Chapter

HYDROXYAPATITE: SYNTHESIS, PROPERTIES, AND APPLICATIONS

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This book chapter is dedicated to Dr Wim Richter
on the occasion of his retirement

ABSTRACT

Hydroxyapatite (HA) has been extensively investigated and used in bone clinical application for more than four decades. The increasing interest in HA is due to its similar chemical composition to that of the inorganic component of natural bone. HA displays favourable properties such as bioactivity, biocompatibility, slow-degradation, osteoconduction, osteointegration, and osteoinduction. HA is commercially available either from a natural source or as synthetic HA. Various methods have been reported to prepare synthetic HA powders which include solid state chemistry and wet chemical methods. For bone applications, pure HA, biphasics with β-tricalciumphosphate (β-TCP) and HA composites have been widely investigated. HA is processed into dense bodies by sintering

and sintering temperature, stoichiometry, phase purity, particle grain size, And porosity are important processing parameters. Furthermore porosity in particular pore size; macro and microporosity; pore interconnectivity; morphology; pore size distribution, and surface properties influence bone remodelling. At high sintering temperatures, HA is transformed primarily into β-TCP which is amorphous and resorbable. Despite the success of HA derived implants one of the major drawbacks of this material is its poor tensile strength and fracture toughness compared to natural bone. This makes HA unsuitable for several load-bearing applications. HA has been reinforced with a number of fillers including polymers such as collagen, metals and inorganic materials such as carbon nanotubes, and HA has also been applied as coatings on metallic implants. To improve the biomimetic response of HA implants, nano-HA powder has been synthesised, and HA nanocomposites containing electrospun nanofibers, and nanoparticles have been produced. Nano-HA displays a large surface area to volume ratio and a structure similar to natural HA, which shows improved fracture toughness, improved sinterability, and enhanced densification. Biological entities such as bone morphogenic proteins (BMP's), stem cells, and other growth factors have also been incorporated into HA nanocomposites. HA implants have been applied in the form of dense and porous block implants, disks, granules, coating, pastes, and cements. Some of the frequent uses of HA include the repair of bone, bone augmentation, acting as space fillers in bone and teeth, and coating of implants. In this book chapter, we will focus on the synthesis and properties of HA powders and HA implants with specific application in bone engineering. We will also share our experience over the past 20 years in dental and craniofacial reconstruction.

1. INTRODUCTION

Due to an ageing population with high prevalence of disease, the need for new biomaterials for improving quality of human life continues to be a major focus for scientists, engineers and clinicians alike. To combat the shortcomings of autographs and allographs which are associated with limited availability of tissue, and morbidity at the donor site; and disease transmission and immunogenic rejection respectively, scientists have started centuries ago implanting artificial or man-made materials in the body to aid and restore functioning to organs or tissues.

Over the past 30-40 years, one of the most significant developments in orthopaedics has been the use of bioceramic materials for bone replacement, reconstruction and repair. Bioceramics are biocompatible ceramic materials,

and commonly include bioglass and calcium phosphates (such as hydroxyapatite (HA), β-tricalcium phosphate (β-TCP)), and biphasic calcium phosphate. Bioceramics were used initially as an alternative biocompatible material to metallic bone implants, however due to their superior performance; bioceramics have now become one of the most widely studied biomaterial for bone clinical applications.

Over the past four decades, the field has seen major advances and a paradigm shift from first to third generation bioceramics [1]:

- 1st generation Bioceramics: "bioinert" such as alumina and zirconia;
- 2nd generation Bioceramics: "bioactive" and "bioresorbable" such as calcium phosphates (hydroxyapatite, and β-tri-calcium phosphates), and bioglass;
- 3rd generation Bioceramics: Porous 2nd generation bioceramics and composites containing biologically active substances such as cells, growth factors, proteins capable of regenerating new tissue.

Of the calcium phosphate bioceramics, HA is the most widely used for orthopaedic and dental reconstruction because it is the predominant component of human bone mineral and teeth enamel. To date, HA implants have been used clinically in the form of powders, granules, cements, dense and porous blocks, biphasics, coatings, and as various composites. Some of the favourable properties of HA include biocompatibility, lack of an immunogenic response, and slow resorption, however it was the phenomenon of "bioactivity" in the 1960's that attracted increasing interest in bioceramics as the material of choice for bone repair.

A material is said to be bioactive[1] when it stimulates a specific biological reaction at the material-tissue interface, occurring with the formation of biochemical bonds between the living tissue and the material" [2].

While the bioinert bioceramics suffered from fibrous encapsulation leading to lack of integration with surrounding tissue, implant migration, and long-term complications, HA implants were able to from direct bonds to native tissue thereby improving the *in vivo* performance of the material. The interaction of bioactive materials with the surrounding tissue is by means of ion-exchange. Tissue repair is induced *in situ* where implanted bioactive materials release chemicals in the form of ionic dissolution products at

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[1] "Bioactivity is the characteristic of an implant material that allows it to form a bond with living tissues" [193].

controlled rates to stimulate native cells, which in turn activates a cascade of biological reactions resulting in new tissue growth [3]. A biologically active carbonate apatite layer forms on the surface of the bioactive implants, which is chemically equivalent to the mineral phase of bone [4].

There are many natural sources for HA which include human bone, bovine bone [5,6], coral [7,8], chitosan [9,10], fish bone [11] and egg shell [12], among others. However a concern with natural HA, is transmission of diseases when proper preparation is not followed to remove all protein [13]. Synthetic HA is more commonly used, since it is more easily available, and free of disease transmission.

Synthetic HA is often stoichiometric with a chemical formula of $Ca_{10}(PO_4)_6(OH)_2$, and a specific atomic Ca/P molar ratio of 1.67. Depending on the synthesis route and HA powder processing conditions, various other calcium phosphates with Ca/P ratio ranging from 2.0 to as low as 0.5 can be produced [14]. HA is generally highly crystalline with the following lattice parameters: (a= 0:95 nm and c = 0:68 nm) and it displays a hexagonal symmetry (S.G. $P6_3/m$) with preferred orientation along the c axis [14]. HA crystals typically display a needle-like morphology.

Although synthetic HA is similar to the inorganic component of natural bone, vast differences exist with respect to the total chemical composition, stoichiometry and structure. Bone which is biological apatite is described as carbonated (3-8 w/t %), calcium deficient HA which is non-stoichiometric, non-crystalline, and ion-substituted. Additionally in bone, HA exists as nanocrystals with dimensions of 4 x 50 x 50 nm whereby the nanocrystals are embedded in an organic collagen fibre matrix which comprises 90% of the protein content [4]. Human bone mineral is ion-substituted HA represented by the chemical formula: $Ca_{8.3}(PO4)_{4.3}(HPO_4,CO_3)_{1.7}(OH,CO_3)_{0.3}$ [15,16]. When CO_3^{2-} and HPO_4^{2-} ions are added, the Ca/P ratio varies between 1.50 to 1.70, depending on the age and bone site [15]. When bone ages, the Ca/P ratio increases, suggesting that the carbonate species increases.

Despite its biocompatibility, the inherent mechanical properties of HA specifically brittleness, low tensile strength, and poor impact resistance have restricted its use in load-bearing applications [17]. HA use is therefore typically limited to non-critical load-bearing applications, such as the ossicles of the middle ear, orthopaedic bone grafting and in dentistry.

The research trends in the field over the past 4 decades includes the application of HA coatings on metallic implants to improve the bioactivity of the latter, development of biphasics with varying ratios of HA: β-TCP for faster bioresorption; development of porous three dimensional (3D) HA

scaffolds for tissue engineering; and development of biomimetic implants consisting of organic/inorganic multiphase HA composites and nanocomposites.

Other interesting trends for HA include applications in drug delivery, cell culture, purification of antibodies on industrial scale, as an artificial blood vessel or trachea, as well as a catheter made of an HA-composite [18,19].

In this review, we will focus on the synthesis and properties of HA and identify some of the most important processing parameters which influence the mechanical and physical properties of HA implants. We will discuss the use of HA as dense compact bodies, coatings on metallic surfaces, as well as its use in composites and nanocomposites for biomimetic and tissue engineering applications with specific focus on craniofacial and bone tissue engineering.

2. SYNTHESIS METHODS FOR HA POWDERS

When manufacturing HA implants, the properties and characteristics of the starting HA powder are crucial. It is important to control the phase purity, stoichiometry, grain size, particle shape and orientation, homogeneity, crystallinity, as well as the agglomeration nature of the powder [20]. The quality of the HA powder is important since it influences the material's physical and mechanical properties and bioactivity since these powders are further processed into HA implants by combining it with polymers for the production of biocomposites, applied as coatings to implants or sintered into green bodies [20].

There are several methods which have been developed to synthesise HA powders and these can be classified as either wet chemistry methods or solid state reactions. An overview of the advantages and disadvantages of each method is shown in Table 1.

2.2. Wet Chemical Methods

A number of wet chemical methods have been reported for synthesis of HA, and these include precipitation [21,22], sol–gel synthesis [23-25], hydrothermal reactions [26-28], emulsion and microemulsion synthesis [29] and mechano-chemical synthesis [20,30,31]. In this review we are focussing on precipitation, sol-gel and hydrothermal methods.

Table 1. Advantage and disadvantages of some of the synthesis methods for HA

	Advantages	Disadvantages	References
Solid-state	Easy to perform; inexpensive; stoichiometric HA formed	Needs high sintering temperature; long treatment times	[32]; [30]; [31]; [20]
Precipitation	Can produce Nano HA particles; industrial production possible; water is the only by-product	Difficulty to obtain stoichiometric HA; need high pH to prevent formation of Ca-deficient HA; need high sintering temperature to form crystalline HA; product very sensitive to reaction conditions such as pH, stirring rate, drying temperature, etc.	[22]; [21]
Sol-gel	Can produce Nano-HA particles; homogenous molecular mixing occurs; low processing temperature's required; increased control over phase purity	Difficulty to hydrolyse phosphate; expensive starting chemicals	[23]; [24]; [25]
Hydrothermal	Well crystallised and homogenous powder; nano-HA has been prepared	Agglomeration of HA powders is common; high pressures required for processing	[26-28]; [33]

2.2.1. Precipitation

Precipitation is the most commonly used synthesis method for HA. Precipitation typically involves a reaction between orthophosphoric acid and dilute calcium hydroxide at pH 9 as shown in equation 1, with the former added drop-wise under continuous stirring.

$$3Ca_3(PO_4)_2 + Ca(OH)_2 \rightarrow Ca_{10}(PO_4)_6(OH)_2 \qquad (1)$$
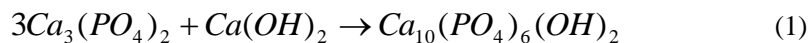
Precipitation occurs at a very slow rate and the reaction temperatures can be varied between 25°C and 90°C. At higher reaction temperatures, a higher crystalline product is formed [34,35].

Ammonium hydroxide, di-ammonium hydrogen phosphate and calcium nitrate can also be used for the production of HA via a precipitation method. The ammonium hydroxide is added to ensure a constant pH and this results in a faster production rate, however after precipitation; the resulting precipitate must be washed to remove nitrates and the ammonium hydroxide [20,36].

For the precipitation method continuous stirring is applied to ensure the slow incorporation of calcium into the apatite structure to reach stoichiometric Ca/P ratio. The morphology of the crystals also changes during this maturation step, from needle-like structures to more block-like. When calcium deficient HA is desired, the process can be carried out at pH's lower than 9 [20,20,34,36].

2.2.2. Hydrothermal Method

In a typical hydrothermal reaction, calcium and phosphate solutions are reacted at very high pressures and temperatures to produce HA particles [26,28,37-40]. A variety of starting calcium and phosphate salts have been reported, and these include calcium hydroxide, calcium nitrate, calcium carbonate and calcium chloride; and calcium hydrogen phosphate and dipotassium and diammonium hydrogen phosphates respectively. A typical hydrothermal reaction is shown in equation 2. The reaction is normally conducted in the range of 60–250°C for 24 h to yield crystalline HA crystals that are usually agglomerated.

$$4Ca(OH)_2 + 6CaHPO_4 \bullet 2H_2O \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 18H_2O \ (2)$$
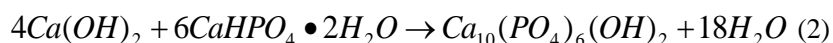
HA nanoparticles, nanorods, and nanowhiskers have been reported by the hydrothermal method [40,41].

2.2.3. Sol-gel Synthesis

Sol-gel materials can be manufactured by three different methods namely: gelation of colloidal powders, hypercritical drying and by controlling the hydrolysis and condensation of precursors and then incorporating a drying step at ambient temperature. [16,42,43] Sol-gel synthesis offers increased control

over formation of particular phases and phase purity while HA synthesis occurs at lower temperatures when compared to hydrothermal reactions for example. Some of the drawbacks of sol-gel techniques have been the difficulty to hydrolyse phosphate and the expensive starting chemicals. [44]. Jillavenkatesa and co-workers examined the possibility to manufacture HA powders by the sol-gel method, by simplifying some of the steps involved and making use of cheaper starting chemicals.

2.2.4. Solid-State Method

This method although less frequently reported, is relatively simple and inexpensive compared to the wet treatment methods. The solid-state method for HA synthesis typically involves combining β-TCP and $Ca(OH)_2$ powders in specific ratios (3:0-3.4), mixing the dry powders in water, wet milling, casting the mixture into bodies, drying and sintering [32] (see equation 3).

$$3Ca_3(PO_4)_2(\beta-TCP) + Ca(OH)_2 \rightarrow Ca_{10}(PO_4)_6(OH)_2 \qquad (3)$$
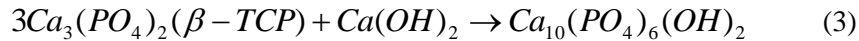
High sintering temperatures of at least 1000°C for 8 hours has been used to achieve phase pure HA with high crystallinity [32]. The β-TCP:$Ca(OH)_2$, sintering temperature was found to be critical for formation of pure HA, while particle agglomeration was influenced by pH [32]. Transformation of HA agglomerates into β-TCP was observed for HA powder prepared by this method [32,45]. The phenomena of HA conversion into β-TCP as a result of sintering has been reported by other groups, and will be discussed in more detail in a later section.

Nano-HA particles have also been produced via the mechanochemical method which is also a solid-state reaction. This synthesis route involves mixing dry powders of calcium hydroxide ($Ca(OH)_2$) and di-ammonium hydrogen phosphate (($NH_4)_2HPO_4$), which are then dry-milled at various rotation speeds and ball to powder ratio's [30,31]. Coreño *et al.* observed that after 2 hours of milling of the powders, HA was formed. When the milling time was increased to 6 hours, they were able to obtain nano-HA powders. The particles observed were between 10 and 50 nm [31].

3. HA PROCESSING PARAMETERS AND MATERIAL PROPERTIES

For clinical applications HA is often applied in the form of dense HA bodies or powder compacts. In recent decades research is being focussed on porous HA scaffolds. For the formation of dense HA bodies, generally the HA powder is firstly calcined i.e. treated at high temperatures in air to remove organic impurities, and volatiles. The calcination process produces pure HA phase with high crystallinity. The calcined pure HA powder is then further processed to produce fine HA powder. This could entail adding binders and deflocculants to a wet mixture and ball-milling. The processed powder is then pressed in a mould (either with or without pressure) to give HA green bodies (pre-sintered). The green bodies are then sintered at high temperatures typically above 1000°C for various periods to produce dense HA bodies.

Over the past several decades, extensive research has been conducted to elucidate the sintering conditions and effect of powder properties on the densification, microstructure, phase stability and mechanical properties of HA bodies [13,20,46,47].

3.1 . Sintering

Sintering of HA bodies has been described as a two stage process [20]. During the initial stage, density increases gradually with the sintering temperature and is associated with particle coalescence and neck formation between the powder particles (see Figure I), as well as removal of moisture, carbonates, and volatiles such as ammonia nitrates, and organic compounds as gaseous products [48]. For stoichiometric HA and calcium deficient HA necking has been reported to occur at 900-1000°C and 1000-1050°C [20].

During the second stage of sintering, densification occurs with removal of maximum porosity in the HA body and subsequent shrinkage (see Figure II). Densification is a process of pore elimination which is driven by a diffusion process involving transfer of matter between particles, from the particle volume or the grain boundary between particles. The changes therefore occurring in HA bodies during densification include increase in grain size, decrease in porosity and surface area, increase in crystallisation, and increase in mechanical properties.
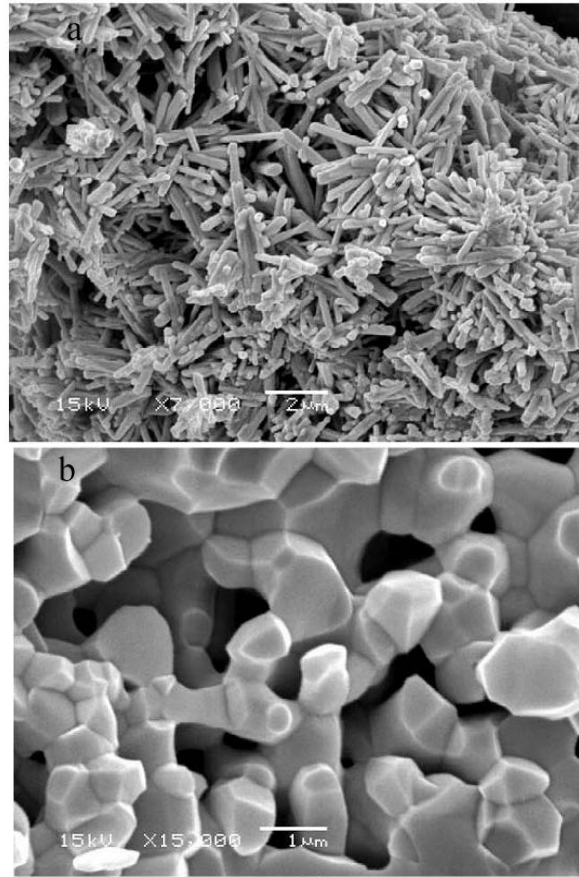
Figure 1. SEM images showing calcium deficient HA a) as synthesized by a precipitation method before sintering, and b) after sintering at 2000°C showing HA neck formation [20]. (Permission obtained from publisher for reprint)

Figure II shows large pores in the sintered bodies at 1050°C, and a significant reduction in porosity at 1250°C. Porosity in implants is needed for bone engineering applications since it facilitates transport of nutrients and oxygen and enables tissue infiltration into the pores. The challenge however is to reach an optimum density which can provide the desirable mechanical properties while still maintaining a porous structure.

Sintering parameters such as temperature, soaking time and atmosphere have been found to directly impact the physical and mechanical properties of HA bodies. Studies have also shown the importance of the Ca/P ratio on the

sintering properties of HA bodies, whereby deviation from stoichiometry, results in lower densification.

Typically the temperature used to sinter dense HA bodies exceeds1000°C. It has been reported that grain size typically increases gradually up to a critical temperature, above which the grain growth phenomena increases exponentially. HA can be sintered to theoretical density of HA is 3.16 g/cm3 between temperatures of 1000-2000°C. However it has been reported that processing at higher temperatures (exceeding 1250-1450°C) results in exaggerated grain growth and decomposition. Thermal instability of HA bodies at high sintering temperatures is influenced by a number of different parameters. These will be discussed in detail in a later section.
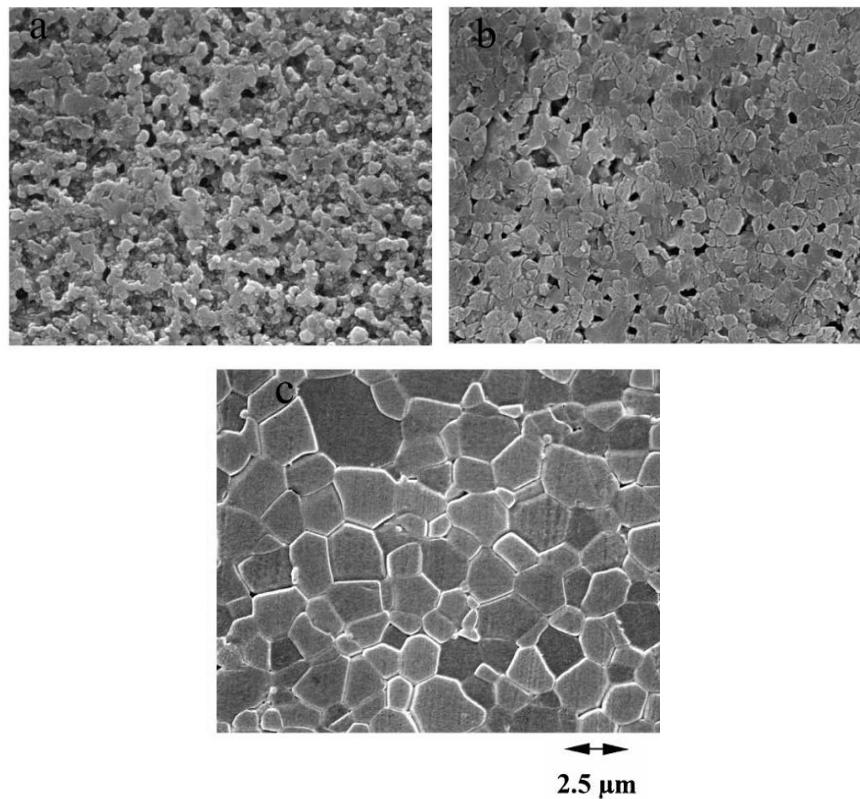

2.5 μm

Figure 2. SEM image of a commercially available HA powder which was cold isostatically pressed at 200 MPa and sintered at a) 1050°C, b) 1150°C and c) 1250°C showing grain growth and gradual removal of porosity in the densified body with an increase in temperature [47]. (Permission obtained from publisher for reprint).
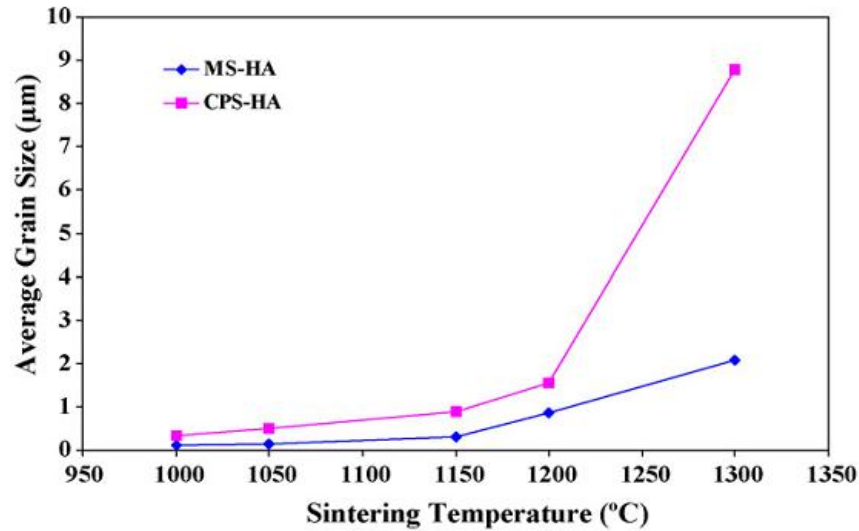
Figure 3. Average grain size increase with sintering temperature for conventional pressureless sintered HA (CPS-HA), and microwave sintered HA (MS-HA) [49]. Particles produced by MS-HA were smaller and resulted in reduced grain growth (Permission obtained from publisher for reprint).

Ramesh *et al* [2008] reports more than a 8 fold increase in grain size when conventional pressureless sintered HA, was heated from 1200°C to 1350°C (see Figure III). Excessive grain growth is associated with failure at the grain boundary, and compromises the mechanical properties at higher sintering temperatures.

The most commonly used sintering method for dense HA bodies is the conventional pressureless sintering. However a major challenge of this method is the high sintering temperatures and long holding times which are required produce highly dense bodies. It has been shown that high sintering temperatures are associated with excessive grain growth and decomposition of HA. Some alternatives which have been proposed include microwave sintering, hot pressing, and hot isotatic pressing. Ramesh *et al* investigated the use of microwave sintering as an alternative to conventional sintering. Smaller, finer particles were produced by microwave sintering which prevented excessive grain growth, and improved the sintering properties of HA bodies [46].

3.2. Thermal Stability of HA

It has been well documented that HA undergoes phase instability at high calcination and sintering temperatures. Several studies have been conducted to investigate the decomposition of HA [20,45,47,50].

There is consensus that the thermal instability of HA occurs in a 4 step process involving dehydroxylation and decomposition [51]:

Step 1: $Ca_{10}(PO_4)_6(OH)_2 \rightarrow Ca_{10}(PO_4)_6(OH)_{2-2x}O_x\square_x + x\ H_2O$
(hydroxyapatite)   (oxyhydroxyapatite)

Step 2: $Ca_{10}(PO_4)_6(OH)_{2-2x}O_x\ \square_x \rightarrow Ca_{10}(PO_4)_6O + (1-x)\ H_2O$
(oxyhydroxyapatite)   (oxyapatite)

Step 3: $Ca_{10}(PO_4)_6O \rightarrow 2\ Ca_3(PO_4)_2 + Ca_4(PO_4)_2O$
(oxyapatite)   (tricalcium phosphate)   (tetracalcium phosphate)

Step 4: $Ca_4(PO_4)_2O \rightarrow 4\ CaO + P_2O_5\uparrow$ or $Ca_3(PO_4)_2 \rightarrow 3\ CaO + P_2O_5\uparrow$
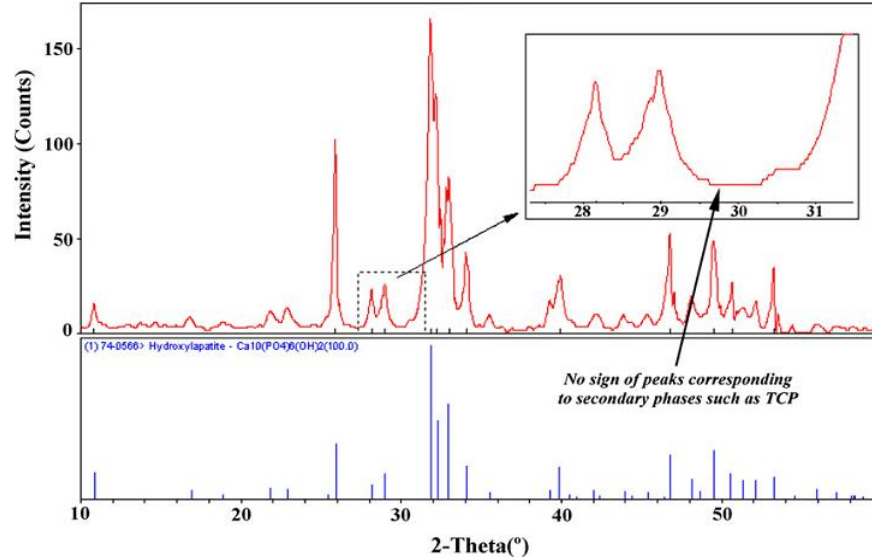
Figure 4. Typical XRD pattern for sintered stoichiometric HA [49]. (Permission obtained from publisher for reprint).

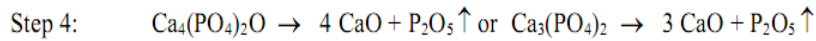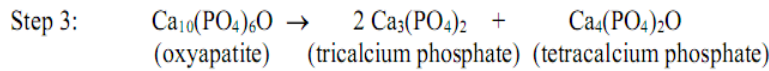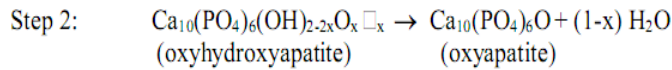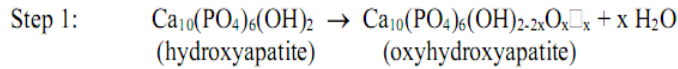Dehydroxylation (steps 1 and 2) involves the loss of water, which proceeds via the interim formation of firstly oxyhydroxyapatite, OHAP and then oxyapatite OHA where ☐ stands for a lattice vacancy in the OH position along the crystallographic c-axis. Decomposition (steps 3 and 4) of OHA then proceeds to secondary phases such as tricalcium phosphate, tetracalcium phosphate and calcium oxide.

The transformation of HA has important consequences in bone engineering and plasma coated implants, since β-TCP is a resorbable calcium phosphate, and while it will enhance resorption of HA implants, decomposition of HA will also reduce the mechanical properties of the material.

To determine the decomposition of HA, typically X-ray diffraction and Fourier-transform Infrared (FTIR) are used. XRD enables determination of the phase purity (Figure IV) while FTIR allows observation of the hydroxyl groups in HA to study dehydroxylation (Figure V).

With XRD, the phase purity of HA is often confirmed by Powder Diffraction File® database (PDF) reference patterns. Pattern JCPDS (File No 74-0566) is commonly used for identification of stoichiometric HA [45,47,49,50]. For pure HA typically three identification peaks at $2\theta = 31.8°$ (211); 32.2° (112); and 32.9° (300) are used.

There exists some controversy in the literature regarding the conditions for HA decomposition. Typical temperatures in the range of 1100-1400°C have been reported for the decomposition of HA [20,47,52,53]; however temperatures as low as 600°C [50] have also been reported. Additionally some studies have shown no HA decomposition even when sintering was conducted at 1000-1300°C [46,49].

There are a number of factors which are believed to control HA decomposition and these include sintering temperature and hold time, powder synthesis method, sintering atmosphere (eg air, water, vacuum), phase purity and Ca/P stoichiometry [20,45,47,50,52].

Processing under vacuum is believed to lead to enhanced decomposition, while a water vapour saturated atmosphere retards decomposition by inhibiting densification. The presence of β-TCP is also known to enhance decomposition of HA. Nilen *et al* [2008] has shown that for a biphasic mixture with pre-sinter HA/β-TCP of 40/60 wt%, approximately 80% of the HA was decomposed to β-TCP during sintering at 1000 °C. It is postulated that β-TCP accelerates HA decomposition due to the thermal expansion coefficient mismatch between the intimately mixed phases [45].
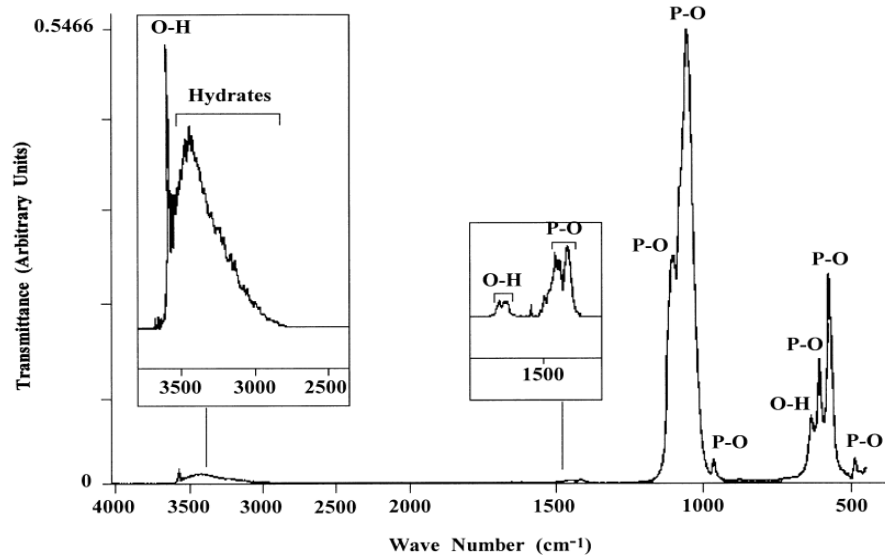
Figure 5. FTIR absorbance spectrum of pure HA showing the OH and PO groups. The HA used is commercial hydroxyapatite powder (Merck 2196, Germany) with stoichiometric Ca/P molar ratio of 1.67 ± 0.002 [47]. (Permission obtained from publisher for reprint).
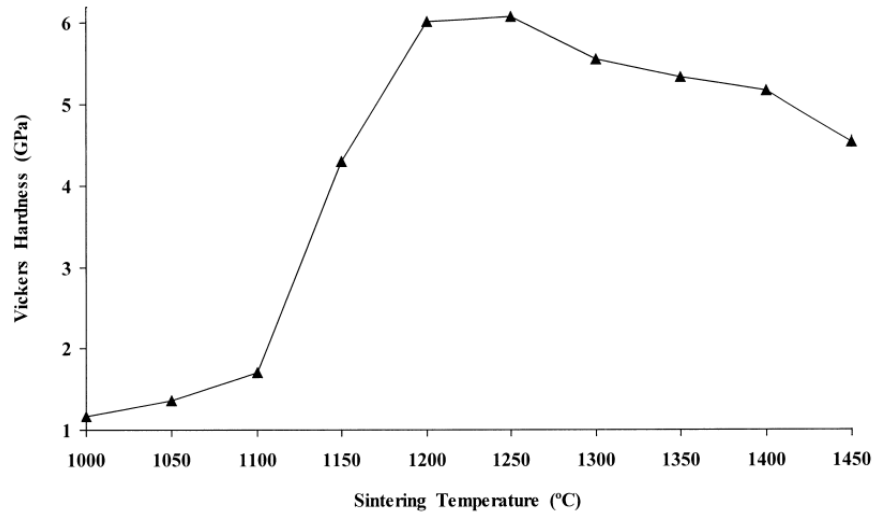
Figure 6. Vickers hardness of a commercially available HA powder was cold isostatically pressed at 200 MPa and sintered at temperatures ranging from 1000 to 1450°C [47]. (Permission obtained from publisher for reprint).

3.3. Mechanical Properties

The mechanical properties of HA should as closely as possible match that of natural bone, to allow for proper bone remodelling at the implant site. HA is typically sintered to enhance the mechanical properties of HA bodies, however the mechanical properties of sintered HA implants is still inferior to natural bone (Table 2).

While various studies report the mechanical properties of natural bone, dentine, dental enamel, and synthetic HA implants, large variations is often seen amongst data which could be attributed to differences in material properties, as well as the limited sensitivity of the measurements hence only average ranges are given in Table 2.

While improved mechanical properties are desired the mechanical properties of HA should not exceed that of natural bone, but rather replicate it as close as possible. Where there is a large gradient in the elastic modulus between implant and the native, this can lead to the so-called stress-shielding phenomenon. During stress-shielding, the load put on the implant during movement is not transmitted by the bone but through the stiff implant leading to atrophic loss of the cortical bone [51]. Bone requires regular movement and tensile loads to be healthy and a lack of this retards the bone regeneration process.

Sintering temperature, porosity, Ca/P stiochiometry, phase purity, and particle grain size are believed to influence the strength of HA bodies. Studies have also shown that Vickers hardness typically increases with sintering temperature (and grain size) up to a critical value beyond which severe loss in hardness is observed [47] (Figure VI). This has been attributed to two phenomena i.e. excessive particle growth and thermal decomposition of HA.

One of the main constraints of HA is its low fracture toughness compared to compact cortical bone which means that HA behaves as a relatively brittle material. Also its mechanical properties diminish in porous implants which typically are required for bone tissue engineering. The elastic modulus of dense HA is in the similar range to cortical bone, dentine, and enamel, but dense bulk HA implants display mechanical resistance of ~100MPa, which is typically 3x higher for natural bone, and mechanical resistance also diminishes drastically in porous bulk HA scaffolds [48]. Due to its high brittleness, the use of HA implants is restricted to non-load bearing applications such as middle-ear surgery, filling of bone defects in dentistry or orthopaedics, as well as coating of dental implants and metallic prosthetics [48].

Table 2.Mechanical properties of bone vs HA [54-56]

Mechanical properties	Cortical bone	Cancellous bone	Dentine	Dental enamel	Dense HA	Porous HA
Compressive strength/ Mpa	100-230	2-12	295	384	120-900	2-100
Flexural/tensile strength /Mpa	50-150	10-20	51.7	10.3	38-300	3
Fracture toughness / $MPa.m^{1/2}$	2-12	NA			1	
Young's/Elastic modulus/GPa*	18-22		18-21	74- 82	35-120	
Vickers Hardness/GPa					3-7	

To improve the mechanical properties of HA implants, reinforcements with various fillers have also been investigated. This includes ceramics, polymers, metals and inorganics. Some researchers have showed that by combining HA and natural or synthetic nanofibers, HA's mechanical strength may be increased. Polymers are more flexible compared to ceramics and therefore aids in increasing the mechanical stability of HA by decreasing its brittleness [57,58]. The concern exists that by combining HA with polymers its osteoinductive property may be masked since the HA particles may be embedded inside the polymer fibre.

In this recent decade, carbon nanotubes (CNT) is gaining increasing interest as a reinforcement for HA implants [54]. However a challenge with CNT's is its natural tendency to agglomerate. Good dispersion of CNT's in polymer matrices is essential to prevent early failure. Much effort has been made in recent years to prepare nanocomposites containing individual and non-aggregated HA nanoparticles involving particle modification [59]. When CNT's were incorporated into HA matrix, fracture toughness was improved by 92% and elastic modulus by 25% compared to a HA matrix without CNT's [60,61]. HA crystal forms a coherent interface with CNT's, resulting in a strong interfacial bond. The uniform distribution of CNT's in the HA matrix, good interfacial bonding and fine HA grain size was crucial to improve fracture toughness thus combining the osteoconductive properties of HA and the excellent mechanical properties of CNT's [62]. However the toxicity of HA-CNT composites is still a concern and this area of research must be fully investigated before HA-CNT's can be considered as a viable option for bone reconstruction.

3.4. Biological Properties of HA

HA bioceramics have been widely used as artificial bone substitutes because of its favourable biological properties which include: biocompatibility, bio-affinity, bioactivity, osteoconduction[2], osteointegration[3], as well as osteoinduction[4] (in certain conditions). HA contains only calcium and phosphate ions and therefore no adverse local or systemic toxicity has been reported in any study.

When implanted, newly formed bone binds directly to HA through a carbonated calcium deficient apatite layer at the bone/implant interface [48,63]. An i*n vitro* method has been developed to determine apatite growth on HA surfaces which is indicative of bioactivity by using simulated body fluid (SBF). The conventional SBF which was developed by Kokubo in 1990, is a solution containing a similar ionic composition and pH to blood plasma. Since then the composition of SBF has been revised for better similarity to blood plasma [64] and also recently has been applied as a biomimetic method for coating metallic surfaces (see section on biomimetic coatings).

A bioactive material develops a bonelike apatite layer *in vitro*, also known as an amorphous calcium phosphate or hydroxycarbonate layer on its surface when treated in SBF. The mechanism of apatite formation on HA surfaces is believed to be due to partial dissolution of HA, and ionic exchange between SBF and HA. The formation of the apatite layer enables an implant to bond directly to host tissue. We have previously shown the growth of a dune-like apatite layer on polyurethane surfaces which were coated with HA using the RSBF [65]. The HA coated PU disks also showed improved cytocompatibility towards fibroblasts cells compared to the uncoated disks.

Osteoconduction and osteoinduction of HA scaffolds is well known. HA surfaces supports osteoblastic cell adhesion, growth, and differentiation and new bone is deposited by creeping substitution from adjacent living bone. HA scaffolds can also serve as delivery vehicles for cytokines with a capacity to bind and concentrate bone morphogenetic proteins (BMPs) *in vivo* [66]. Finally, osteogenesis occurs by seeding the scaffolds before implantation with cells that will establish new centers for bone formation, such as osteoblasts

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[2] *Osteoconduction* "This term means that bone grows on a surface. An osteoconductive surface is one that permits bone growth on its surface or down into pores, channels or pipes." [194]

[3] Osteointegration: "Direct anchorage of an implant by the formation of bony tissue around the implant without the growth of fibrous tissue at the bone–implant interface." [194]

[4] *Osteoinduction: "*This term means that primitive, undifferentiated and pluripotent cells are somehow stimulated to develop into the bone-forming cell lineage. One proposed definition is the process by which osteogenesis is induced." [194]

and mesenchymal cells that have the potential to commit to an osteoblastic lineage [67].

Osteoinduction occurs because of the stimulation of the host's mesenchymal stem cells in surrounding tissues. These stem cells then differentiate into bone-forming osteoblasts. Extensive studies have been conducted over the past several years to better understand the osteoinduction potential of HA. Osteoinduction has been seen in several independent studies in various hosts such as dogs, goats and baboons [7,68-70].

Porous HA seeded with undifferentiated stromal stem cells was able to differentiate into mature bone forming cells and lamellar bone in ectopic sites (subcutaneous) [66]. This process was underlined by increased expression of alkaline phosphatase (a marker of early osteogenic development and an initiator and regulator of calcification [71]). Additionally bone GIa protein also known as osteocalcin (responsible for calcium ion binding and a marker of bone mineralization [72]), and collagen I mRNA was detected comparable to natural bone [73]. These findings were further confirmed by histological and immune-histochemical analysis of the HA bone interface. Osteoblasts appeared on HA surfaces and partially mineralized bone (osteoid) was formed directly on these surfaces [72,74]. It was demonstrated that osteoblast response toward HA is initially mediated by activation of focal adhesion components, culminating on actin-rearrangement executed by cofilin activation via rac-1. HA implants have also shown up-regulation of certain osteoblast gene expression profiles that was observed as early as 24 hours of implantation where it up-regulated osteoblast expression of at least ten genes (including proliferin 3, Glvr-1, DMP-1, and tenascin C) and down-regulated 15 genes (such as osteoglycin) by more than 2-fold compared with plastic surfaces [75]. HA gene expression differs from one animal species to another with highest levels reported in primates as compared to rabbit and dog animal models [76]. It is also affected by surface texture of HA, whereby porous HA showed more alkaline phosphatase positive cells than smooth dense HA surfaces and more than other calcium phosphates in the study, indicating increased differentiation potential of mesenchymal cells on porous HA [77]. HA gene expression pattern explained the basis of its biocompatibility and bioactivity.

Yuan *et al*. observed in their study that bone was formed in dog muscle inside the porous calcium orthophosphate which had microporosity on the surface. They however did not observe bone formation when implants with a dense macroporous surface were used. [69].

A 3D printed calcium phosphate brushite implant with controlled geometry was produced and implanted into Dutch milk goats by Habibovic *et*

al. Their results showed that calcium phosphate brushite and monetite implants were able to induce ectopic bone formation [70].

Ripamonti *et al*. have conducted extensive work on the long-term use (1 year) of HA implants in the non-human primate *Papio ursinus* [7,68]. Their studies indicate spontaneous bone formation in non-osseous sites. In one study they used coral-derived calcium carbonate that was converted to HA by a hydrothermal reaction [7]. Constructs of HA and calcium carbonate (5% and 13% HA) exhibiting different morphologies (rods and disks) were implanted into the heterotopic *rectus abdominus* or into orthotopic calvarial defects respectively. Different time points were assessed during this 1 year study and in all instances, induction of bone in the concavities of the matrices was detected. After a year, resoption of the calcium carbonate/HA was visible as well as deposits of newly formed bone [7]. Ripamonti's group also had success with biphasic HA/TCP biomimetic matrices with ratios of 40/60 and 20/80 when implanted into non-osseous sites in the Chacma baboon, *Papio ursinus*. The induction of *de novo* bone formation was detected in the concavities of the HA/TCP scaffolds without the application of osteogenic proteins. Dissolution of the implanted scaffolds was also observed in the 20/80 biphasic scaffolds after 1 year [68]. In a very recent study Riamonti reports on an 8 month *in vivo* trial in *P. ursinus* using HA coated Ti implants where osteoinduction was also observed [78].

4. HA COATINGS

Despite the biocompatibility and bioactivity of HA implants, it is well known that HA displays poor mechanical properties, i.e. poor tensile strength and fracture toughness hence for many years the clinical applications for HA implants was limited to non-load bearing applications. Traditionally metallic implants such as titanium and its alloys have been the material of choice for load bearing applications such as dental implants, joint replacement parts (for hip, shoulders, wrist etc.) and bone fixation materials (plates, screws etc.). However long-term complications with Ti- based implants has been well documented which include severe wear resulting in inflammation, pain and loosening of the implants which has restricted the lifespan of the conventional Ti implants to 10-15 years [79].

One of the major innovations in bone reconstruction in the past 20 years has being to apply HA as a surface coating on mechanically strong metallic implants such as titanium implants and its alloys, in an attempt to improve

bone fixation to the implant and thus increase the lifetime of metallic implants. Furthermore the bioceramic coating protects the implant surface from environmental attack. The rationale in using HA coatings as a mean of fixation for orthopedic and dental implants has been known as early as 1980s [80]. The application of HA coatings on metallic implant devices offer the possibility of combining the strength and ductility of metals and bioactivity of bioceramics.

Studies have reported higher osteoblast activity *in vitro* and increased collagen levels for cells growing on HA-coated Ti surfaces compared to the uncoated Ti controls [81], and *in vivo* HA coated titanium implants resulted in higher bone implant contact area [82]. Bioactive HA coatings on bioinert titanium implants encouraged the in-growth of mineralized tissue from the surrounding bone into the implant's pore spaces and improved biological fixation, biocompatibility and bioactivity of dental implants [83].

Several methods have been reported in the literature to coat metallic implants with HA and include plasma spraying, sputtering, electron beam deposition, laser deposition, electrophoretic deposition, sol–gel coating, or biomimetic coating [83]. The advantages and disadvantages of some of the conventional coating methods appears in Table 3. With an exception of biomimetic coating, all of these methods require post-heat treatment processing to obtain HA crystallization in a vacuum chamber, because the uncrystallized HA coating is typically easily dissolved and can prevent bone formation [83].

Plasma spraying and biomimetic coatings are discussed in more details in the following sections.

4.1. Plasma Spraying

Plasma spraying is one of the most well developed commercially available methods for coating metallic implant devices with HA. Plasma spraying offers advantages of good reproducibility, economic efficiency, and high deposition rates. Initially HA plasma-sprayed implants resulted in improved bone response compared with conventional titanium implants however, long-term clinical results were less favourable and associated with failure [83,84]. It has not been clarified whether the initial positive bone response was due to the proposed bioactivity of HA, or to possible alterations in surface topography or to a greater press fit of the thicker HA-coated implants when screwed in the same size defects as the controls. Conventionally HA coatings using the plasma spraying method were relatively thick and porous, and their uneven

structure and low-bonding strength have been responsible for a number of clinical failures [85].

Table 3. Summary of the various techniques for coating implants with HA [55]

Technique	Thickness	Advantages	Disadvantages
Plasma spraying	30–200 mm	High deposition rates; low cost	Line of sight technique; high temperatures induce decomposition; rapid cooling produces amorphous coatings; relatively thick coatings
Sputter coating	0.5–3 mm	Uniform coating thickness on flat substrates; dense coating	Line of sight technique; expensive time consuming; produces amorphous coatings
Pulsed laser deposition	0.05–5 mm	Coating with crystalline and amorphous phases; dense and porous coating	Line of sight technique
Dynamic mixing method	0.05–1.3 mm	High adhesive strength	Line of sight technique; expensive; produces amorphous coatings
Dip coating	0.05–0.5mm	Inexpensive; coatings applied quickly; can coat complex substrates	Requires high sintering temperatures; thermal expansion mismatch
Sol–gel	< 1 mm	Can coat complex shapes; Low processing temperatures; relatively cheap as coatings are very thin	Some processes require controlled atmosphere processing; expensive raw materials
Electrophoretic deposition	0.1–2.0mm	Uniform coating thickness; rapid deposition rates; can coat complex substrates	Difficult to produce crack-free coatings; requires high sintering temperatures

Technique	Thickness	Advantages	Disadvantages
Hot isostatic pressing	0.2–2.0mm	Produces dense coatings	Cannot coat complex substrates; high temperature required; thermal expansion mismatch; elastic property differences; expensive; removal/interaction of encapsulation material
Electrochemical deposition	0.05-0.5mm	Uniform coating thickness; Rapid deposition rates; can coat complex substrates; moderate temperature; low cost	Poor adhesion with substrate
Biomimetic coating	<30 mm	Low processing temperatures; can form bonelike apatite; can coat complex shapes; can incorporate bone growth stimulating factors	Time consuming; requires replenishment and a constant pH of simulated body fluid; poor adhesion with substrate

It is well documented that HA coatings prepared by plasma spraying are typically composed of varying percentages of crystalline HA, TCP, and amorphous calcium phosphate [86]. This can be attributed to the thermal decomposition of HA during the high processing temperature during plasma treatment. It has been shown that the dissolution rate of HA coating is correlated with the biochemical calcium phosphate phase of the coating [87], such that the more crystalline HA the implant coating contains, the more resistant the coating is to dissolution. Conversely, increased concentrations of amorphous calcium phosphate and TCP are thought to predispose HA coatings to dissolution and in extreme cases even failure [88]. It has been suggested that the dissolution of calcium phosphate from the surface of the implant in the human body is responsible for the bioactivity of the HA surface, at the same time, if the dissolution rate is faster than bone growth or implants stabilization, the coating would be useless. Studies suggested that both amorphous and crystalline phases in the coatings are desirable to promote a more stable interface with the biological environment [89,90].

Studies on varying the HA coating thickness has been conducted and suggests that thinner HA layers, in the nanometer range, revealed increased cellular response [91-93], increased bone formation *in vivo* and slightly higher

removal torque analysis. Although finite element analysis (FEA) indicated that bone stress distributions at bone-implant interface decreased with the increase of the HA coating thickness but coatings ranging from 60 to 120 μm were reported to be an optimum choice for clinical application than increased thickness of 200μm [94].

4.2. Biomimetic Coatings

Biomimetism is the study of the formation, structure, or function of biologically produced substances and materials, and biological mechanisms and processes for the purpose of synthesizing similar products by artificial mechanisms which mimic natural ones. In many cases, biomimetic strategies do not set out to copy directly the structures of biological materials but aim to abstract key concepts from the biological systems that can be adapted within a synthetic context [95]. Thus, biomimetic materials are invariably less complex than their biological counterparts and, to date, hierarchical complex architectures, such as those observed in bone; remain outside the current technologies [96].

The biomimetic methods, applied to produce HA coatings, have attracted considerable research attention in the last decades [97,98]. As mentioned previously the biomimetic coating method commonly involves immersing metal implants in SBF at physiological pH and temperatures, which results in the formation of an apatite layer on metal surfaces [99,100]. This technique allowed nano fibrous polymer pore walls to be mineralized without clogging the larger pores and the interpore openings. Similarly, electrospun fibrous scaffolds from various synthetic and natural polymers also were mineralized using the SBF technique, although it is reported as being a slow process, lasting days to weeks [100]. Similarly, biomimetic nano-apatite coatings of porous titanium scaffolds resulted in enhanced human osteoblast culture as well as greater bone formation in a canine bone in growth chamber [101].

He *et al* [102], developed an electrode deposition process that reduced the mineralization time to under an hour. Using an electrolyte solution and varying parameters like temperature and voltage, a control over the surface topography and Ca/P ratio was achieved. SEM/EDS elemental mapping for Ca, P, C and O revealed needle-like phases were deposited at 80°C. TEM examinations revealed further details of the deposits formed that were mainly composed of needle-like HA crystals.

An alternative coating method based on biomimetic techniques was designed to form a crystalline hydroxyapatite layer very similar to the process for the formation of natural bone on the surface of titanium alloy pretreated with NaOH. Two types of solutions were used: supersaturated calcification solution (SCS) and modified SCS (M-SCS). M-SCS was prepared by adding appropriate quantities of vitamin A (A) and vitamin D2 (D) with A/D ratio of 4.5. The vitamin A and D were included in minor amounts in M-SCS solution to modify the physical structure of the final product and to enhance the osteoinductive and biochemical properties of coatings. The proposed biomimetic method represented a simple way to grow HA coatings on titanium substrates at room temperature [103].

Biomimetic HA-polymer composite scaffolds have been widely explored for bone regeneration [104,105]. The mineral not only adds to the structural integrity of the scaffold, but it can also be actively osteoconductive. Biomimetic scaffolds will be discussed in more details in the sections on tissue engineering and nanophase HA.

5. TISSUE ENGINEERING

The approach of tissue engineering is to use various disciplines to control the interaction between scaffolds (materials), cells and growth factors in order to generate suitable environments for the regeneration of functional tissues and organs [106,107]. Research in tissue engineering is focussed at mimicking the extracellular matrix (ECM) with respect to scaffold structure and composition. One of the key components in tissue engineering for bone regeneration is the scaffold that serves as a template for cell interactions and for the formation of bone-extracellular matrix to provide structural support to the newly formed tissue. For bone tissue engineering, the scaffold, should display some of the following properties: three-dimensional scaffold; high volume of open and interconnected pores; bioresorbable scaffold with controlled resorption; suitable mechanical properties; biocompatibility; bioactivity and contain suitable signal molecules to induce new bone tissue formation.

In recent years extensive studies have been conducted to develop biomimetic materials for bone tissue engineering applications. Some of the most important scaffold properties are discussed below.

5.1. Porosity

There is consensus in the literature that a 3D porous scaffold is required for tissue engineering. Within these 3D structures the pore structure and size, surface area to volume ratio, texture and surface topography should be carefully controlled to enhance cell shape, alignment and organisation [107,108].

A 3D porous morphology is one of the most crucial factors affecting bone biological activity because pores allow migration and proliferation of osteoblasts and mesenchymal cells, as well as vascularisation [109]. Pores should be open, interconnected, uniformly distributed, with high pore-to-volume and surface area ratios. It has been shown that both micro and macropores are essential for bone engineering. The larger macropores are required for cell attachment, proliferation, tissue formation and ingrowth, while microporosity is essential for vascularisation and the transport of oxygen, nutrients, ions, and metabolic waste to and from the implant. Microporosity results in a larger surface area that is believed to contribute to higher bone inducing protein adsorption as well as to ion exchange and bone-like apatite formation by dissolution and reprecipitation [55,110].

Due to the lack of bone in-growth into dense HA blocks, porous bodies and granules of HA bioceramics have been developed and have been widely used in clinical settings. The challenge of conventional porous HA was represented by the non-uniform pore geometry and low inter-pore connections, that prevented pores to become completely filled with newly formed host bone [111]. Techniques to produce porous HA bioceramics with highly interconnecting structures were developed to promote osteoconduction to occur deep inside such ceramics [112].

Porous HA implants can be manufactured from a variety of methods including processing of natural bone, ceramic foaming, sintering with porogens starch consolidation, microwave processing, slip casting and electrophoretic deposition [113]. It is also possible to make use of bicontinuous water-filled microemulsion or a combination of slurry dipping and electrospraying to produce HA foams as potential matrices [55,114].

Various porogens can be used i.e. either volatile (these materials release gases at higher temperatures) or soluble materials which include sucrose, naphthalene, gelatine, hydrogen peroxide etc. Removal of organic porogens can either be conducted by physical processes like vaporation and sublimation or chemical reactions like combustion and pyrolysis [113].
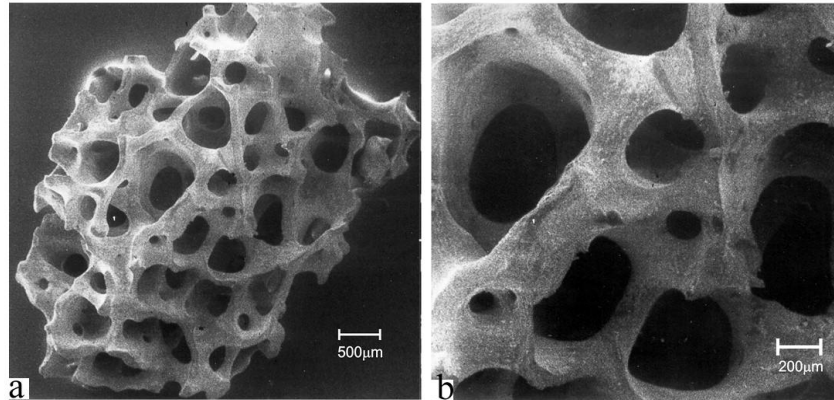
Figure 7. SEM image showing porous structure of Endobon® with is HA granules with various pore sizes and pore size distribution [120]. (Permission obtained from publisher for reprint).

There exists some discrepancy in the literature however regarding the optimum pore sizes of HA implants for bone engineering. This is largely due to the scaffold design and porosity structure. In general pore sizes smaller than 1 µm increases the bioactivity and ensures interaction with proteins, while pores between 1 – 20 µm determines the cell type that is attracted to the scaffold, assist with cellular development and vascularisation and orientates and direct the cellular in-growth. Cellular-growth, and bone in-growth occurs in pores between 100 – 1000 µm. Pores larger than 1000 µm ensure the functionality, shape and aesthetics of the implant [55,110].

High porosity content enhances bone formation, but pore volumes higher than 50% may lead to a loss of biomaterial's mechanical properties hence a careful balance is needed with respect to porosity, degradation and mechanical properties [115].

Figure VII shows the porous interconnected structure of Endobon® (Biomet UK Ltd), which is a commercially available porous HA implant which is highly osteoinductive [113]. The pores in Endobon® are created by removal of the organic component from natural cancellous bones with pore size ranging from 100 µm to 1500 µm.

Dr Wim's group has over the past 15 years developed a variety of 3D HA and biphasic scaffolds with various porosities and surface topographies [45,65,68,116-119]. Highly porous sintered biphasic HA disks were formed by the inclusion of stearic acid spheres (0.7 to 1.0 mm in diameter) with the bioceramic powder's during processing, whereby the spheres melted out

during sintering to give macropores of 700-1400µm [119]. Very positive osteoinductive results were obtained *in vivo* studies with the biphasic disks.

5.2. Composite Scaffolds

There is need to engineer multiphase materials i.e. composites that combine the advantages of each component to produce a superior material than its individual components and with a structure and composition more closely resembling that of natural bone. The aim of tissue engineering is to help the body heal naturally by implanting a resorbable and porous scaffold to serve only as a temporary matrix that would degrade over time while allowing regeneration of the host tissue at the implant site. The rate of degradation of HA implants however should match the regeneration rate of native tissue, and this currently is one of the major challenges in this field. Degradation depends on the particle size, crystallinity, porosity, the composition and preparation conditions as well as the environment at the implantation site.

Extensive work has been conducted regarding development of biphasic bioceramics with improved resorption rates. From experimental results it was determined that the biodegradation of β-TCP proceeds the fastest, followed by unsintered bovine bone apatite, sintered bovine bone apatite, coralline HA and then synthetic HA [48]. It was observed by Podaropoulos *et al.* that implants in dogs of β-TCP completely resorbed within 5-6 months. The rate of absorption does depend on the species, the phase purity of the implant as well as the health state of the patient [121]. More dense HA bodies is known to resorb at a slower rate compared to porous HA, due to the larger surface area and ability of infiltration of blood vessels, and easier access of nutrients and molecules in the latter. When biphasic implants such as HA/TCP is used, the degradation rate is dependant on the HA/TCP ratio. When the ratio is high, the degradation rate is slow and visa versa. [48,63]. A faster resorbable material may allow soft-tissue cells to prematurely intrude into the defect, while a nonresorbable or slowly resorbing material that remain for a long time may inhibit new bone formation [121]. The ratio of biphasic implants must be carefully controlled to get the desired bioresorbtion rate of the implant whilst allowing adequate time for the body to produce new bone at the implant site.

In addition of HA and β-TCP a number of other materials have also be included in biomimetic HA composites for bone tissue engineering and commonly include natural HA, polymers, proteins (such as collagen, hyaluronic acid, gelatine) and biological signal molecules which include

growth factors such as bone morphogenic proteins (BMP's), stem cells, etc. Since natural bone is a composite material containing both an inorganic and organic component, a composite material can more closely replicate natural bone compared to just HA alone.

A variety of HA-polymeric composites have been developed. While HA provides bioactivity, the incorporation of a polymeric matrix improves the materials mechanical properties in particular brittleness, tensile, and fracture toughness. Composites of HA with polymers such as polymethyl methacrylate, poly (3-hydroxybutyrate-co-3-hydroxyvaleate), and polyacrylic acid, have been developed which showed improved mechanical properties, as well as good biocompatibility and bioactivity [122].

HA/chitosan-gelatin composites with most pores between 300 and 500 μm have also been produced [123]. These scaffolds supported the proliferation and mineralization of rat calvarial osteoblasts *in vitro*. Porosity in these scaffolds can be increased by decreasing the chitosan-gelatin concentration and increasing the chitosan-gelatin/ hydroxyapatite ratio [123].

Coating hydroxyapatite with a hydroxyapatite/ poly(ε-caprolactone) produced composite scaffolds with 87% porosity and 150–200 μm pore size, and of improved mechanical properties: higher amounts of the composite coating (more polymer) increased compressive strength (maximum 0.45 versus to 0.16 MPa for no coating) and elastic modulus (maximum 1.43 versus 0.79 for no coating) [124].

Collagen scaffold of pores 30–100 μm, porosity 85% was combined with hydroxyapatite where it enhanced osseintegration by the formation of surface bioactive apatite layer that supported attachment and proliferation of rabbit periosteal cells [125].

Chen et al [126], combined three materials for use in bone tissue engineering, where synthetic poly (DL-lactic-*co*-glycolic acid) (PLGA), naturally derived collagen, and inorganic apatite were hybridized to form scaffolds with 91% porosity and 355–425 μm pores.

A direct rapid prototyping process called low-temperature deposition manufacturing was proposed to fabricate poly (a-hydroxy acid)-tricalcium phosphate (TCP) composite scaffolds [127]. This process integrated extrusion/jetting and phase separation and can therefore fabricate scaffolds with hierarchical porous structures, creating an ideal environment for new tissue growth. The interconnected computer-designed macropores allowed tissue ingrowth throughout the scaffold. Moreover, the micropores allow nutritional components in, and metabolic wastes out.

Recent advances in composite materials for bone engineering is based on nanotechnology and involves the development of nanocomposites, containing nanofibers, HA nanoparticles, carbon nanotubes etc. HA nanocomposites will be discussed in the following section.

6. NANOPHASE HA – THE NEXT GENERATION BIOCERAMICS FOR BONE ENGINEEIRNG

Bone is an example of a nanostructured material. Bone is structurally divided into three levels that orientate themselves into heterogeneous and anisotropic bone: 1) the nanostructure, 2) the microstructure and 3) the macrostructure that includes cortical and cancellous bone [128-130]. With the advent of nanotechnology exciting new possibilities are now available for development for the ideal bone implant.

Nanotechnology has been defined as ''the creation of functional materials, devices and systems through control of matter on the nanometer length scale (1–100 nm), and exploitation of novel phenomena and properties (physical, chemical, and biological) at that length scale'' (National Aeronautics and Space Administration) [131].

Nanotopography has been shown to influence cell adhesion, proliferation, differentiation and cell specific adhesion. Related changes in chemistry and nanostructure impart important chemical changes and permit biomimetic relationships between alloplastic surfaces and tissues. Nanoscale modification of an implant surface could contribute to the mimicry of cellular environments to favour the process of rapid bone formation [131]. The result may be changes in physical properties including enhanced magnetic, catalytic, optical, electrical, mechanical, and biological properties when compared to conventional formulations of the same material. Nanostructured surfaces possess unique properties that alter cell adhesion by direct (cell–surface interactions) and indirect (affecting protein–surface interactions) mechanisms. The changes in initial protein–surface interaction are believed to control osteoblast adhesion [132,133].

Engineering of nanomaterials can thus meet current challenges in bone replacement therapies. [57,134] It appears that the novelty of nanomaterials lies in their primary interaction with proteins that are responsible for subsequent cell responses. Proteins like fibrinonectin, vitronectin, laminin and type I collagen will firstly absorb onto the biomaterial's surface within

milliseconds after implantation. These adsorbed proteins may interact with certain cell membrane receptors which in turn may either enhance or inhibit cellular adhesion and growth [129,135,136]. The type of plasma protein adsorbed to the surface, as well as the concentration, conformation and bioactivity of the proteins, may depend on the surface chemistry, hydrophobicity, hydrophilicity, charge, roughness, topography and/or energy of the biomaterial [135,137]. This protein-interaction characteristic, as well as the fact that human tissue consist of nanofibers that are arranged in different patterns, makes biomimetic nanofibers for tissue regeneration possible [138].

In addition to the improved bioactivity, nano crystalline HA powders exhibit improved sinterability and enhanced densification due to greater surface area, which may improve fracture toughness, as well as other mechanical properties [139,140]. This is explained by the fact that the distances transported by the material during the sintering become shorter for ultrafine powders with a high specific surface area, resulting in densification at lower temperature [141].

To mimic the size scale of mineral crystals in bone and other mineralized tissues, nano-HA has been compounded with synthetic polymers or natural macromolecules to fabricate nano composite scaffolds [142,143]. Nano-HA/polymer composite scaffolds have not only improved the mechanical properties of polymer scaffolds, but also significantly enhanced protein adsorption over micro-sized HA/polymer scaffolds and eventually improved cell adhesion and function [144].

There are various techniques available to manufacture nano-HA particles. These methods include wet chemical precipitation [145], sol-gel synthesis [146], co-precipitation [147], hydrothermal synthesis [148], mechano-chemical synthesis [149], mechanical alloying [150], ball milling [151], radio frequency induction plasma [152], electro-crystallization [153], microwave processing [154], hydrolysis of other calcium orthophosphates [155], double step stirring [156], and other methods.

Electrospinning is a technique used to generate a controllable continuous nanofiber scaffold that is similar to ECM. [57,138,157]. This provides a special environment in the spaces between the cells. Electrospun nanofibers have biomimetic properties and are ideal for tissue engineering and scaffolds since these fibers' dimensions, porosity, interconnectivity and surface area can be controlled by controlling the solution conditions and electrospinning process [57,157]. We have recently in our lab produced nanofibers of electrospun biphasic HA/TCP scaffolds combined with various ratios of

gelatine, ranging from 3% w/t to 10% w/t. The best nanofibers were observed when gelatine was added between 7-10% w/t (unpublished data).

Nanocomposites produced from gelatine and HA nanocrystals are conducive to the attachment, growth, and proliferation of human osteoblast cells. Collagen-based, polypeptidic gelatine has a high number of functional groups and is currently being used in wound dressings and pharmaceutical adhesives in clinics. The flexibility and cost effectiveness of gelatine can be combined with the bioactivity and osteoconductivity of hydroxyapatite to generate potential engineering biomaterials. The traditional problem of hydroxyapatite aggregation can be overcome by precipitation of the apatite crystals within the polymer solution. The porous scaffold generated by this method exhibited well-developed structural features and pore configuration to induce blood circulation and cell in-growth [158].

Jegal *et al* [159], incorporated HA into electrospun nanofibers, but used a gelatine apatite precipitate homogenized in an organic solvent with poly(lactide-co-caprolactone) (PLCL) to make the mineral surface more available. Venugopal and co-workers designed an electrospun nanofibrous scaffold and concluded that it had potential as a biomaterial [160]. They focused on the two major solid components of natural bone, namely collagen and HA to construct the scaffold. The scaffolds were fabricated with collagen as well as collagen and HA (1:1). The results of the collagen/HA electrospun nanofibrous scaffold suggested that the scaffold was a highly porous structure, providing more adhesion space, cell proliferation and the mineralisation of osteoblasts. It also allowed for the efficient movement of nutrients and waste products within the structure. Culturing of osteoblasts on the scaffold was possible and cells showed normal proliferation, increased level of mineralisation and a slight increase in aneral homeostasis [160].

A study has been conducted to investigate the effect of treating bone defects by nano HA (40 nm particle sized), versus micro HA (53-124 μm), in bone defects in rat. Histological and histomophometric analysis showed that osteoconductive properties of nano HA were significantly higher than micro HA particles, where active osteoblastic and osteoclastic activity indicated active bone remodeling. Nano HA demonstrated rapid bone ingrowth and accelerated bone formation within and around the implanted material [161].

Fathi *et al*. [162] endeavoured production of synthetic nano-HA powder with improved bioresorption abilities. Crystallinity and pore size increased with increasing sintering temperatures. When sintered at 600°C, the HA nanopowder exhibited the characteristics closely resembling human bone. The HA nanopowder was also more readily resorbed *in vitro* when compared to

conventional HA. The reason for this can be due to the high surface area as a result of nanostructure processing [162].

3D porous HA nanocomposites containing synthetic PA (polyamide) were developed and demonstrated compressive strength comparable to the upper value strength of natural cancellular bone. The interconnective pore structure of the scaffold allowed for easy cell seeding and spatially even distribution of transplanted cells. Subsequently, a critical defect on rabbit mandible was employed for *in vivo* evaluation of pure nano-HA/PA scaffolds and MSCs hybridized scaffolds. According to the histological and microradiographic results, in short term after implantation hybrid scaffolds presented better biocompatibility and enhanced osteogenesis than pure nano-HA/PA scaffolds, while in long term both pure scaffolds and hybrid ones exhibited good biocompatibility and extensive osteoconductivity with host bone [163].

In recent years several studies are focusing on the development of HA-CNT nanocomposites with improved mechanical properties [60-62]. Reports are also available on the processing of HA–CNT composite coatings for orthopedic implants through plasma spraying [60], laser surface alloying [164], electrophoretic deposition [165] and aerosol deposition [166]. In addition to conventional sintering [167,168] and hot isostatic pressing [169] and spark plasma sintering (SPS) [170] has also been employed to fabricate free-standing HA–CNT composites.

7. CLINICAL CRANIOFACIAL APPLICATIONS

Since its introduction in the mid-1980s, HA has been investigated extensively for its clinical viability in various bone defects conditions to improve the clinical outcome for the patient and shorten the healing time.

Investigators and clinicians have to answer several questions to approve the use of HA as a potential bone graft material and as an alternative to autographs. For craniofacial reconstruction, three primary questions which are still controversial in the field are given below.

1. Will the titanium oxide surface of endosseous implant undergo osteointegration when it is placed within a mass of HA undergoing bony replacement? If the answer to this question is yes, then simultaneous HA-endosseous implant placement could eliminate the need for either pre-implantation bone grafting or simultaneous bone grafting.

2. Will HA graft containing bone morphogenic protein or platelet-derived growth factor undergo bony replacement significantly faster than would a pure HA graft? If the answer to this question is yes, then HA could prove to be the ideal carrier, especially in applications in which the carrier is exposed to displacement forces and a particular three-dimensional osseous shape is required, such as augmentations or reconstructions of bones of the facial skeleton.
3. Can a HA graft successfully bridge a mandibular discontinuity defect, and is the presence of native periosteum required? If the answer to this double question is yes, then immediate and anatomically correct HA graft reconstruction of a non-irradiated mandibular defect could be achieved, provided that a sufficient soft tissue bed is available [171].

We share our experiences in clinical dental reconstruction over the past 20 years in an attempt to improve our understanding of the field.

Resorbed edentulous mandible remains a daunting clinical problem. This condition significantly compromises the stability of a mandibular prosthesis. The advent of the endosseous titanium-dental-implant-retained prosthesis has revolutionized the care of the edentulous patient. Since HA-coated dental implant was initially introduced to the clinic in the mid-1980s, it has been successfully used to replace missing teeth, and it showed an earlier integration than implants with other kinds of implant surfaces. Many researchers have demonstrated a better initial osseo-integration and a high short-term success rate [172-174]. Coating porous-surfaced titanium implants (35% porosity and 50–200 μm pore size) with calcium phosphate resulted in earlier and greater bone ingrowth and enhanced mechanical properties for implants retrieved from rabbit femoral [174].

Long-term survivability of HA-coated dental implants is still significantly controversial because many times the remaining bone stock compromises the placement of the implants and consequently compromises the prosthesis. [175]. In a prospective clinical study, Watson et al [176] reported cervical bone loss of more than 4 mm in 5 of 33 HA-coated dental implants in a long-term follow-up and suggested that the cervical bone level adjacent to the HA-coated implant failed to establish a steady state. On the contrary, 429 HA-coated cylindrical omniloc implants by McGlumphy et al [177] demonstrated 96% survival after 5 years in function. Longer periods of follow up indicated initial high rate of success according to definite criteria up to 5-years of function, a rate that declined thereafter up to 10-years of function.

Interestingly despite the reduced rate of success, these implants were considered to be functional and clinically surviving [178,179].

Clinical and radiographic evaluation of immediate loaded HA coated implants in the mandible and splinted by bar attachment and connected to an over denture by plastic clips, revealed clinical stability at one year of function, absent periapical radiolucency and a decline in rate of crestal bone loss by the end of the year. In the maxilla, immediate loading single root form HA coated implant placed in premolar region, reported 100% rate of success after 3-year post loading with minimum bone loss around the implant [180].

We investigated the use of natural HA for augmentation of resorbed mandibular ridges in 12 human clinical trials where the results were very satisfactory. We utilized HA graft of grain size 600-1000 μm for augmentation and cylindrical titanium endoseous implants that were placed at the same time and allowed for loading after three months. Clinical and radiographic evaluation showed complete merging of HA graft with surrounding bone, increased mandibular ridge with and osseointegration of titanium implants to new remodelled bone (Figure VIII).

HA cement and granular HA grafts were used to augment the edentulous atrophic canine mandible. On histologic examination, the HA cement grafts showed osteoconduction and subperiosteal and endosteal osteonal bone formation, whereas the granular HA grafts showed only osteoconduction. Neither graft material showed chronic or acute inflammation [171]. Despite the fact, some reported the failure of nano crystalline hydroxyapatite paste in preservation of ridge dimensions when implanted in fresh extracted sockets in dogs [181].

Huang *et al* [182], reported the treatment of 33 patients complaining of different bony problems; ridge augmentation, sinus lifting, repair of periodontal disease, and repair of radicular cysts. The porous BonaGraft (Biotech One, Taipei, Taiwan) implant with ratio of poorly crystalline HA: β-TCP of 60:40 was used. All patients reported satisfactory clinical outcomes without major material-related side effects, radiographic evaluation revealed enhanced bone formation which was difficult to distinguish from the surrounding bone. These results support the use of HA in different clinical situations.

In case of reconstruction of a severely atrophied maxilla, sinus augmentation with a mixture of HA granules and autologous cancellous bone was performed to support plasma-sprayed HA coated implants and the patient gave consent for postmortem analysis of his upper jaw. Ten years after successful reconstruction of his maxilla and functional implant loading, the

region of augmentation showed stable contours both radiographically and histologically. In 10 years, HA granules had only minimally degraded and it outlined the grafted area to protect it against resorption. The plasma-sprayed HA coating on the dental implant was intact, showing 48% contact with the surrounding bone [183]. HA as a synthetic bone in sinus bone grafting was superior in terms of bonding with host bones as well as induction and integration with new bone compared to the allografts [184].
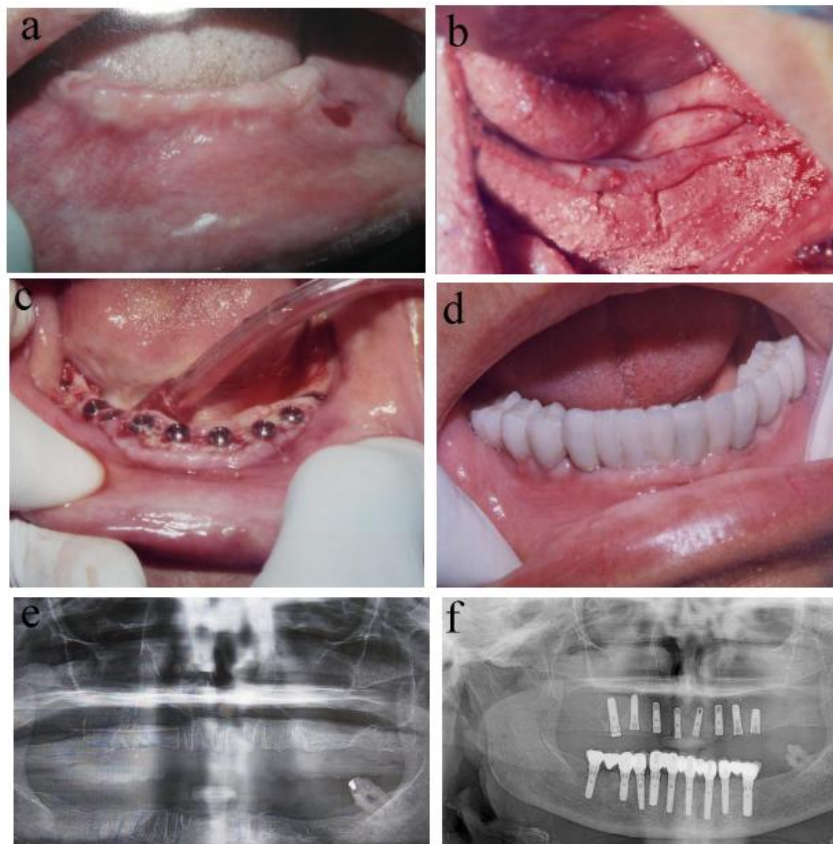
Figure 8. Mandibular ridge augmentation with natural HA; a: intraoral preoperative view, b: HA bone graft placed in position, c: intraoral view after insertion of eight endoseous dental implants to support and retain a subsequent fixed partial denture, d: fixed partial denture after insertion: ,e: preoperative panoramic x-ray showing edentulous atrophied maxilla and mandible with remaining mandibular left second molar which was extracted later, f: postoperative panoramic x-ray showing osseointegration of the dental implants with fixed partial dentures as the superstructure.

To answer the second question, mixed BMP/HA group showed the highest level of bone induction, especially compared to the BMP group and followed by pure HA when critical-size defect of 4 mm was made using a trephine in the calvarial bone o rat and, after that, BMP and/or HA was applied to the defect according to the grouping. Defects were evaluated radiographically and histologically 4 weeks postoperatively [185].

Large, cylindrical implants of a porous HA calcium phosphate ceramic (instead of the easily displaced granular forms), were used to replace larger than critical-size sections; 35 mm long and 20mm in diameter of the left tibial diaphysis of sheep and yet proved suitable for osteoconduction [186]. The use of particulate HA grafting material reported a notable finding in which a 9-fold increase in the modulus of elasticity of the defect implanted with synthetic HA material after 26 weeks, was observed when compared to the modulus of the normal trabecular bone at the site [187].

In an attempt to test the significance of HA in medically compromised patients, four patients with severe mandibular osteoradionecrosis requiring segmental mandibulectomy; and contraindicated for long anesthesia and/or surgery such as microsurgery, underwent a two-stage reconstruction with iliac autologous cancellous bone graft mixed with a macro porous biphasic ceramic bone substitute composed of 75% HA and 25% β-TCP in a ratio of 50-50%. No clinical or paraclinical complications were noted, and CT scans at 6 months showed that two patients had a favourable outcome with cortico-cancellous bone [188].

The short and long term adverse affects of HA implants after ridge reconstruction was investigated by following 637 patients for a period of 1 to 10 years (mean 6.0_+2.6 years) who had received the implants. Major loss of HA was seen in 17 patients (2.7%). Long term follow-up revealed a high percentage of patient satisfaction (97%) [189].

A composite of natural HA/chitosan composite when used in repairing bone defects revealed mild inflammatory infiltration observed 2 weeks after surgery. Fibrous tissue became thinner 2 ~8 weeks after surgery and bony connections were detected 12 weeks after surgery. The new bone was the same as the recipient bone by the 16th postoperative week [190].

A novel application of HA was proposed by Mehrotra *et al*, where the feasibility of using pre-shaped HA/collagen condyles as carriers for platelet-rich plasma after gap arthroplasty in 19 patients with temporomandibular ankylosis was evaluated. Aesthetic and functional outcomes and the possibility of neocondylar regeneration were assessed. Synthetic condyles were fixed to the ramus with a titanium mini plate, and temporal fascia was placed in

between. All patients showed appreciable improvements in mouth opening and excursion of the jaw. There were a few complications such as mild fever, and temporary involvement of the facial nerve, which improved with time. No open bite or recurrence was reported during the 18 months' follow up. Radiographic evaluation at 3 months showed a less opaque condyle, but the opacity at 18 months was more defined, suggesting a newly formed condyle [191].

A synthetic, resorbable HA (R-HA) was applied to augment the subantral area in 10 consecutive sinus lift procedures in humans. Implants were simultaneously placed in eight patients; in the remaining two, where residual bone height was less than 3 mm, a two-stage surgical approach was carried out. In the simultaneous technique, radiopaque grafted mineral surrounded the implants. In the two stage technique, R-HA particles filled the augmented site and were confined to the subantral area. At the uncovering phase, all implants (n = 36) were stable, with no clinical bone resorption around the cervix [178].

We used HA in inducing bone formation in angular bone defect of the mandible associated with dentigerous cyst around impacted second molar which was discovered accidently during routine radiographic examination for orthodontic treatment (Figure IX).

Figure 9. Repair of large bony defects associated with impacted mandibular second molar with natural HA; a: preoperative CT axial section of the mandible showing bilateral bone defects where outer and inner cortical plates are nearly absent or very thin especially in the right side defect, b: preoperative 3-d model of the mandibles retrieved from CT axial cuts, c: CT axial cut of the mandible six months postoperative where coarse grain sized graft matrial (600-1000μm) were used on the right side to reduce rate of graft degradation allow for adequate bone remodelling while finer grain size was used on the left (250-600μm) due to smaller size and reduced time of healing, d: 3D model of the mandible six months post operatively where incomplete and merging of the new bone is shown, e: 2 years post operative CT of the mandible showing nearly complete merging of the new bone with the old one and complete degradation of the graft aterial, f: 3-d model of the mandible 2 years post operative showing complete reconstruction of the mandible.

We conclude from our long experience with HA, that resorbable natural HA is the best osteoconductive material especially hexagonal crystal structure prepared at low temperature not exceeding 650 °C. This HA maintains the properties of internal porosity and nano crystallite structure of natural bone in a way that approaches the gold standard of autogenous bone grafts.

CONCLUSION

Over the past 4 decades, HA has proven itself as a significant biomaterial for bone reconstruction and repair, and today it is one of the most commonly used bone grafts for clinical surgery. The third generation HA bioceramics are bioresorbable, bioactive, osteoconductive, and osteoinductive. HA implants of today are porous multiphase 3D scaffolds consisting of biphasics and/or inorganic/organic composite's which are intended for bone tissue engineering applications. Despite the progress which has been made in the field there is still great potential for new innovation to address some of the challenges with HA implants. These include: 1) improvement of the *mechanical properties* of HA implants such that it can be used effectively in load-bearing applications; 2) improving the *resorption profile* of biphasics and HA composites to match the bone remodelling rate such that as the HA implant degrades, new bone tissue is regenerated; 3) controlling the *release of growth factors and cytokines* from HA implants such that long-term bone regeneration is possible; 4) improving the bioactivity of HA in terms of *gene activation*; 5) improving the long-term performance of *HA coated metallic implants* and reducing early failure; 6) and development of *nanophase biomimetic HA* more closely replicating the structure, composition and performance of natural bone.

Despite the many successful reports on osteoinduction and osteointegration of HA implants in animal models this has not always translated to clinical studies although many successful trials have been seen in humans. It is known that the bone remodelling process differs amongst species, and the outcome is also dependent on a number of factors such as patient age, implantation site, patient's state of health and previous history of surgeries and medical conditions. Furthermore much of the work which has been conducted to date on osteoinduction has been empirical, and based on trial and error. A better understanding is therefore needed of the fundamental processes involved in the bone remodelling process. We need to better understand how bone is remodelled in the human body, and what role does each of the signalling molecules (i.e osteoblasts, osteoclasts, stem cells, specific ions, growth factors, cytokines, proteins, etc.) play in this complex phenomena. When we have a better understanding of the bone remodelling process then we will be able to design better bioceramics for the future [48].

The potential for nanotechnology in developing the ideal HA bone graft should not be underestimated. Since HA exists as a nanostructured material in bone, in the drive to develop the perfect HA implant, current trends lean towards nano-HA to better mimic natural bone. However for adoption of a

nanoHA solution, the toxicity and biological response much be studied and better understood (e.g. with respect to carbon nanotubes).

As the population ages, the demand for easily available bone graft materials will increase. The road-map for HA towards the perfect biomaterial is far from over, and over the next decades will require further collaboration with multidisciplinary teams of material scientists, biologists, chemists, engineers and clinicians, and a better fundamental understanding of the intricacies and complexities of natural bone and the bone remodelling process. When all factors such as genetics, chemical synthesis, protein biochemistry and immunology are taken into account, it is possible to design a smart HA implant that will be able to simulate complex biological signals, replace mechanical function, induce the formation of new bone tissue, and respond to stimuli from the host [107,192].

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