IMPACT OF DIFFERENT ULTRASONIC FREQUENCIES ON PRECIPITATION PROCESS OF DICALCIUM PHOSPHATE DIHYDRATE COATED HYDROXYAPATITE GRANULAR SURFACES

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DECLARATION

I hereby declared that I have conducted, completed the research work and written the dissertation entitles: "**Impact of Different Ultrasonic Frequencies on Precipitation Process of Dicalcium Phosphate Dihydrate Coated Hydroxyapatite Granular Surface**". I also declared that it has not been previously submitted for the award for any degree or diploma or other similar title of this for any other examining body or University.

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LIST OF ABBREVIATIONS

Al_2O_3	Alumina
APS	Atmospheric plasma spraying
ASD	Anodic spark deposition
CaCO3	Calcium carbonate
CaPs	Calcium phosphate
CDHA	Calcium deficient hydroxyapatite
cd-HAp	Calcium-deficient hydroxyapatite
Co	Cobalt
CP	Calcium phosphate
Cr	Chromium
DCPA	Anhydrous dicalcium phosphate
DCPD	Dicalcium phosphate dihydrate
DFDBA	Demineralized freeze-dried bone allografts
ECM	Extracellular matrix
ED	Electrodeposition
EDX	Energy Dispersive X-ray
EPD	Electrophoretic deposition
FDBA	Freeze-dried bone allografts
Fe	Iron
FTIR	Fourier Transform Infrared Spectroscopy

GF	Growth factors
HAp	Hydroxyapatite
HBSS	Hank’s Balanced Salt Solution
Ksp	Solubility product constant
MAO	Micro arc oxidation
MCPM	monocalcium phosphate monohydrate
Mo	Molybdenum
Nb	Niobium
Ni	Nickel
OCP	Octacalcium phosphate
PCL	Polycaprolactone
PLA	Polylactic acid
PLD	Pulsed laser deposition
PS	Plasma spraying
PSA	Particle Size Analysis
SEM	Scanning Electron Microscope
Ta	Tantalum
TCP	Tricalcium Phosphate
TTCP	tetracalcium phosphate
TZ	Yttria-stabilized zirconia
UV-Vis	Ultraviolet-visible infrared spectroscopy
W	Tungsten
WPS	Wet powder spraying

XRD	X-Ray Diffraction
ZrO2	Zirconia oxide
α-TCP	Alpha-tricalcium phosphate
β-TCP	Beta-tricalcium phosphate

KESAN FREKUENSI ULTRASONIK YANG BERBEZA TERHADAP PEMBENTUKAN SALUTAN DIKALSIUM FOSFAT DIHIDRAT (DCPD) PADA PERMUKAAN GRANUL HIDROKSIPATIT

ABSTRAK

Projek ini membentangkan proses pembentukan salutan dikalsium fosfat dihidrat (DCPD) pada permukaan granul hidroksipatit menggunakan kaedah larutan-mendapan dengan bantuan getaran frekuensi tunggal (360kHz) dan frekuensi berganda (58/132 kHz) menggunakan mesin ultrasonik (creast ultrasonic). Pembentukkan tulang baru bagi hidroksiapatit perlahan berbanding pembentukkan tulang asli. Oleh yang demikian, salutan DCPD dicadangkan pada permukaan hidroksiapatit untuk meningkatkan kadar pembentukkan tulang baru. Kaedah baru salutan DCPD menggunakan teknologi ultrasonik untuk memperoleh lapisan salutan dalam tempoh masa yang singkat, kos yang rendah dah berkesan berbanding kaedah lain yang mengambil masa yang lama dan memperuntukkan suhu yang tinggi untuk memperoleh lapisan salutan CaP. Beberapa kaedah pencirian seperti Pembelauan Sinar-X (XRD), Mikroskop Elektron Imbasan (SEM), Tingkah Laku Pembubaran dan analisis pH telah dijalankan terhadap granul hidroksiapatit dengan salutan dikalsium fosfat dihidrat untuk memahami sifat-sifat spesimen. Hasil daripada kaedah pencirian menunjukkan bahawa jumlah kristal DCPD yang terbentuk pada permukaan granul hidroksiapatit meningkat apabila kepekatan kalsium fosfat berasid ditingkatkan pada frekuensi tunggal dan frekuensi berganda. Kepekatan kalsium fosfat berasid bertambah (0.025M,0.050M,0.075M,0.100M) maka jumlah pembentukkan kristal DCPD juga meningkat. Walau bagaimanapun, frekuensi tunggal memperoleh pembentukkan salutan DCPD yang tinggi berbanding frekuensi

berganda. Justeru, kajian ini menunjukkan bahawa jumlah penghasilan salutan DCPD pada permukaan granul hidroksiapatit menggunakan frekuensi ultrasonik dapat dikawal dengan mengubah kepekatan larutan kalsium fosfat berasid atau jenis frekuensi.

IMPACT OF DIFFERENT ULTRASONIC FREQUENCIES ON PRECIPITATION PROCESS OF DICALCIUM PHOSPHATE DIHYDRATE COATED HYDROXYAPATITE GRANULAR SURFACES

ABSTRACT

This project presents the fabrication of DCPD-coated HAP granules using the dissolution-precipitation method with the aid of sonication of single frequency (360kHz) and double frequency (32/158kHz) using the ultrasonic machine (crest ultrasonic). The new bone formation rate for hydroxyapatite is relatively slow compared to the growth of the natural bone, especially in a large bone defect area. Therefore, DCPD layer coating is proposed on the surface of HAp granules to increase the new bone formation rate. A new approach on DCPD coating using ultrasonic technology to obtain a coating layer in a short period, low cost and effective compared to other methods that are time consuming and involve high temperature to obtain CaP coating layer. Several characterisation methods were carried out, such as X-Ray Diffraction (XRD), Scanning Electron Microscope (SEM), dissolution behaviours of calcium ions, and pH analysis. The results of characterisations showed that the amount of DCPD crystals precipitated on the HAp granule surface increased as the acidic calcium phosphate solution concentration increased for single and double frequencies. As the concentrations of acidic calcium phosphate increased (0.025M,0.050M,0.075M,0.100M), the DCPD formation also increased. However, single frequency produced higher DCPD formation than double frequency. Therefore, this study showed that the amount of DCPD coated on HAp granule surfaces could be regulated by changing the concentration of acidic calcium phosphate solution or type of ultrasonic frequency.

CHAPTER 1

INTRODUCTION

1.1 Background of Research

Bioceramics, as reported by Ginebra et al., 2018 is essential and plays a significant role in bone tissue engineering and can be utilised as a vehicle for local delivery of active ions due to its ability to trigger specific biological responses. Calcium phosphate (CaP) bioceramics have been utilised to replace and encourage new bone production as a bone substitute material. As reported by Kucko et al., 2019 CaP compounds contain Ca^{2+} and PO_4^{3-} ions that exist naturally in the inorganic mineral phase of teeth and bones that have excellent biological properties as a bone substitute material for dental and orthopaedic applications. Hydroxyapatite (HAp) is one of the most biocompatible synthetic biomaterials used as bone transplants. HAp has excellent biocompatibility with hard tissues and high osteoconductivity and bioactivity (Bizari et al., 2014). However, the new bone formation rate for bone substitute materials that is hydroxyapatite is relatively slow compared to the growth of the natural bone, especially in a large bone defect area. Therefore, DCPD layer coating is proposed on the surface of HAp granules to increase the new bone formation rate. A previous study showed that initiating an appropriate amount of DCPD coating layer on the β-TCP granular through the dissolution-precipitation process can stimulate the new bone formation rate (Shariff et al., 2017). Likewise, Fukuda et al., 2019 reported that the formation of the DCPD layer on β-TCP granular cement shows promise in the reconstruction of bone defects due to β-TCP granules bridge with dicalcium phosphate dihydrate (DCPD) crystals (Fukuda et al., 2019). Moreover, there is no study has ever been reported to understand the effect of ultrasonic frequency in inducing precipitation of DCPD on bioceramic materials.

Therefore, the effect of different frequencies in inducing the coating process of hydroxyapatite granules will be elaborated in this project.

1.2 Problem Statement

HAp bioceramic granules have been used as bone substitute material in clinical application because it shows a good bone formation rate after being implanted in the bone defect. However, its new bone formation rate is relatively slow compared to the growth of the natural bone, especially in a large bone defect. If the new bone formation rate of HAp bioceramic granules can be enhanced, the range of its clinical application will be increased. Therefore, another approach should be identified to improve the new bone formation rate of HAp bioceramic granules. DCPD coating technique on the HAp granular surfaces is crucial to understand its effectiveness when implanted in the bone defect area.

Eliaz et al., 2017 reported that variety of methods can produce synthetic CaP coatings, including plasma spraying (PS), high-velocity oxygen-fuel (HVOF) thermal spraying, sputter coating, radio-frequency magnetron sputtering, pulsed laser deposition (PLD), ion-beam deposition, frit enamelling, hot isostatic pressing (HIP), metallo-organic chemical vapour deposition (CVD), derivation from sol-gels, electrophoretic deposition (EPD), chemical deposition and electrodeposition (ED). In addition, several approaches for DCPD coating technique on biomedical Mg alloy were fabricated through chemical deposition, anodic oxidation, electroless plating, biomimetic approach and conversion coating (Cheng et al., 2014). Drawback of one of these techniques, it is harder to obtained good CaP coating by process that involved high temperature and the crystalinity are hard to control (Eliaz et al., 2017). Biomimetic approach is also low-cost process of inducing CaP coating but the time

taken for the coating process is time consuming and took days to obtained (Eliaz et al., 2017).

A previous study reported by Khairul et al. stated that the DCPD coating layer on the β-TCP granular surface was formed using the shaking technique and dissolution-precipitation process (Shariff et al., 2017). Although this method successfully coated the β-TCP granular surface with DCPD, a more practical and cost-effective coating method should be implemented when scaling up this material to an extensive production process. Furthermore, sonochemical synthesis is a low-cost, rapid, and straightforward approach and it has been demonstrated that the sonochemical process is a very promising coating technology (Gedanken et al., 2018). Therefore, to meet this expectation, ultrasonic technology was introduced in this study to see whether this method efficiently produces a similar amount of DCPD coating in a short period. It was expected that this study would reveal the impact of different ultrasonic frequencies in fabricating the DCPD coating layer on HAp granular surfaces. The findings from this research will give a new insight to all bioceramic researchers' regarding the potential of ultrasonic technology in fabricating DCPD coating layers on bioceramic granules.

1.3 Research objectives

The objective of this project is:

1. To investigate the effect of DCPD coating on HAp granules using single frequency in DCPD fabrication process.
2. To observe the effect of DCPD coating on HAp granules using double frequencies in the DCPD fabrication process.

1.4 Research Approach

This project presents the fabrication of DCPD-coated HAP granules using the dissolution-precipitation method with the aid of sonication of different frequencies from an ultrasonic bath. The amount of DCPD coating on HAP granule depended on the concentrations of MCPM during the exposure process. The fabrication of HAp granule starts with mixing calcium carbonate powder with DCPD powder according to the calcium to phosphate ratio (Ca/P) of 1.67 for HAp granule. By utilising a planetary ball milling machine for 6 hours, calcium carbonate and DCPD are mixed homogenously in a wet milling process with ethanol 95% as the medium. After that, the mixture was dried in the oven at 60°C for one hour. The dried mixture was then pressed into a compacted form of powder, a pellet, using a hand press machine at 50 MPa. These pellets underwent a sintering process for 6 hours at 1000℃ for HAp. The sintered sample was then crushed with a mortar and pestle before the sieving process that obtained the granules with granular size between 600-1000 μm. Preparation of different concentrations of acidic calcium phosphate solution begins with different concentrations of MCPM powder that are subject to be dissolved in a 0.025 mol/L diluted phosphoric acid. Different amount of MCPM to obtain 0.025 mol/L, 0.050 mol/L, 0.075 mol/L and 0.100 mol/L concentration of acidic calcium phosphate solution. HAP granules were exposed to different acidic calcium phosphate solution concentrations throughout the coating process in a glass beaker. The glass beakers were then placed in the ultrasonic bath and sonicated using single (360kHz) and double (58/132kHz) frequencies for 30 minutes. The samples were then filtrated and rinsed with acetone before being dried in an oven at 60℃ for 1 hour. Specimen characterisation XRD and SEM were conducted for compositional analysis and surface morphology. pH analysis was conducted to observe the pH

value by immersing the samples in HBSS solution for 1,3, and 7 days at room temperature and measured using a pH meter. Dissolution behaviour was done to observe calcium ions released by immersing the samples in HBSS solution for 1,3, 5 and 7 days at room temperature and measured using a calcium ion meter. The procedure for fabrication of DCPD-coated HAP granules and its characterisation were summarised in Figure 1.1.

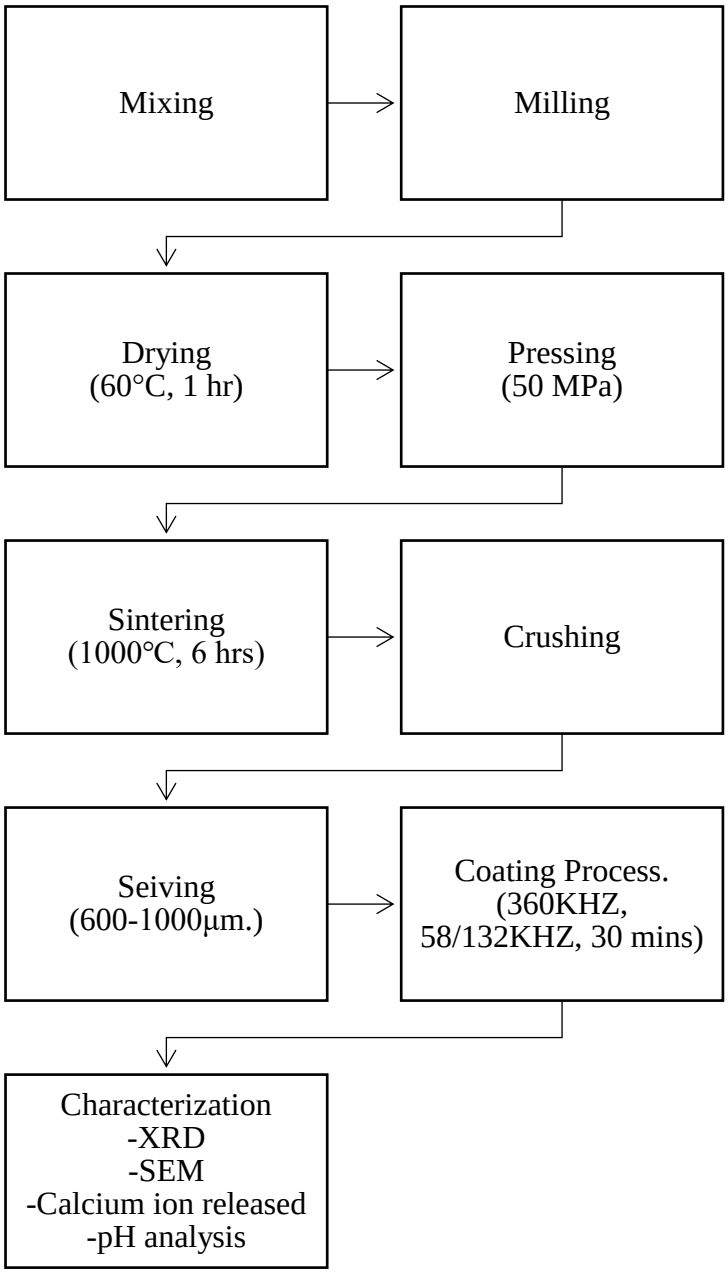

Figure 1.1: Fabrication process of DCPD-coated HAp granules and its characterisation

CHAPTER 2

LITERATURE REVIEW

2.1 Classification of biomaterials

Biomaterials come in two different varieties, natural and synthetic. It is a synthetic chemical designed to interact with several biological system components. Typically, biomaterials are created for use in therapeutic environments. Their main duties are assisting people in getting well and restoring function after illnesses or injuries (Parida et al., 2012). The separation of biomaterials into distinct categories is summarised in Figure 2.1 below.

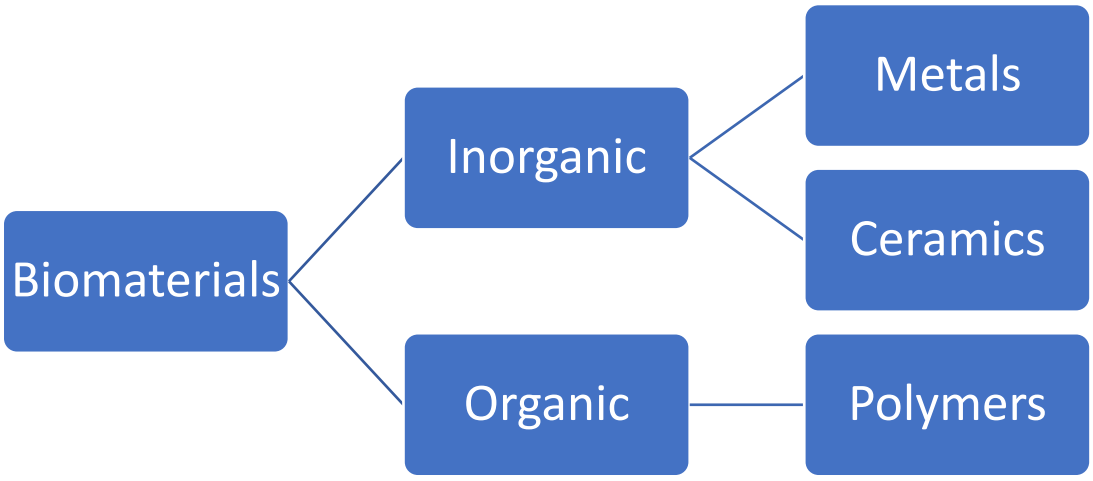

Figure 2.1:Biomaterials (Parida et al., 2012).

2.1.1 Metallic biomaterials

The most frequent metals used for implants are tungsten (W), tantalum (Ta), nickel (Ni), titanium (Ti), chromium (Cr), cobalt (Co), niobium (Nb), and iron (Fe) (W). However, our bodies can only withstand very little amounts of metallic implants since they can corrode in a physiological setting. The rusting parts destroy our body tissue and organs (Parida et al., 2012).

2.1.2 Ceramic biomaterials

There are three types of ceramic materials used to make implants: inert, semi-inert, and non-inert. Non-absorbable ceramics like carbon, silicon nitrides, zirconia, and alumina are examples of inert bioceramics. Certain glass ceramics are among the bioactive ceramics known as semi-inert bioceramics. Ceramics that can be reabsorbed and biodegraded are classified as non-inert bioceramics. Resorbable ceramics include, for example, calcium phosphates and calcium aluminates (Parida et al., 2012).

2.1.3 Polymeric biomaterials

For use in medical applications such as dental materials, implants, prosthetic materials, and tissue engineered goods, synthetic polymeric biomaterials are produced. The benefit of being easily produced in a variety of shapes is a property of polymeric biomaterials. Materials like polyethylene, polypropylene, polyvinyl chloride, and polymethyl methacrylate are examples of polymeric biomaterials (Parida et al., 2012).

2.1.4 Composite biomaterials

Composite biomaterials differ significantly from their base materials in terms of their physical characteristics. The interface of the composite cannot be resorbable by the body, and each component must be biocompatible. Applications like orthopaedic implants, bone cement, composite dental fillings, and others frequently use composite biomaterials (Parida et al., 2012).

2.2 Biomaterials for bone regeneration in tissue engineering

Biomaterials having desired characteristics such as biocompatibility, biodegradability, and osteoconductivity have been conceived and developed to better function as bone transplants (Yu et al., 2015). Clinically used bone transplants can alternatively be classed as biological (autografts, allografts, and xenografts) or synthetic (Yu et al., 2015). Autograft refers to a chunk of bone removed from the patient's own body and transplanted in another place of the same patient. They have exceptional osteogenic, osteoconductive, and osteoinductive qualities and generate no immunogenic reaction, making them the gold standard for bone repair (Yu et al., 2015).

2.2.1 First generation biomaterials in bone regeneration

In the 1960s, the first generation of biomaterials was created to «match the repaired tissue's physical properties with minimal host toxicity». These materials are biologically inert and have minimal interaction with human tissue. They did not stimulate bone development, but they did generate fibrous tissue. The first successful joint prosthesis for Charnley was made of stainless steel (Yu et al., 2015). Chromium imparts corrosion resistance to stainless steel. Cobalt-chromium alloys were created to enhance the wear resistance of stainless steel. These materials are corrosion and wear resistant. Their modulus of elasticity was tenfold that of human cortical bone (Yu et al., 2015). The high modulus of the implant protects the adjacent bone from stress. Bone resorption and implant failure resulted from a lack of mechanical stimulation.

In the 1940s, Branemark invented osseointegration for implants, which is the direct connection between a load-bearing implant and host bone tissue without the growth of soft tissue. He demonstrated that titanium implants may become

irreversibly incorporated into bone, rendering them incapable of being removed without fracture (Yu et al., 2015). Surfaces etched with acid improved titanium implant integration following insertion. This improved the device's long-term performance, lowering implant loosening and failure. Charnley developed poly methyl methacrylate (PMMA) bone cement for prosthesis fastening of the femoral head. Due of its inert nature, PMMA may be effective for initial fixation but not for subsequent biological fixation (Yu et al., 2015). Ultrahigh molecular weight polyethylene was also utilised for arthroplasties due to its high abrasion resistance, low friction, unparalleled toughness, ease of production, and biocompatibility. The combination of irradiation for sterilisation and oxygen results in oxidative degradation, which decreases wear resistance and mechanical characteristics (Yu et al., 2015).

2.2.2 Second generation biomaterials in bone regeneration

The biomaterials of the second generation included synthetic and naturally derived biodegradable polymers, calcium phosphates, calcium carbonate, calcium sulphates, and bioactive glasses (Yu et al., 2015).. Compared to synthetic polymers, naturally generated polymers such as collagen and hyaluronic acid can provide innate biological informational guidance to cells, resulting in superior cell attachment and chemotactic responses. Larry Hench invented the first artificial bioactive substance, Bioglass, in 1969. Bioglass was the first synthetic osseointegrative substance designed to make a direct chemical contact with bone (Yu et al., 2015).

Midway through the 1980s, the use of "bioactive materials" in a variety of dental and orthopaedic applications was introduced with the intention of producing bioactive components that may elicit a beneficial biological response in the physiological environment (Yu et al., 2015). In addition, the requirement for materials with particular physical, chemical, biological, biomechanical, and degrading qualities led to the development of biodegradable materials. The phrase "bioactive material" refers to a substance that, when introduced within the human body, interacts with the surrounding tissue to form a bond by triggering a specific biological response at the material interface. Albee and Morrison reported the first application of calcium phosphates for bone healing in 1920 (Yu et al., 2015).

The chemical formula and Ca/P ratio of calcium phosphate ceramics such as hydroxyapatite, tricalcium phosphate, and octacalcium phosphate vary. TCP has a Ca/P ratio of 1.5 and a rapid dissolving rate that speeds up material absorption. The biological apatites, such as bone mineral, dentine, and tooth enamel, contain multiple replacements with 2-hydrogenophosphate and carbonate, etc., which confers unique biological, functional, and chemical properties (Yu et al., 2015). Calcium phosphate bioceramics have better properties for stimulating bone growth and bone bonding due to their compositional similarity with bone mineral (Yu et al., 2015).

2.2.3 Third generation biomaterials in bone regeneration

Biomaterials of the third generation are intended to integrate instructional signals to produce desirable biological responses, such as enhanced cell survival, directed cell differentiation, and lineage commitment (Yu et al., 2015). To enhance material/cell interactions, nutrient/oxygen infiltration, and vascularization, materials

with the right physical properties, such as high porosity and interconnectivity, have been created and engineered. It was proven that these matrices regulate oxygen-tension within the pore structure, allowing the matrix to sustain osteogenic and vasculogenic cell survival even deep within the matrix pore structure, so promoting large-area bone regeneration (Yu et al., 2015). The usage of nanocrystalline calcium phosphates, which exhibit faster breakdown and better bone cell activities compared to micron grain size calcium phosphate, is an example of bone tissue engineering using the exceptional features of nanomaterials (Yu et al., 2015).

2.3 Design of biomaterials for bone regenerative engineering

The bone is the basic and essential part of the human skeletal system. Bone is a dynamic and highly vascularized tissue that remodels throughout an individual's lifespan. It performs an essential function in mobility, ensures that the skeleton has appropriate load-bearing capacity, and protects the body's delicate internal organs (Molly et al., 2008). In addition to its structural tasks, bone is closely involved in homeostasis by storing calcium and phosphate ions and controlling the blood content of essential electrolytes (Molly et al., 2008).

The three main functions of bone are to protect internal organs, support the body’s structure, and allow for movement. The two primary types of bones are cancellous (also known as trabecular) and cortical (also known as compact). Most bones have a cancellous bone as their internal structure and a cortical bone as their external shell. Extracellular matrix (ECM), which includes cells, hydroxyapatite, collagen fibrils, and bound materials, is another component of bone (Kalsi et al., 2021).

Once there is insufficient bone tissue in a specific area of the body, it is known as a bone defect. The main causes of bone defects are trauma, congenital abnormalities, and tissue removal because of cancer. Bone can only partially repair large bone defects, but it can repair small bone defects (Kiernan et al., 2018). Therefore, bone grafting is necessary to address this problem. Bone grafting is a procedure that uses the transplanted bone to rebuild and correct bone defects. In addition to the bone from your body or a donor, an artificial bone graft is also an option. The process of moving tissue from one area of the same person to another without inducing vein bleeding is known as an autograft. Autografts have the benefit of not being immunologically rejected. It differs from other types of bone grafts in that it undergoes osteogenesis without inducing an immune response. Due to their non-immunogenicity, osteoconductivity, and osteoinductivity, autografts are the preferred option. Even so, autografts are limited because they necessitate additional surgical procedures and could result in pain and morbidity at the donor site (Xiao et al., 2020).

Bone transplantation between genetically different people is referred to as allograft. The allograft-induced new bone formation will take longer because it requires adaptation and triggers an immune response. Bone grafts called xenografts are created from different species of living things. Because of their strong immune response, poor biomechanics, and foreign body reaction, xenografts are less popular than allografts despite being less expensive (Mahsut Dinçel, 2018). Theoretically, xenografts and allografts could take the place of autografts, but they are unethical and susceptible to immune rejection. Therefore, it is essential to create artificial materials for bone grafting (Xiao et al., 2020). Figure 2.2 illustratre different types of bone graft (Xiao et al., 2020).

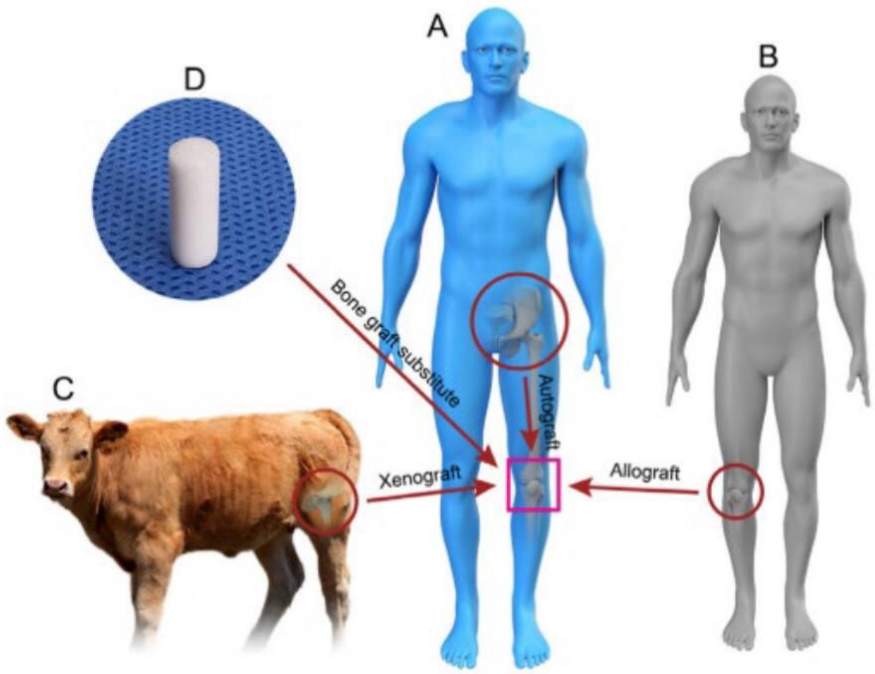

Figure 2.2: Different types of bone graft (Xiao et al., 2020).

The main objective of bone repair using bone graft is to incorporate bone progenitor cells and growth factors to stimulate cell formations. The implanted bone graft is to mimic both the structure and the function of the extracellular matrix (ECM) of the original bone, which provides an environment for cell proliferation, differentiation, and adhesion. Following that, the process of bone regeneration will be accelerated. New tissue will develop after a bone graft is implanted in a defected bone space, but the implant will progressively deteriorate (Kalsi et al., 2021). The implanted bone graft will eventually be completely replaced by the new tissue. Bone grafts made of various organic and synthetic biomaterials can mimic the microenvironment of the bone. Figure 2.3 consist of process of bone tissue engineering to heal large bone defect (Kalsi et al., 2021)

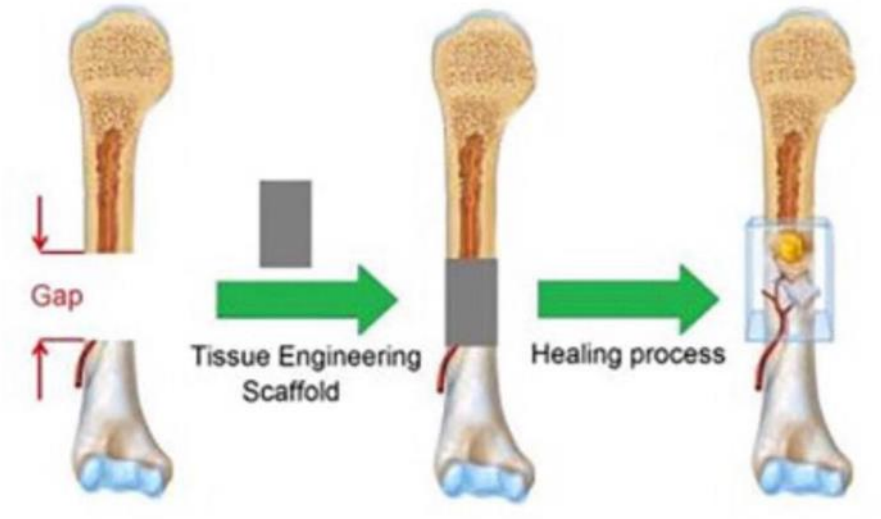

Figure 2.3: Process of Bone Tissue engineering to heal large bone defect (Kalsi et al., 2021)

2.3.1 Polymer

Some natural polymers are suitable for use in applications involving bone regeneration because they exhibit good bioactivity and biocompatibility properties. Engineering methods can also be used to alter their characteristics. Natural polymers used include silk, alginate, peptides, chitosan, collagen, and hyaluronic acid (Kalsi et al., 2021). Collagen is the protein that is most prevalent in the body. It strengthens the tissues of the body. As a result, it has the ability to grow bone-producing cells. Because they can increase cellular adhesion to their surface, collagen-based bone grafts are very bioactive. A variety of stem cells have been raised on 3D collagen scaffolds for various tissue engineering applications (Alaribe et al., 2016).

2.3.2 Ceramic

Another name for ceramics used in medical applications is bioceramics. They’ve been used in the replacement and reconstruction of broken bone fragments. A few popular bioceramics used for bone repair or regeneration include bioglass, calcium phosphates, alumina, and zirconia. Bioglass is suitable for use in biomedical

applications due to its high calcium to phosphorus ratio. This enables it to promote the development of apatite crystals on its surface after being implanted in the body. Additionally, bioglass has high osteoconductivity and bioactivity and regulates the rate of degradation. But it has some shortcomings, such as a lack of toughness and strength (Kalsi et al., 2021).

Calcium phosphate is one of the most widely used bioceramics for tissue regeneration (CaP). This is because their chemical composition closely resembles that of bone tissue. CaP exhibits the properties of bioactivity, resorbability, and osteoconductivity in accordance with its chemical composition. CaP examples include hydroxyapatite (HAp), beta-tricalcium phosphate (β-TCP), and biphasic calcium phosphate, which combines HAp and β-TCP (Dolcimascolo et al., 2019).

Alumina is a bioceramic with a crystalline structure (Al_2O_3). The increased mechanical strength of the material is a result of the small grain size and low porosity. Alumina exhibits good wear resistance despite being fragile. Zirconia has a polymorphic structure and a hard surface. Because of its high breaking load and excellent biocompatibility, it is appropriate for bone grafting (Dolcimascolo et al., 2019).

2.3.3 Metal

Due to their excellent mechanical qualities, such as high fracture toughness, high yield strength, and high ductility, metallic biomaterials are used in the field of bone repair and regeneration. These characteristics enable them to support the load without deforming. Cobalt alloys, stainless steel, and titanium alloys are a few commonly used metals. There are some restrictions for metallic biomaterials, however, due to the toxic nature of the metallic ions released during corrosion. These

metallic ions may result in allergic reactions and bodily inflammation (Kalsi et al., 2021).

Alpha, beta, or biphasic titanium alloys are different categories. Alpha alloys that contain alpha stabilisers like gallium or aluminium have good strength. Good ductility can be found in beta alloys that contain beta stabilisers like vanadium, niobium, and molybdenum. Alpha and beta stabilisers are combined to create biphasic alloys. Ti6Al4V is found to be the best titanium alloy for biomedical applications. Ti-6Al-4 V is an (α + β) titanium alloy that also contains stabilisers Al and β stabilizer element V. It has good mechanical properties, which make it an excellent material for joint replacement (Heng et al., 2010). Stainless steels are iron-based alloys with a high chromium content and low carbon content. Although the carbon content enhances the mechanical characteristics, it also leads to the formation of carbides, which makes stainless steel corrode in biological environments.

2.4 Introduction on ceramic as biomaterials

A ceramic is an inorganic solid made of either metal or non-metal compounds. It is first shaped at a high temperature before being densified. They are typically hard, brittle, and corrosion-resistant. They may consist solely of crystalline phases or may contain both glassy and crystalline phases. The two main subcategories of ceramics are traditional and advanced ceramics. The main components of traditional ceramics are clay and cements that have been heated to high temperatures and then hardened. Advanced ceramics require modern processing techniques, which leads to their use in the engineering and medical industries. Figure 2.4 Structure of human body and ceramics as biomaterials in replacement (Shanmugam et al., 2018).

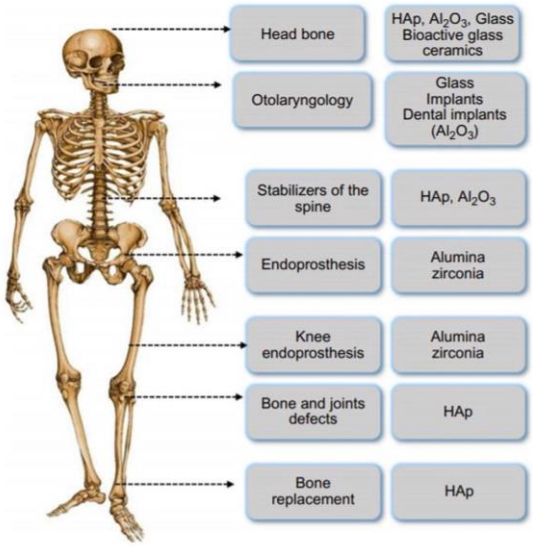

Figure 2.4: Structure of human body and ceramics as biomaterials in replacement (Shanmugam et al., 2018).

The most frequent ceramics used in biomedical applications are silicates, metallic oxides, carbides, sulphides, refractory hydrides, selenides, and carbon-based materials like diamond and graphite. Calcium phosphate, such as hydroxyapatite, is the main inorganic component of bone and tooth enamel (Eliaz et al, 2017). Classification of selected bioceramics according to their type and applications shown in Table 2.1.

Table 2.01: Bioceramics according to their type and applications (Heimann et al., 2015).

Type of bioceramics	Application	Material	References
Bioinert	Femoral balls, inserts of acetabular cups, artificial heart valves, dental roots, bone screws, endoscope	Alumina	Heness and Ben-Nissan (2004)
	Femoral balls, dental veneers, tooth inlays	Zirconia (Y-TZP)	Heness and Ben-Nissan (2004)
	Anti-wear coating of femoral balls and knee prostheses, coating for coronary stents	Titanium nitride, Zirconium nitride	Staia et al. (1995)
Bioactive	Bone cavity fillings, ear implants, vertebrae	Hydroxyapatite (HAp)	Cao and Hench (1996)

	replacement, hip implant coatings, bone scaffolds		and Lobel and Hench (1998)
	Bone replacement, ear implants	Bioglasses	Hench (1991)
Bioresorbable	Dental cement, Bone replacement, UV-absorbing sunscreens	Low-crystalline HAp, β-tricalcium phosphate (β-TCP), α-TCP, Tetracalcium phosphate (TeCP), Octacalcium phosphate	Barounian, Hesaraki and Kazemzadeh (2012) Heness and Ben-Nissan (2004)

2.5 Calcium phosphate ceramic as bone substitute materials

Calcium phosphate is one of the primary minerals found in bone (CaP). Calcium phosphate (CaP) is made up of calcium cations (Ca^{2+}) orthophosphate (PO_3^4), metaphosphate (PO_3), or pyrophosphate ($P_2O_4^7$) anions as well as hydrogen (H^+) or hydroxide (OH^-) ions (Eliaz et al, 2017).

2.5.1 Properties required by calcium phosphate groups

Bioactivity, which is related to the dissolution process, is one of the characteristics needed for calcium phosphate ceramics. This is due to the fact that cellular or extracellular activity, which initiates the in vivo partial dissolution and ion release, is the main mechanism underlying bioactivity. The partial dissolution will result in a rise in calcium and phosphate ion concentration. Apatite microcrystals precipitate and form on the surface as the saturation level in the microenvironment that surrounds the bone defect area rises. The incorporation of proteins and growth factors (GFs) from the microenvironment is increased by surface precipitation, which may encourage cell adhesion and the formation of new bone (Eliaz et al., 2017). The new bone that develops on the surface of the CaP implant will remodel

the bone defect once the host bone and the CaP bioceramics are connected (Eliaz et al., 2017).

The substances most frequently used in clinical and hospital applications, particularly bone substitutes, are calcium phosphates. Because calcium phosphates are soluble and osteoinductive. This property allows the released ions to dissolve and be incorporated into the developing bone stock (Jones, 1996). As others have mentioned, ion release and ion degradation are both necessary for the calcium phosphate to be bioactive. These activities increase the concentration of calcium and phosphate ions in the region, promoting bone mineral development on calcium phosphate surfaces.

Table 2.2 shown properties required from calcium phosphates as bone substitute in clinical applications (Eliaz et al., 2017). Biocompatibility is a crucial requirement for all implantable materials because the body might react negatively with inflammation or a foreign body reaction without it. Researchers found that calcium phosphates are biocompatible after implantation in the body. This is due to their availability in both dissolved and abundant solid form.

Table 2.2: Properties required as bone substitute for CaP in clinical application (Eliaz et al., 2017)

Property	Functions
Bioactivity	The ability of a CaP to participate in specific biological reactions or influence living tissues
Biocompatibility	The ability of a material to perform with an appropriate host response in a specific application
Bioactive fixation	Reactive surfaces form chemical bonding with bone, thus minimizing the fibrous capsule formation
Biostability	The ability of a material to maintain its properties in vivo
Osteoconduction	Ability to provide a scaffold for the formation of new bone

Osteoconduction and osseointegration are essential for cell adhesion and integration in the CaP. The ability of calcium phosphate to promote bone growth on their surfaces is known as osteoconduction, as opposed to the ability of calcium phosphate to induce the growth of progenitor cells (Jeong et al., 2019). Progenitor cells can differentiate into various cell types like stem cells. The task of replacing damaged or deceased cells falls to progenitor cells. The osteoinduction and osteoconduction processes of calcium phosphates are crucial as they facilitate cell adhesion and proliferation since cell adhesion depends on the ability of calcium phosphates to bind to extracellular matrix proteins (ECM proteins). The properties of calcium phosphates, such as their surface roughness, solubility, phase content, grain size, particle size, surface charge, and surface energy, determine their capacity to do so (Eliaz et al., 2017). Resorption describes the process by which the CaP bioceramic is absorbed by cells or through dissolution. It is believed that the ideal rate of resorbability runs concurrently with the growth of bone tissue.

2.6 Type of calcium phosphate groups

Calcium phosphate groups are classified as α-tricalcium phosphate (α-TCP), hydroxyapatite (HAp), dicalcium phosphate dihydrate (DCPD), anhydrous dicalcium phosphate (DCPA), tetracalcium phosphate (TTCP), β-tricalcium phosphate (β-TCP), calcium-deficient hydroxyapatite (HAp), and monocalcium phosphate monohydrate (MCPM) (Kucko et al., 2019). Hydroxyapatite (HAp) and β-tricalcium phosphate (β-TCP) are common medical materials.

In a physiological setting, HAp is the least soluble calcium phosphate, making it the most stable calcium phosphate. Furthermore, the surface of HAp can serve as a site for forming new bone minerals. Contrarily, β-TCP degrades more

quickly and is more soluble than HAp. The fact that HAp and β-TCP don't cause inflammatory reactions and are structurally stable can be inferred as the reason for their widespread clinical use. The different types of calcium phosphate are listed in Table 2.3, along with information about their solubility and Ca/P ratios.

Table2.3: Types of calcium phosphate groups (Kucko et al., 2019)

Chemical Name	Formula	Ca/P	Solubility at 25°C (mg/L)
Monocalcium phosphate monohydrate (MCPM)	$Ca(H_2PO_4)_2.H_2O$	0.5	~18,000
Dicalcium phosphate dihydrate (DCPD)	$CaHPO_4.2H_2O$	1.0	~88
Octacalcium phosphate (OCP)	$Ca_3(HPO_4)_2(PO_4)_4.5H_2O$	1.33	~8.1
α -Tricalcium phosphate (α -TCP)	α -$Ca_3(PO_4)_2$	1.5	~2.5
β -Tricalcium phosphate (β -TCP)	β -$Ca_3(PO_4)_2$	1.5	~0.5
Hydroxyapatite (HAp)	$Ca_{10}(PO_4)_6(OH)_2$	1.67	~0.3
Tetracalcium phosphate (TTCP)	$Ca_4(PO_4)_2O$	2.0	~0.7

Because of their different Ca to P ratios, calcium phosphates (CaPs) are a class of bioceramics with varying solubility and bioactivity. The majority of CaP ceramics degrade biologically when exposed to physiological conditions. The ability to build new bones is directly correlated with the rate of calcium phosphate degradation in a physiological setting. The Ca/P ratio of the CaP bioceramics determines how quickly they degrade (Bal et al., 2020). As the Ca/P ratio falls, the rate of degradation rises. Additionally, the solubility of the materials and the pH in the area have an impact (Shanmugam et al., 2018).

2.6.1 Monocalcium phosphate monohydrate (MCPM)

$Ca(H_2PO_4)_2$. The chemical name for the substance known as monocalcium phosphate monohydrate is H_2O. (MCPM). Among the calcium phosphate groups, it

has the lowest Ca/P ratio (0.5). A compound with a low Ca/P ratio, it is water soluble. It is the most acidic member of the CaP family. It is frequently used as a sealer and a component of self-hardening CaP cements in dental applications (Eliaz et al., 2017).

2.6.2 Dicalcium phosphate dihydrate (DCPD)

The chemical formula for brushite, also known as dicalcium phosphate dihydrate (DCPD), is CaHPO42H2O. It is a stable phase at 25 °C and pH values of 4.5 to 4.3. DCPD contains water molecules and has a Ca/P ratio of 1 under physiological conditions. Additionally, it is very soluble. It has biodegradable, biocompatible, and osteoconductive properties. DCPD serves as an intermediary in the precipitation of HA and bone mineralization. DCPD crystals can be produced by neutralising phosphoric acid with calcium hydroxide at a pH range of 3 to 4 at room temperature (Eliaz et al., 2017). Usually, DCPD calcium phosphate cement is used for bone replacement and repair (Wang et al., 2014). In physiological fluid, DCPD dissolves more easily than hydroxyapatite. Therefore, DCPD tends to resorb more quickly than HAp both in vitro and in vivo (Nosrati et al., 2019). DCPD can increase the amount of calcium and phosphate ions released near a bone implant, stimulating the activities of cells resembling bone. This is because higher solubilities of DCPD and rising ion concentrations can impact protein adhesion. Because of this, the protein adhesion will result in cell adhesion and control how quickly bones regenerate (Jeong et al., 2019).

2.6.3 Hydroxyapatite (HAp)

Hydroxyapatite is one of the highly compatible synthetic biomaterials utilised as bone grafts for bone regeneration (HAp). Hydroxyapatite is a specific type of

calcium phosphate that naturally occurs in human bone tissue. $Ca_{10}(PO_4)_6(OH)_2$ is the chemical formula for HAp, and its Ca/P ratio is 1.67. It is believed that HAp is the calcium phosphate that dissolves the least in physiological environments. A bioactive and osteoconductive biomaterial called HAp's surface may serve as a location for synthesizing new bone minerals (Farid, 2019). This implies that bone tissues and HAp can directly bond. The biocompatibility, non-toxicity, lack of immune response, lack of inflammation, and lack of postoperative morphological change are additional advantages of HAp (Pokhrel, 2018).

Artificial HAp-based pastes, cements, and granules have all been researched for use in bone regeneration procedures, but HAp has the lowest solubility of the calcium phosphate groups, indicating a slow drug release and degradation rate (Ghiasi et al., 2019). Many studies have been conducted to assess the mechanical properties of HAp in order to create porous scaffolds with sufficient mechanical strength to withstand the loads that act during the first hours and days of the healing process. Porous HAp scaffolds should only be used in non-load bearing applications. (Dantas et al., 2016). Meanwhile, Pokhreal et al. 2018 reported that HAp has crucial biological qualities such as a lack of immunological reactivity and no postoperative morphological change, making it ideal for filling bone deficiencies in orthopaedic applications

2.6.4 Tricalcium Phosphate (TCP)

Another calcium phosphate substance frequently investigated as a bone substitute is tricalcium phosphate (TCP), which has the chemical formula $Ca_3(PO_4)_2$. It is broken down into two distinct phases: the α and β. At temperatures of 1125 °C or higher, α-TCP can form during sintering, and β-TCP is formed at temperatures of 900–1100 °C. TCP has a stable structure and a good biodegradation rate due to its

Ca/P ratio of 1.5. Below 1125°C, β-TCP has a more stable structure and a faster biodegradation rate. Excellent cell adhesion is another benefit of the β-TCP structural design. Therefore, β-TCP is frequently applied to bone regeneration. It is less stable than HAp but dissolves more easily and degrades more quickly (Jeong et al., 2019).

2.6.5 Octacalcium phosphate (OCP)

The octacalcium phosphate (OCP) chemical formula is $8H_2(PO_4)_6.5H_2O$. It is produced when the more stable calcium phosphates separate from aqueous solutions. Similar to HAp, OCP has atomic configurations divided by water molecules. OCP is crucial for the in vivo formation of apatitic biominerals, especially for dental applications (Kuffner et al., 2017). OCP has been applied as a coating in medical applications. It has been discovered that the OCP coating has osteoinductive properties.

2.7 Biological process induced by calcium phosphate after implantation

After implantation there are biological process induced by calcium phosphate. Biological activities that take place at the calcium phosphate surface after implantation shown in Figure 2.5 and Figure 2.6 schematic diagram of phenomena that occur on the surface of calcium phosphate after implantation (Bertazzo et al., 2010).