T.C.
MARMARA UNIVERSITY
INSTITUTE FOR GRADUATE STUDIES IN
PURE AND APPLIED SCIENCES

PRODUCTION AND CHARACTERIZATION OF
BIOCERAMIC NANOPOWDERS OF NATURAL-
BIOLOGICAL ORIGIN

A. Umut TÜYEL

THESIS
FOR THE DEGREE OF MASTER OF SCIENCE
IN
BIOENGINEERING

SUPERVISOR
Assoc.Prof Ebru TOKSOY ÖNER

İSTANBUL 2008

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ABSTRACT

PRODUCTION AND CHARACTERIZATION OF BIOCERAMIC NANOPOWDERS OF NATURAL BIOLOGICAL ORIGIN

The never-ending call for new techniques and materials to repair damaged musculoskeletal tissues, bone defects, and worn out body parts makes bioceramics to be an important research focus. Having a calcium-phosphorus ratio similar to natural bone mineral, there is an increasing interest in calcium phosphates for biomedical applications. For preparing nano-powders of calcium phosphates, naces are considered as suitable natural materials for dental and bone restorations. Besides their worldwide availability and low cost, they are able to initiate and induce mineralized tissue formation by human osteoblasts *in vitro* and also exhibit osteogenic and osteoinductive features by developing bonds with bones. With the ultimate objective of preparing inexpensive calcium phosphate bioceramic particles, various raw materials of natural-biological origin including the bones of cuttlefish *Sepia officianalis*, Chinese sweet water Pearl Powder, the Pacific Kumamoto oyster *Crassostrea sikamea*, the bivalve mollusc *Venus verrucosa* and the common European oyster *Ostrea edulis* were obtained and used in this study. The classical hydrothermal and a new sonochemical synthesis method were both applied to the raw materials and the obtained bioceramics were characterized. Besides particle size distribution, the morphology of the powders was examined by a scanning electron microscope and the crystalline phases of the nano-powders were identified by a high resolution XRD and FT-IR. Biocompatibility studies were also performed and experimental results of the *in vitro* tests with osteoblasts demonstrated the suitability of the produced bioceramics for biological uses.

Keywords: Calcium phosphate, bioceramics, hydroxyapatite, tri-calcium phosphate, hydrothermal conversion, nacre.

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ÖZET

DOĞAL BİOLOJİK KAYNAKLARDAN BİYOSERAMİK NANO-TOZLARIN ÜRETİMİ VE KARAKTERİZASYONU

Zarar görmüş muskuloskeletal dokuların, kemik defektlerinin, yıpranmış vücut parçalarının tedavi edilebilmesi için yeni teknikler ve malzemelerin bitmeyen arayışı biyoseramikleri önemli bir araştırma odağı haline getirmektedir. Kemğin mineral yapısına benzer kalsiyum fosfat oranına sahip olduğundan kalsiyum fosfatlar biyomedikal uygulamalar için artan bir ilgiye sahiptir. Kemik ve diş restorasyonları için nano kalsiyum fosfat tozlarının hazırlanmasında sedefler uygun doğal kaynak olarak düşünülmüştür. Dünya çapındaki bulunabilirliklerinin ve düşük maliyetlerinin yanı sıra, insan osteoblastlarında in-vitro olarak mineralize doku formasyonunu başlatıp indükleyebilmeleri ve kemikler ile bağ oluşturarak osteogenik ve osteoindüktif özellikleri bulunmaktadır. Nihai amaç olarak ucuz kalsiyum fosfat biyoseramik partiküllerinin üretimi için; tatlı su incisi tozu, pasifik kumomoto istiridyesi kabuğu Crasostrea Sikamea, iki yanaklı kabuklu Venus Verrucosa, genel Avrupa istiridyesi kabuğu Ostrea Edulis’ide içeren çeşitli doğal biyolojik kaynak bu çalışmada elde edilmiş ve kullanılmıştır. Klasik hidrotermal ve yeni sonokimyasal metotlar elde edilen tüm hammaddeler uygulanmış ve elde edilen biyoseramikler karakterize edilmiştir. Partikül boyutu dağılımının yanı sıra tozların morfolojileri taramalı elektron mikroskopu ile incelenmiş ve nano tozların kristal fazları yüksek çözünürlüklü XRD ve FT-IR ile incelenmiştir. Biyouyumluluk çalışmaları da gerçekleştirilmiştir ve osteoblastlar ile yapılan in-vitro deneysel sonuçlar üretilen biyoseramiklerin biyolojik uygulamalar için uygun olduğunu kanıtlamıştır.

Anahtar Kelimeler: Kalsiyum fosfat biyoseramikler, hidroksiapatit, tri-kalsiyum fosfat, hidrotermal çevirim, sedef.

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LIST OF SYMBOLS AND ABBREVIATIONS

CJD	: Creutzfeldt Jacob Disease
FA	: Fluoroapatite
MPa	: Mili Pascal
ANOVA	: Analyzes of Variance
SDS	: Sodium Dodecil Sulphate
SD	: Standart Deviation
NBT	: Nitro-Blue Tetrazolium Chloride
BCIP	: 5-Bromo-4-Chloro-3'-Indolyphosphate p-Toluidine Salt
PBS	: Phosphate Buffer Solution
ELISA	: Enzyme Linked Immune Sorbent Assay
MTT	: [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]
FBS	: Fetal Bovine Serum
RPMI	: Roswell Park Memorial Institute (Mediums develotion place)
EDTA	: Ethylenediaminetetraacetic acid
JCPDS	: Joint Committee on Powder Diffraction Standards
SEM	: Scanning Electron Microscopy
BSE	: Bovine Spongiform Encephalopathy
HIV	: Human Immunodeficiency Virus
HT	: Hydrothermal Transformation
SPARC	: Osteonectin
SPPI	: Secreted Phosphoprotein-I
HA	: Hydroxyapatite
TCP	: Tri-calcium phosphate
μ	: micron
DTA/TGA	: Differential thermal analyzes / Thermal gravity analyzes

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PART I

INTRODUCTION AND OBJECTIVES

The regeneration potential of human bone is limited in the cases of repair of large bone defects, such as those associated with comminuted fractures or bone tumor resection. In most cases, autogenous and allogenic bones are used as bone grafts. However, the amount of autogenous and allogenic bones that can be collected is severely limited. Furthermore, there is a risk of donor site morbidity for autografts, and transmission of infection for allografts (Kenneth et al., 2007).

Living in the era of life control and prolongation, artificial implants of hydroxyapatite (HA), are very popular for hard tissue (e.g., bone) restorations because they accelerate bone growth around the implant (Oktar et al., 2006). Having a calcium-phosphorus ratio similar to natural bone mineral, low-density, highly porous Beta-Tricalcium Phosphate (beta-TCP) resembles cancellous bone in both porosity and structure. Due to their resorbable nature, beta-TCP have been proposed as potential bone defect fillers where they fill the void and gradually dissolve away, being replaced by bone. Hence there is an increasing interest in calcium phosphates for biomedical applications.

Nacres are considered as suitable natural materials for dental and bone restorations because they are not only able to initiate and induce mineralized tissue formation by human osteoblasts *in vitro* but also exhibit osteogenic and osteoinductive features by developing bonds with bones (Lemos et al 2006.,). The present work aims at preparing inexpensive nano-sized hydroxyapatite (HA) and beta-TCP particles from various raw materials of natural-biological origin. Hence a new approach has been proposed for developing highly bioactive bone-scaffolds of

HA from various natural biological origins via a new ultrasonic method and classic hydrothermal transformation method.

The raw materials used in this study include the bones of cuttlefish *Sepia officianalis*, Chinese sweet water Pearl Powder, the Pacific Kumamoto oyster *Crassostrea sikamea*, the bivalve mollusc *Venus verrucosa* collected from Turkish and Portuguese beaches and the common European oyster *Ostrea edulis*. Each sample was reduced to sub100µm particle size and Differential thermal analysis (DTA/TG) was employed to determine their exact CaCO₃ content. Besides the classical method of hydrothermal transformation followed by sintering, sonochemical method was also applied for the synthesis of the bioceramics. The morphology of HA and TCP powders was examined by a scanning electron microscope. The crystalline phases of the powders were identified by a high resolution X-ray diffraction analyzer and Fourier transform infrared spectroscopy (FT-IR). Biocompatibility studies were performed by isolating osteoblasts from the calvaria of 1- to 5-day old neonatal wistar rats and incubating the cells in the presence of the bioceramic samples. Then, osteoblasts' morphology, viability/proliferation and secretion capability of alkaline phosphatase were tested.

PART II

GENERAL BACKGROUND

II.1 BIOMATERIAL

Any substance or combination of substances synthetic or natural in origin which can be used for any period of time as a system or a part of a system that treats, augments, or replaces any tissue; organ or function of the body is called as biomaterial (Medical devices in Scotland, 2008). Theoretically, any material can be “biomaterial” as long as it is used for medical or surgical purposes (Vanrecum et al., 1995).

II.1.1 Properties of Biomaterials

The nature of bonding determines the properties of materials. Metallic bonds allow high electrical and heat conductivity due to the free electrons, which act as the medium. On the other hand ionic materials are insulator of heat and electricity since their electrons are tightly held by the ions. The non discriminative nature of metal atoms for their neighbors makes it easy to change their positions under load resulting in yield point. Whereas ionic materials have a limited number of slip planes due to repulsion of like charged ions, making such materials brittle. Covalent bonds share valence electrons with neighboring atoms. Generally covalent compounds show poor electrical and thermal conductivity as for ionic bonding. Besides the primary bonds, the secondary bonds such as dipole-dipole interactions, hydrogen bonds, van der Waals interactions also play the major role in determining the property.

In order of strength, these bonds may be classified as follows: Ionic >metallic >covalent >hydrogen >van der Waals. Thus elastic moduli of biomaterials are in the following order: ceramic >metallic >polymeric (Bhat, 2008 a).

II.1.2 Structure of Biomaterials

On the basis of structure, materials can be divided into two broad categories, namely, crystalline and non crystalline or amorphous. In crystalline solids, the atoms,

ions or molecules comprising the individual crystal are arranged in a three dimensional lattice following a precise periodic patterns with relatively minor defects disturbing the general order of the array.

The arrangements of atoms in a crystalline solid are represented by a three dimensional space lattice which can be described as a repeating unit called a unit cell or basic cell. Depending on axial length and angles of unit cell there are 7 crystal systems. The cubic and hexagonal systems are the most common for implant metals and ceramics. Most materials used for implants are made of more than two elements. Thus the structure of these materials will depend on type and number of sites occupied. Frequently the number of nearest neighbors each atom has in a particular structure provides an indication of the structure. This number is called coordination number (Bhat, 2008 b).

II.1.2.1 Phase Changes

Some elements or compounds can exhibit two or more phases in the solid states. This phenomenon is termed as allotropy. The process of temperature induced phase change is called allotropic phase transformation. In binary system of two metals we observe different structural types or phases at different composition (Serdar and Elgun, 1999).

II.1.2.2 Non Crystalline Solids

A solid that does not possess a long-range order of crystallinity is called non crystalline or amorphous. Some substances never crystallize in cooling experiments, but instead they remain permanently in the under cooled super cooled state. Such substances frequently are called glasses but the term amorphous material or vitreous material is increasingly preferred (Zallen, 2008).

II.1.2.3 Surface Properties

Surface Energy: Surface energy is a measure of the extent to which bonds are unsatisfied at the surface of material. At the surface there is an asymmetric force field, which results in a net attraction of surface atoms into bulk. This tends to deplete the surface of atoms putting in surface tension. Metals and ceramics have surfaces with high surface energies in contrast most polymers have much smaller surface energies. Surface energy derives from the unsatisfied bonding potential of

molecules at a surface, giving rise to 'free energy'. This is in contrast to molecules within a material which have less energy because they are subject to interactions with like molecules in all directions. Molecules at the surface will try to reduce this free energy by interacting with molecules in an adjacent phase. When one of the bulk phases is a gas, the free energy per unit area is termed the surface energy for solids, and the surface tension in liquids. One manifestation of surface energy is a state of tension at the surface of a liquid, which is why work is required to increase the surface area of a liquid, hence the above physical definition. However, when both phases are condensed, (i.e. solid-solid, solid-liquid and immiscible liquid-liquid interfaces) the free energy per unit area of the interface is called the 'interfacial energy'. The term surface energy is also closely linked with surface hydrophobicity. Whereas surface energy describes interactions with a range of materials, surface hydrophobicity describes these interactions with water only. Because water has a huge capacity for bonding, a material of high surface energy (i.e. high bonding potential) can enter into more interactions with water and consequently will be more hydrophilic (Jeremy Webb, 1998).

Contact Angles: When a liquid drop is placed onto a solid surface or another liquid surface two things may happen. The liquid may sit on the surface in the form of a droplet or it may spread out over the entire surface which event occurs depend on the interfacial free energies of the two substances. The contact angle can be affected greatly by the surface roughness and adsorption of polar gases or organic species or contamination by dirt. If the liquid is very strongly attracted to the solid surface (for example water on a strongly hydrophilic solid) the droplet will completely spread out on the solid surface and the contact angle will be close to 0° . Less strongly hydrophilic solids will have a contact angle up to 90° . On many highly hydrophilic surfaces, water droplets will exhibit contact angles of 0° to 30° . If the solid surface is hydrophobic, the contact angle will be larger than 90° . On highly hydrophobic surfaces, the surfaces have water contact angles as high as 150° or even nearly 180° . On these surfaces, water droplets simply rest on the surface, without actually wetting to any significant extent. These surfaces are termed superhydrophobic and can be obtained on fluorinated surfaces (Teflon-like coatings) that have been appropriately micropatterned. This is called the Lotus effect, as these new surfaces are based on lotus plants' surface (which has little protuberances) and would be superhydrophobic even to honey. The contact angle thus directly provides

information on the interaction energy between the surface and the liquid (Wikipedia.: “Contact angle”, 2008).

Critical Surface Tension: The critical surface tension of a material is determined by measuring the different values of contact angle θ formed by liquids with different values of $\gamma_{L/G}$ a plot of $\cos \theta$ versus γ is usually a straight line. The $\gamma_{L/G}$ at which $\cos \theta = 1$ is defined as the critical surface tension. The more hydrophobic the surface, the lower is γ_c blood compatibility of material surfaces has been shown to vary in the same order as the critical surface tension. The amount of thrombus formation increases and blood clotting time decreases as γ_c increases (Ulusoy et al., 2004).

Electrokinetic Theory: When a material with a charged surface is placed in a solution with ions a diffused layer of oppositely charged ions appears close to surface. Surfaces may be charged for a variety of ions appears close to surface. Surfaces may be charged for a variety of reasons. For example most ceramics have a net negative charge on the surface due to the breaking of oxide bonds, which leaves unsatisfied oxygen ion charges on the surface. The presence of the electrical double layer gives rise to electrokinetic phenomena when either the particles of the medium moves. The streaming potential and electro osmosis owe their existence to the electrical double layer.

The surface properties are among the most important material properties that a biomaterial possesses. Thus is due to the fact that when a device is implanted to tissues, the surface chemistry will determine to a large extent how the material and tissues or fluids interact. The surfaces of metal implant corrode by liberation metallic ions into solution. Polymeric materials leach constituents such as plasticizers, lubricants and monomers from their interior. Inorganic glasses and clays may undergo a process called ion exchange. Ion in solution can diffuse in the material. Thus proper surface properties are important to have desirable biocompatibility of implants (Stanislav et al., 2005).

II.1.3 Calcium Phosphates as Biomaterials

Calcium Phosphate is the name given to a family of minerals containing Calcium ions (Ca^{+2}) together with orthophosphates (PO_4^{-3}), metaphosphates or pyrophosphates ($\text{P}_2\text{O}_7^{-4}$) and occasionally hydrogen or hydroxide ions. Seventy percent of bone is made up of (HA), a calcium phosphate mineral. Tooth enamel is

also largely calcium phosphate (Wikipedia: “Calcium phosphate”, 2008). Calcium phosphate has been used in the form of artificial bone. This material has been synthesized and used for manufacturing various forms of implants, as well as for solid or porous coatings on other implants (Park et al., 1992 a). Calcium phosphate can be crystallized into salts such as (HA) and β -whitlockite depending on the Ca:P ratio, presence of water, impurities, and temperature. In a wet environment and at lower temperatures ($<900^{\circ}\text{C}$), it is more likely that (HA) will form, while in a dry atmosphere and at a higher temperature, β -whitlockite will be formed (Park et al., 1992 b). Both forms are very tissue compatible and are used as bone substitutes in a granular form or a solid block. The apatite form of calcium phosphate is considered to be closely related to the mineral phase of bone and teeth. The mineral part of bone and teeth is made of a crystalline form of calcium phosphate similar to (HA) $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$ (Park et al., 1992 c). Hard tissue such as bone, dentin, and dental enamel are natural composites which contain (HA) (or a similar mineral), as well as protein, other organic materials, and water. Enamel is the stiffest hard tissue (Park et al., 1992 c). Many different methods have been developed to make precipitates of (HA) from an aqueous solution of $\text{Ca}(\text{NO}_3)_2$ and NaH_2PO_4 . There has been successful use of modifications to Jarcho and colleagues wet precipitation procedure for synthesizing (HA)s for use as bone implants (Jarcho et al., 1979; Bajpai et al., 1985) and drug delivery devices (Abrams et al., 1994; Arar et al., 1992; Bajpai et al., 1994; Parker.,1993).

II.1.3.1 Use of Calcium Phosphates

Calcium phosphates have a very wide range of usage in various industries. They are used for the production of phosphoric acid and fertilizers. For example, calcium phosphates are utilized in the odda process which is a method for the industrial production of nitrogen fertilizers. The raising agent with E number E 341 is a calcium Phosphate with chemical formula $\text{Ca}(\text{H}_3\text{PO}_4)_2$ and CaHPO_4 (Wikipedia.: “Tricalcium phosphate Uses”, 2008). They are used in a variety of dental products for remineralization (Gianluca et al., 2007). Another practical application of these compounds are their use in gene transfection of cells. It is not too well understood, but the calcium phosphate precipitate and DNA form a complex that is thought to help the DNA enter the cell. Calcium phosphate forms an insoluble precipitate with

DNA, which attaches to the cell surface and is taken to the cells by endocytosis (Cold Spring Harbor Protocols., 2008).

II.1.3.2 Tri Calcium Phosphate

Tri-Calcium Phosphate (TCP) is a compound with formula $\text{Ca}_3(\text{PO}_4)_2$. It is also known as calcium orthophosphate, tertiary calcium phosphate, tribasic calcium phosphate or bone ash. Calcium phosphate being one of the main composition products of bone, it has an alpha and beta crystal form. As rock it is found in whitelockite. TCP is used in powdered spices as an anticaking agent, is an important raw material for phosphoric acid and fertilizers. Used in cheese products, is a mineral salt found in rocks and bones, used as nutritional supplement.

Commonly used in porcelains and dental powders, medically as an antacid or calcium supplement, although calcium carbonate is more common in this regard (Wikipedia: “Tricalcium phosphate General Cases and Uses”, 2008).

β -TCP is stable up to 1125°C and above this, up to 1430°C, α -TCP is the stable phase. Beyond 1430°C, the super α -TCP form becomes stable until the melting point of 1756°C. It is found in nature as a rock in Morocco, Israel, Philippines, Egypt, and Russia and in smaller quantities in some other countries. The natural form is not completely pure, and there are some other components like sand and lime which can change the composition, in terms of P_2O_5 , most calcium phosphate rocks have a content of 30%- 40% P_2O_5 in weight (Wikipedia.: “Tricalcium phosphate General Cases and Uses”, 2008).

II.1.3.3 Hydroxyapatite

Hydroxyapatite (HA) is a form of Calcium Phosphate which is found in skeletal system of most of the vertebrate animals. Its chemical formula is $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ and it is also called as HA, HAp or OHAp. Its stoichiometric ratio is 1,67.

One of the most extensively studied bioactive ceramics is HA. HA is chemically similar to the mineral component of bone and other hard tissues in mammals. It is composed of the same ions that construct the mineral part of teeth and bones. It shows excellent biocompatibility not only with hard tissues, but also with soft tissues, such as skin and muscles (Donglu et al., 2006 a). There are lots of different methods described for the synthesis of HA bioceramics. Bento et. al (2006)

parts of the body, particularly bone. Thus, the ceramics used for the latter purposes are classified as bioceramics. They have started to be used in dentistry as dental crowns because of their relative inertness to the body fluids, high compressive strength, and esthetically pleasing appearance. Some carbons have found use as implants especially for blood interfacing applications such as heart valves. Due to their high specific strength as fibers and their biocompatibility, ceramics are also being used as reinforcing components of composite implant materials and for tensile loading applications such as artificial tendon and ligaments (Scott et al., 2006). In order to be classified as a bioceramic, the ceramic material must meet or exceed the properties listed in Table II.1. Ceramics used in fabricating implants can be classified as nonabsorbable (relatively inert), bioactive or surface reactive (semi-inert) (Hench et al., 1991-1993), and biodegradable or resorbable (non-inert) (Hentrich et al., 1971). Alumina, zirconia, silicone nitrides, and carbons are inert bioceramics. Certain glass ceramics and dense (HA)s are semi-inert (bioactive) and calcium phosphates and calcium aluminates are resorbable ceramics (Joyce et al., 2007).

Although Plaster of Paris was used in 1892 as a bone substitute (Peltier et al., 1961), the concept of using synthetic resorbable ceramics as bone substitutes was introduced in 1969 (Hentrich et al., 1969). Resorbable ceramics, as the name implies, degrade upon implantation in the host. The resorbed material is replaced by endogenous tissues. The rate of degradation varies from material to material.

II.2.1 Tricalcium Phosphate (TCP)

A multicrystalline porous form of β -tricalcium phosphate [β -Ca₃(PO₄)₂] has been used successfully to correct periodontal defects and augment bony contours (Metsger et al., 1982). X-ray diffraction of β -tricalcium phosphate shows an average interconnected porosity of over 100 μ m (Lemons et al., 1979). Often tribasic calcium phosphate is mistaken for β -tricalcium phosphate. According to Metsger et al. (Metsger et al., 1982), tribasic calcium phosphate is a nonstoichiometric compound often bearing the formula of HA [Ca₁₀(PO₄)₆(OH)₂]. β -tricalcium phosphate is prepared by a wet precipitation procedure from an aqueous solution of Ca(NO₃)₂ and NaH₂PO₄ (Bajpai et al., 1988). The precipitate is calcined at 1150°C for 1 h, ground, and sieved to obtain the desired size particles for use as bone substitutes (Bajpai et al., 1988; Bajpai et al., 1990), and for making ceramic matrix drug delivery systems (Morris et al., 1989; Moldovan et al., 1994). These particles are used as such or

pressed into cylindrical shapes and sintered at 1150 to 1200°C for 36 h to achieve the appropriate mechanical strength for use as drug delivery devices (Morris et al., 1989; Benghuzzi et al., 1991). The composite sets and hardens on the addition of water. TCP is usually more soluble than synthetic (HA) and, on implantation, allows good bone ingrowth and eventually is replaced by endogenous bone.

Table II.1 Desired Properties of Implantable Bioceramics

1. Should be nontoxic
2. Should be noncarcinogenic
3. Should be nonallergic
4. Should be noninflammatory
5. Should be biocompatible
6. Should be biofunctional for its lifetime in the host

II.2.2 Bioactive Ceramics

In order to substitute the function of lost or damaged hard tissues, such as bone and tooth, tissue implants and synthetic devices have been utilized. Tissue transplants can be autologous, allogenic or xenogenic. However the use of autologous tissue involves additional surgery and donor site morbidity and the use of allogenic or xenogenic tissue involves the risk of immunrejection and disease transmission. Therefore, synthetic hard tissue implants are very necessary. All categories of biomaterials including metals, ceramic, composites and even polymers are investigated as candidates for tissue replacements. Upon implantation in the host, surface reactive ceramics form strong bonds with adjacent tissue. One of their many uses is the coating of metal prostheses. This coating provides a stronger bonding to the adjacent tissues, which is very important for prostheses. A list of the uses of surface-reactive ceramics is shown in Table II.2.

Table II.2 Uses of Surface Reactive Bioceramics

1. For coating of metal prostheses
2. In reconstruction of dental defects
3. For filling space vacated by bone screws, donor bone, excised tumors and diseased bone loss
4. As bone plates and screws
5. As replacements of middle ear ossicles
6. For lengthening of rami
7. For correcting periodontal defects
8. In replacing subperiosteal teeth

II.2.3 Bioceramic Manufacturing Techniques

In order to fabricate bioceramics in more and more complex shapes, scientists are investigating the use of old and new manufacturing techniques. These techniques range from the adaptation of an age old pottery technique to the latest manufacturing methods for high temperature ceramic parts for airplane engines. No matter where the technique is perfected, the ultimate goal is the fabrication of bioceramic particles or devices in a desired shape in a consistent manner with the desired properties. The technique used to fabricate the bioceramic device will depend greatly on the ultimate application of the device, whether it is for hard tissue replacement or the integration of the device within the surrounding tissue (Joyce et al., 2007 b).

Advanced techniques, such as rapid prototyping or the use of foaming agents have been employed to produce porous scaffolds of synthetic HA, but beyond expensive apparatuses needed, there is still poor control of the internal porous architecture (Silva et al., 2005).

II.3 STRUCTURE OF BONE TISSUE

There are two types of bone tissue: compact and spongy. The names imply that the two types differ in density, or how tightly the tissue is packed together. There are three types of cells that contribute to bone homeostasis. Osteoblasts are bone-

forming cell, osteoclasts resorb or break down bone, and osteocytes are mature bone cells. Equilibrium between osteoblasts and osteoclasts maintains bone tissue (Seers training web site, 2008).

Compact Bone & Spongy (Cancellous Bone)

Figure II.2: Structure of Compact and Cancellous Bone Tissue

II.3.1 Cortical Bone (Compact Bone)

The main constituents are the mineral (HA), the fibrous protein collagen, and water. There is also some non-collagenous organic material. In the highly mineralized bone (petrosal bone of some non-human mammals), the main organic component is collagen. About %10 of the organic material is non-collagenous. Mainly non-collagenous proteins are Osteocalcin or Bone Gla protein, Osteonectin or SPARC, Osteopontin or secreted phosphoprotein-I (SPPI) and bone Sialoprotein.

The Mineral has a plate like habit, the crystals being impure and extremely small, about 4nm by 50 nm by 50nm. In particular there is about %4-6 of carbonate replacing the phosphate groups. The cement line rout haversion system contains less calcium and phosphorus and more sulphur than nearby parts of bone (Jonathan Black.; Garth W. Hastings..., 1998).

II.3.2 Cancellous Bone (Spongy Bone)

Trabecular bone consists primarily of lamellar bone, arranged in packets that make up an interconnected irregular array of plates, and rods, called trabeculae. These trabeculae, on average, have thicknesses in the range of 100-200 microns. The space between trabeculae is filled with bone marrow, and the precise architectural arrangement of the trabecular plates and rods also depends on anatomic site, making trabecular bone a highly heterogeneous material. Individual trabeculae have relatively uniform compositions that are slightly less mineralized and slightly more hydrated than cortical tissue (Wikipedia: “Bone, Trabecular Bone”, 2008).

II.3.3 Dentin-Enamel

The permanent adult human dentition normally consists of 32 teeth, of which 16 are located in mandible and 16 in the maxilla.

There is a generic heterogeneous structure for these teeth where enamel forms an exterior layer over the underlying dentin. From the cervix to the apex of the root, the exterior of the dentin is covered by cementum to which the periodontal ligament attaches the tooth to alveolar bone. Dental enamel is dense, highly mineralized, hard and brittle. It contains prism like structures that span from the enamel surface to the junction of enamel and dentin, the dentino-enamel junction. The prisms are comprised of (HA) crystallites and contain very little organic matrix. These properties make dental enamel an excellent material for cutting and masticating food (Jonathan Black.; Garth W. Hastings., 1998 b).

Figure II.3: Structure of Human Tooth.

II.4 HARD TISSUE REPLACEMENT

II.4.1 Materials Used in Orthopedics

In the field of biomaterials, research has to follow two different but complementary paths:

On one hand, the characteristics and performance of the material have to be studied in accordance with its role in the body. On the other, its biocompatibility has to be studied.

Biomaterials used in orthopedic surgery have developed a great deal in recent years. There has been a better understanding of the advantages they bring and their limitations. Steels corrode (vitallium) and cobalt chromium alloys wear. The complications connected with intolerance to the debris of metallic wear have meant that metal-metal prostheses are no longer used. The combination of metal and polyethylene also produces wear debris, which plays a decisive role in physiopathology of the loosening of prostheses, and the ceramic-ceramic joint may become blocked if the slightest particle enters the interface. Plastics, such as polyethylene, which cover the sliding surfaces of many joint prostheses become deformed, creep, and break down, tending to limit the life of these prostheses. Cements made of methyl methacrylate, which are used to fix some joint prostheses in the bone, have a high polymerization temperature if they are used in large quantities, and for this reason cause bone necrosis.

In 10% of cases, allografts produce considerable immune reactions and they are assimilated by the skeleton only slowly and incompletely. Bone substitutes are not necessarily successful in mechanical terms and at present can only be used to a limited extent. Many materials have disappeared completely from our arsenal of therapeutic options and one may ask what materials will replace the currently used biomaterials (Dominique et al., 2004 a).

II.4.2 Bone Replacement Materials

II.4.2.1 Autografts

Autografts are bone graft taken directly from the patient. They can not be used to replace large segments of bone or an osteocartilaginous segment forming part of a joint. Being bone-forming, they alone can induce the formation of new bone and

help in the healing of a fracture of the assimilation of an allograft (bone graft taken from another person) (Dominique et al., 2004 a).

II.4.2.2 Allografts

Since 1979, the attention has been turned to Marseilles (France), where allogenic bone fragments are stored in liquid nitrogen at -196 °C with cryopreservatives and preserved in tissue banks (Dominique et al., 2004 b).

Currently used in traumatology or in oncology, these allografts make it possible to reconstruct a bone segment, which has been destroyed by a tumor or an accident. These allografts are well tolerated by the body and only in exceptional cases (10% of cases) do immunological rejection phenomena occur. They can therefore be used easily in anybody requiring this type of operation (Dominique et al., 2004 b).

II.4.2.3 Xenografts

Xenografts are tissue or organs from an individual of one species transplanted into or grafted onto an organism of another species, genus, or family. A common example is the use of pig heart valves in humans (Northwestern University, 2004). Xenografts were used several decades ago by French teams (Judet-Sichard). The large number of rejection phenomena experienced with them (more than 50%) led to people refusing to use them. Because of the current shortage of human grafts, new attempts using different sterilization, preparation, or treatment techniques (lyophilization, ceramization, irradiation, heating) try to mitigate the inadequacies of this type of graft (Dominique et al., 2004 b).

II.4.3 Bone Substitutes

Derivatives of artificial HA (a combination of hydroxapatite-collagen, (HA) cement, corals or madrepores, vitroceramics or bioglasses) are undergoing in depth mechanical and experimental studies to see how well they are tolerated *in-situ* and how they can be used. Even if some bone substitutes really are “colonized” by the bone of the host, their mechanical properties are still inadequate which in turn means that large fragments can not be used in human clinical medicine. Furthermore, these structures, which are uniquely bone-conducting, do not form new bone, and tend to break down rapidly (Seers training web site, 2008).

II.5 BIOCERAMICS OF NATURAL-BIOLOGICAL ORIGIN

Natural species of sea origin, such as corals and naces, have always been of special interest not only in biomaterials but also in other fields of science and technology. Their bio-mineralization mechanism has been investigated and documented extensively. It is interesting to note that nacreous dental implants have been found in prehistoric Mayan skulls.

In the particular case of oyster shells, the brick-like structure of nacre (mother of pearl) is made up of pure aragonite crystallized in an organic matrix. HA is the most well known and studied biocompatible bioceramic for use as bone and tooth implants since the 1960's, with more than 1000 articles appearing in the literature every year, due to its chemical and structural similarity with human hard tissues (Goller et al., 2006).

HA can be produced chemically or from natural resources like corals or human and animal bones. HA materials can be prepared from bovine and human resources by treating them with diluted HCl acid, which demineralizes and freeze-dries the tissues (Goller et al., 2004). The other most popular source to get HA is autografts. Because these kinds of grafts would create cause for an additional surgery for the patient, autografts are no longer practical (Ozyegin et al., 2004). Calcination method of various bony tissues (human tooth, bovine bone, human bone) is another very wide available and practical way (Goller et al., 2002). Preparation of bioceramics by hydrothermal transformation method of calcite structures from corals (Ben-Nissan et al., 2003), eggshells, various seashells (naces) (Umut et al., 2007), and cuttle fish (Rocha et al., 2005), are also reported in literature.

Hydrothermal transformations (HT) of corals from the Pacific Ocean have gained interest since 1970s because the resultant materials are composed of HA and feature similar microstructure to the inorganic mineralized structure of natural bones (Hench et al 1991; 1994). Several processing routes, reactors and set-up approaches have been proposed. However, beyond special apparatus and high temperatures needed, these methods require the use of corals, which are not available worldwide or are even species in danger. To produce HA from nacre and cuttle fish (Kannan et al., 2007), is a newly investigated approach. Rocha et al reported their promising work about producing HA scaffolds from cuttlefish bones. Another paper presents

the HA and TCP composites of bioglass by using human teeth (Goller et al., 2002). Y.Genç and F.N. Oktar have used human teeth to produce composites of HA and TCP and they have reported improved endurance and tissue compatibility when compared with any other bioglass (Erkmen et al., 2007). In addition, bovine derived bone ash has also been used as HA and TCP raw material successfully by Özyegin et al. In that report they pointed at the risks of BSE (Ozyegin et al., 2004). In another work reported in literature, bovine femoral bone was used to produce HA (Seers training web site, 2008). Risks associated with HIV and BSE contamination in the human teeth derived ceramic production have been pointed out by F.N Oktar (F.N. Oktar et al., 2007). Lemos et al has presented the use of oyster shells in biomedicine by hydrothermally transforming them into nano-powders of (HA) (Lemos et al., 2006). Also egg shells are reported as HA and TCP precursor molecules in the literature. (D.Siva et al., 2007)

Production of bioceramics from pearl powder is a new application. Pearls are composed partly of the mineral substance calcium carbonate and partly of a tough, horny substance of organic nature called conchiolin. The shell of the pearl-bearing mollusc is also composed of these two substances. Calcium carbonate may crystallize in either of two forms, calcite or aragonite. In the outer portions of the shell of the pearl oyster the calcium carbonate is in the form of calcite, but in the inner nacreous lining and in the pearl itself the mineral is present as aragonite. This is deposited by the mollusk in very thin crystalline layers in the horny layers of conchiolin, so that the lining of the shell is built of approximately parallel layers of mineral and of animal substance. In the normal shell this is all that takes place, but in the case of a mollusk whose interior is invaded by any small source of irritation, such as a borer, or a grain of sand, or other bit of foreign material, a process of alternate deposit of conchiolin and of aragonite goes on upon the invading matter, thus forming a pearl (JJ Kent Incl ., 2004).

PART III

EXPERIMENTAL WORK

III.1 MATERIALS

III.1.1 Raw Materials

Five different raw materials were used in this study for the production of calcium phosphate bioceramics. These materials include Chinese sweet water Pearl Powder and the Pacific Kumamoto oyster *Crassostrea sikamea* obtained from China and Japan, respectively. The bones of cuttlefish *Sepia officianalis*, the bivalve mollusc *Venus verrucosa* and the common European oyster *Ostrea edulis* were collected from Turkish and Portuguese beaches.

III.1.2 Chemicals

All chemicals and solutions used in this study were supplied by MERCK (Germany), SIGMA (USA), DIFCO (USA) and FLUKA (Switzerland). All experiments were carried out in the Biotechnology Laboratories of Bioengineering Department at Marmara University.

III.1.3 Culture Media

PBS Isolation Medium

Phosphate Buffered Saline 10x Concentrate (SIGMA)	100 ml
Deionized and Distilled water	to 1 Liter

RPMI-1640 Culture Medium

RPMI-1640 Medium (SIGMA)	1 L
Fetal Bovine Serum	100 ml
Penicillin	100 units/ml
Streptomycin	0.1 mg/ml

Amphoterycin B

0.25 mg/ml

III.2 LABORATORY EQUIPMENT

Analytic Balances (0.1 Mg, 1 Mg, 10 Mg)	Ab204-S, Pg403-S, Pg40002-S (Mettler Toledo, Sweden)
Autoclaves	Mod3870 Elv (Systec, Germany) OT 032 Nuve, Turkey
Automatic Pipettor Sets	4500080, (Finipipette, Finland) 4500110, (Finipipette, Finland)
Centrifuges	Highspeed Centrifuge 3K30 Benchtop Refrigerated Centrifuge, 8K10 (Sigma, Germany)
Deepfreezes	-20°C, (Arcelik, Turkey) -20°C, (Heto Holten, Denmark)
Heating Magnetic Stirrer	Mr3003s, (Heidolph, Germany)
Laminar Flow Cabinet	Class II Safety Cabinet, Safe 2010 0.9, (Heto Holten, Denmark)
Ovens	Oven Bd115 Pasteur Oven, Ed115 CO ₂ Oven CB150 (Binder, Germany)
pH Meter	Mp220k, (Mettler Toledo, Germany)
Refrigerators	+ 4 °C, (Arçelik, Turkey)
Vortex	Heidolph, Germany

Water Bath	Masterheat 29l, (Heto Holten, Denmark)
Water Purification Systems	Primary Grade Water Purification System, Purelab Prima 30 Ultrapure Water System, P.Maxima Ls, (Usf Elga, U.K.)
X-Ray Diffractometer	Rigaku Miniflex (USA)
Fourier Transform Infra Red Spectroscope	Perkin Elmer – Spectrum One
Scanning Electron Microscopes	JEOL JSM LV-5910 JEOL JSM T-330
High Temperature Furnaces	Nabertherm (L9/11 – LT9/13) (Germany)
Particle Size Analyzer	Mastersizer 2000 (Malvern U.K.)
Sieve	50-100µm diameter
DTA/TGA Analyzer	PerkinElmer Pyris 6 TGA

III.3. EXPERIMENTAL PLAN AND TECHNIQUES

III.3.1. Preparation and Analysis of Raw Material Powders

Five different raw materials, namely pearl powder, cuttlefish bone, Kumamoto oyster, sand mussel and common european oyster were obtained from several different markets and beaches all over the world. The oyster shells and bones of cuttlefishes were washed under distilled water for two times to eliminate any

contaminants and dried in oven. In order to prepare the raw material powders, dry shells and cuttlefish bones were pounded by hand milling in an agate mortar and sieved with 100µm and 50µm pore sized sieves. Powder samples with particle sizes less than 100 µm were used for further studies.

Differential thermal analysis (Perkin Elmer Pyris 6 TGA) was employed to evaluate the exact CaCO₃ content of the powder samples. According to these results, for each batch of powdered sample, the required volume of an aqueous solution of H₃PO₄ was calculated in order to set the stoichiometric molar ratio of Ca/P = 10/6 for HA and Ca/P = 3/2 for TCP. While the chemical equation (1) describes the (HA) synthesis from calcium carbonate and phosphoric acid, equation (2) shows the reaction for TCP formation.

III.3.2. Synthesis of Bioceramics

Two different chemical synthesis methods, each with sintering temperatures of 450°C and 850°C were applied to five different raw materials making up a total of twenty different biomaterial products.

Method-1: The raw powder was suspended in distilled water. The suspension was put on a hot plate and the temperature was set to 80°C for 15 minutes and then equivalent H₃PO₄ was added drop by drop into the solution. The reaction continued for 2 hours, then to evaporate the liquid part, the mixture was put into the incubator at 100°C for 24 hours and the resulting dried sediment was collected.

Heat treatment was performed to the powder sediment by putting into nabertherm furnace. The furnace temperature was increased 5°C per minute until reaching to 450°C and 850°C in two independent experiments. The temperature was kept constant for 4 hours at that temperature and then it is cooled.

Figure III.1 Synthesis step in Hotplate

Method-2: The raw powder was suspended in distilled water. The suspension was put on an ultrasonic bath and the temperature set to 80°C for 15 minutes then equivalent H_3PO_4 is added drop by drop into the solution. After 2 hours of reaction, the mixture was put into the incubator at 100°C for 24 hours to evaporate the liquid part and then the resulting dried sediment was collected. Heat treatment of the sediment was performed as explained above in Method 1. The samples obtained from the two methods were characterized by SEM, FT-IR, XRD, and Particle Size Analyzer.

Figure III.2 Synthesis step in Ultrasonic Bath.

III.3.3 Particle Size Distribution Analysis

Particle size analyzes were performed with Malvern Mastersizer 2000 analyzer. To measure the size distribution of the particles in the samples, a certain

amount of sample was suspended in 200 ml distilled water and agitated with ultrasound while the measuring continues.

III.3.4 X-Ray Diffraction Analysis

The crystalline phases of the bulk scaffolds were identified by X-ray diffraction analysis (XRD, Rigaku Geigerflex D/Mac, C Series, Cu K α radiation, Japan). Copper K α radiation ($\lambda = 1.5406$ nm) produced at 30 kV and 25 mA scanned. The range of diffraction angles (2θ) between 10° and 80° with a 2θ step of $0.02^\circ/\text{s}$. Identification of the phases was performed by comparing the experimental XRD patterns to standards complied by the Joint Committee on Powder Diffraction Standards (JCPDS) using the cards 71–2396 for aragonite, 09–0432 for hydroxylapatite and 09–0169 for whitlockite (TCP).

III.3.5 Fourier Transform Infra-Red Spectroscopy

Other structural and chemical features of the grown HA were assessed by Fourier Transform Infra-Red transmission spectroscopy (FT-IR, Perkin Elmer – Spectrum One) using fine powdered samples.

III.3.6 Scanning Electron Microscopy

The microstructure of the produced powder was observed by field emission scanning electron microscopy (JEOL JSM LV-5910, 30kV acceleration voltage, beam current 10 μA) under secondary electron mode, using gold-coated samples.

III.3.7 Biocompatibility Studies

III.3.7.1 Sterilization

Sterilization is the process by which items are rendered free of almost all viable microorganisms. The purpose of sterilization is to provide the end user with a sterile product. Sterilization process must be monitored to ensure that all parameters and specifications have been met to assure sterility of items. Throughout this study, sterilization was carried out by killing the microorganisms using steam. The Laboratory equipment which is used in the experiments was sterilized in autoclave. This process, which is known as steam autoclave sterilization, has advantages of short cycle time, good penetration and low price.

Sterilization of Media: The isolation media and the cultivation media have been sterilized separately by 22µm pore sized filter. Autoclave sterilization is not chosen because of high temperature its denaturing effect on the proteins. Most of the proteins may resist until 44-50°C and the sterilization conditions are much higher than that degree. The usual sterilization temperature in autoclave is 121°C and 1.06 bar which is almost twice the denaturation temperature of the proteins. The culture media were sterilized under laminar flow and with hands which are cleaned gloved and sprayed with ethanol.

III.3.7.2 Cultivation Conditions

For the *in vitro* tests with osteoblasts, the following media were used: RPMI (Gibco BRL, NY, USA) medium supplemented with 10% FBS and 1% antibiotic–antimycotic (Sigma, St. Louis, USA); Dulbecco's phosphate-buffered saline, trypsin–EDTA, [3(4,5dimethylthiazol-2yl)2,5diphenyltetrazoliumbromide] MTT, BCIP–NBT (Gibco, Burlington, Ontario, Canada); Crude bacterial collagenase (Boehringer, Biberach, Germany); RPMI Cell culture medium (Sigma, St. Louis, USA); T25 culture flasks and 24-well plates (Nunc products, Naperville, USA).

The cells were seeded into 5ml of culture media in T-25 tissue culture flasks and led to grow in a controlled 5% CO₂, 95% humidified Binder's CO₂ incubator at 37°C.

III.3.7.3 Preparation of Osteoblast Cell Culture

Statements on the biological effects of a biomaterial require the use of cell cultures of the related tissue. The primary culture osteoblast system meets the international criteria, in terms of quality and accuracy of the results obtained, offering possibility of examining the influence of the material on cell biology.

Osteoblasts were isolated from the calvaria of 1- to 5-day old neonatal Wistar rats. The calvaria dissected, freed from soft tissue, cut into small pieces and rinsed in phosphate-buffered saline without calcium and magnesium. The calvaria pieces were incubated with 1% trypsin–EDTA for 5 min followed by four sequential digestions with 2% collagenase at 37°C for 45 min each. The supernatant of the first collagenase digestion, which contains a high proportion of periosteal fibroblasts, was discarded. The other digestions produced a suspension of cells with high proportion

of osteoblasts. After centrifugation at $1000 \times g$ for 5 min, each pellet was resuspended in 5 ml of RPMI medium supplemented with 10% FBS and 1% antibiotic–antimycotic. The cells were seeded into 25 ml tissue culture flasks and led to grow in a controlled 5% CO₂, 95% humidified incubator at 37°C. Osteoblasts were plated 1×10^5 cell density, at passage 2, in 24-well plates. After 2 h, the medium was changed to a medium containing powders at a concentration of 0.005 g/ml. After 72 h of incubation in the presence of each of the tested powders, osteoblasts' morphology, viability/proliferation and secretion capability of alkaline phosphatase were tested.

III.3.7.4 MTT Assay

MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay, first described by Mosmann in 1983, is based on the ability of a mitochondrial dehydrogenase enzyme from viable cells to cleave the tetrazolium rings of the pale yellow MTT and form dark blue formazan crystals which are largely impermeable to cell membranes, thus resulting in its accumulation within healthy cells. Solubilization of the cells by the addition of a detergent results in the liberation of the crystals which are solubilized. The number of surviving cells is directly proportional to the level of the formazan product created. The color can then be quantified using a simple colorimetric assay. The results can be read on a multi well scanning spectrophotometer (ELISA reader). To assess the viability of the osteoblast cells in the presence of the sample powders, 60µl of MTT (5 mg/ ml) was added to each well. After two hours of incubation at 37°C, the cell morphology was analyzed by inverted optical microscopy, and formazan salts were solubilized with 100µl SDS 10% HCl. After further incubation for 18 h, optical density was measured at 595 nm.

III.3.7.5 ALP Assay

The alkaline phosphatase production was evaluated by the BCIP–NBT assay. This assay is based on a chromagenic reaction initiated by the cleavage of the phosphate group of BCIP (5-Bromo-4-Chloro-3'-Indolylphosphate p-Toluidine Salt) by alkaline phosphatase present in the cells. This reaction produces a proton which reduces NBT (Nitro-Blue Tetrazolium Chloride) to an insoluble purple precipitate. In brief, the supernatant of each well was removed, and the cell layer was rinsed twice with PBS. Then, 200µl of BCIP–NBT solution, prepared according to the

manufacturer protocol, was added to each well. After 2 h of incubation, the cells were observed by optical microscopy, and the insoluble purple precipitates were solubilized with 210µl of SDS 10% HCl and incubated for 18 h. The optical density was measured at 595 nm. The results are presented as means $\pm$ SD. The statistical significance was measured by ANOVA and Bonferroni's post-test (confidence level was 95%).

III.3.7.6 Collagen Assay

The osteoblast collagen production was analyzed by SIRCOL assay in the cultures supernatant and complemented with the analysis described by Tullberg (Stanislav S. et al.2005) in the fixed cells. These methods are based on the selective binding property of the syrius- red dye to the [Gly–X–Y] tripeptide end sequence of mammalian collagen. The solubilized collagen was measured by an optical density analysis at 595 nm. The amount of collagen was calculated based on a linear regression from previously known concentrations of type I collagen and their optical density measurement.

PART IV

RESULTS AND DISCUSSION

In last two decades, remarkable advances in the field of biomaterials have led to the development of bioceramics of various compositions for bone repair and prostheses applications. (HA), Tricalcium phosphates and their composites are widely used due to their good biocompatibility and osteointegrative properties.

Many *in vitro* and *in vivo* studies have suggested naces as suitable natural materials for dental and bone restorations because they demonstrate ability to initiate and induce mineralized tissue formation by human osteoblasts *in vitro* and exhibit osteogenic and osteoinductive features by developing bonds with bones (Lemos et al 2006).

With the ultimate objective of preparing inexpensive calcium phosphate bioceramic particles, five different raw materials of natural-biological origin, namely, pearl powder, cuttlefish bone, Kumamoto oyster, sand mussel and common european oyster were obtained from several different markets and beaches all over the world and used in this study. Two different synthesis methods were applied and the obtained biomaterials were analyzed in terms of their morphology, surface characteristics, chemical composition, particle size distribution and biocompatibility.

IV.1 Synthesis of Bioceramics

The raw materials used in this study were the bones of cuttlefish *Sepia officianalis*, Chinese sweet water pearl powder, the Pacific Kumamoto oyster *Crassostrea sikamea*, the bivalve mollusc *Venus verrucosa* and the common european oyster *Ostrea edulis*. After removing any contaminants, they were pounded in an agate mortar and sieved to obtain powder samples with particle sizes less than 100 μm .

IV.1.1 DTA/TGA Analysis of the Raw Materials

HA and TCP are produced chemically by using different calcium and phosphate sources. As a calcium source CaO and CaCO₃; as a phosphate source diammoniumnitrogenphosphate ((NH₄)H₂PO₄) and phosphoric acid (H₃PO₄) are the most commonly used ones. It is known that naces and bones used in this study mainly consist of CaCO₃; and H₃PO₄ was chosen as a phosphate source for the preparation of the biomaterials.

To calculate the required amount of H₃PO₄ in order to set the stoichiometric molar ratio of Ca/P = 10/6 for HA and Ca/P = 3/2 for TCP, Differential thermal analysis was employed to evaluate the exact CaCO₃ content of the powder samples. DTA results are shown as percent mass loss in Table IV.1. Assuming complete decomposition of CaCO₃ to CaO, % mass loss data obtained from thermal analysis were used to calculate the mass percentage of CaO. The bioceramic preparation methods used in this work were applied to a fixed initial mass of two grams of each raw material powder. Therefore, the number of moles of Ca in 2 grams of sample was calculated (Table IV.1).

Table IV.1 DTA Results and calcium contents of Raw Materials

Raw Material	Mass loss (%)	Mass % of CaO	Moles of Ca in 2 g
<i>Pearl Powder</i>	49,7	51,3	0,018294
<i>Sepia Officianalis</i>	44,4	55,6	0,019828
<i>Crasostrea Sikamea</i>	43,4	56,6	0,020180
<i>Venus Verrucosa</i>	44,0	56,0	0,019970
<i>Ostrea Edulis</i>	39,9	60,1	0,021430

Using the specifications of the %85 H₃PO₄ solution (specific density of 1.71) used in this study, the required volume of acid solution were calculated for the stoichiometric ratios of HA (Ca:P = 10:6) and TCP (Ca:P = 3:2) . Table IV.2 shows the H₃PO₄ amounts required for the experiments per 2 grams of raw material sample.

Table IV.2 Phosphoric Acid Amounts Used for Bioceramics Preparation

	HA (Ca:P = 10:6)		TCP (Ca:P = 3:2)	
Origin	Moles of P	H ₃ PO ₄ µl/2g	Moles of P	H ₃ PO ₄ µl/2g
<i>Pearl powder</i>	0,0109764	638 µl	0,012196	710 µl
<i>Sepia officianalis</i>	0,0118968	692 µl	0,013219	768 µl
<i>Crasostrea sikamea</i>	0,0121080	704 µl	0,013453	782 µl
<i>Venus verrucosa</i>	0,0119820	697 µl	0,013113	774 µl
<i>Ostrea edulis</i>	0,0128580	748 µl	0,014287	832 µl

IV.1.2 Production of (HA) and TCP

Two different chemical synthesis methods, each with sintering temperatures of 450°C and 850°C were applied to five different raw materials making up twenty different biomaterial products. The first method is the classical hydrothermal synthesis which is one of the most practical processes to obtain nanosized powders with narrow particle size distributions, elimination/reduction of agglomerates and high purity powders at relatively low temperature (<350°C) and pressure (<50 MPa) (Ryu1 et al., 2008). The second method is sonochemical synthesis method. Ultrasonic irradiation is known stimulate the reactivity of chemical species through particle size reduction and surface activation by intensive stirring, resulting in the acceleration of the heterogeneous reactions between liquid and solid reactants effectively (Kim and Saito, 2001)

In both methods, first, the raw material powders were suspended in distilled water. Whereas in the first method, the suspension was stirred on a hot plate, in the latter method, it was subjected to irradiation of ultrasound. In both methods, H₃PO₄ was added to the suspensions at the same reaction temperature (80°C). After 2 hours of reaction, the reaction products were dried and subjected to heat treatment at two different sintering temperatures of 450°C to yield TCP and 850°C to yield HA.

In order to analyze the mass yields, the final dry mass of the products were determined. Table IV.3 shows the amounts of bioceramic powders after sintering per 2 grams of starting material.

Table IV.3. Amounts of Bioceramic Powders After Sintering

	HA (Ca:P = 10:6), 850°C		TCP (Ca:P = 3:2), 450°C	
Origin / Synthesis method	hydrothermal	sonochemical	hydrothermal	sonochemical
<i>Pearl Powder</i>	1.3824	1.745	2.053	2.071
<i>Sepia officianalis</i>	1.3326	1.648	1.742	1.818
<i>Crasostrea sikamea</i>	1.3911	1.820	1.995	2.034
<i>Venus verrucosa</i>	1.6050	1.755	2.230	1.962
<i>Ostrea edulis</i>	1.7448	1.844	2.070	2.377

When we compare the dry masses of the final products, the highest yield was obtained from *O. edulis* that was subjected to ultrasonic method and sintered at 450°C. On the other hand, the lowest yield was obtained from *S. officianalis* that was subjected to hydrothermal synthesis method and sintered at 850°C. As expected, biomaterials exposed to higher sintering temperatures gave lower mass yields.

IV.2 Characterization of the Bioceramic Powders

Twenty different bioceramic samples obtained in this study were also analyzed for the characterization studies. Firstly, the particle distribution of the HA and TCP powders were determined. Then, their morphology was examined by a scanning electron microscope. The crystalline phases of the powders were identified by a high resolution X-ray diffraction analyzer and Fourier transform infrared spectroscopy (FT-IR).

IV.2.1 Particle Size Distribution

Particle size analyzes of the powder suspensions were performed with Malvern Mastersizer 2000 analyzer and the results are shown in Table IV.4. Sample obtained from *V. verrucosa* by hydrothermal synthesis method and sintered at 850°C had the minimum on mean particle size. *Crasostrea sikamea* subjected to hydrothermal synthesis method and sintered at 450°C showed the maximum particle size.

Table IV.4 Particle Size Distrubution

Origin	Synthesis method	Sintering Temperature	Mean Particle Size (nm)	Min Particle Size (nm)	Max Particle Size (nm)
<i>Pearl powder</i>	sonochemical	450°C	605	458	1247
		850°C	622	460	1269
	hydrothermal	450°C	609	461	1386
		850°C	551	410	1123
<i>Sepia Officianalis</i>	sonochemical	450°C	857	622	1860
		850°C	473	357	765
	hydrothermal	450°C	722	533	1811
		850°C	557	410	1170
<i>Crasostrea Sikamea</i>	sonochemical	450°C	679	527	1660
		850°C	488	352	1192
	hydrothermal	450°C	906	622	2745
		850°C	605	432	1145
<i>Venus Verrucosa</i>	sonochemical	450°C	621	464	1323
		850°C	527	396	947
	hydrothermal	450°C	507	394	823
		850°C	451	340	774
<i>Ostrea Edulis</i>	sonochemical	450°C	630	468	1360
		850°C	491	366	846
	hydrothermal	450°C	622	460	1269
		850°C	561	415	1026

IV.2.2 Fourier Transform Infrared Spectroscopy (FT-IR)

Fourier transform infrared spectroscopy shows the vibration of specific bonds in the presence of infrared light. The characteristic peaks of calcium phosphate based materials are the OH ($3570\text{-}3568\text{ cm}^{-1}$), CO₃ ($1300\text{-}1650\text{ cm}^{-1}$ and $877\text{-}872\text{ cm}^{-1}$) and

PO₄ (1190-976 cm⁻¹, 961- 962 cm⁻¹, 660-520 cm⁻¹). The FT-IR spectrum of each sample was analyzed for the presence of these peaks and the results are summarized in Table IV.5 The FT-IR spectra of the samples are given in the Appendix A

Table IV.5 Summary of FT-IR Spectra

Origin	Method / Sintering Temperature	OH 3570-3568 cm ⁻¹	CO ₃ 1300-1650 cm ⁻¹	PO ₄ 1190-976 cm ⁻¹	PO ₄ 961- 962 cm ⁻¹	CO ₃ 877-872 cm ⁻¹	PO ₄ 660-520 cm ⁻¹
<i>Pearl Powder</i>	Hydrothermal 850°C	+	+	+	+	+	+
	Hydrothermal 450°C	-	+	+	+	+	+
	Sonochemical 850°C	-	-	+	+	+	+
	Sonochemical 450°C	-	+	+	+	+	+
<i>Sepia Officinalis</i>	Hydrothermal 850°C	-	+	+	+	+	+
	Hydrothermal 450°C	-	-	+	+	+	+
	Sonochemical 850°C	-	-	+	+	+	+
	Sonochemical 450°C	-	+	+	+	+	+
<i>Crasostrea Sikamea</i>	Hydrothermal 850°C	+	+	+	+	+	+
	Hydrothermal 450°C	-	+	+	+	+	+
	Sonochemical 850°C	-	-	+	+	+	+
	Sonochemical 450°C	-	+	+	+	+	+
<i>Venus Verrucosa</i>	Hydrothermal 850°C	+	+	+	+	+	+
	Hydrothermal 450°C	-	+	+	+	+	+
	Sonochemical 850°C	-	+	+	+	+	+
	Sonochemical 450°C	-	+	+	+	-	+
<i>Ostrea Edulis</i>	Hydrothermal 850°C	-	-	+	+	+	+
	Hydrothermal 450°C	-	-	+	+	+	+
	Sonochemical 850°C	-	-	+	+	+	+
	Sonochemical 450°C	+	-	+	+	+	+

The spectra of samples prepared by the hydrothermal synthesis method and sintered at 850°C using the *Crasostrea Sikamea*, *Venus Verrucosa* and pearl powder as raw material contained all the characteristic peaks. Absence of some specific bands may be due to the presence of acidic remains within the powder samples.

IV.2.3 X-Ray Diffraction (XRD)

Each material has a specific crystal structure and when a material is subjected to x-ray radiation; it gives specific peaks related to their crystal structure. The X-ray diffraction patterns of each sample was analyzed and identification of the phases was performed by comparing the experimental XRD patterns to standards for aragonite, hydroxylapatite and whitlockite (TCP). The results are summarized in Table IV.6 The XRD spectra of the samples are given in the Appendix B

Table IV.6 XRD Results

Origin	Method / Sintering Temperature	FA	CaO	HA	α -TCP	β -TCP
<i>Pearl Powder</i>	Hydrothermal 850°C	+	+	+	+	+
	Hydrothermal 450°C	+	+	-	-	+
	Sonochemical 850°C	+	+	+	+	+
	Sonochemical 450°C	+	-	-	-	+
<i>Sepia Officianalis</i>	Hydrothermal 850°C	+	-	+	+	+
	Hydrothermal 450°C	+	+	+	-	+
	Sonochemical 850°C	+	+	+	-	+
	Sonochemical 450°C	+	+	-	-	+
<i>Crasostrea Sikamea</i>	Hydrothermal 850°C	+	+	+	+	+
	Hydrothermal 450°C	+	+	-	-	+
	Sonochemical 850°C	+	+	+	+	+
	Sonochemical 450°C	+	+	+	-	+
<i>Venus Verrucosa</i>	Hydrothermal 850°C	+	+	+	+	+
	Hydrothermal 450°C	+	+	-	-	+
	Sonochemical 850°C	+	+	+	+	+
	Sonochemical 450°C	+	+	+	-	+
<i>Ostrea Edulis</i>	Hydrothermal 850°C	+	+	+	+	+
	Hydrothermal 450°C	+	-	+	-	+
	Sonochemical 850°C	+	+	+	-	+
	Sonochemical 450°C	+	+	+	-	+

The XRD analysis of the materials have shown the co-existence of different types of calcium phosphate phases, namely, fluorapatite (FA), (HA), beta and alpha TCP, most probably due to the complex nature of the organic raw material. The sintering temperature was found to affect the active phase of the bioceramics. As sintering temperature increased, the stabile phase changed from HA to alpha TCP.

FA is an important constituent of the tooth enamel. (Wikipedia.: “Fluorapatite” 2008). However, the toxic effects of the fluoride (70mg/kg body mass) which include GIS effects, Neurologic and Cardiovascular effects and Electrolite abnormalities have to be taken into consideration, too. (Wikipedia: “Fluoride poisoning” 2008)

IV.2.4 Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) is used for determining the surface structure, crystal structure and particle sizes. All materials have different crystal packages and it defines its physical properties such as porosity, hardness, endurance, and interactions between materials. The microstructure of the gold-coated bioceramic powders were observed by field emission scanning electron microscopy (SEM) under secondary electron mode. Four SEM images are shown below and the images of other samples may be found in the Appendix C.

Elemental distribution of the samples were also analyzed and they were found to be composed of C, O, Ca, Na, Mg, Cl, F and P. Peaks for toxic elements like Pb or As were not present in the obtained spectra. While C, O and Ca are the major components of the organic molecules; Na, Cl and Ca have very important roles in the signal transduction. P containing molecules are majorly used as energy storage but also they join the structure of the organic molecules. Mg has cofactor functions for some enzymes and F is an important element for the tooth enamel. A very important result of the elemental analysis is that the powder samples were free from any heavy metals and any heavily toxic minerals.

Figure IV.1: *C. sikamea* derived product (850°C and Hydrothermal method) 4kX magnification.

Figure IV.2: *V. verrucosa* derived product (450°C and Hydrothermal method) 4kX magnification.

Figure IV.3: *S. officianalis* derived product (450°C and Sonochemical method) 4kX magnification.

Figure IV.4: *O. edulis* derived product (850°C and Sonochemical method) 4kX magnification.

IV.3 Biocompatibility of the Bioceramic Powders

Biocompatibility was described as "it refers to the ability of a biomaterial to perform its desired function with respect to a medical therapy, without eliciting any undesirable local or systemic effects in the recipient or beneficiary of that therapy, but generating the most appropriate beneficial cellular or tissue response in that specific situation, and optimizing the clinically relevant performance of that therapy" (Williams, 2008). Osteoblasts are the cells that support synthesis, secretion and mineralization of extracellular bone matrix. Therefore, the behavior of osteoblasts in the presence of biomaterials developed for applications in hard tissue replacements is an important parameter for evaluating the materials' biocompatibility. Therefore, the powder samples obtained in this thesis were subjected to a series of tests in order to assess their biocompatibility. For that, osteoblasts were isolated from the calvaria of 1- to 5-day old neonatal wistar rats and the obtained cells were incubated in the presence of the bioceramic samples. Then, osteoblasts' morphology, viability/proliferation and secretion capability of alkaline phosphatase were tested.

IV.3.1 Cell viability and proliferation

In the presence of the sample powders, osteoblasts' viability was evaluated by the MTT assay, based on the reduction of tetrazolium salt to formazan crystals by dehydrogenase present in living cell mitochondria. The formazan salt formation is directly related to the amount of dehydrogenase, providing an indirect measurement of cell proliferation. As shown in Figure IV.5 when sonochemical method was used, better viability values were obtained with *C. sikamea* and *O. edulis* derived HA powders. In the classical hydrothermal method, *O. edulis* derived TCP powder showed better viability when compared with others and control. Generally, the viability and proliferation of osteoblasts were found to increase considerably in the presence of the investigated powders.

Figure IV.5: Cell viability and proliferation Results of The Products

IV.3.2 Collagen Secretion

Collagen is a very important molecule in the connective tissues and bone. The osteoblasts are one of the cells, which can produce collagen, and it is important to produce it to make connections and perform a scaffold for cells to localize. The results of collagen secretion in the presence of the samples are shown in Figure IV.6

Figure IV.6: Collogen Secretion Results of The Products.

Both synthesis methods yielded better collagen secretion when Pearl powder was used as the raw material. In the hydrothermal synthesis method, *C. sikamea* also showed a good collagen secretion ability when it was sintered at 850°C.

IV.3.3 Alkaline Phosphatase Secretion (ALP)

Alkaline phosphatase is an essential enzyme, which is responsible from calcium and phosphate organization in the mineralized tissues. The enzyme is secreted from osteoblasts to organize the bone matrix.

A- Pearl powder; B- *S.Officinalis*; C- *C.Sikamea*; D- *V.Verrucosa*; E- *O.Edulis*

Figure IV.7: ALP Assay Results.

As can be seen in Figure IV.7 *Sepia officinalis* showed better alkaline phosphatase secretion ability than the other 850°C sintered products. On the other hand, when 450°C sintered powders were compared, *ostrea edulis* showed better results than the others. Generally, the production of alkaline phosphatase was not altered remarkably in the presence of the powders.

PART V

CONCLUDING REMARKS AND RECOMMENDATIONS

V.1 Concluding Remarks

HA and TCP ceramics derived from several types of materials are being used for the coating materials to the orthopedic devices since several years. There are lots of publications about production of those ceramics from several sources. The corals, bovine bones, tooth, are used for those production process, but there are some limitations about those sources. First of all, corals are rare in nature and the growth and reproduction of corals need very long time. They are very essential for the wild life of the oceans, however, their existence is vanishing day by day and therefore, they must be selected as protected species.

Secondly, the bovine bones and tooth which are used as implantation materials may cause several diseases. An important one of those diseases is a prion related disease called BSE (Bovine spongiform encephalopathy). BSE or the human form of the disease called as CJD (Creutzfeldt Jacob Disease) is a long term period prion related disease which cause a spongiform necrotic plaques in the brain of the patients. When a misfolded prion comes into contact with a healthy prion, reportedly, it can sometimes force the normal prion to change shape. The process continues, like a nuclear chain reaction, until the brain is destroyed. It was recently proved beyond doubt that the new variant CJD was a human form of mad cow disease (Goller et al. 2004) . Laboratory studies provide insight into prions' ability to survive in their environment. These agents are especially hardly and remain infectious for long periods of time in a dried state. For example, when buried in soil in a petri dish, half of the original infectivity remained after three years. Formalin-fixed tissue provides the greatest challenge because the chemical increases prions' resistance to heat, making them almost indestructible (Goller et al. 2004).

Products from nacre origin have no risks for the prion related diseases and also they are very highly reproductive raw materials which may be fabricated. They are very interesting and promising materials, which are highly biocompatible, and there are no malfunctions reported about the naces yet. In addition, naces are very cheap materials to find as a raw material so they have economic advantages than the other materials.

Biocompatibility is an important issue for the end use of the produced ceramics. Experimental results of the *in vitro* tests with osteoblasts demonstrated the suitability of the produced bioceramics for biological uses. An important subject of biocompatibility is the orthopedic usage of the biomaterials. Although the implant technologies are developing rapidly the clinical reports about the biomaterial survival is not at the point expected. Implant materials especially hip joint implants have a maximum of 20 years of survival *in-vivo* and they have to be replaced not to poison the host after that time. Another important problem for the implantation is the tissue with the contact of implanted material may become necrotic tissue because of the long time presence of non-compatible materials. Because of that, issue after the extraction of the implant material there may be no existing tissue, which you should fix. With the knowledge of those issues, the clinicians prefer not to implant any materials if the patients are young and if they have any survive chance after 20 years. Because of those issues the implant survival is very important and if the *in-vivo* period of the material may be longed lots of patients with orthopedic problems may have the chance to not to wait for several years before the implantation is performed.

As a final remark, orthopedic research in bioceramics continues to advance at a rapid pace as new techniques and materials are applied to repair damaged musculoskeletal tissues, bone defects, and worn out body parts. Thus, new biomaterials and better scaffolds to target major diseases such as fracture healing, soft tissue repair, osteoporosis, and osteoarthritis continue to be an important research focus. Some of the most exciting new materials, porous ceramic scaffolds for bone repair and bony ingrowth, are now on the market and will soon be common in the orthopedic arena, improving the expected lifespan of implants and other surgical procedures to last for the life of the patient. Eventually, one can expect routine total joint replacement to become a procedure of the past, as tissue engineering and products such as bioceramic scaffolds become commonplace.

V.2 RECOMMENDATIONS

In the light of this study, several recommendations can be drawn for future work related to the calcium phosphate biomaterials derived from raw materials of natural-biological origin.

Firstly, the raw materials used and the bioceramic powders obtained in this study should also be analyzed in terms of their physical properties like density, toughness and porosity not only to improve the methods used for their preparation but also to make a better judgement on their end use.

The mechanical properties like the compressive strength and Young's modulus of the end products should be measured in order to assess their suitability for bone tissue engineering applications.

For the sintering of the biomaterials, a microwave furnace can be used and morphological, mechanical and biocompatibility properties of the bioceramic products can be compared with those of the powders obtained in this study.

Different types of wastes from the food industry can be screened for the presence of valuable biological raw materials such as eggshells and nacre. These wastes can be used for the production of bioceramics in a more environmentally friendly and economical way.

Biomaterials should be tested *in vivo* to understand their effects on the surrounding tissues, organs and blood. The information gained from those investigations can be used for improving the method to control the particle size and the structure of the products.

The biocompatibility results obtained in this study suggested the use of these samples for clinical and tissue-engineering applications therefore further investigations on this issue is also recommended.

Composites of these bioceramic powders can be prepared using biopolymers of various nature such as proteins and polysaccharides. The material properties of these composites can be elucidated to find their potential application areas.

Finally, the products or their composites can be used for drug targeting studies for different types of bone diseases and also their potential use as coating materials for implants and as dental cements should be investigated.

REFERENCES

- [1] Abrams L.; Bajpai P.K.: “Hydroxyapatite ceramics for continuous delivery of heparin”, *Biomed. Sci.Instrum.* 30 (1994) 169–174.
- [2] Arar H.A.; Bajpai P.K.: “Insulin delivery by zinc calcium phosphate (ZCAP) ceramics”, *Biomed.Sci. Instrum.* 28 (1992) 172–178.
- [3] Bajpai P.K.; Fuchs C.M.; Strnat M.A.P.: “Development of Alumino-Calcium Phosphorous Oxide (ALCAP) Ceramic Cements. In: Biomedical Engineering IV Recent Developments.”, *Proceedings of the Fourth Southern Biomedical Engineering Conference*, Pergamon Press, Editors.; Jackson, M.S. and B. Sauer New York, NY (1985) 22–25.
- [4] Bajpai P.K.: “Ceramic drug delivery systems”, *Biomedical Materials Research in The Far East* (I). Kyoto, Japan. (1994) 41–42.
- [5] Bajpai P.K.; Fuchs C.M.; McCullum D.E.: “Development of tricalcium phosphate ceramic cements.”, *Quantitative Characterization and Performance of Porous Implants for Hard Tissue Applications*, American Society for Testing and Materials ASTM STP, J.E. Lemons (Ed.), Philadelphia, PA. 953 (1988) 377–388.
- [6] Bajpai P.K.: “Ceramic amino acid composites for repairing traumatized hard tissues.”, *Handbook of Bioactive Ceramics*, Vol. II: *Calcium Phosphate and Hydroxylapatite Ceramics*. T. Yamamuro, (1990).
- [7] Ben-Nissan B.: “Natural bioceramics: from coral to bone and beyond. Curr Opin Solid State” *Mater Sci*,7 (2003) 283 8.
- [8] Benghuzzi H.A.; Giffin B.F.; Bajpai P.K.; England B.G.: “Successful antidote of multiple lethal infections with sustained delivery of

difluoromethylornithine by means of tricalcium phosphate drug delivery devices.”, Trans. Soc. Biomater. 24 (1991) 53.

- [9] Ben-Nissan B.; Milev A.; Vago R.: “Morphology of sol–gel derived nanocoated coralline hydroxyapatite.”, Biomater 25 (2004) 4971 5.
- [10] Black J.; Garth W. Hastings.: “ Cortical Bone, Organic”, *Handbook of Biomaterial Properties* Chapman and Hall, (1998) 3. (a)
- [11] Black J.; Garth W. Hastings.: “ Structure of Human Dentination”, *Handbook of Biomaterial Properties* Chapman and Hall, (1998) 24.(b)
- [12] C.C. Silva.; H.H.B. Rocha.; F.N.A. Freire.; M.R.P. Santos.; K.D.A. Saboia.; J.C. Góes.; A.S.B. Sombra.: “Hydroxyapatite screen-printed thick films: optical and electrical properties”, Materials Chemistry and Physics, 92 1(2005) 260-268.
- [13] Cold Spring Harbor Protocols “Gene Transfection” (2008), <http://www.cshprotocols.org/cgi/content/full/2006/2/Pdb.prot3871>
- [14] Dominique G. Poitout.; Reinat Kotz.; Karl-Göran Thorngren.: “Biomechanics and Biomaterials in Orthopedics”, (2004) 4.(a)
- [15] Dominique G. Poitout.; Reinat Kotz.; Karl-Göran Thorngren.: “Biomechanics and Biomaterials in Orthopedics”, (2004) 5.(b)
- [16] Donglu Shi.: “General Structure and Chemistry of Hydroxyapatite”, *Introduction to Biomaterials ISBN: 981-259-627-9*, 2nd Edition; Tsinghua University press., (2006) 14.(a)
- [17] Donglu Shi.: “General Structure and Chemistry of Hydroxyapatite”, *Introduction to Biomaterials ISBN: 981-259-627-9*, 2nd Edition; Tsinghua University press., (2006) 21.(b)

- [18] D. Siva Rama Krishna.; A. Siddharthan.; S. K. Seshadri.; T. S. Sampath Kumar.: “A novel route for synthesis of nanocrystalline hydroxyapatite from eggshell waste”., J Mater Sci: Mater Med. 18 (2007) 1735–1743
- [19] Elgun S.Z.: “Polymorphism and Allotropy” (1999)
<http://info.lu.farmingdale.edu/depts/met/met205/allotropy.html>
- [20] F.N. Oktar.: “Microstructure and mechanical properties of sintered enamel hydroxyapatite”., Ceramics International 33 (2007) 1309–1314.
- [21] Goller G.; F. N. Oktar.; S. Agathopoulos.; D. U. Tulyaganov.; J. M. F. Ferreira.; E. S. Kayali.; I. Peker.: “Effect of sintering temperature on mechanical and microstructural properties of bovine hydroxyapatite (BHA)”, J Sol-Gel Sci Techn, 37 (2006) 111–115.
- [22] Goller G.; Oktar F.N.; Ozyegin L.S.; Kayali E.S.; Demirkesen E.: “Plasma-Sprayed Human Bone-Derived Hydroxyapatite Coatings: Effective and Reliable”, Mater Lett, 58 (2004) 2599-604.
- [23] Goller G.; Oktar F.N.: “Sintering Effects on Mechanical Properties of Biologically Derived Dentine Hydroxyapatite.”, Mater Lett 56 (2002) 142 7.
- [24] Goller. G.; Faik Nüzhet Oktar.: “Sintering effects on mechanical properties of biologically derived dentine hydroxyapatite”, Materials Letters, 56 3(2002) 142-147.
- [25] Gianluca Plotino.; Nicola M. Grande.; Rossella Bedini.; Cornelis H. Pameijer.; Francesco Somma.: “Flexural properties of endodontic posts and human root dentin”, Dental Materials, 23, 9,(2007) 1129-1135.
- [26] Graves G.A.; Jr, Hentrich R.L.; Jr, Stein H.G.; Bajpai P.K.: “Resorbable Ceramic implants in bioceramics.”, In: *Engineering and Medicine (Part I)*. Interscience Publishers New York, C.W. Hall.; S.F. Hulbert.; S.N. Levine.; F.A. Young. (1972) 91–115.

- [27] Hench L.L.: “Bioceramics: From concept to clinic.”, *J. Am. Ceram. Soc.* 74 (1991) 1487–1510.
- [28] Hench L.L. “Bioceramics: From concept to clinic.”, *Am. Ceram. Soc. Bull.* 72 (1993) 93–98.
- [29] Hentrich R.L.; Jr, Graves G.A.; Jr, Stein H.G.; Bajpai P.K.: “An evaluation of inert and resorbable ceramics for future clinical applications.”, *J. Biomed. Mater. Res.* 5 (1971) 25–51.
- [30] Hentrich R.L. Jr Graves G.A.; Jr Stein H.G.; Bajpai P.K.: “An evaluation of inert and resorbable ceramics for future clinical applications.”, Fall Meeting, Ceramics-Metals Systems, Division of the American Ceramic Society, Cleveland, Ohio.(1969).
- [31] In-Young Ryu1.; Dae-Joon Kim.; Jung-Suk Han.; Myung-Hyun Lee.: “Influence of Two-Step Sintering Variables on Phase Stability of Hydrothermally Prepared Hap Nano Powders.”, *Key Engineering Materials*, 361-363 (2008) 91-94.
- [32] Jarcho M.; Salsbury R.L.; Thomas M.B.; and Doremus R.H.: “Synthesis and fabrication of β -tricalcium phosphate (whitlockite) ceramics for potential prosthetic applications”. *J. Mater. Sci.* 14 (1979) 142–150.
- [33] Jeremy Webb.: “Surface energy – meaning”, *School of Biological Sciences University of Manchester* (1998),
<http://www.bio.net/bionet/mm/biofilms/1998-September/000364.html>
- [34] Joseph D. Bronzino.: “Ceramic Biomaterials, Introduction”, *Biomedical Engineering Fundamentals*, 3rd Edition Taylor & Francis, (2006) 39-1.
- [35] Joyce Y. Wong.; Joseph D. Bronzino.: “Resorbable Ceramics”, *Biomaterials*, CRC Press. (2007) 2-2. (a)

- [36] Joyce Y. Wong.; Joseph D. Bronzino.: “Bioceramic Manufacturing Techniques.”, *Biomaterials*, CRC Press. (2007) 2-23. (b)
- [37] Kannan S.; Rocha J.H.G.; Agathopoulos S.; Ferreira J.M.F.: “Fluorine-substituted hydroxyapatite scaffolds hydrothermally grown from aragonitic cuttlefish bones”, *ACTA BIOMATERIALIA*, 3 2 (2007) 243-249.
- [38] Kenneth S.; Vecchio .; Xing Zhang .; Jennifer B.; Massie .; Mark Wang .; Choll W. Kim.: “Conversion of sea urchin spines to Mg-substituted tricalcium phosphate for bone implants”, *Acta Biomaterialia* (2007)
- [39] Kingery W.D.; Bowen H.K.; Uhlmann D.R.: “Introduction to Ceramics”, 2nd ed John Wiley NewYork., (1976) 368.
- [40] Kurkcu M.; Benlidayi M.E.; Ozsoy S.; L.S Ozyegin.; Oktar F.N.; Kurtoglu C.: “Histomorphometric Evaluation of Implants Coated with Enamel and Dentine Derived Hydroxyapatite”, *J Mater Sci: Mater in Med*, 19 (2008) 59-65.
- [41] Lemos.A.F.; J.H.G. Rocha.; S.S.F. Quaresma.; S. Kannan.; F.N. Oktar.; S. Agathopoulos.; J.M.F. Ferreira.: “(HA) nano-powders produced hydrothermally from nacreous material”, *Journal of the European Ceramic Society*, 26 16 (2006) 3639-3646
- [42] Lemons J.E.; Niemann K.M.W.: “Porous Tricalcium Phosphate Ceramic for Bone Replacement.”, 25th Annual O.R.S., Meetings, San Francisco, CA, February, (1979) 162.
- [43] Medical devices in Scotland.; Glossary.: Biomaterials(2008)
<http://mdis.org/library/glossary/B.htm>

- [44] Metsger S.; Driskell T.D.; and Paulsrud J.R.: “Tricalcium phosphate ceramic A resorbable bone implant: review and current status.” *JADA* 105 (1982) 1035–1038.
- [45] Moldovan K.; Bajpai P.K.: “A ceramic system for continuous release of aspirin.”, *Biomed. Sci. Instrum.* 30 (1994) 175–180.
- [46] Morris L.M.; Bajpai P.K.: “Development of a resorbable tricalcium phosphate (TCP) amine antibiotic composite.”, *Mat. Res. Soc. Symp.* 110 (1989) 293–300.
- [47] Nagy E.A.; Bajpai P.K.: “Development of a ceramic matrix system for continuous delivery of azidothymidine.”, *Biomed. Sci. Instrum.* 30 (1994) 181–186.
- [48] Northwestern University Department of Biochemistry, Molecular Biology and Cell Biology Holmgren Lab.; Mark Lefers.: “Definition: Xenograft”, (2004)
<http://www.biochem.northwestern.edu/holmgren/Glossary/Definitions/Def-X/Xenograft.html>
- [49] Oktar FN.; Valerio P.; Goller G.; Agathopoulos S.; Goes AM.; Leite MF.: “Biocompatibility of The Outer Prismatic and The Inner Nacreous Layers of Four Different Molluscs”, *Key Engineer Mater*, (2006); 309-311: 449-52
- [50] Oktar FN.: “Microstructure and mechanical properties of sintered enamel hydroxyapatite”, *Ceram Int*, 33 (7) (2007) 1309-14.
- [51] Ozyegin L. S.; Faik N. Oktar.; Gultekin Goller.; E. Sabri Kayali.; Tokay Yazici.: “Plasma-sprayed bovine hydroxyapatite coatings”, *Materials Letters* 58 (2004) 2605– 2609.

- [52] Park J.B.; Roderic S. Lakes.: “Calcium Phosphate”, *Biomaterials an Introduction*, 2nd Edition.; Park, Joon B.; Lakes, Roderic S.;Plenum press., New York and London, (1992) 122. (a)
- [53] Park J.B.; Roderic S. Lakes.: “Structure of Calcium Phosphate”, *Biomaterials an Introduction*, 2nd Edition.; Park, Joon B.; Lakes, Roderic S.;Plenum press., New York and London, (1992) 122. (b)
- [54] Park J.B.; Roderic S. Lakes.: “Structure of Calcium Phosphate”, *Biomaterials an Introduction*, 2nd Edition.; Park, Joon B.; Lakes, Roderic S.;Plenum press., New York and London, (1992) 123. (c)
- [55] Park J.B.; Roderic S. Lakes.: “Properties of Hydroxyapatite”, *Biomaterials an Introduction*, 2nd Edition.; Park, Joon B.; Lakes, Roderic S.;Plenum press., New York and London, (1992) 123. (d)
- [56] Parker D.R.; Bajpai P.K.:“Effect of locally delivered testosterone on bone healing” *Trans. Soc. Biomater.* 26 (1993) 293.
- [57] Peltier L.F.: “The use of plaster of Paris to fill defects in bone.”, *Clin. Orthop.* 21 (1961) 1–29.
- [58] Richard Zallen.: “Amorphous solid” (13.03.2008)
<http://www.britannica.com/eb/article-9110300/amorphous-solid>
- [59] Rocha J.H.G.; Lemos A.F.; Agathopoulos S.; Valeiro P.; Kannan S.; Oktar F.N.; Ferreira J.M.F.: “Scaffolds for Bone Restoration from Cuttlefish.”, *Bone*, 37 (2005) 850-7.
- [60] Scott A. Guelcher.; Jeffrey O. Hollinger.: “Definition of Ceramics” *An Introduction to Biomaterials*, Taylor & Francis,(2006) 312.
- [61] Seers training web site, Anatomy.: “Structure of Bone Tissue”,(2008)
http://training.seer.cancer.gov/module_anatomy/unit3_2_bone_tissue.html

- [62] S. Daglilar.; M.E. Erkan.; O. Gunduz.; L.S. Ozyegin.; S. Salman.;S.Agathopoulos., F.N. Oktar.: “Water resistance of bone-cements reinforced with bioceramics”, Materials Letters 61 (2007) 2295–2298.
- [63] Stanislav S. Dukhina.; Ralf Zimmermann.; Carsten Werner.: “Electrokinetic phenomena at grafted polyelectrolyte layers”, Journal of Colloid and Interface Science., 286, 2, (2005) 761-773.
- [64] Sujata V.BHAT.: “Biomaterials” ISBN: 0-7923-7058-9 (2002) 13, http://books.google.com/books?id=P0M_eYBRukAC&dq=biomaterials&hl=t&r&source=gbs_book_other_versions_r&cad=1_1 (04.03.2008). (a)
- [65] Sujata V.BHAT.: “Biomaterials” ISBN: 0-7923-7058-9 (2002) 14-15, http://books.google.com/books?id=P0M_eYBRukAC&dq=biomaterials&hl=t&r&source=gbs_book_other_versions_r&cad=1_1 (04.03.2008). (b)
- [66] T. M. Coelho.; E. S. Nogueira.; A. Steimacher.; A. N. Medina.; W. R. Weinand.; W. M. Lima.; M. L. Baesso.; A. C. Bento.: “Characterization of natural nanostructured hydroxyapatite obtained from the bones of Brazilian river fish”, Journal of Applied Physics 100, 094312 (2006).
- [67] The Structure of Pearls. JJ Kent Inc.: (2004)
<http://www.jjkent.com/articles/structure-pearl.htm>
- [68] Tuyel U.; Ebru Toksoy Öner.; Sevgi Özyegin .; Faik N.Oktar.: “Production and characterization of bioceramic nanopowders of natural-biological origin.”, Journal of Biotechnology 131 (2007) 65 68.
- [69] Ulusoy U.; C. Hiçyılmaz.;M. Yekeler.: “Role of shape properties of calcite and barite particles on apparent hydrophobicity” Chemical Engineering and Processing., 43, 8, (2004) 1047-1053.

- [70] Vanrecum,A,F.; La Berge,M.: “Education Goals for Biomaterials Science and Engineering perspective View” Journal of Applied Biomaterials, 6 (1995) 137-144.
- [71] Wantae Kim and Fumio Saito.: “Sonochemical synthesis of **hydroxyapatite** from H_3PO_4 solution with $\text{Ca}(\text{OH})_2$ ”, Ultrasonics Sonochemistry 8, 2 , (2001), 85-88
- [72] Wikipedia.: “Biocompatibility”, (2008)
<http://en.wikipedia.org/wiki/Biocompatibility>
- [73] Wikipedia.: “Bone, Trabecular Bone”,(2008)
<http://en.wikipedia.org/wiki/Bone>
- [74] Wikipedia.: “Calcium phosphate” (2008)
http://en.wikipedia.org/wiki/Calcium_phosphate
- [75] Wikipedia.: “Contact angle” (2008)
http://en.wikipedia.org/wiki/Contact_angle
- [76] Wikipedia.: “Fluorapatite” (2008)
<http://en.wikipedia.org/wiki/Fluorapatite>
- [77] Wikipedia.: “Fluoride poisoning” (2008)
http://en.wikipedia.org/wiki/Fluoride_poisoning
- [78] Wikipedia.: “Tricalcium phosphate Uses” (2008)
http://en.wikipedia.org/wiki/Tricalcium_phosphate
- [79] Wikipedia.: “Tricalcium phosphate General Cases and Uses” (2008)
http://en.wikipedia.org/wiki/Tricalcium_phosphate

- [80] Z. E. Erkmen.; Y. Genç.;F. N. Oktar.: “Microstructural and Mechanical Properties of Hydroxyapatite–Zirconia Composites”, J. Am. Ceram. Soc., 90 [9] (2007) 2885–2892

Appendix A

FT-IR Results of The Products.

HA Hotplate (Pearl Powder)

TCP Hotplate (Pearl Powder)

HA Ultrasound (Pearl Powder)

HA Hotplate (Cuttlefish bone)

TCP Ultrasound (Pearl Powder)

HA Ultrasound (Cuttlefish bone)

TCP Hotplate (Cuttlefish bone)

TCP Hotplate (*C.Sikamea*)

TCP Ultrasound (Cuttlefish bone)

TCP Ultrasound (*C.Sikamea*)

HA Hotplate (*C.Sikamea*)

HA Hotplate (*V.Verrucosa*)

HA Ultrasound (*C.Sikamea*)

HA Ultrasound (*V.Verrucosa*)

TCP Hotplate (*V. Verrucosa*)

TCP Hotplate (*O. Edulis*)

TCP Ultrasound (*V. Verrucosa*)

TCP Ultrasound (*O. Edulis*)

HA Hotplate (*O. Edulis*)

HA Ultrasound (*O. Edulis*)

Appendix B

XRD Results of The Products.

HA Ultrasound (*Cuttlefish bone*)

HA Ultrasound (*O.Edulis*)

HA Ultrasound (*C.Sikamea*)

TCP Hotplate (*Pearl Powder*)

HA Ultrasound (*V.Verrucosa*)

TCP Hotplate (*Cuttlefish bone*)

TCP Hotplate (*C.Sikamea*)

TCP Ultrasound (*Pearl Powder*)

TCP Hotplate (*V.Verrucosa*)

TCP Ultrasound (*Cuttlefish bone*)

TCP Hotplate (*O.Edulis*)

TCP Ultrasound (*C.Sikamea*)

TCP Ultrasound (*V.Verrucosa*)

TCP Ultrasound (*O.Edulis*)

Appendix C

SEM Images of The Products

(Pearl Powder Hotplate HA)

(Cuttlefish Bone Hotplate HA)

(*V.Verrucosa* Hotplate HA)

(*C.Sikamea* Hotplate HA)

(*O. Edulis* Hotplate HA)

(Cuttlefish Bone Ultrasound HA)

(Pearl Powder Ultrasound HA)

(*V. Verrucosa* Ultrasound HA)

(*C. Sikamea* Ultrasound HA)

(*O. Edulis* Ultrasound HA)

(Cuttlefish Bone Hotplate TCP)

(Pearl Powder Hotplate TCP)

(*C.Sikamea* Hotplate TCP)

(*O.Edulis* Hotplate TCP)

(*V.Verrucosa* Hotplate TCP)

(Pearl Powder Ultrasound TCP)

(Cuttlefish Bone Ultrasound TCP)

(*C.Sikamea* Ultrasound TCP)

(*O.Edulis* Ultrasound TCP)

(*V.Verrucosa* Ultrasound TCP)

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Publications

- Umut Tüyel., Ebru Toksoy Öner., L. Sevgi Özyeğin., Faik N. Oktar., Production and Characterization of Bioceramic Nanopowders of Natural-Biological Origin. ECB13 European Congress on Biotechnology 16-19 September 2007, Barcelona, Spain. [Poster Presentation]
- Umut Tüyel., Ebru Toksoy Öner., L. Sevgi Özyeğin., Faik N. Oktar., Doğal Yapılardan Nano-Biyoseramik Tozların Üretilmesi ve Karakterizasyonu. 15th National Biotechnology Conference, 28-31 October 2007, Antalya Turkey[Oral Presentation]
- Umut Tüyel., Ebru Toksoy Öner., L. Sevgi Özyeğin., Faik N. Oktar., A new use for Cuttlefish bone; Production of TCP by hot-plate method. 5th International Conference on Nanosciences and Nanotechnologies, 14-16 July 2008, Thessaloniki, Greece. [Poster Presentation]