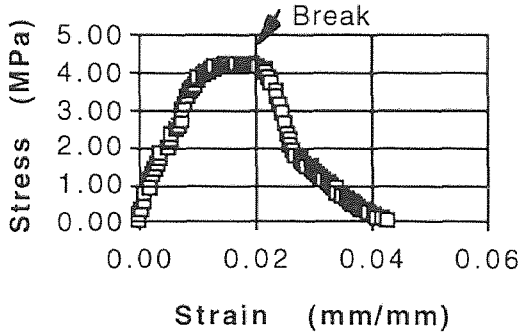


Figure 34 The HA-500 Composite



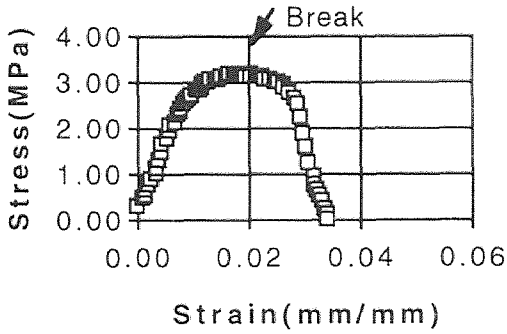


Figure 35 The OsteoGen Composite **Figure 36** The Modified OsteoGen Composite

3.2.3 Discussion

As materials were tested at room temperature, the DTE/HA-500 composites, DTE/unmodified and modified OsteoGen HA composites (moduli : 1.47-2.09 GPa) were stiffer then the DTE polymer alone (modulus : 1.1 GPa), but were less than the DTE/CaP fiber composites reported previously[29] (modulus : 2.24 GPa). The tensile strengths of the composites in this study were much lower than the DTE polymer alone (Fig.38), but the tensile strength of CaP fiber composites reported previously[29] were slightly lower (~10%) than the DTE polymer. This is probably due to the poor coupling of particles with the polymer and voids in the composites caused by poor fabrication.

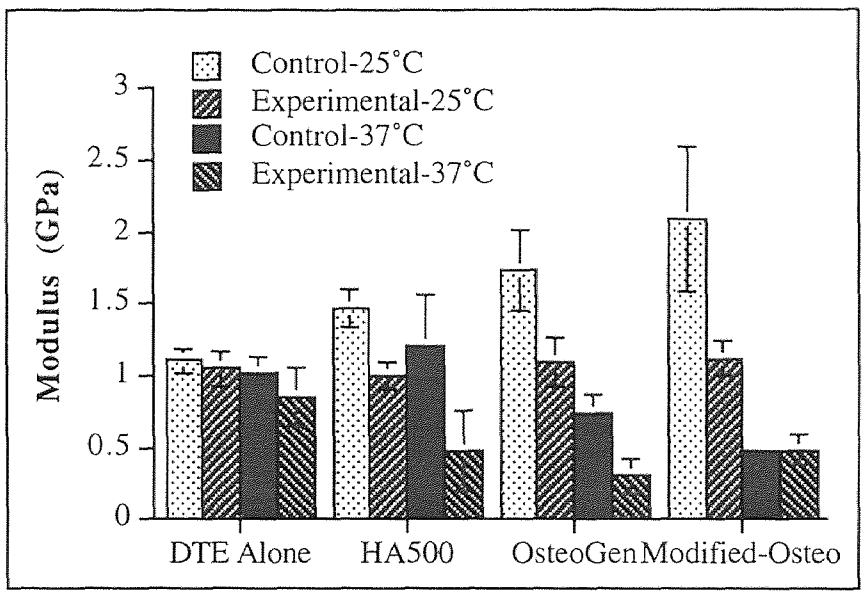


Figure 37 The tensile moduli of composites in all testing conditions.

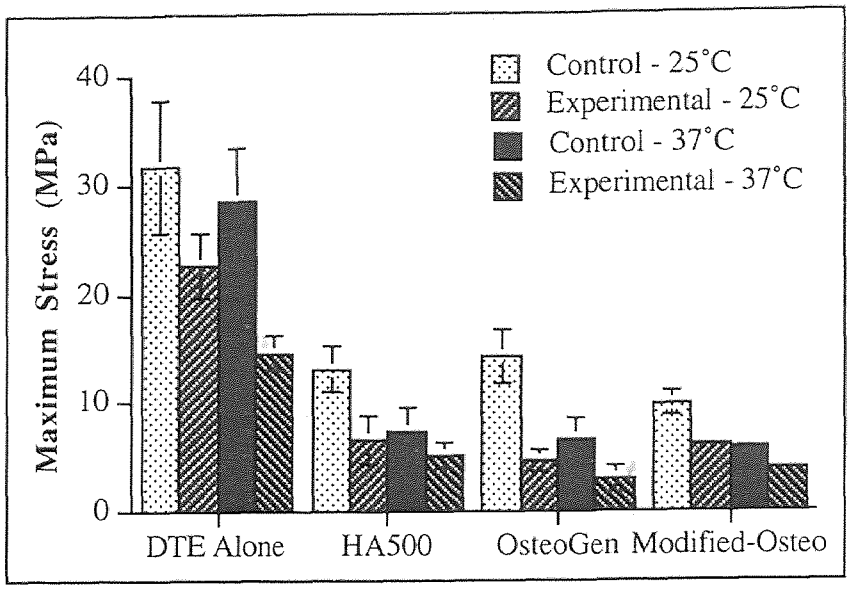
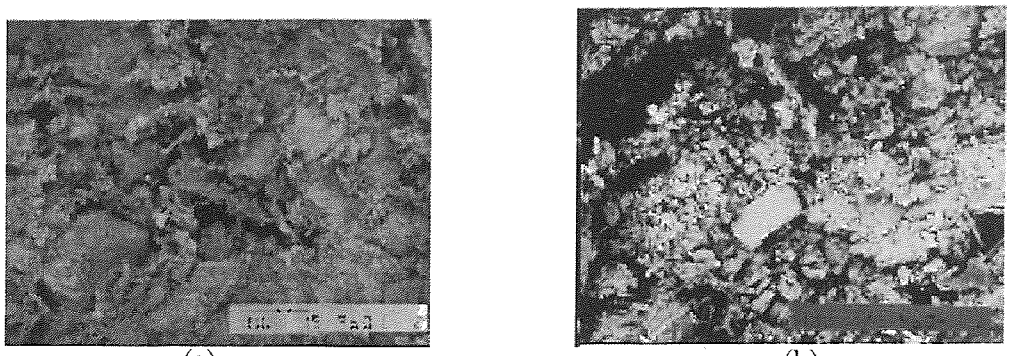


Figure 38 The breaking strengths of composites in all testing conditions.

As the materials were tested after being soaked in the SBF solution and in the 37°C water environment, the moduli and tensile strengths of the composites in this study decreased sharply compared to the composites tested at room temperature in dry conditions (Fig.37, 38). These decreases did not account for the degradation of the composites which was verified by the result of the *in vitro* degradation part in this study. Instead, they were due to water diffusion, higher temperature, poor binding and the voids and defects inherent in the composite manufacturing process such as poor packing, poor mixing, or a flow problem during the compression molding. Also, the stress-strain curves showed that the HA particles did not carry the load, so the curves of the composites were similar to that of the DTE polymer (Fig.29-36). The SEM micrographs indicated that there were holes in the unmodified and modified OsteoGen HA composites (Fig.39). In addition, the mechanical properties of the samples might be altered due to the tightening of the grips that caused the samples to be damaged during stabilization. This is why the mechanical results of the modified and unmodified OsteoGen HA composites tested in the 37°C water environment were not as expected. Even before they

were soaked in the SBF solution, they did not show the higher modulus seen in materials tested at room temperature in dry condition.



(a) (b)

Figure 39 (a) Cross Sectional View of OsteoGen Composite (200x)

(b) Cross Sectional View of Modified OsteoGen composite (200x)

CHAPTER 4

CONCLUSIONS AND RECOMMENDATIONS

4.1 Conclusions

At short time intervals (i.e., 5 days), the *in vitro* response of various biodegradable materials to simulated body fluid (SBF) were investigated. These included individual poly-(desaminotyrosyl-tyrosine ethyl ester)-carbonates (DTE polymer), calcium phosphate (CaP) glass fibers and synthetic ceramic and non-ceramic hydroxyapatite (HA), and their respective composites.

To mimic an *in vivo* environment, the titroprocessor system was programmed to maintain a constant pH of 7.4 in a closed isothermal environment of 37°C. The chemical kinetics, or the nature of materials degradation was evaluated by the amount of added acid or base, and the changes in calcium ion concentration. The mechanical properties of these materials were obtained via tensile testing at room temperature and in a 37°C environment. Also, the scanning electron microscopy (SEM) analysis was observed.

Although the CaP fiber composite had a tensile modulus 54% higher than that of the DTE polymer[29] (i.e., better mechanical properties), the composite degraded in an acid manner and a calcium and phosphate precipitate was formed in the experiment. The result was paralleled to an *in vivo* evaluation which revealed formation of a precipitate and decreased bone ingrowth.[39] As a result, this CaP fiber composite is unacceptable as a biodegradable internal fixation device.

The *in vitro* degradable evaluation of the HA-500 composite (synthetic ceramic) in this study indicated the material was inert and inactive. This is a poor choice for a biodegradable material, because its *in vivo* degradation is too slow to be useful. Also, HA-500 particles just reinforced the DTE polymer slightly (the modulus of the HA-500

composites was 33% higher than the DTE polymer). The mechanical properties of the composites were not strong enough for bone fixation.

The result of *in vitro* degradable evaluation indicated that the OsteoGen HA composite appeared to be osteoconductive, supported by the HA formation on the composites. OsteoGen HA particles behaved like a nucleating substrate for the formation of HA. The modulus of this composite obtained at room temperature increased the modulus of the DTE polymer by more than 56%.

Plasma surface modification of OsteoGen HA particles did not influence the ability of nucleation for HA formation and provided a moderate improvement in the modulus of the OsteoGen HA composites. The modified OsteoGen HA composites had a modulus 90% higher than that of the DTE polymer. The breaking strength was not improved by this coupling method.

However, the moduli of modified and unmodified OsteoGen composites decreased sharply after the materials were soaked in the SBF solution for 5 days or tested in the 37°C water environment. SEM micrographs demonstrated that there were holes in these composites. It is believed that the moduli decreases are due to poor fabrication processes, not the actual degradation of materials.

Although the results of the tensile testing in the 37°C water environment were not as expected, this experiment revealed important information that the mechanical properties of materials tested in the 37°C water environment were different from those at room temperature in the dry condition. As a result, the mechanical properties of biodegradable materials used in the internal fixation should be evaluated in the 37°C water environment, since the material will be in contact with body fluid.

In conclusion, the modified OsteoGen HA composite shows the most promise as a biodegradable material for use in internal fixation. The modified OsteoGen HA composite, however, is not suitable for high load application such as the fixation of femurs. A modified OsteoGen HA composite may be used as bone defect fillers, a pin

and a low load screw for the fixation of bimalleolar fractures of the ankle, intra-articular fractures of the elbow joint and for bony avulsions, or a low load maxillofacial implant. DTE/modified OsteoGen HA composites could provide better bone ingrowth and stiffness long enough to support the healing process for these applications.

4.2 Recommendation for Future Work

Due to the limitation of time, funds, and materials, more experiments could have been done for a greater understanding of biodegradable materials used in bone fixation.

Better fabrication techniques (such as using a mechanical mixer, extrusion or injection molding) should be employed in the future to make better qualities of composites to improve the mechanical properties of these materials, and to reduce the rate of degradation.

With regards to the mechanical tests, ASTM standard sized specimens should be employed in the future tests as more materials and a larger mold become available. Not only the tensile testing but also the flexural testing should be done to obtain the mechanical properties of materials. The strain rate effect on the mechanical test results should be studied in more detail. As the tensile testing was set-up in the 37°C water environment, specific grips were used. These grips could be redesigned to prevent the specimen from being damaged by inadequate grips, which occurred in this study. In addition, different volume fraction , like 20%, 40%, of HA particles in the composites could be tested for mechanical properties.

The human body has an infinite reservoir of calcium ions, but in this study the calcium ions in the SBF solution were finite. When trying to mimic *in vivo* conditions for long term experiments, an infinite reservoir of calcium ions could be provided. For example, exchange the SBF solution everyday.

To evaluate the degradation of the OsteoGen HA composite, the *in vitro* response of the material to the SBF solution and the *in vivo* experiment for a long time period should

be investigated. GPC (gel permeation chromatography) should be performed before and after the material is soaked in the SBF solution or with *in vivo* conditions. Also, mechanical tests in the 37°C water environment should be done to understand the mechanical properties after degradation. In addition, other particles and fibers, such as fibrous forms of HA, and a less acidic CaP continuous fiber, for composite reinforcement and suitable biodegadation, could be investigated. These suggestions may help to obtain better evaluations when analyzing biodegradable composites as internal fixation devices.

APPENDIX I

EXPERIMENTAL RESULTS DATA

Table 8 Tensile test results for the DTE polymer specimens in control group at room temperature.

DTE polymer - Control / 25°C			
Sample No.	Young's Modulus (GPa)	Max. Stress (MPa)	Elongation (%)
1	1.255	25.85	4.2
2	1.127	25.85	3.3
3	1.044	27.50	3.6
4	1.099	40.01	4.5
5	1.130	36.80	4.2
6	0.996	34.40	4.8
Mean	1.109	31.735	4.098
Std. Dev.	0.089	6.139	0.556

Table 9 Tensile test results for the DTE polymer specimens in experimental group at room temperature.

DTE Polymer - 5days/wet/25°C				
Sample No.	Young's Modulus (GPa)	Max. Stress (MPa)	Elongation* (%)	Elongation# (%)
1	1.182	16.60	2.20	
2	1.200	23.59	3.16	
3	0.984	23.23	2.70	5.0
4	1.120	23.84	4.10	3.8
5	0.885	24.60	2.97	7.9
6	0.992	25.29	3.37	6.1
7	0.971	21.02	2.66	4.5
Mean	1.048	22.596	3.022	5.478
Std. Dev.	0.120	2.962	0.608	1.644

Elongation* - at the maximum stress
Elongation# - after the sample had reached total failure

Table 10 Tensile test results for the DTE/HA-500 composite specimens in control group at room temperature.

DTE/HA-500 Composites - Control / 25°C				
Sample No.	Young's Modulus (GPa)	Max. Stress (MPa)	Elongation* (%)	Elongation# (%)
1	1.512	14.63	1.43	4
2	1.413	10.50	1.10	
3	1.681	11.40	1.20	
4	1.361	15.53	1.46	4
5	1.525	15.43	1.36	4
6	1.329	14.30	1.26	4.1
Mean	1.470	13.632	1.302	4.025
Std. Dev.	0.130	2.148	0.140	0.050

Elongation* - at the maximum stress
Elongation# - after the sample had reached total failure

Table 11 Tensile test results for the DTE/HA-500 composite specimens in experimental group at room temperature.

DTE/HA500 Composite - 5days/wet/25° C				
Sample No.	Young's Modulus (GPa)	Max. Stress (MPa)	Elongation* (%)	Elongation# (%)
1	0.927	4.00	1.20	
2	1.117	4.80	0.70	
3	1.090	6.51	0.85	2.0
4	0.899	8.45	1.02	3.2
5	0.969	9.01	0.89	1.88
Mean	1.000	6.555	0.932	2.360
Std. Dev.	0.098	2.193	0.188	0.730

Elongation* - at the maximum stress
Elongation# - after the sample had reached total failure

Table 12 Tensile test results for the DTE/OsteoGen HA composites specimens in control group at room temperature.

DTE/OsteoGen HA Composite - Control / 25°C			
Sample No.	Young's Modulus (GPa)	Max. Stress (MPa)	Elongation (%)
1	1.627	11.67	0.70
2	1.707	15.00	1.00
3	2.375	11.25	0.45
4	1.580	12.76	0.79
5	1.552	14.25	1.20
6	1.846	18.19	1.10
7	1.875	13.01	1.09
8	1.669	15.15	0.91
9	1.355	17.15	1.50
Mean	1.732	14.269	0.97
Std. Dev.	0.287	2.364	0.306

Elongation - at the maximum stress and immediately total failure

Table 13 Tensile test results for the DTE/OsteoGen HA composite specimens in experimental group at room temperature.

DTE/OsteoGen HA Composite - 5days/wet/25°C			
Sample	Young's Modulus (GPa)	Max. Stress (MPa)	Elongation (%)
1	0.827	4.20	0.40
2	1.305	5.82	0.37
3	1.003	4.81	0.46
4	1.241	5.32	0.36
5	1.137	5.12	0.39
6	1.073	3.05	0.40
Mean	1.098	4.720	0.398
Std. Dev.	0.172	0.982	0.034

Elongation - at the maximum stress and immediately total failure

Table 14 Tensile test results for the DTE/Modified OsteoGen HA composites specimens in control group at room temperature.

DTE/Modified OsteoGen HA Composite - control/25°C				
Sample No.	Young's Modulus (GPa)	Max. Stress (MPa)	Elongation* (%)	Elongation# (%)
1	1.627	8.656	0.552	0.704
2	2.006	10.784	0.481	0.748
3	2.637	10.394	0.410	0.621
Mean	2.090	9.9445	0.481	0.691
Std. Dev.	0.510	1.133	0.071	0.065

Elongation* - at the maximum stress
Elongation# - after the sample had reached total failure

Table 15 Tensile test results for the DTE/Modifed OsteoGen HA composite specimens in experimental group at room temperature.

DTE/MOdified OsteoGen HA Composite - 5days/wet/25°C				
Sample No.	Young's Modulus (GPa)	Max. Stress (MPa)	Elongation* (%)	Elongation# (%)
1	1.253	6.333	0.37	0.919
2	1.096	5.742	0.57	1.466
3	1.014	6.127	0.603	1.307
Mean	1.121	6.067	0.514	1.231
Std. Dev.	0.122	0.300	0.126	0.281

Elongation* - at the maximum stress
Elongation# - after the sample had reached total failure

Table 16 Tensile test results for the DTE polymer specimens in control group in 37° water envirnment.

DTE Polymer - Control/37°C				
Sample No.	Young's Modulus (GPa)	Max. Stress (MPa)	Elongation* (%)	Elongation# (%)
1	0.938	22.86	5.00	7.00
2	1.090	24.70	4.00	12.50
3	1.194	30.08	4.11	6.85
4	0.921	35.09	4.09	
5	0.912	30.06	5.61	7.07
Mean	1.011	28.558	4.562	8.356
Std. Dev.	0.125	4.863	0.713	2.764

Elongation* - at the maximum stress
Elongation# - after the sample had reached total failure

Table 17 Tensile test results for the DTE polymer specimens in experimental group in 37° water envirnment.

DTE Polymer - 5 days/wet/37°C				
Sample No.	Young's Modulus (GPa)	Max. Stress (MPa)	Elongation* (%)	Elongation# (%)
1	0.682	9.70	2.76	14.2
2	1.030	13.88	1.70	9.7
3	0.731	12.29	2.50	
4	1.101	15.65	3.05	8.5
5	0.701	16.47	2.78	14.0
6	0.573	14.76	4.31	9.1
7	0.612	16.70	4.26	9.4
Mean	0.776	14.207	3.050	10.810
Std. Dev.	0.206	2.511	0.941	2.579

Elongation* - at the maximum stress
Elongation# - after the sample had reached total failure

Table 18 Tensile test results for the DTE/HA500 composite specimens in control group in 37° water envirnment.

DTE/HA500 Composite - Control/wet/37°C				
Sample No.	Young's Modulus (GPa)	Max. Stress (MPa)	Elongation* (%)	Elongation# (%)
1	1.715	9.88	0.75	3.45
2	0.871	4.02	0.72	2.70
3	1.567	9.38	0.73	3.58
4	1.204	5.39	0.70	2.80
5	1.051	7.73	1.37	4.54
6	0.886	7.14	1.361	3.93
Mean	1.216	7.257	0.939	3.499
Std. Dev.	0.355	2.262	0.331	0.693

Elongation* - at the maximum stress
Elongation# - after the sample had reached total failure

Table 19 Tensile test results for the DTE/HA500 composite specimens in experimental group in 37° water envirnment.

DTE/HA500 Composite - 5 days/wet/37°C				
Sample No.	Young's Modulus (GPa)	Max. Stress (MPa)	Elongation* (%)	Elongation# (%)
1	0.643	6.44	2.01	10.0
2	0.494	4.59	1.38	7.8
3	0.845	6.70	1.48	10.8
4	0.252	4.15	2.07	4.7
5	0.154	4.05	2.78	5.8
Mean	0.478	5.187	1.942	7.803
Std. Dev.	0.282	1.282	0.559	2.641

Elongation* - at the maximum stress
Elongation# - after the sample had reached total failure

Table 20 Tensile test results for the DTE/OsteoGen HA composite specimens in control group in 37° water envirnment.

DTE/OsteoGen HA Composite / Original / 37C				
Sample No.	Young's Modulus (GPa)	Max. Stress (MPa)	Elongation* (%)	Elongation# (%)
1	0.593	7.850	3.50	7.40
2	0.871	8.750	1.31	5.61
3	0.626	4.056	0.70	3.15
4	0.701	4.790	3.18	5.69
5	0.865	8.494	1.21	6.06
6	0.823	5.564	0.79	2.30
Mean	0.747	6.584	1.782	5.035
Std. Dev.	0.1223	2.029	1.233	1.920

Elongation* - at the maximum stress
Elongation# - after the sample had reached total failure

Table 21 Tensile test results for the DTE/OsteoGen HA composite specimens in experimental group in 37° water envirnment.

DTE/OsteoGen HA Composite - 5 days/wet /37°C				
Sample No.	Young's Modulus (GPa)	Max. Stress (MPa)	Elongation* (%)	Elongation# (%)
1	0.417	4.34	1.70	3.02
2	0.393	4.25	1.81	4.27
3	0.294	3.59	1.51	3.72
4	0.410	2.68	1.38	2.26
5	0.170	1.47	2.00	3.18
6	0.154	1.36	1.67	2.93
Mean	0.306	2.947	1.676	3.230
Std. Dev.	0.120	1.328	0.217	0.693

Elongation* - at the maximum stress
Elongation# - after the sample had reached total failure

Table 22 Tensile test results for the DTE/Modified OsteoGen HA composite specimens in control group in 37° water envirnment.

DTE/Modified OsteoGen HA Composite - Control/wet/37°C				
Sample No.	Young's Modulus (GPa)	Max. Stress (MPa)	Elongation* (%)	Elongation# (%)
1	0.501	6.095	2.21	9.09
2	0.482	5.753	2.57	10.62
3	0.464	5.745	3.00	8.70
Mean	0.482	5.864	2.593	9.470
Std. Dev.	0.018	0.200	0.396	1.015

Elongation* - at the maximum stress
Elongation# - after the sample had reached total failure

Table 23 Tensile test results for the DTE/Modified OsteoGen HA composite specimens in experimental group in 37° water envirnment.

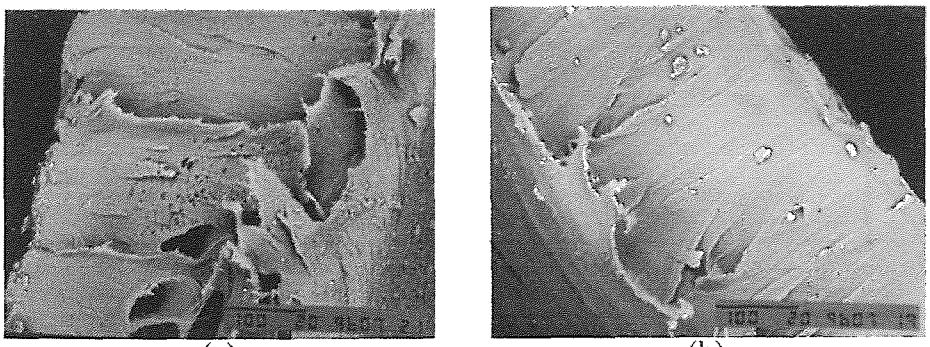
DTE/Modified OsteoGen HA Composite - 5 days/wet /37°C				
Sample No.	Young's Modulus (GPa)	Max. Stress (MPa)	Elongation* (%)	Elongation# (%)
1	0.625	3.730	0.80	2.70
2	0.513	4.699	1.31	2.55
3	0.467	3.962	2.10	3.80
4	0.322	3.194	1.69	3.42
5	0.477	4.622	3.10	6.84
Mean	0.481	4.041	1.797	3.862
Std. Dev.	0.109	0.631	0.872	1.742

Elongation* - at the maximum stress
Elongation# - after the sample had reached total failure

APPENDIX II SEM MICROGRAPHYS

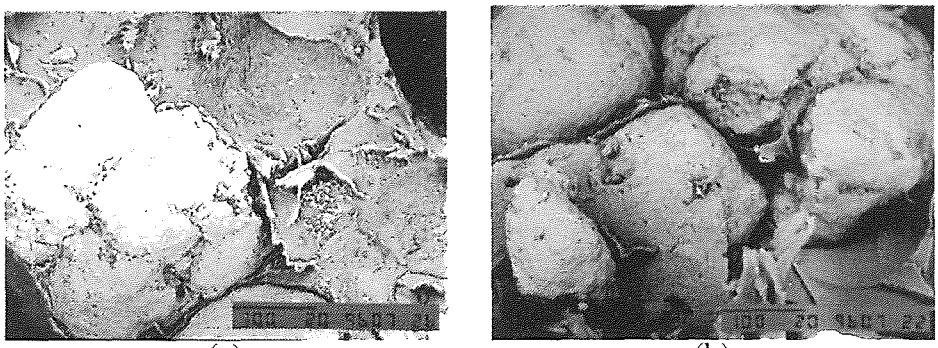
Cross sectional view of materails

after tensile testing at room temperature (Figure 40-43)



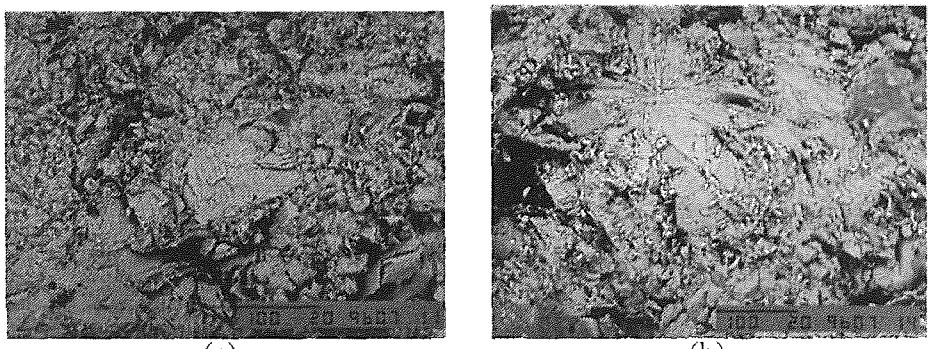
(a) (b)

Figure 40 DTE Polymer , 200x (a) Control (b) Experimental.



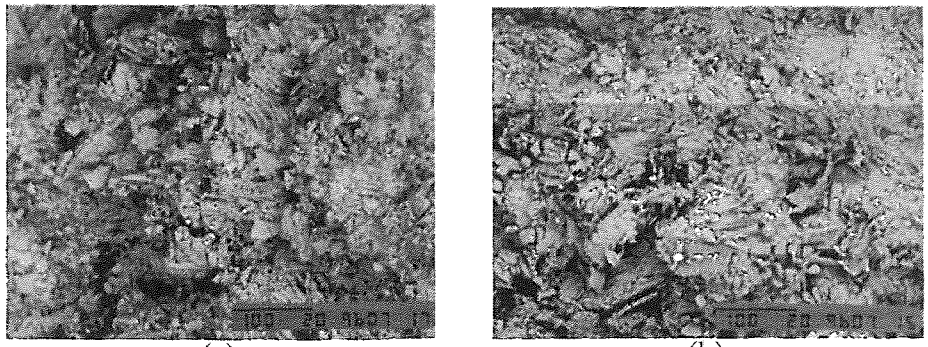
(a) (b)

Figure 41 DTE/HA-500 Composites, 200x (a) Control (b) Experimental.



(a) (b)

Figure 42 DTE/OsteoGen Composites, 200x (a) Control (b)Experimental .

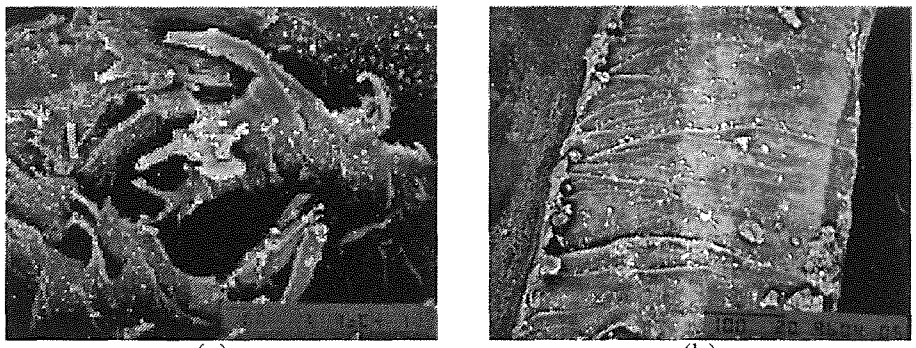


(a) (b)

Figure 43 DTE/Modified OsteoGen Composites, 200x (a) Control (b)Experimental.

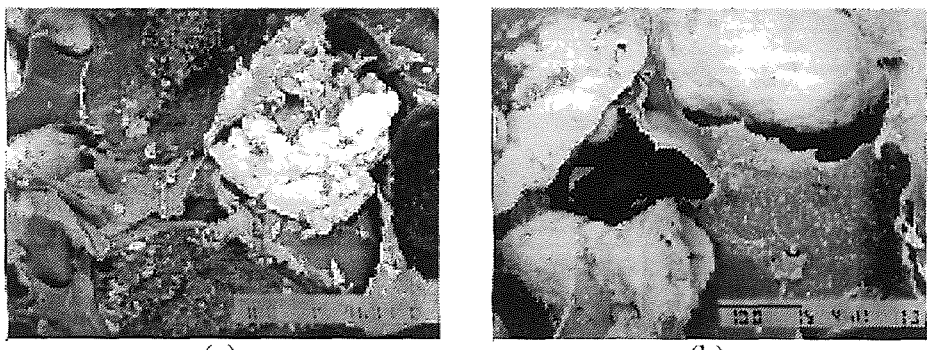
Cross sectional view of materails

after tensile testing in the 37°C water environment (Figure 44-47)



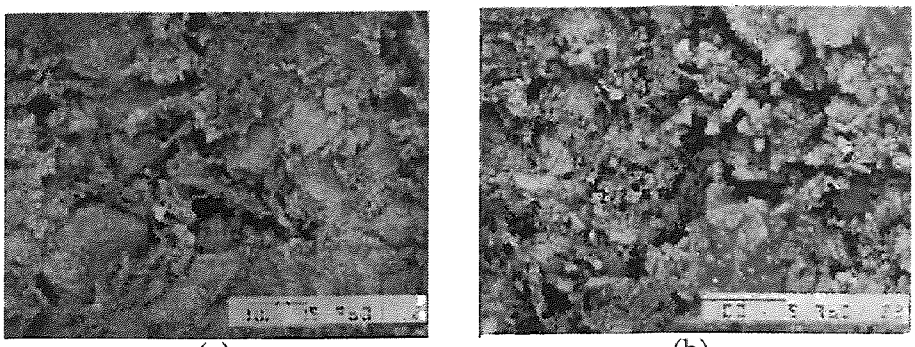
(a) (b)

Figure 44 DTE Polymer , 200x (a) Control (b) Experimental.



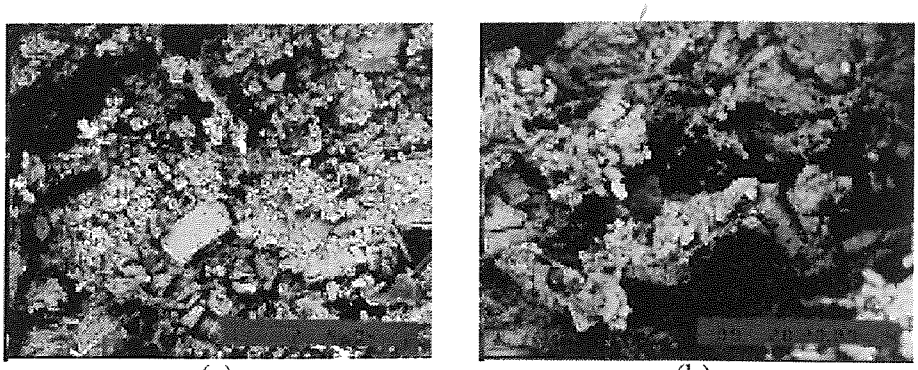
(a) (b)

Figure 45 DTE/HA-500 Composites, 200x (a) Control (b) Experimental.



(a) (b)

Figure 46 DTE/OsteoGen Composites, 200x (a) Control (b)Experimental .



(a) (b)

Figure 47 DTE/Modified OsteoGen Composites, 200x (a) Control (b)Experimental.

REFERENCES

1. Perren S. M., "Physical and Biological Aspects of Fracture Healing with Special References to Internal Fixation", *Clinical Orthopaedics and Related Research*, vol. 138, pp. 175-196, 1979.

2. Tencer A. F., K. D. Johnson, *Biomechanics in Orthopedic Trauma - Bone Fracture and Fixation*, J.B. Lippincott Company, Philadelphia, PA, 1994.

3. Starr C., *Biology Concepts and Applications*, Wadsworth Publishing Company, CA, 1991.

4. Tortora G. J. , N.P. Anagnostakos, *Principles of Anatomy and Physiology*, Sixth Edition, Harper Collins Publishers, New York, NY, 1990.

5. Currey J. D., *The : Mechanical Adaptations of Bone.*, Princeton University Press, Princeton, NJ, 1984.

6. Cruess R.L., J. Dumont, "Current Concepts - Fracture Healing", *Can J. Surg.* , vol. 18 , pp. 403-413, 1975.

7. Knowles J.C., "Development of a Natural Degradable Polymer for Orthopaedic Use", *Journal of Medical Engineering & Technology*, vol. 17, pp. 129-137, 1993.

8. Daniels A.U., K.O. Mellssa, Chang, and P.A. Kirk, "Mechanical Properties of Biodegradable Polymers and Composites Proposed for Internal Fixation of Bone", *Journal of Applied Biomaterials*, vol. 1, pp. 57-78, 1990.

9. Hollinger J. O., *Biomedical Applications of Synthetic Biodegradable Polymers*, CRC Press, Boca Raton, Florida,1995.

10. Pulapura S., and J. Kohn, "Trends in the Development of Bioresorbable Polymers for Medical Applications." *J. of Biomaterials Applications* , vol. 6, pp. 216-250, 1992.

11. Bostman O. M., "Current Concepts Review: Absorbable Implants for the Fixation of Fracture", *The Journal of Bone and Joint Surgery*, vol. 73 A, pp. 148-153, 1991.

12. Kulkarni R. K., E. G. Moore, A.F. Herfyeli, and F. Leonard, " Biodegradable poly(lactic acid) Polymers.", *J. Biomed. Mater. Res.*, vol. 5, pp. 169-181, 1971.

13. Getter l., D. E. Cutright, S. N. Bhaskar, and J. K. Augsburg, "A Biodegradable Interosseous Appliance in the Treatment of Mandibular Fractures." *J. Oral. Sug.*, vol. 30, pp. 344-348, 1972.

14. Zhou J., S.I. Ertel, H.M. Buettner, and J. Kohn, "Evaluation of Tyrosine-derived pseudo-poly (amino acids) : *In vitro* Cell Interactions." *The 20th Annual Meeting of the Society of Biomaterials*, pp. 371, 1994.

15. Bostman O.M., E. Hirvensalo, J. Markinen, and P. Rokkanen, "Foreign-body Reactions to Fracture Fixation Implants of Biodegradable Synthetic Polymers." *J. Bone Jt. Surg.*, vol. 72B, pp. 592-596, 1990.

16. Suganuma J., H. Alexander, and J.L. Ricci, "Biological Response of Intramedullary Bone to Poly-L-lactic acid." *Mater Res. Soc. Simp. Proc.*, vol. 252, pp. 131-146, 1992.

17. Daniels A. U., M. S. Taylor, K. P. Andriano and J. Hellwe, "Toxicity of Absorbable Polymers Proposed for Fracture Fixation Devices." *Trans 38th Ann. Mtg. Orthop. Res. Soc.*, vol. 17, pp. 88, 1992.

18. Nunes R.W., J.R. Margin, J.F. Johnson, "Influence of Molecular Weight and Molecular Weight Distribution on Mechanical Properties of Polymer", *Polymer Engineering Science*, vol. 22 -4 , pp. 205-228, 1982.

19. Ertel S.I., and J. Kohn, "Evaluation of a Series of Tyrosine-derived Polycarbonates as Degradable Biomaterials." *J. Biomed. Mater. Res.* , vol. 28 , pp. 1-12, 1994.

20. Anderson J.M., "Perspectives on the In Vivo Responses of Biodegradable Polymer.", *Biomedical Applications of Synthetic Biodegradable Polymer*, CRC Presss Inc., Boca Raton, Florida, 1995.

21. McCrum N.G., C.P. Buckley, and C.B. Bucknall, *Principles of Polymer Engineering,* Oxford University Press, New York, NY, 1981.

22. Ertel S.I., R. Parsons, and J. Kohn, "Investigation of a Tyrosine-derived Polycarbonate as a Potential Orthopaedic Implant." *Trans 19th Ann Mtg Soc Biomater.*, p. 17, 1993.

23. Choueka J., H. Alexander, J. L. Charvet, K. J. Koval, W. S. Green, K.A. Hooper, S. I. Ertel, K. James, and J. Kohn, "Canine Intramedullary Bone Response to Tyrosine-derived Polycarbonates.", *Fifth World Biomaterials Congress*, p. 563, 1996.

24. Frenkel S. R., J.R. Ricci, G. Chen, " Comparative Cytotoxicity of Poly(DTE carbonate) and Poly(lactic acid)." , *Fifth World Biomaterials Congress*, p. 27, 1996.

25. Macon N. D., A. Schwartz, K. James, J. Charvet, and J. Ricci, "Material Properties of Poly(desaminotyrosyl-trosine ethyl ester carbonate) at Two Molecular Weight after Subcutaneous Implantation in the Rat.", *Fifth World Biomaterials Congress*, p. 317, 1996.

26. Strong A. B., *Fundamentals of Composites Manufacturing : materials, methods, and applications.*, Society of Manufacturing Engineers, Dearborn, Mich., 1989.

27. Jones R. M., *Mechanics of Composite Materials.*, Scripta Book Co., Washington D.C., 1975.

28. Schwartz M. M., *Composite Materials Handbook*, Mc-Graw-Hill Book Company, New York, NY, 1984.

29. Perez B. J., "Mechanical Properties of a Discontinuous Random Fiber Composite for Totally Bioabsorbable Fracture Fixation Devices", *Thesis of New Jersey Institute of Technology, 1994.*

30. Boeree N.R., J. Dove, J.J. Cooper, J. Knowled, and G.W. Hastings, "Development of a Degradable Composite for Orthopaedic use: Mechanical Evaluation of an Hydroxyapatite-polyhydroxybutyrate Composite Material", *Biomaterials*, Vol. 14 (10), pp. 793-796, 1993.

31. Zimmerman M. C., "The Design and Analysis of Absorbable and Semi-absorbable Composite Fracture Fixation Devices." *Ph.D. Dissertation Rutger University* , 1985.

32. Parsoms J. R., J. L. Ricci, and H. Alexander, "Osteoconductive Composite Grouts for Orthopedic Use.", *Bioceramics : Material characteristic versus in vivo behavior*, Annals New York Academy of Sciences, vol. 523, pp. 190-207, 1988.

33. Verheyen C.C.P.M., J. R. de Wijn, C. A. van Blitterswijk, and K.de Groot, "Evaluation of Hydroxyapatite/poly(l-lactide) Composite: Mechanical behavior", *Journal of Biomedical Materials Research*, Vol. 26, pp. 1277-1296, 1992.

34. Lin S. T., S.L. Krebs, S. Kadiyala, K.W. Leong, W.C. LaCourse and B. Kumar, "Development of Bioabsorbable Glass Fibers.", *Biomaterials* , vol. 14, pp. 1057-1061, 1994.

35. Parsons J.R., J.L. Ricci, p. Liebrecht, R. Salsbury and H. Alexander, "Enhanced Stabilization of Orthopedic Implants with Spherical Hydroxyapatite Particulate.", *Biological and Biomedical performance of Biomaterials*, Elsevier Science Publishers B.V., pp. 477-482, 1986.

36. Ricci J.L., N.C. Blumenthal, J.M. Spivak, and H. Alexander, "Evaluation of a Low-temperature Calcium Phosphate Particulate Implant Material: Physical-chemical Properties and *In vivo* Bone Response.", *J. Oral Maxilloface Surg.*, vol. 50, pp. 969-978, 1992.

37. Tretinnikov O.N., K. Kato, and Y.Ikada, " *In vitro* Hydroxyapatite Deposition on to a Film Surface-grafted with Organophosphate Polymer.", *Journal of Biomedical Materials Research*, Vol. 28, pp. 1365-1373, 1994.

38. Yamamda S., T. Nakamura, T. Kokubo, M. Oka, and T. Yamamuro, "Osteoclastic Resorption of Apatite Formed on Aoatite- and Wollastonite-containing Glass-ceramic by a Simulated Body Fluid.", *Journal of Biomedical Materials Research*, Vol. 28, pp. 1357-1363, 1994.

39. Department of Bioengineering Hospital for Joint Diseases, The Report of The Second Quarter Activities, NIST-ATP Award 70NAB4H1502, Aprial 1 - June 30, 1995.

40. Kokubo T., T. Hayashi, S. Sakka, T. Kitsugi, T. Yamamuro, M. Takagi and T. Shibuya, " Surface Structure of a Load-bearable Bioactive Glass Ceramic A-W", *Ceramics in Clinical Application*, P. Vincenzini (ed.), Elservier, Amsterdam, pp. 175-184, 1987.

41. Department of Bioengineering Hospital for Joint Diseases, The Report of The Fourth Quarter Activities, NIST-ATP Award 70NAB4H1502, September 1 - December 31, 1995.