LITERATURE REVIEW

Hydroxyapatite (HA)

HA Properties

There are many apatite compounds, including fluorapatite, chlorapatite, carbonate-apatite, and hydroxyapatite (Oliveira *et al* 2006). Hydroxyapatite is chemically similar to the mineral component of bones and hard tissues in mammals; its chemical formula is $Ca_{10}(PO_4)_6(OH)_2$ (Fernandes & Laranjeira 1999). The chemical nature of hydroxyapatite lends itcellsf to substitution, meaning that it is common for non-stoichiometric hydroxyapatites to exist. The most common substitutions involve carbonate, fluoride and chloride substitutions for hydroxyl groups, while defects can also exist resulting in deficient hydroxyapatites.

Hydroxyapatite is bioactive material; the ability to integrate in bone structures and support bone ingrowths, without breaking down or dissolving. Hydroxyapatite is a thermally unstable compound, decomposing at temperature from about 800-1200°C depending on its stoichiometry. Hydroxyapatite is a calcium phosphate including hydroxide, and its Ca/P ratio is represented as 1.67. The structure of hydroxyapatite is hexagonal, which has unit cell size, a = 9.418 Å and c = 6.883 Å (Shi *et al* 2004). This structure can be assumed as ideal hexagonal crystal structure (closed-packed) from PO_4^{-3} ion, which is inserted by Ca^{+2} ion and OH^- ion among the empty space of PO_4^{-3} ions (Figure 1) (Shi *et al* 2004).

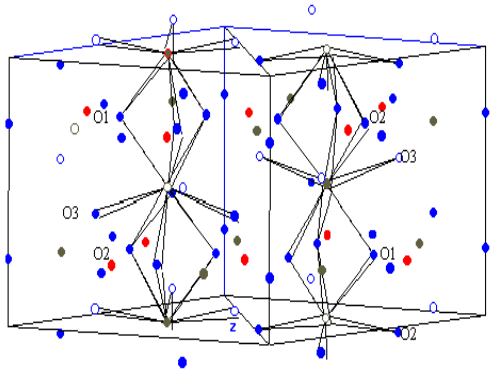

Figure 1 The crystal structure of hydroxyapatite (Shi *et al* 2004).

Table 1 Family of calcium phosphate compounds (Shi *et al* 2004).

Mineral name	Chemical Name	Chemical Formula	Ca/P
Monetite	Dicalcium phosphate (DCP)	$CaHPO_4$	1.00
Brushite	Dicalcium phosphate dehydrate (DCPD)	$CaHPO_4.2H_2O$	1.00
Whitlockite	Octacalcium phosphate (OCP)	$Ca_8(HPO_4)_2(PO_4)_4.5\ H_2O$	1.33
		$Ca_{10}(HPO_4)(PO_4)_6$	1.43
	Tricalcium phosphate (TCP)	$Ca_3(PO_4)_2$	1.50
Hydoxyapatite	Hydroxyapatite (HA)	$Ca_{10}(PO_4)_6(OH)_2$	1.67
Hillinstockite	Tetracalcium phosphate (TTCP)	$Ca_4P_2O_9$	2.00

There are different phases of calcium phosphate ceramics that can be used in medicine, depending on whether a bioactive or a resorbable material is desired (Table 1) (Shi *et al* 2004). Generally, dense hydroxyapatite does not have the mechanical strength to enable it to succeed in long term load bearing applications. But, hydroxyapatite may be employed as bone fillers in forms such as powders, porous blocks or beads to fill bone defects or voids. These may arise when large sections of bone have had to be removed (such as bone cancers) or when bone augmentations are required (such as dental applications). The bone filler would provide a scaffold and encourage the rapid filling of the void by naturally forming bone and provides an alternative to bone grafts. It would also become part of the bone structure and would reduce healing times compared to previous bone prostheses.

HA Synthesis

Hydroxyapatite in particulate form can be produced by using a variety of methods, such as wet method, dry method and hydrothermal method (Shi *et al* 2004). In this study, hydroxyapatite synthesis was being performed through wet method that is precipitation. Santos *et al* (2004) have been succeeding in synthesizing hydroxyapatite through wet precipitation method based on the chemical reaction below:

$$10Ca(OH)_2 + 6H_3PO_4 \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 18\ H_2O$$

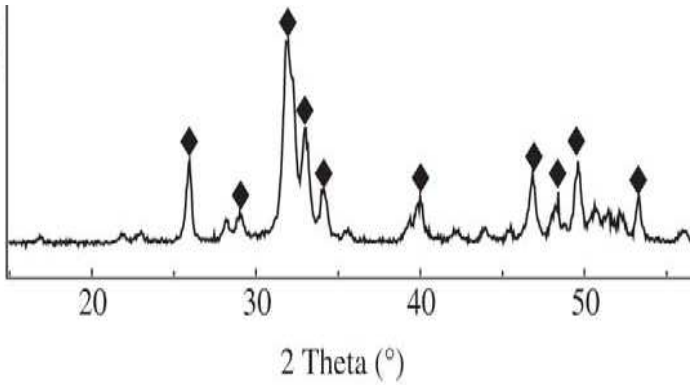

Figure 2 XRD pattern of HA from wet precipitation method (◆ HA) (Santos *et al* 2004).

The 0.5 M $Ca(OH)_2$ suspension was prepared using $Ca(OH)_2$ powder. The suspension was degassed, vigorously stirred and heated for one hour at 40°C temperatures. The 0.3 M H_3PO_4 solution was dropped into the $Ca(OH)_2$ suspension at same temperature for approximately one hour at the rate 6 mL/min. The pH was adjusted become pH = 7 by addition of 1 M NH_4OH solution at the end of the precipitation process. The XRD result showed below match to the hydroxyapatite pattern (Santos *et al* 2004).

Biphasic Calcium Phosphate (BCP)

BCP Properties

Development of biphasic calcium phosphate (BCP), especially with hydroxyapatite (HA: $Ca_{10}(PO_4)_6(OH)_2$) and tricalcium phosphate (TCP: $Ca_3(PO_4)_2$) has drawn considerable attention. HA and TCP, although have similar chemical composition, they differ in their biological resorbing capacity. The dense HA ceramics when used as bone implant as almost resorbable and bio-inert. While the porous β-TCP containing ceramics displays affinity for high speed biological degradation, they are bioactive and bioresorbable materials. The main attractive feature of bioactive bone graft materials such as BCP ceramics is their ability to form a strong direct bond with the host bone resulting in a strong interface compared to bioinert or biotolerant materials which form a fibrous interface. The

bioactivity relies on physical and chemical properties of biphasic calcium phosphate ceramics (Victoria & Gnanam 2002).

It is also noted that the presence of small amount of β-TCP 1100°C and 1200°C may be associated with the partial decomposition of HA phase. If HA is annealed in air at 1200°C, it decomposed into the β-TCP phase according to chemical reaction below (Ooi *et al* 2007).

$$Ca_{10}(PO_4)_6(OH)_2 \rightarrow 3\beta\text{-}Ca_3(PO_4)_2 + CaO + H_2O_{gas}$$

BCP Synthesis

Kumar *et al* (2005) was succeed in synthesizing BCP from sintering process. Firstly, the BCP granules were synthesized by the microwave. Calcium hydroxide and diammonium hydrogen ortho phosphate (DAP) were used as raw materials. The amounts of reactants used for the reaction were calculated based on the Ca/P molar ratio of 1.58. Weighed amounts of the starting granules were dissolved in water and the DAP solution was added to the calcium hydroxide solution. The solution is then exposed to 900°C microwave irradiation in a microwave oven during 20 minutes. The product was then dried in an oven. The result of XRD has a major peak indicating TCP that is (0 2 0) peak as shown below.

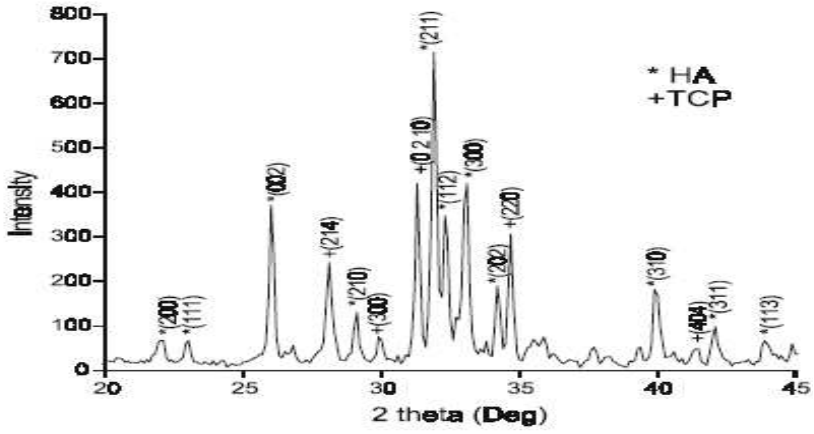

Figure 3 BCP X-Ray Diffraction Pattern from 900^0C sintering (Kumar *et al* 2005).

In vitro study

Biocompatibility testing *in vitro* often involves the detection of cell damage and death as like as cytotoxicity. Coelho *et al* (2000) was used the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to a purple formazan product in the MTT assay to estimate cell viability. The screening test is useful to detect over toxic effects of a test material in showing incompatibility. For instance, the rate of growth, proliferation and differentiation of cells on a material may be dependent on successful initial attachment and spreading of the cells on the surface of the implant materials. In this respect, the initial and short-term responses of cells to an implant material *in vitro* may provide valuable indicators of the long-term biocompatibility *in vivo* (Lin *et al* 1997).

Riberio *et al* (2009) have been succeed in showing cell attachment to the BCP implant materials within 2 days of immersion in osteoblast cells as SEM pictures show below.

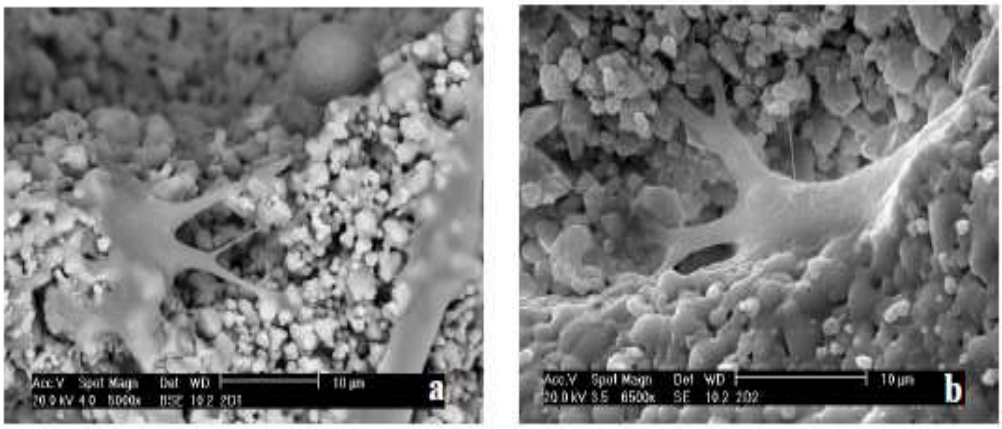

Figure 4 SEM picture showing the morphology of osteoblast cells on BCP implant materials after 2 days immersion *in vitro* (Ribeiro *et al* 2009).