CHARACTERISATION OF HYDROXYAPATITE COATED AND UNCOATED TITANIUM AND EFFECTS TOWARDS SAOS-2 CELLS

MOHAMMAD IDHAM BIN ABD HAMID

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by

MOHAMMAD IDHAM BIN ABD HAMID

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LIST OF SYMBOLS

a.u	Arbitrary unit
°	Degree
°C	Degree Celsius
g	Gram
θ	Incidence Angle Of X-Ray Beam
μm	Micrometer
%	Percentage
wt.%	Weight percentages

LIST OF ABBREVIATIONS

CO2	Carbon Dioxide
DMEM	Dulbecco's Modified Eagle Medium
DNA	Deoxyribo Nucleic acid
EDX	Energy Dispersive X-ray spectroscopy
HA	Hydroxyapatite
IPS	Institut Pengajian Siswazah
Mm	Milimeter
PBS	Phosphate Buffer Saline
Rpm	Rotate Per Minute
SBF	Simulated Body Fluid
USM	Universiti Sains Malaysia
VEGF	Vascular Endothelial Growth Factor

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PENCIRIAN TITANIUM DILITUP HIDROKSIAPATIT DAN TIDAK DILITUP SERTA KESAN TERHADAP SEL SAOS-2

ABSTRAK

Titanium tulen dan titanium berasaskan aloi telah dikenal pasti mempunyai potensi sebagai material terbiodegradasi kerana ciri mekanikalnya yang baik seperti mempunyai ketumpatan yang rendah tetapi sangat kuat, boleh menahan ketegangan apabila menghadapi penetapan dalaman dan mempunyai daya ketahanan yang tinggi. Dalam kajian ini, sampel titanium tidak terlitup dan terlitup dengan hidroksiapatit (HA) telah digunakan. Sampel terlitup dengan hidroksiapatit disediakan dengan melitup HA ke atas Ti6Al4V menggunakan kaedah pipetting (*solvent casting).* Sampel yang tidak dilitup telah disediakan dengan sampel Ti6Al4V dikisar dan digilap. Pencirian pertama telah dilakukan dengan menggunakan mikroskop pengimbas elektron (FESEM) terhadap kedua-dua sampel. Pencirian pembelauan sinar-X (XRD) dan Spektroskopi Inframerah Transformasi Fourier (FTIR) dijalankan ke atas litupan sampel yang terlitup dengan hidroksiapatit. Seterusnya, ujian biokompatibiliti material dilaksanakan ke atas sel Saos-2 dengan menggunakan cerakin MTT, LDH dan Sircol Collagen. Pada pencirian FESEM, litupan HA terhadap sampel Ti6Al4V menunjukkan permukaan morfologi yang tidak sekata berbanding sampel Ti6Al4V yang tidak terlitup. Analisis XRD menunjukkan keberadaan fasa kristal yang ingin dicapai pada Ti6Al4V yang dilapisi HA. FTIR menunjukkan keberadaan kompaun karbon dan fosfat pada permukaan Ti6Al4V yang dilapisi HA. Reaksi positif oleh sel Saos-2 terhadap Ti6Al4V dibuktikan melalui ujikaji menggunakan cerakin MTT, LDH dan Sircol Collagen secara kaedah tidak langsung pada 7 hari pertama. Pemerhatian di bawah FESEM menunjukkan filopodia sel Saos-2 lebih mudah berkembang ke atas permukaan tidak rata pada

Ti6Al4V terlitup dengan HA walaupun terdapat kerpasan semula litupan ke atas permukaan sel. Hal ini membuktikan bahawa sampel tersebut memberikan sokongan yang baik untuk pelekatan sel dan mengesahkan kebolehan material Ti6Al4V terlitup dengan HA sebagai templat (scaffold) kepada tisu.

CHARACTERISATION OF HYDROXYAPATITE COATED AND UNCOATED TITANIUM AND EFFECTS TOWARDS SAOS-2 CELLS

ABSTRACT

Titanium and its alloys have been recently emphasised as potential biocompatible metals due to their good mechanical properties such as being low density but stronger, withstanding strain during internal fixation and having good durability. This study focused more on the biocompatibility study. In this study, uncoated and hydroxyapatite (HA) coated samples were used. HA-coated samples were prepared by coating HA onto a titanium alloy (Ti6Al4V) using the solvent casting method. The uncoated samples were prepared by grinding and polishing the Ti6Al4V samples. First characterisation was performed using Field Emission Scanning Electron Microscope (FESEM) on both types of samples. X-ray diffraction (XRD) and Fourier Transform Infrared spectroscopy (FTIR) analyses were conducted with the coating of HA-coated samples. The biocompatibility of the samples was evaluated using Saos-2 cells using MTT, LDH and Sircol collagen assay. On FESEM characterisation, the coating of HA onto Ti6Al4V, achieved through the solvent casting method, led to irregularities in the morphological surface characteristics compared to uncoated Ti6Al4V. Analysis using X-ray Diffraction (XRD) demonstrated the presence of crystalline phase in the HA-coated Ti6Al4V. Fourier Transform Infrared (FTIR) analysis indicated the presence of phosphate and carbon organic compounds on the surface of HA-coated Ti6Al4V. A positive cell response to the titanium ions was revealed during the first 7 days of the biocompatibility study using the indirect method such as MTT, LDH and Sircol collagen assay. Observation under a Field Emission Scanning Electron Microscope (FESEM) reveals that the filopodia of the Saos-2 cells preferred to develop onto the

irregular surface of HA-coated Ti6Al4V, although there was not a clear image as the reprecipitation of the coating covered the cell surfaces. This study provided evidence of a good cell-material interaction on Ti6Al4V that may confirm the feasibility of using HA-coated Ti6Al4V as well as Ti6Al4V as hard tissue scaffolds.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Implant is a medical device that was developed to either enhance an existing biological structure that has been damaged or replace a biological structure that has been lost. When designing an implant device, the biocompatibility of the material used is of the utmost importance. This ensures that the material is not toxic or otherwise harmful to the human body.

In clinical practice, dental implants are one of the most utilised medical implants nowadays. The surgical procedure involves the insertion of a dental implant into the maxilla and mandibular region, wherein the tooth roots are substituted with metallic, screw-shaped posts. This facilitates the replacement of impaired or absent teeth with synthetic teeth that closely resemble and perform similarly to natural teeth. Dental implant presents a viable alternative to ill-fitting dentures or bridgework, particularly in cases where the absence of natural tooth roots prevents the construction of denture or bridgework tooth replacements.

While fixed partial dentures and dentures effectively address the aesthetic concerns associated with tooth loss, dental implants offer superior levels of security and stability. They do not induce gingival discomfort and exhibit superior hygiene compared to dentures. Furthermore, they facilitate the execution of entirely typical masticatory processes.

For an implant to achieve functionality, numerous factors play a pivotal role in ensuring successful implant placement. These factors encompass the patient medical condition and medications that has been taken, type of implant utilised, whether the implant placement is conducted simultaneously or delayed, the initial

height of the bone prior to treatment, the level of oral hygiene maintained, and the individual's smoking habits. The selection of the implant type is a crucial determinant for achieving successful implant placement through the initiation of osseointegration.

The implant devices commonly utilised in medical applications are primarily composed of titanium, cobalt and its alloys, and stainless steel. These materials are preferred due to their exceptional biocompatibility and favourable mechanical characteristics (Paital and Dahotre, 2009).

An additional prerequisite for a medical implant is its ability to facilitate tissue regeneration. The utilisation of the metals in isolation is insufficient to meet such a requirement. Extensive scientific investigations spanning multiple decades have consistently demonstrated that the utilisation of hydroxyapatite (HA) possesses the ability to facilitate osseointegration, which promotes the development of new bone tissue. Furthermore, this application of HA has been shown to enhance the mechanical fixation and stability of metallic implants (Acton, 2013). HA can be utilised as a thin film deposited onto the surface of the implant (coating), or it can function as a substance that fills in gaps within bone structures. In the past few decades, plasma spraying has emerged as a prominent technique for applying HA coatings onto metallic implants (Kweh, 2000; Sun, 2003; Heimann, 2006).

Despite the simplicity of plasma spraying of HA, it is characterised by a high rate of deposition, a low temperature of the substrate, variability in coating porosity, and variability in phase and structure (Shi, 2006). The primary issue lies in the elevated temperature experienced during the spraying process, which can reach up to 10,000ºC. This high temperature has the effect of modifying the structure of HA as well as the phases of the metal substrate. Consequently, it leads to dehydroxylation,

reduced crystallinity, and phase decomposition, resulting in a combination of different HA compositions. The alteration in the phase composition results in a reduction of its biocompatible characteristics.

Another challenge associated with the application of shaping and implanting HA is its inherent weak and brittle mechanical properties. In previous studies conducted by Jo et al. (2012), and Mohd Yusoff et al. (2014), a potential solution to address the brittleness problem was proposed. This involved the application of a poly ε-caprolactone, (PCL) binder to coat HA particles. According to reports, the inclusion of PCL as a binder in hydroxyapatite coatings results in enhanced stability and flexibility, preventing the occurrence of cracks or delamination (Jo et al, 2012 and Mohd Yusoff et al., 2014).

It is essential to investigate the biocompatibility of novel devices. Cell adhesion is a critical factor in assessing the biocompatibility of a material when it is implanted in the human body. This role will provide additional insights into the processes of cell migration, differentiation, and proliferation, thereby confirming the suitability of the titanium-based implant for bone repair and regeneration.

The primary focus of this research is osseointegration because of its significance to the accomplishment of successful implant placement. Surface treatment is critical for osseointegration, tissue reformation, and implant biocompatibility. This factor plays a crucial role in achieving a positive rapid cell response and early osseointegration, thus preventing implant failure. Surface topography appears to affect the initial stages of bone formation, the performance of osseointegration, and the capacity to retain the initial blood clot.

Previous research has investigated the biological activity of osteoblast mainly Saos-2 cells on titanium implants. However, the precise molecular process that

underlies the interaction of Saos-2 cells with the hydroxyapatite-covered titanium surface is still a conundrum. The hormonal signals such as VEGF and BMP are believed have some role in inducing osseointegration by angiogenesis under ischemia.

One of the post-translational products of this hormonal signal is collagen. Collagen is essential in wound healing processes as it is an important component of the extracellular matrix to facilitate wound healing. This collagen is produced by cells such as osteoblasts, macrophages and endothelial cells during wound healing responding to hormonal signals such as VEGF and BMP (Jośko et al., 2000).

1.2 Problem Statement

The *in vitro* and *in vivo* performances of bio-implantable titanium and its alloys have been significantly improved because of the vast development of a variety of surface modification techniques. However, with emerging clinical bio-implantable titanium and its alloys, there is still a gap because of issues with the rate and quality of osseointegration in these implants (Li et al., 2014). The researchers are still investigating deeper into the factors influencing the process of osseointegration due to their increasing understanding of its cellular mechanisms. This has contributed to the improvement of the osseointegration process by applying the favourable aspects and occurrences related to this biological mechanism.

Few reports have been written about HA coatings made with the casting technique. In earlier studies, HA was found to coat titanium. Sohmura was the first researcher to come up with this method (Sohmura et al., 2001). HA-coated implants were made by pouring medical-grade titanium into investment and graphite moulds that had been coated with HA. Other researchers have said that they used this method

to coat cobalt-based alloys with HA and biphasic calcium phosphate (BCP). They also studied the effect of pre-heating the mould and the post-sintering process on the improvement of bioactive implants (Escobedo, 2006; Almanza, 2006; and Minouei, 2011). The researcher might be able to use a paint brush to coat the implant according to the Rodrigues et al., (2019) method, but this method uses the oil of turpentine as a solvent that may be harmful to the cells and this method does not record its biocompatible study (Rodrigues et al., 2019).

An *in vitro* study required a very affordable sample preparation as a prototype before being modified to an advanced process (Novák et al., 2023). All these techniques above require high equipment and services thus maximising the cost of a sample. A simple technique is needed to solve the problem and at the same time fulfil the biocompatibility criteria for the *in vitro* studies.

It is essential to address advanced molecular studies such as the post-translational molecular production of cells during osseointegration. Many signalling factors such as VEGF, BMP and others may contribute to the osseointegration pathway. Their post-translational products such as collagen, reflected the signalling products and interactions that were required during osseointegration process (Ferrara et al., 2003). Thus, the molecular perspective can be considered while resolving the osseointegration issues either from the implant or the patient itself.

1.3 Research Questions

1. What is the surface topography of HA-coated Ti6Al4V on the solvent casting method by FESEM?

2. What is the other physical biocompatible HA-coated Ti6Al4V on the solvent casting such as chemical composition by EDX, its crystallographic structure by XRD and the functional group by FTIR?
3. What are the cell viability responses of Saos-2 cells on uncoated Ti6Al4V and HA-coated Ti6Al4V-conditioned medium using MTT Assay, the cell cytotoxicity responses using the LDH Assay and cell adhesion responses by using FESEM?
4. What is the level of collagen synthesis of Saos-2 cells on uncoated Ti6Al4V and HA-coated Ti6Al4V in normal medium and serum-deprived conditioned medium?

1.4 Objectives

1.4.1 General objective

1. To evaluate the biological behaviour of Saos-2 cells on HA-coated Ti6Al4V and uncoated Ti6Al4V.

1.4.2 Specific objectives

1. To modify Ti6Al4V surface topography by coating with HA using the solvent casting method and characterise the coating's surface topography by FESEM.
2. To characterise the HA-coating layer for its physical biocompatible properties by using EDX for chemical composition, crystallographic structure by XRD and functional group by FTIR.
3. To evaluate the biocompatibility responses of Saos-2 cells towards HA-coated Ti6Al4V and uncoated Ti6Al4V using the MTT Assay for cell viability, LDH Assay for cell cytotoxicity and FESEM for cell adhesion.

4. To assess the collagen synthesis of Saos-2 cells towards HA-coated Ti6Al4V and uncoated Ti6Al4V using the Sircol Collagen Assay.

1.5 Hypothesis

1.5.1 Null hypothesis

1. Uncoated Ti6Al4V surface topography has no significant difference from the HA-coated method using the solvent casting method.
2. The chemical composition and crystallographic structure of HA-coated method have no significant difference with standard HA powder.
3. Cell viability and cell cytotoxicity Saos-2 cells towards HA-coated Ti6Al4V has no significant difference compared to uncoated Ti6Al4V.
4. The collagen synthesis of Saos-2 cells towards HA-coated has no significant difference compared to uncoated Ti6Al4V.

1.5.2 Alternate hypothesis

1. The uncoated Ti6Al4V surface topography has a significant difference with HA-coated method using the solvent casting method.
2. Chemical composition and crystallographic structure of the HA-coated method has significant difference with standard HA powder.
3. Cell viability and cell cytotoxicity Saos-2 cells towards HA-coated Ti6Al4V have a significant difference compared to uncoated Ti6Al4V.
4. The collagen synthesis of Saos-2 cells towards HA-coated Ti6Al4V has a significant difference compared to uncoated Ti6Al4V.

1.6 Justification of the study

In most previous biocompatibility studies, the biocompatibility of uncoated Ti6Al4V and HA-coated titanium alloys was assessed using simulated body fluid (SBF). Most researchers employed plasma-sprayed titanium alloy samples coated with HA, subjecting them to elevated temperatures that induce alterations in the preferred chemical composition of hydroxyapatite (Rahimi et al., 2022). In this study, specimens of solvent-casting HA-coated Ti6Al4V were employed, avoiding the use of elevated temperatures. These investigations also employ *in vitro* cell culture as opposed to other studies that primarily utilise SBF. This study also encompasses the investigation of collagen synthesis, which is slightly found in other publications.

CHAPTER 2

LITERATURE REVIEW

2.1 Dental implant

The area of dentistry that is undergoing development nowadays is dental implantology, which is a specialised subfield of dentistry that focuses on the rehabilitation of the malfunctioning masticatory apparatus that occurs because of the loss of teeth (Velasco-Ortega et al., 2010). Dental implantology is the second oldest dental profession, with exodontia (oral surgery) being the oldest (Block, 2018). One of the dental implantology subfields is a dental implant. It is a structure made of synthetic biomaterials that is placed in the mouth to support a dental prosthesis. Dental implants gained significant importance due to Dr. Branemark's discovery of osseointegration, which involves directly attaching the implant to the bone without any unwanted tissue in between (Rutkowski et al., 2022).

Dental implants are indicated to replace teeth that have been lost due to accident, ageing or due to the pathological conditions. These implants are in demand today because of aesthetics' benefits and to improve even a slight masticatory performance (Mohajerani et al., 2017). Dental implants are recommended for the patients that is edentulous and have intermediate gaps or free end edentulism. These implants are advised to these patients if they dissatisfied with an unstable partial prosthesis to maintain it for becoming stable with an implant to secure the prosthesis (Praveen et al., 2012).

A patient's root-bearing mandible or maxilla can be surgically augmented to accommodate the insertion of one or more of these implants, and these implants can be placed in any one of several different locations (Lim et al., 2022). The success and long-term prognosis of the implant depend on the osseointegration process's ability to

fix the implant in the maxilla or mandibular bone (Tolstunov, 2006). It is now widely used to treat complete and partial tooth loss and ais considered an important treatment option in dentistry.

A high success rate (above 97% for 10 years) is one of the most important advantages of dental implants over traditional fixed partial dentures (70-90%) (Seong & May, 2019; Gupta et al., 2023). Other advantages include reducing risk of tooth decay and endodontic issues in neighbouring teeth. Previous study has shown implant improved preservation of bone in areas without teeth. Moreover, implants decrease the sensitivity in adjacent teeth compared to the dentures (Raikar et al., 2017).

2.2 Surgical preparation and treatment

Implant procedures require sterile surgical preparation. Sharp and new osteotomy drills must be used for osteotomy under copious cool saline (Arakji et al., 2022). These precautions are needed to prevent different types of unwanted injuries such as thermal and mechanical to the bone. Osteotomy must be done at slow speed and high torque with an incremental drill sequence. During an osteotomy, the bone temperature should be limited to 47 ºC to prevent changes that is irreversible such as osseointegration failure and bone tissue necrosis (Stanford, 2007).

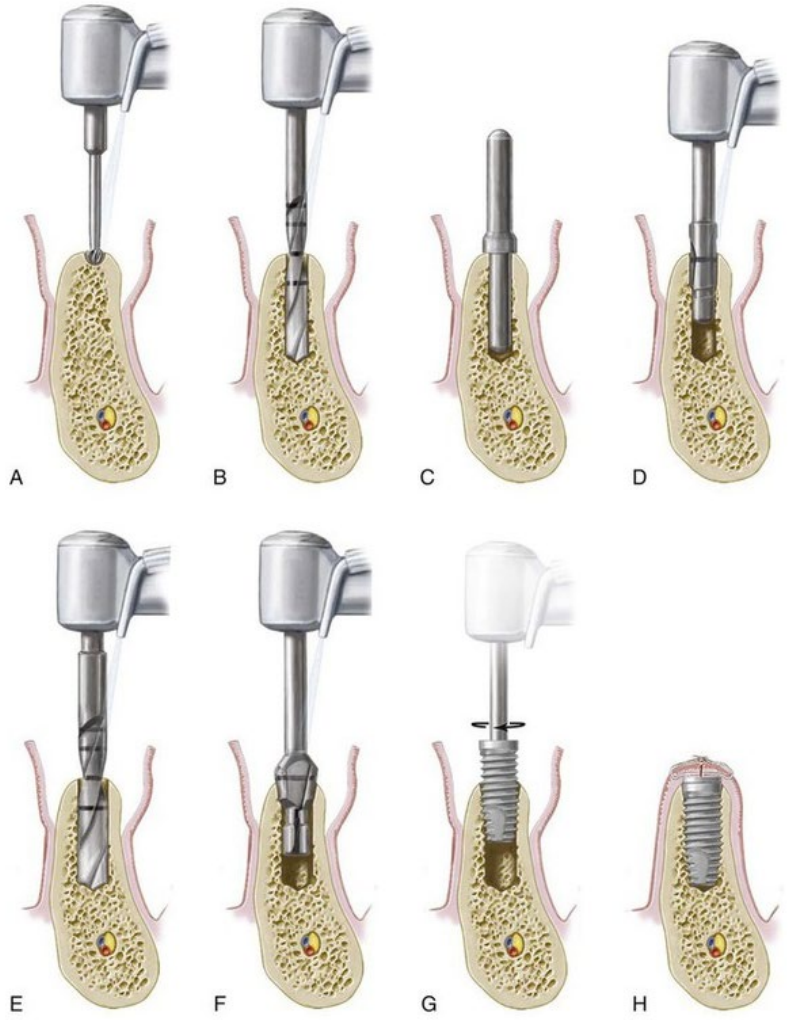

Figure 2.1 Implant site preparation (osteotomy) for dental implant. A, Initial marking or preparation of the implant site with a round bur. B, Use of a twist drill to establish depth and align the implant. C**,** Guide pin is placed in the osteotomy site to confirm position and angulation. D, Pilot drill is used to increase the diameter of the coronal aspect of the osteotomy site E, Final drill used to finish preparation of the osteotomy site. F, Countersink drill is used to widen the entrance of the recipient site and allow for the subcrestal placement of the implant collar and cover screw. G, Implant is inserted into the prepared osteotomy site with a handpiece or handheld driver. H,Cover screw is placed and soft tissues are closed and sutured. Adapted from. Klokkevold et al., (2015).

Three surgical approaches have been applied over the years: (1) two-stage, (2) one-stage, and (3) immediate-loading. The first stage of a two-stage surgical procedure is to place the implant body below the soft tissue until the bone begins to heal. The healing process usually takes 2 to 3 months for the mandible and 3 to 6 months for the maxilla (Ebenezer et al., 2021). During the second stage of surgery,

soft tissues are reflected to attach a permucosal element or abutment. In a one-stage surgical approach, the implant body in the bone and the permucosal element above the soft tissue are both placed simultaneously until the occurrence of initial bone maturation. The abutment of the implant then replaces the permucosal element without the need for secondary soft tissue surgery. The immediate-restoration approach places the implant body and the prosthetic abutment at the initial surgery, and restoration (mostly transitional) is then attached to the abutment (Stanford, 2007).

2.3 Complication of surgery

Various complications and problems can be encountered during surgery and postoperatively. Perforated buccal or lingual plates can be observed during the procedure. In the case of an elliptical or eccentric surgical site preparation, a wider implant should be used if possible. If a wider implant does not present, the osteotomy must be packed with an autogenous graft, compress the graft, and the implant must be placed again (Ebenezer et al., 2021). Bleeding on the floor of the mouth can occur from the lingual artery or facial artery injury. Therefore, absolute care must be taken during osteotomy preparation. Nerve injury can lead to altered nerve sensation in the form of anaesthesia, paraesthesia or hyperesthesia. Consequently, the surgical landmark is often set conservatively 2 mm above the mandibular canal (Stanford, 2007).

Incision line opening is the most common postoperative complication (Stanford, 2007). Epithelial margin trimming can be done if granulation process extends for more than two weeks. No attempt should be made to cover them with tissue if implants become exposed during the healing period (Misch & Wang, 2008). The probability of implant mobility is unusual but may occur, mostly accompanied by

a radiolucent zone around the implant. This mobile implant should be removed (Ebenezer et al., 2021).

Among the signs and symptoms of failure for an implant are: horizontal mobility > 0.5 mm; bone loss that is rapidly progressive; pain on percussion; an uncontrolled exudate; generalised radiolucency around the implant; more than one half of the bone is lost around the implant; and implants inserted in poor positions, making them have no function for prosthetic support (Ebenezer et al., 2021).

A success rate of 85% at the end of the 5-year period and 80% at the end of the 10-year period are the minimum criteria for success (Stanford, 2007). Factors contributing to successful implant placement are a healthy bone, a non-smoker patient, hygiene, and the skill of the dental surgeon involved such as prosthodontist, and oral and maxillofacial surgeon. The type of implant used as a dental implant also contributes (Nelson, 2011).

2.4 Types of implants

There are three types of implants: endosteal, subperiosteal, and transosteal. Endosteal implants pierce only one cortical plate of the maxilla and mandible. The most frequently used endosteal implant is a root-form implant (Ebenezer et al., 2021). The subperiosteal implant has an implant substructure and a superstructure where a custom-cast frame is placed directly beneath the periosteum. A transosteal implant crosses through both cortical plates (Stanford, 2007).

Implants can be categorised based on the type of material, which is metal, ceramic, or polymer. Examples of metals used for implants are stainless steel and titanium. Hydroxyapatite and bioglass are some examples of ceramics that have been used as implants. This ceramic usually serves as a coating on a metal implant for

synergistic biomaterial properties. In addition to that, polymers are commonly utilised in the field of biomedical implantation, including soft and hard tissue implants, orthopaedic devices, and dental implants (Duran et al., 2023). Some common examples of polymers used for implants include polycaprolactones, teflon, silicone rubbers, and polyethylene (Mohd Yusoff et al., 2014; Duran et al., 2023).

2.5 Overview of titanium and its alloy

Titanium (Ti) is one of the biomaterials that has a wide range of qualities that make it biocompatible (Ronoh et al., 2022). It is the metal of promise for research in biomaterial science because it is resistant to corrosion, has a high resistance, and does not have an allergic response to it (Namanloo et al., 2022). Ti6Al4V, Ti6Al7Nb, and pure titanium are the three alloying systems that see the most widespread application in commercial settings (Xue, 2018). These alloy systems collectively make up a sizable portion of the market for titanium biomaterials.

Titanium and titanium alloys are resistant to corrosion because of the spontaneous development of titanium oxide which is just a few nanometers thick(Schenk, 2001). The titanium oxide shields the metal from further oxidation. Several surface treatments that are based on chemical and physical alteration can be used to increase the corrosion resistance, biocompatibility, bioactivity, and osseointegration of a material (Karaman et al., 2016).

2.5.1 Surface modification of titanium alloy

Surface modification can increase biocompatible properties and thus enhance osseointegration (Nobles et al., 2021). Surface topography can be modified on multiple levels from the macroscopic design or shape of an implant to the introduction of microscopic, submicron, or nano-textures superimposed on one another. Surface

topography can be modified by coating, grit blasting, acid etching and anodising (Mohajerani et al., 2017).

Hydroxyapatite coating can increase the biocompatibility of its bioactive components like bone. There are several methods of coating such as sol-gel, chemical vapour deposition, cold spraying, thermal, plasma spraying, solvent casting and other (Sasikumar et al., 2019). The hydroxyapatite-solvent casting method is the method of choice that increases the biocompatibility of the implant as well as the practicality properties and affordable (Mohd Yusoff et al., 2014).

2.6 Hydroxyapatite (HA)

Calcium (Ca) and phosphate (P) are the main elements of the bones and teeth. There are many coatings that can be developed from Ca/P (Table 1) (Vladescu et al., 2015) but hydroxyapatite is the bioceramic of choice due to its resemblance to the bone composition Ca/P ratio, 1.67.

Table 2.1 Phosphate coating types (Vladescu et al., 2015).

Type of phosphate coating	**Chemical formula**	**Ca/P ratio**
Stoichiometric hydroxyapatite	$Ca_5(PO_4)_3OH$ or $Ca_{10}(PO_4)_6(OH)_2$	1.67
Calcium-deficient hydroxyapatite	$Ca_{10-x}(HPO_4)_x$ $(PO_4)_{6-x}$ $(OH)_{2-x}$, with 0 < x_1	1.50 < Ca/ P < 1.67
Carbonate hydroxyapatite	$Ca_5(PO_4)_{2.5}(CO_3)_{0.5}(OH)$ or $Ca_5(PO_4, CO_3)_3(OH)$	2.00
Fluorapatite	$Ca_5(PO_4)3$ F	1.67
Tricalcium phosphate	$Ca_3(PO_4)_2$	1.5

Tetracalcium phosphate	$Ca_4P_2O_9$	2.00
Octacalcium phosphate	$Ca_8H_2(PO_4)_6*5H_2O$	1.33
Rhenanite	$NaCaPO_4$	1.00
Oxyapatite	$Ca_{10}(PO_4)_6O$	1.67

Hydroxyapatite (HA) is a calcium phosphate that has biocompatibility qualities. It goes by its chemical formula $Ca_{10}(PO_4)_6(OH)$. HA ceramic is a porous structure that allows cell penetration (Liu et al., 2022). However, because HA ceramics are too brittle to be used as bulk materials, they are typically applied as a coating to the surface of metallic implants (Ishikawa et al., 2003). This allows manufacturers to combine the mechanical strength of metal with the superior biological qualities of HA ceramics.

There are evidence demonstrating that HA ceramics exhibit excellent biocompatibility in bone (Mala & Ruby Celsia, 2018; Pina et al., 2018; Gul et al., 2020). However, there have been reports of biological reactions, possibly due to proteins that attach to the ceramic particles (Sammons, 2015; Hayashi et al., 2016). Further research is required before HA ceramic may be considered an optimal candidate for usage as a biomaterial in dental and medical implants.

2.6.1 HA coating application in dental implants.

To improve osseointegration and durability, HA coating application on dental implants has been extensively studied. Studies on HA-coated dental implants found substantial results. These implants integrate faster and stronger than uncoated implants, according to clinical investigations (Ong et al., 2000). Implant success depends on osseointegration, the link between biological bone and an artificial

implant. Secondly, HA-coated implants may speed jawbone integration, reducing healing time and treatment length.

Thirdly, long-term study demonstrated that HA-coated implants are more stable and durable, improving long-term outcomes and reducing implant failure (Almeida et al., 2023). In one study, HA-coated dental implants beat uncoated implants in stability and functionality (Pandey et al., 2022). Clinical study shows that HA's biocompatibility and resemblance to bone mineral improve tissue integration and minimise inflammatory reactions or rejection (Ong et al., 2000). HA-coated implants may promote bone regeneration and remodelling, especially for bone-deficient patients.

Comparative studies of HA-coated implants to other surface treatments reveal their efficacy and performance (Rahimi et al., 2022). Clinical data highly recommends HA-coated dental implants for tooth restoration due to their increased osseointegration, quicker healing, stability, and long-term effects (Ong et al., 2000; Rahimi et al., 2022). Nevertheless, ongoing research is being conducted to enhance implant design, surface characteristics, and clinical procedures to maximise the advantages of HA coating in dental implant therapy.

2.7 Poly (ε-Caprolactone) (PCL)

Poly (ε-Caprolactone) (PCL), a polyester (Mohd Yusoff et al., 2014), melts at 60 °C and decomposes at 350 °C. It is commonly utilised for temporary medical implants that are eventually absorbed and replaced by natural tissue, such as suture components (Lemos et al., 2012), and tissue-engineered skin. Additionally, it serves as a scaffold for the growth of fibroblasts and osteoblasts (Barbarisi et al., 2015).

In vitro degradation tests showed a 50% hydrolysis loss of PCL strength after 8 weeks (Yoon & Ji, 2005). This polymer has been approved by the Food and Drug Administration (FDA) for use in biomedical implant devices and drug delivery after extensive biocompatibility and efficacy testing (Xiao et al., 2019).

PCL's weak mechanical qualities limit its usage as a rigid tissue scaffold. Thus, PCL-based scaffolds need mechanical performance enhancement. Many studies stress PCL's co-polymerisation with hydroxyapatite (Mohd Yusoff et al., 2014; Chen et al., 2022; Solechan et al., 2023). This is to lower production costs with natural biomaterials and at the same time increase cell development and osteogenesis.

2.8 Biomaterial and biocompatibility

2.8.1 Biocompatibility

Biocompatibility is the capability of a substance to perform its intended function in conjunction with an appropriate response (Nandal et al., 2011). The degree to which the substance of the implant is compatible with the body has become the most important consideration when choosing an implant. The substance must possess qualities that are harmless to the local biological system (Crawford et al., 2021).

2.8.2 Biomaterial

The term biomaterial refers to any non-living component of a medical device that is designed to have some sort of interaction with living organisms (Kuhn, 2005). Biomaterial is defined as a material designed to take a form that can direct, through interactions with living systems, the course of any therapeutic or diagnostic procedure. This definition has been agreed upon by a series of biomaterial expert panels at a conference held in Chengdu, China (Mozafari, 2020). In the field of biomaterials, the emphasis has been placed on the synthesis, characterisation, and biology of the host

material. Most biomaterials cause a foreign-body reaction, which prompted an investigation of the meaning of the term biocompatibility.

There are three biologic responses when implants are rooted in the host tissue that are biotolerant, bioinert and bioactive. Biotolerant that is surrounded by fibrous tissue, for example stainless steel. Bioinert direct rigid attachment, for example titanium and its alloys and bioactive which allows the formation of bone on their surface for example hydroxyapatite.

The response or the host's response specifically, is necessary for the implant to operate well. A poor or non-existent host response might cause the implant to be rejected and can cause injury to the patient (Bostanci et al., 2022). A simple healing process that is resistant to bacterial colonisation is an example of an optimal host response. Dental implant tooth fixation serves as an example of a specific use of biocompatibility (He et al., 2021). *In vitro* and *in vivo* investigations are carefully regulated by the choice of materials, cells, and metabolic and biomechanical conditions to access the biocompatibility of biomaterials. If the biomaterial is biocompatible, the desired host response is initiated an osseointegration can take place.

2.8.3 Cell line for biocompatibility study

In vitro studies need a suitable cell line for testing the biocompatibility properties of certain biomaterials. The cell types chosen are determined by the target organ application. Human osteoblasts are more suitable for dental implant studies. These *in vitro* studies using human osteoblast will closely replicate the actual implant condition by involving direct contact between the implant and the bone tissue. Various comparative studies show that human cells react differently than cells from other mammals (Horie et al., 2022; Hwang et al., 2023).

For biocompatibility testing for bone implant, there are a lot of cell line that can be utilised such as MG-63, MC3T3-E1, Human Osteoblast cell (HOb) cells and Saos-2 cells. The Saos-2 cell has some features of an osteoblast. This cell has a calcitriol receptor. They also have the receptor for parathyroid hormone (PTH) and make cyclic AMP when treated with PTH. When these cells are injected under the skin of mice without immune systems, they do not cause tumours to grow. This cell makes a mineralised matrix when implanted intra-peritoneally into immunocompromised mice, which is a characteristic of osteoblastic cells (Rodan SB, 1987).

This osteoblast cell line is a suitable candidate for *in vitro* study of the implant as this osteoblast cell-like rapidly proliferates to minimise the lag phase of cell growth, as well as its osteoblastic feature which involves most of the cells during the osteogenesis process (Czekanska et al., 2014).

2.8.4 Direct and indirect technique for cell seeding

2.8.4(a) Direct technique for cell seeding

Direct technique of cell seeding is one of the most straightforward methods for assessing biocompatibility of the material. In this approach, the sample is directly exposed to the designated cells for *in vitro* cell culture. Following an incubation period, the cell culture surrounding the samples is examined using either an inverted microscope or an electron microscope to assess cell morphology, viability, and attachment to the sample (Ayobian-Markazi et al., 2012).

2.8.4(b) Indirect technique for cell seeding

In addition to the direct contact technique, the indirect method for cell seeding is widely utilised in *in vitro* biocompatibility testing. This approach is aimed at assessing the cytotoxicity and biocompatibility of leachable compounds from

biomaterials. Samples are immersed in cell culture media, such as Dulbecco's Modified Eagle Medium (DMEM), which closely mimics the biological environment. Detailed guidelines for this technique can be found in ISO 10993:5 (X. Liu et al., 2018).

2.9 Host inflammatory response

The term osseointegration refers to the process by which an implant and the human body can be made to function as a single integrated unit. For researchers to obtain the optimum biocompatibility of the biomaterial, as was previously discussed, they need to understand the entire process of host inflammatory response, which includes the injury as well as the implant itself, which induces the inflammatory response (Lee & Bance, 2019).

2.9.1 Introduction of host inflammatory response

It has been commonly accepted that inflammation, wound healing, and the reaction to a foreign body are all components of the tissue or cellular responses to injury (Anderson, 2015). When it comes to how it responds to injury at the tissue level, the human body is very similar to that of lower animals. Phagocytosis and regeneration were the earliest reactions to injury to arise in animal phyla. These processes can be found in amoebas, hydras, sponges, and other organisms (Anderson, 2015). At the level of these creatures, phagocytosis refers to the process in which a cell consumes a solid particle (White et al., 2022). This process requires no more than the simple detection of damage as the particle is foreign and the action required to reject the particle.

A more complex level of response is seen in larger multicellular animals, such as invertebrates, in which the presence of a vascular system enables the mobilisation

and transport of specialised inflammatory cells (phagocytes) to the injury site (Ranganath & Rao Nagashree, 2001). This higher level of response is seen in larger animals. This nonspecific acute inflammatory response extends beyond basic identification and phagocytosis to include chemotaxis, which is the movement of cells in response to a chemical concentration gradient, as well as alterations in the microcirculatory system (Hannoodee and Nasuruddin, 2022a). There is a highly specialised immune response that may be found in vertebrates. This response improves the efficiency of phagocytosis as well as the initial inflammatory response that occurs after an injury.

This augmentation is made possible by the presence of cells known as lymphocytes that remember one encounter with a harmful agent and create a stronger, more specific, and faster reaction when they encounter that agent again. The immunological response can be distinguished from the acute inflammatory reaction by a triad of characteristics known as specificity, memory, and amplification (Hamad & Mangla, 2022).

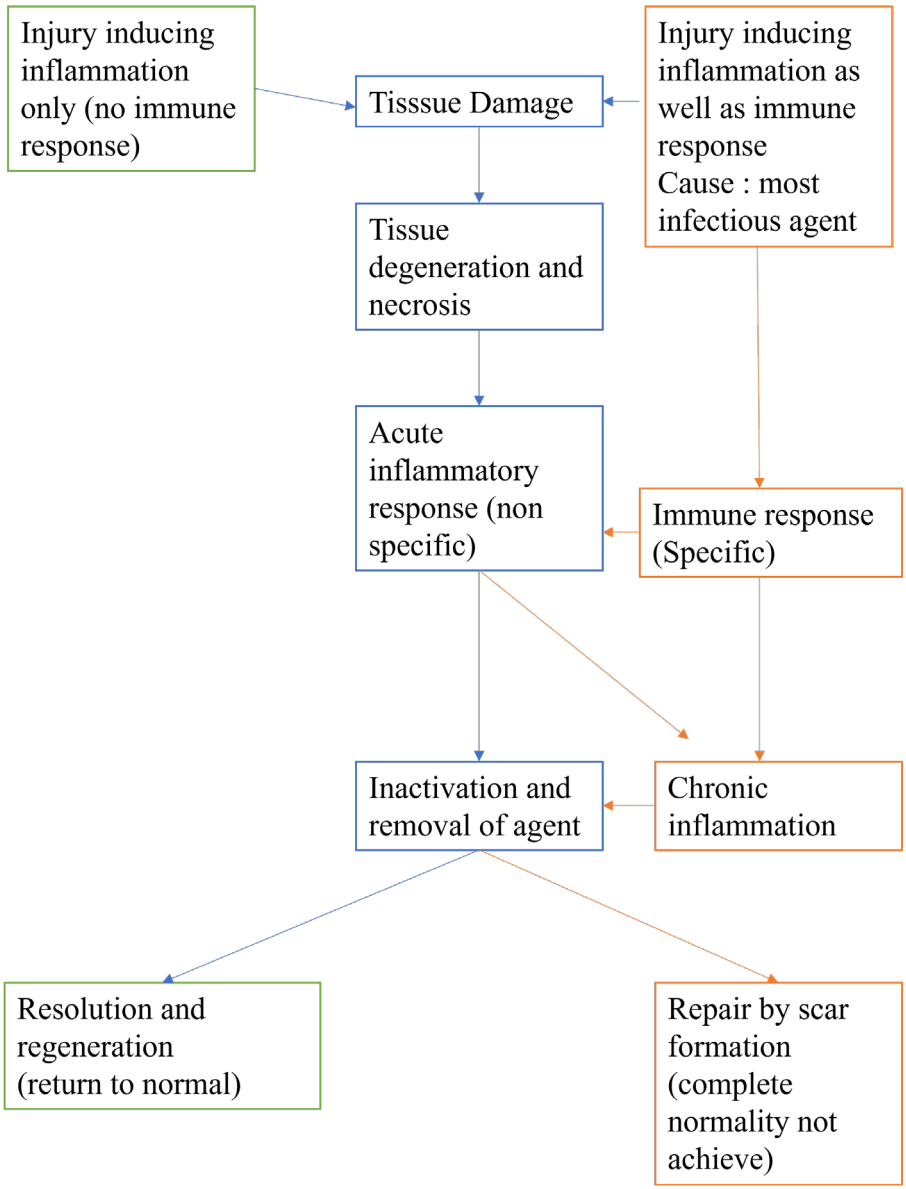

Figure 2.2 Host Inflammatory Response, adapted from Anderson (2015).

2.9.2 Acute inflammatory response

Tissue damage causes acute inflammation. Any non-lethal injury can cause it. Acute inflammation is the first defence against injury, characterised by microcirculation alterations that exude fluid and emigrate leukocytes from blood vessels to the injury site (Miller & Zachary, 2017). Acute inflammation is short-lived and aims to remove the harmful substance before the immune response develops.

Acute inflammation was considered a disease until the late 18th century. John Hunter was the first to recognise that acute inflammation was a healthy response to injury: "But if inflammation arises, regardless of the reason, it is still an effort whose objective is to return the organs to their normal duties" (Hannoodee & Nasuruddin, 2022b).

2.9.3 Wound healing

Following tissue injuries, the occurrence of inflammation is typically accompanied by the subsequent initiation of the healing process. Prior to initiating any therapeutic intervention, it is imperative to perform the necessary elimination of inflammatory and necrotic cellular remnants. Following a transient injury, such as a solitary minor traumatic event, the phenomenon of wound healing occurs expeditiously. If the trigger is rapidly deactivated by the host's immunological or inflammatory responses, the process of healing is also accelerated. In instances of persistent low-level tissue injury, the process of tissue repair occurs concurrently with the presence of persistent chronic inflammation (Hunt et al., 2000).

Resolution is the process of returning the tissue to its pre-injury normal state, which is the desirable outcome of healing. If the injury was slight, clearing the inflammation-related debris will be enough to return the tissue to its normal state. Regeneration has the potential to replace any necrotic parenchymal cells with brand-new cells of the same type of parenchymal tissue (Nagle et al., 2022).

2.10 Osseointegration in dental implant

For osseointegration to take place, a response must be initiated from the host inflammatory response and end with a good healing mechanism. A good and desirable