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Comprehensive Review of Preparation Methodologies of Nano Hydroxyapatite

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Abstract

The present paper provides a snapshot review of nano hydroxyapatite (HAP), its importance in biomedical and orthopedic fields and its various preparation methodologies. Most recent research related to these preparation methods are also reviewed comprehensively.

Key words: *Hydroxyapatite; Hydrothermal; Mechanochemical; Microwave; Sol gel; Ultrasonic.*

1. INTRODUCTION

It is a well-established fact that the Human bone consists of 20% of collegen fibrils, 69% of nano size crystalline inorganic phase and 9% of water (Mollazadeh *et al.* 2007; Nejali *et al.* 2009) These Nano sized crystalline composite ingredients mainly resemble hydroxyapatite (HAP) – $Ca_{10}(PO_4)_2(OH)_2$ basically a type of calcium phosphate, with structural dimensions similar to a rod or a needle (length 40-60 nm, width 10-20 nm and thickness 1-3 nm) (Luis C Mendes *et al.* 2012). Further, it is also considered as one of the most significant human implantable materials on the basis of the degree of its biocompatibility, bioactivity and Osteroconductivity (Alessandra Bianco *et al.*2007). In addition, its affinity to create quick bonds with neighboring bones makes it also a designer material for bone repair or artificial bone substitute. The chemical, structural and morphological properties of HAP bioceramic are highly sensitive to change in physical properties, chemical composition and processing temperature.

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Scientific literature finds mention of several methods of preparation of Nano HAP. The most frequently used methods among these are

(i) Co-Precipitation Method (Zhang *et al.* 2003; Dan Nicolae Ungureanu *at al.* 2011; Jianping Zhu *et al.* 2011; Luis C Mentes *et al.* 2012; Rozita Ahmad Ramil *et al.* 2011)
(ii) Hydrothermal Method (Nasser Y Mustafa *et al.*2005; Earl *et al.* 2006; Jing Bing Liu et al. 2003; Delia *et al.* 2012; Mehmaz Salarian *et al.* 2008)
(iii) Ultrasonic Assisted Irradiation Method (Sahebali Manafi *et al.* 2008; Gerand Eddy Pioneer *et al.* 2009; Kojima *et al.* 2012; Eny Kusini *et al.* 2012; Coa Li Yun *et al.* 2005)
(iv) Mechano Chemical Method (Tomohiro Iwasaki *et al.* 2013; Adzila *et al.* 2011; Radzali Othman *et al*; Yeong *et al.* 2001; Greta Gergely *et al.* 2010)
(v) Microwave Irradiation Method (Gobi *et al.* 2013; Siddharthan *et al.* 2006; Sahil Jalota *et al.* 2004; Mohammad Bhilal Khan *et al.* 2011; Samar kalita *et al.* 2010) and
(vi) Sol Gel method (Aldona *et al.* 2006; Anbalagan *et al.* 2006; Santosh *et al.* 2009; Changsheng Liu *et al.* 2001; Khelendra Agarwal *et al.* 2011).

A review on most recent work done in these methods is provided below.

2. NANO HYDROXYAPATITE PREPARATION METHODS

2.1 Co-Precipitation Method

This is the one of the most widely adopted methods due to its simplicity, rapid preparation as well as easy control of particle size, composition and various possibilities to modify overall homogeneity of the product. The first stage consist of mixing the Anion solution e.g. calcium source with Cation solution e.g. phosphorous solution followed by formation of nucleation, precipitation and filtration. The final stage consists of calcinations under desired temperature Fig. 1.

Table 1 shows the recent review of papers related to Co-Precipitation method in the last two decades in this method. Due to the simultaneous occurrence of nucleation and crystal growth, the reaction in this method requires a sharp fine tuning to optimize morphology and minimal crystal growth

2.2 Hydrothermal Method

This method involves the usage of water as a solvent heated in a sealed vessel. Table 2 shows the recent review of papers related to the hydrothermal method. The initial stage in the

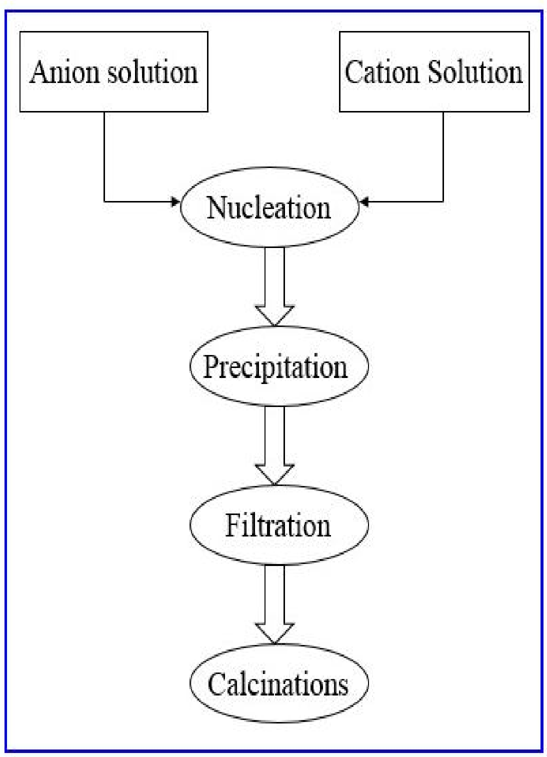

Fig. 1: Co-Precipitation Method

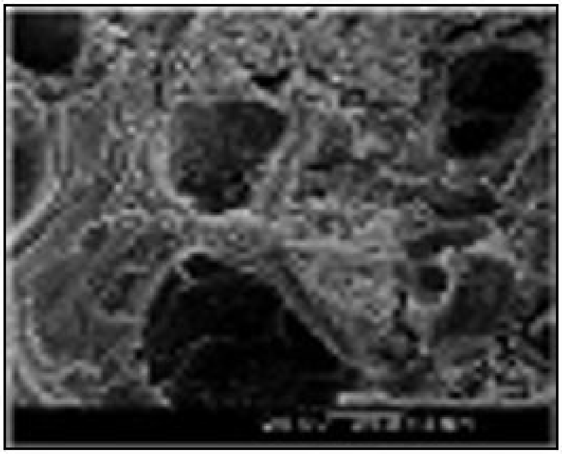

Fig 2: SEM Image by Z.M. Zhang

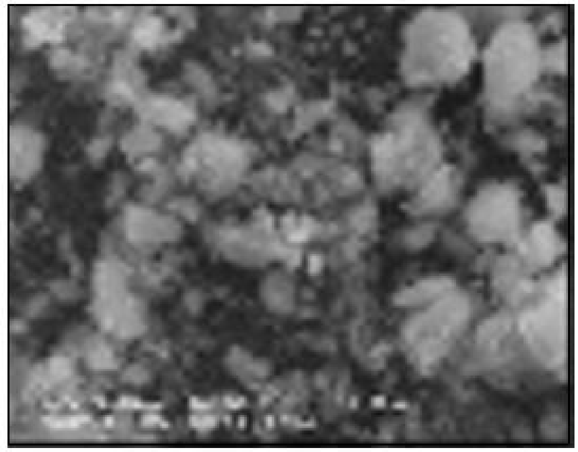

Fig 3: SEM Image by D. N. Ungureanu

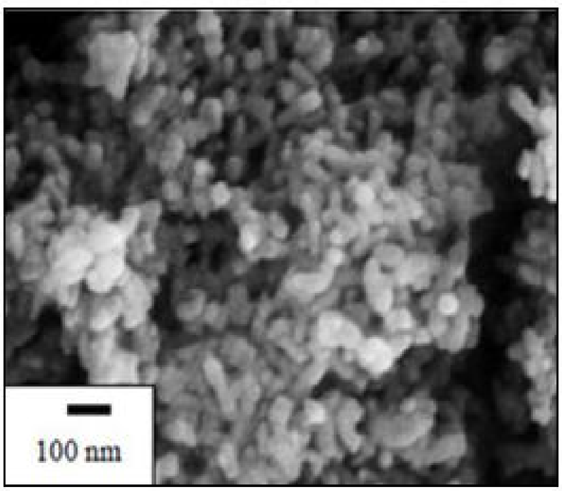

Fig 4: SEM Image by Jianping Zhu

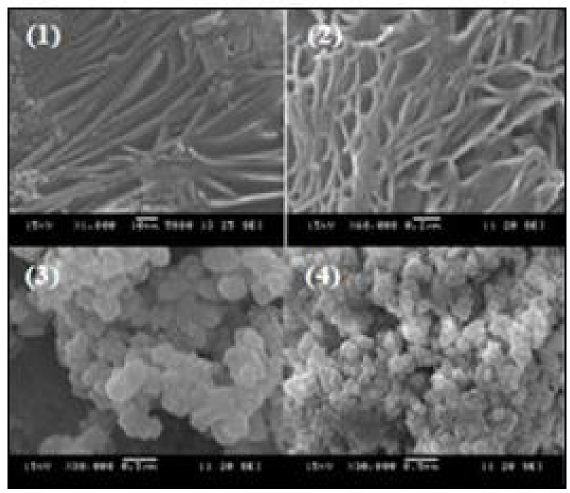

Fig 5: SEM Image by R.A. Ramli

synthesis of Nano HAP in this method is choosing the Calcium and Phosphorous precursor followed by mixing the two by maintaining the Ca/P ratio at a constant value of 1.67 under hydrothermal reactor mechanism Fig. 6. The mixing is then allowed to age, and subsequently washed and filtered. Finally it is dried in an oven and calcined using muffle furnace. Table 2 shows the review of hydrothermal method work done in the last two decades.

The change in the solvent and reactant properties at an extreme temperature means that experimental variables can be controlled to a higher degree in this method. This regulates the crystal.

Table 1. Co Precipitation Method

S.No	Author Year	Precursor/ Ca/P Ratio/pH	Stirring/ Aging time	Washing Solvent	Drying/ Calcinations	Characterization	Remarks
1	Zhang et.al 2005	Calcium Chloride &Ortho Phosphoric acid / 1.67/7	48 hrs	Water	-	XRD-No significant changes after adding alginate SEM- Alginate composite shows good cell affinity Fig(2)	Mechanical properties improved by adding sodium alginate
2	DanNicolae Ungureanu et al 2011	Calcium Hydroxide & Ortho phosphoric acid1.67/9.5-10	48 hrs	Water & ethanol	130 °C, 200 °C, 600 °C and 1200 °C for 2 hrs	XRD - The presence of <2% of CaO peaks SEM - Spherical shape fine nano particle Fig (3)	Rheological study conclude that ultra-fine powders have high specific surface
3	Jianping Zhu et al 2011	Calcium Nitrate tetraHydrate & Diammonium hydrogen phosphate 1.67	40 °C at 3.5hrs 60 °C at 3.5hrs 80 °C at 4 hrs	-	90 °C for 72hrs 660° C for 2 hrs	XRD- Narrow diffraction peaks SEM - Fibrous, scaly and spherical shape Fig(4)	Different nano structures are synthesized
4	Luis C. Mentes et al 2012	Calcium nitrate & Diammonium hydrogen phosphate 1.89 & 2.38 /10	80 °C at 40 min	Freeze	-	SEM -Strong action of collagen on HAP morphology	Role of collagen plays a vital role in the synthesis of nano HAP
5	Rozita Ahmad Ramli et al 2011	Calcium hydroxide & Ortho Phosphoric acid /1.67 /11	24 hrs	-	80 °C for 24 hrs 800 °C for 2 hrs	XRD - Pure HAP with no carbonated ions SEM - Nano size porous form Fig (5)	Pure HAP was synthesized at 800 °C

Table 2. Hydrothermal Method

S. No	Author	Precursor	Hydrothermal reactor mechanism	Drying & Calcinations	Characterization	Remarks
1	Nasser Y Mustafa et al 2011	Calcium Carbonate & Ortho phosphoric acid	Reflex condenser protected from atmosphere by CO2 absorbing trap and ports for introducing N_2 as titrant	105 °C for 6 and 24 hrs/600 °C for 6 and 24 hrs	XRD - 141.3nm agglomerated image Fig (7,8)	Different routes tried
2	Earl et al 2006	Calcium Nitrate tetra hydrate & Diammonium hydrogen phosphate	Teflon line hydrothermal reaction for 24 and 72 hrs at 200 °C	50 °C for 4 hrs/ -	XRD - Well defined sample SEM - Nano rod like structure 100-600nm length and 10-60nm dia Fig (9,10)	Nano rod synthesized
3	Jing Bing Liu et al 2003	Calcium hydroxide & Calcium hydrogen phosphate dihydrate	-	60 °- 140 °C / -	SEM - Whiskers morphology of 40 and 600nm dia and length	Well elongated particles were absorbed on the condition that pH as 7 and temp 120 °C
4	Detia et al 2012	Calcium chloride & Sodium phosphate	-	-/ 650 °C for 6 hrs	XRD –Data good agreement with HAP SEM - Nano rod formation	Used in bio medical applications
5	Mehmaz salarian et al 2008	Calcium nitrate tetra hydrate & Diammonium hydrogen phosphate + CTAP	90 °C, 120 °C, 150 °C for 6 and 22 hrs	90 °C/ -	XRD – Characterization of HAP with difference SEM - Nano rod formation	Morphology and size of the particle controlled by dopant

2.3 Ultrasonic Irradiation Method

This method produces nano HAP by irradiating the mixture of Ca Source and P (Fig 11) with a source of ultrasonic radiation of varying frequencies and power. The obtained mixture is a well-defined product with high purity. Table 3 highlights the recent review of papers related to this method. Physical and chemical properties of the obtained mixture have been found to change with variation in frequency and power.

Mixing Ca and P precursor, maintaining Ca/P ratio and pH as a constant value is the first and foremost step in the synthesis of Nano HAP by this method followed by the passage of ultrasonic waves of desired frequency and power for a specific irradiation time. Drying and calcinations are followed by ultrasonic treatment. Table 3 shows the review of ultrasonic irradiation method work done from 2003 to 2012.

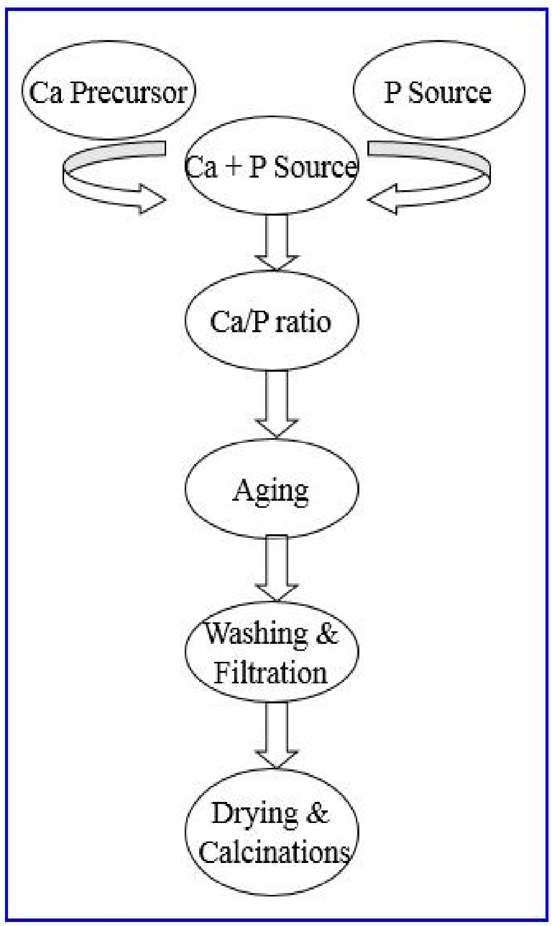

Fig. 6 : Hydrothermal Method

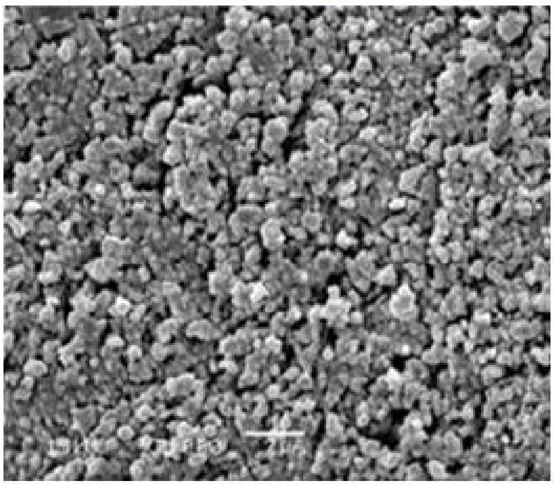

Fig 7 SEM Image by N.Y. Mostafa

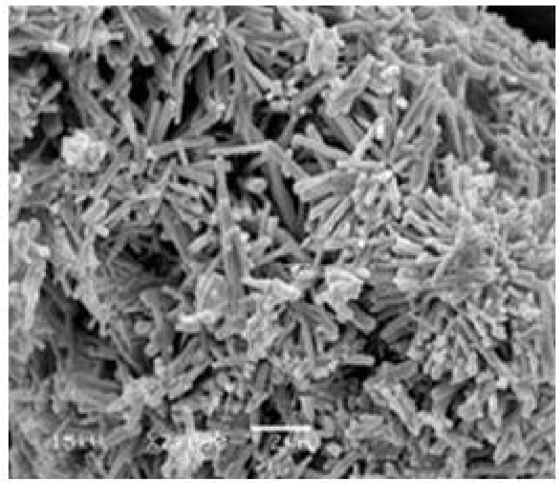

Fig 8 SEM Image by N.Y. Mostafa

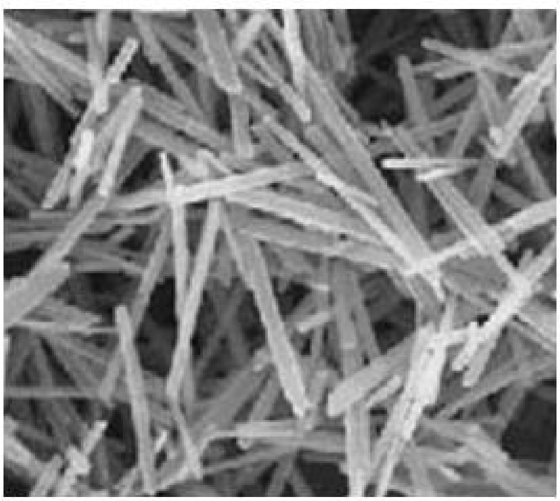

Fig 9 SEM Image by J.S. Earl

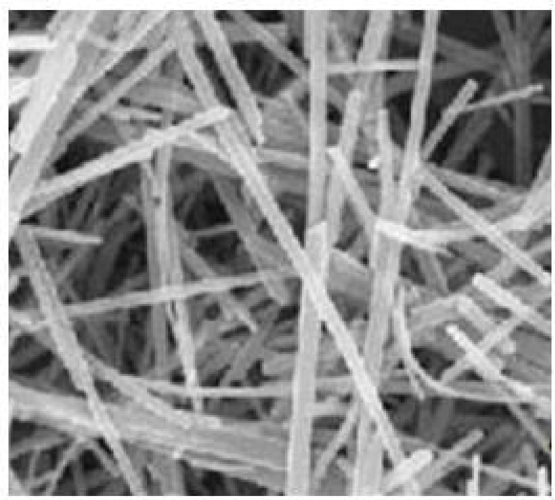

Fig 9 SEM Image by J.S. Earl

Table 3. Ultra sonic irradiation method

S. No	Author Year	Precursor/ Ca/PRatio/pH	Ultrasonic irradiation	Drying	Characterization	Remarks
1	Sahebali Manafi et al 2008	Calcium Nitrate & Diammonium hydrogen phosphate /1.67/ 10	30, 60, 90 and 120 min	150 °C for 24 hrs	XRD - Matches well with the standard values SEM - agglomerated image changes to rod like structure Fig (12)	Surface morphology varies with variation in ultrasonic irradiation
2	Gerand Eddy Pioneer et al 2009	Calcium nitrate tetra hydrate & Potassium hydrogen phosphate/ 1.67/-	0-50W, 30KHz	100-400 °C	XRD- Crystallite size decreases with radiation dose increases Fig(13,14)	Ultrasonic power of 50W and temp 400 °C was sufficient to produce nano HAP
3	Kojima et al 2012	Calcium Chloride & Tri sodium phosphate 1, 1.5, 1.67 and 2	20 or 40 KHZ	70 °C	XRD - Particle size of 30-50nm Fig (15)	very promising method for biomedical and ion exchange maintenance
4	Eny Kusrini et al 2012	Bovine bone	20, 40, 60 and 180 min.	-	XRD - Crystal size 36.31nm for aqua Bides and 40.67nm for ethanol	Crystallinity of Hap has no effect on adding sonification media
5	Coa Li Yun et al 2005	Calcium nitrate & Ammonium dihydrogen Phosphate 1.2-2.5	600 W	-	XRD - Well defined peaks	HAP can be synthesized by Cs^{2+} = 0.01-0.1 mol/l, 600W, Ca/P ratio = 1.2-2.5 and temp = 313-353K

2.4 Mechano Chemical Method

The Mechano Chemical method is the combination of mechanical and chemical phenomena on a nano scale solid material. Here nanomaterials are synthesized by mechanical activation and in this method, ball milling is a widely used technique wherein the powder mixture is placed in a ball mill and is subjected to high energy collision from the balls and thus mechanical force is used to achieve chemical processing and transformation. Contamination, long processing time, no control on particle morphology, agglomerates, and residual strain in the crystallized phase are the other disadvantages of high-energy ball milling process. However, the method is famous for its results, various applications and potential scientific values. Table 4 shows the recent review of papers related to nano HAP with Mechano Chemical method.

The method consists mainly of mixing Ca and P, maintaining Ca/P ratio and pH (Fig 16). The highlight of this method is choosing a mechanical milling with selected milling media such as Zirconia,

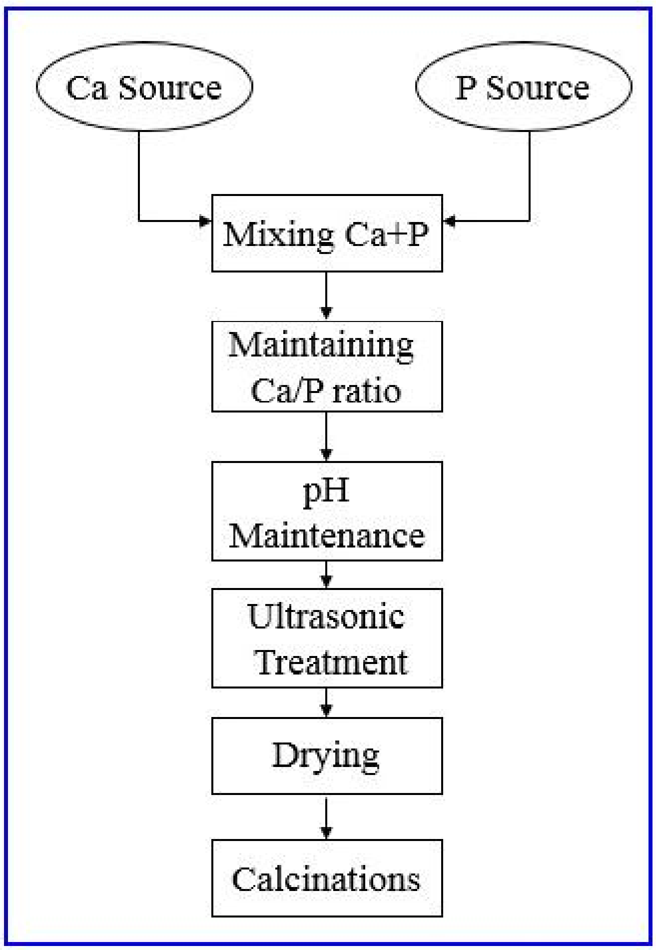

Fig. 11: Ultrasonic Irradiation Method

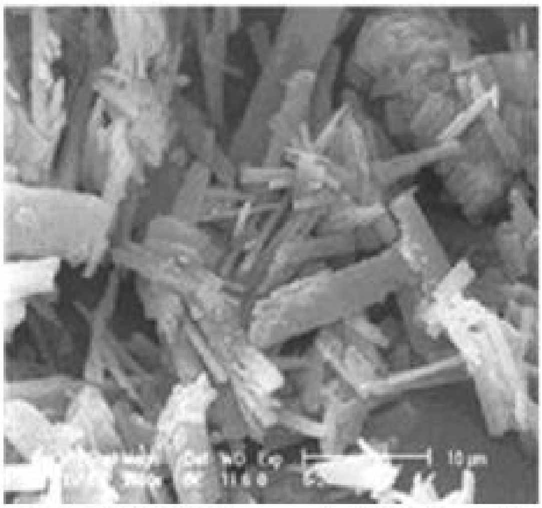

Fig 12 SEM Image by S. Manafi

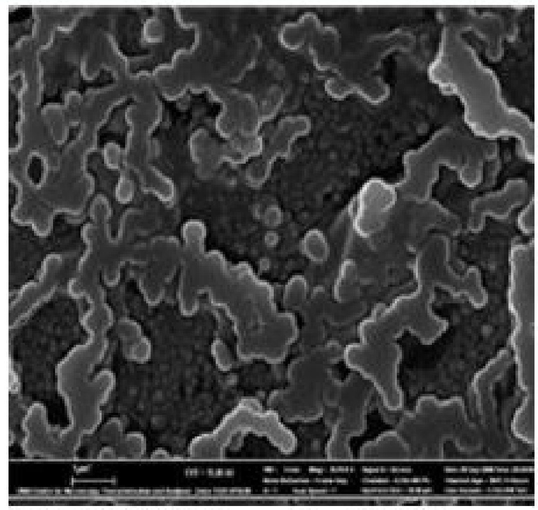

Fig 13 SEM Image by G.E. Pioneer

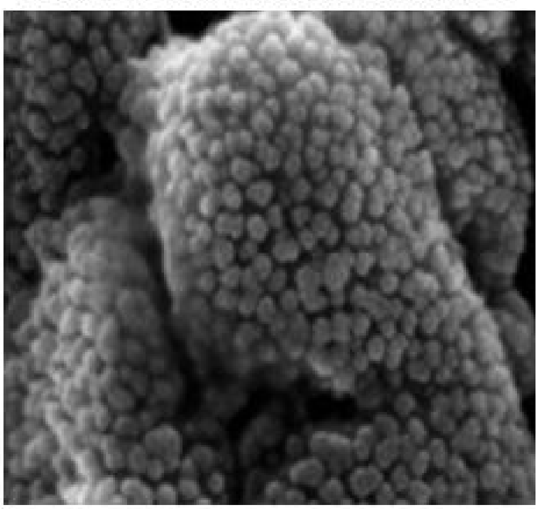

Fig 14 SEM Image by G.E. Pioneer

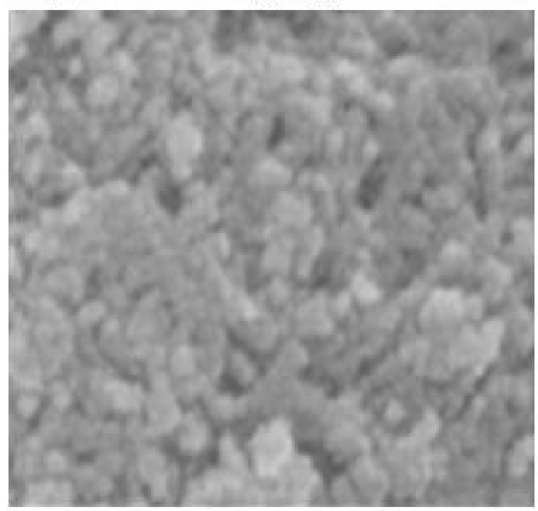

Fig 15 SEM Image by Y. Kojima

Table 4. Mechano Chemical Method

S.No	Author	Precursor	Milling Media/ hours/Speed/ mass ratio	Characterization	Remarks
1	Tomohiro Iwasaki et al 2011	Calcium Carbonate & Hydrated Calcium Hydrogen Phosphate	Zirconia/ 1 hrs, 3 hrs/ 140 rpm	XRD - High Crystalline nano Hydroxyapatite SEM-As the milling time increases, the coarse particle disappears and the member of hydroxyapatite nano particle increases.Fig (17)	The combination of precursors can produce HAP in a short period of time
2	Adzila et al 2011	Calcium Hydroxide & Diammonium hydrogen phosphate	Stainless steel/ 15hrs/ 170rpm 270rpm, 370rpm/1/6	XRD- Crystallite size was disagreement to the milling speed	Crystallinity increases with increases in the milling speed
3	Radzali Othman et al	Calcium Hydroxide & Hydrated Calcium Hydrogen Phosphate	Agate, Alumina Stainless steel Zirconia/ 15hrs,8hrs,3hrs, 1hrs/ 20:1	XRD –A single phase HAP SEM -Stainless media produce a much finer powder as compared to agate media Fig (18)	Stainless steel or agate media with 2hrs milling and HAP BPR as 10:1 is more suitable for single crystal
4	Yeong et al 2001	Calcium Oxide & Calcium hydrogen phosphate	Zirconia balls	XRD –A single phase HAP of high crystallinity was attained by less than 20 hrs of mechanical activation SEM- Average particle size of 25nm Fig (19)	20 hrs of mechanical activation is enough for pure HAP formation
5	Greta Gergely et al 2010	Sea Shell & Egg Shell Ortho phosphoric acid	Alumina & Zirconia/ 5hrs/ 4000rpm -	SEM - 100nm particle size obtained Fig(20)	Attrition milling was much more effective than ball milling

Table 5. Microwave Assisted Irradiation Method

S.No	Author Year	Precursor/ Ca/P ratio/ pH	Microwave irradiation	Drying & Calcinations	Characterization	Remarks
1	Gobi et al 2013	Calcium nitrate tetra hydrate Di potassium hydrogen phosphate/9	2.45GHz, 900W	80 °C for 6 or 12 hrs 900 °C for 2 hrs	XRD- Formation of pure HAP SEM- Nano sized spheres, rod and fibers structure Fig(22,23,24)	CTAB was used as surfactant
2	Siddharthan et al (2005	Calcium Nitrate tetra hydrate Ortho phosphoric acid/ 1.5	2.25GHz, 800W for 15 min	500 °C, 600 °C, 650 °C for 2 hrs	XRD - CDHA transform to a TCP at 650C SEM- Needle like morphology Fig (25)	CDHA can be prepared at rapid rate using microwave irradiation
3	Sahil Jalota et al 2004	Calcium Nitrate tetra hydrate Potassium dihydrogen phosphate	600W, 2.45GHz for 5 min	-	XRD- TCP, Single phase HA, Biphasic HAP, TCP sample SEM- Nano whiskers	Presence of tricalcium phosphate
4	Mohammad Bhilal Khan et al 2011	Calcium Nitrate tetra hydrate Di potassium hydrogen phosphate	600W, 1000W for 1 – 5 min	900°C and 1100 °C	XRD-Minimum limit for microwave exposure to get thermally stable HAP inferred with appearance of additional identifiable peaks	Minimum exposure of radiation is needed to produce pure HAP
5	Samar J Kalita et al 2010	Calcium Nitrate tetra hydrate Sodium Phosphate dibasic anhydrous9	600W	-	XRD- Average crystalline size of 12nm SEM- Elliptical and rod shape morphology	Varity of nano structures produced

agate, alumina, stainless steel etc. with particular speed and duration as well as critically maintaining the ball mass ratio. The samples are given heat treatment. Table 4 shows the review of Mechano Chemical method work done in the last two decades.

2.5 Microwave Assisted Irradiation Method

This method is one of the advanced methods for the preparation of nano HAP with an associated disadvantage that the procedure is extended in comparison with other methods. Microwave irradiation provides an efficient, environmentally friendly and economically viable method of heating due to its increased reaction kinetics and rapid initial heating coupled with reduced reaction times when compared to conventional heating methods finally culminating in products in powder form that are well defined, of high purity and homogeneous. Table 5 shows the recent review of papers related to microwave assisted irradiation method.

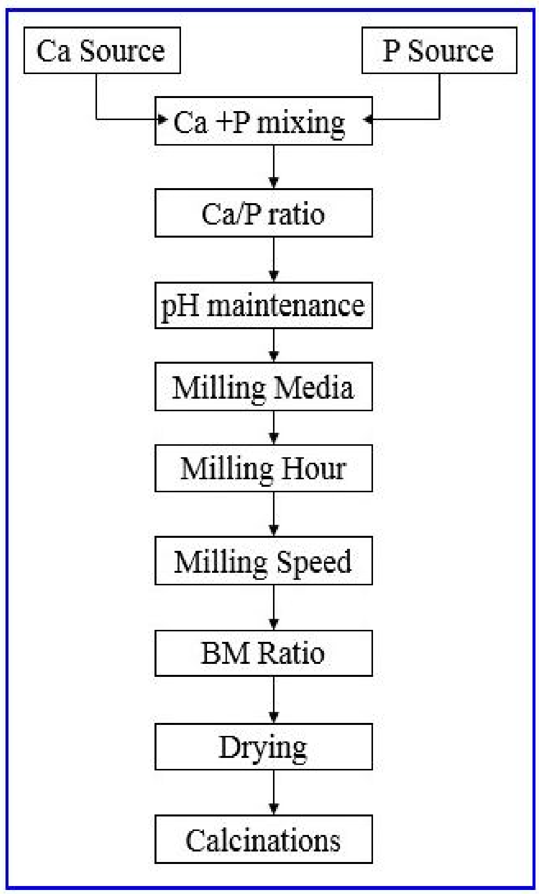

Fig. 16 : Mechano Chemical Method

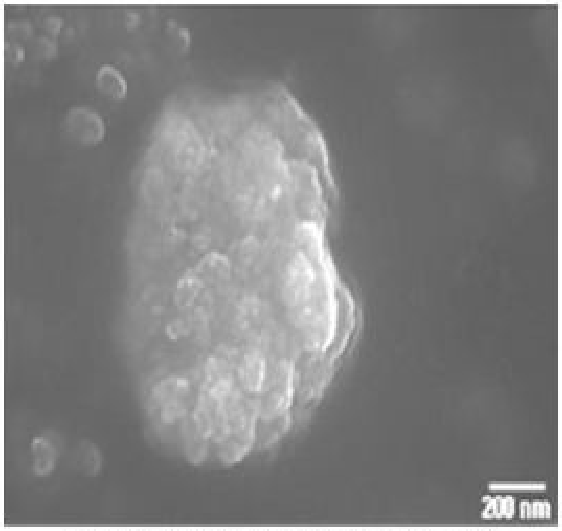

Fig 17 SEM Image by T. Iwasaki

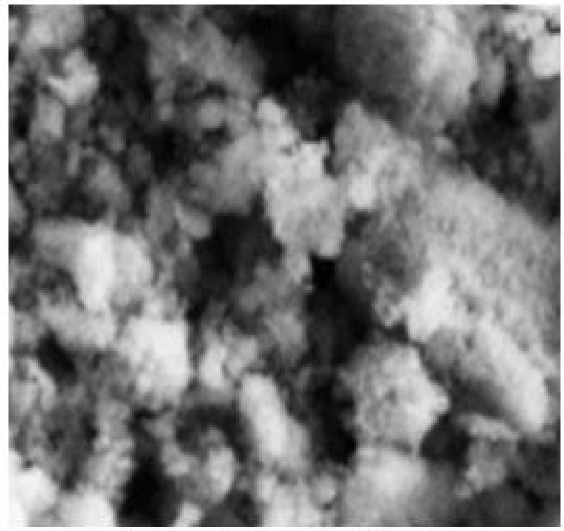

Fig 18 SEM Image by R. Othman

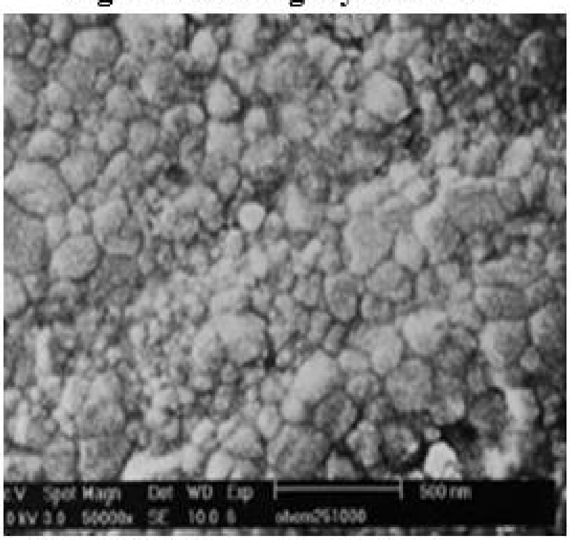

Fig 19 SEM Image by K.C.B. Yeong

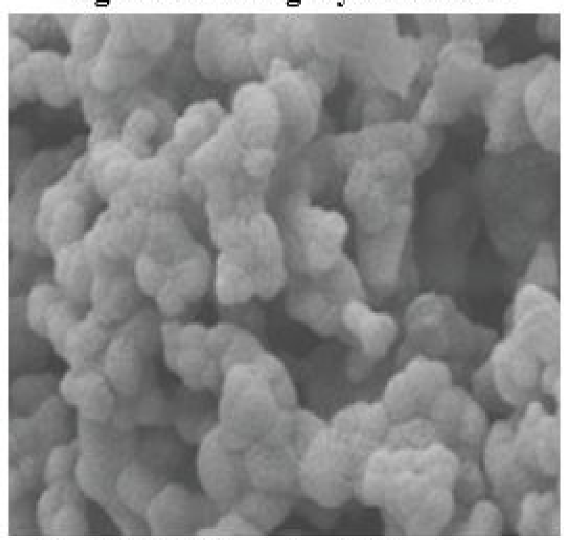

Fig 20 SEM Image by G. Gergely

The usual steps of mixing Ca+P, maintaining Ca/P ratio and pH are all followed. The second step to be followed is passing microwave radiation for a specific time followed by heat treatment Fig 21. Table 5 reviews work done in this method in the last 20 years.

2.6 Sol Gel Method

The Sol Gel method has of late attracted dedicated attention by a majority of researchers due to its many special features such as low temperature growth, low cost, homogeneous molecular products and the ability to produce nano sized particles easily when compared with other methods. Table 6 shows the recent review of papers related to Sol gel method

The first step to be followed in sol gel method is the choosing a Ca precursor and Phosphor precursor Fig 26.The second step is mixing the above and fixing the pH with ammonia or ammonium hydroxide, followed by ageing, filtration, drying and calcinations. Table 6 shows the review of sol gel method work done in the last 20 years.

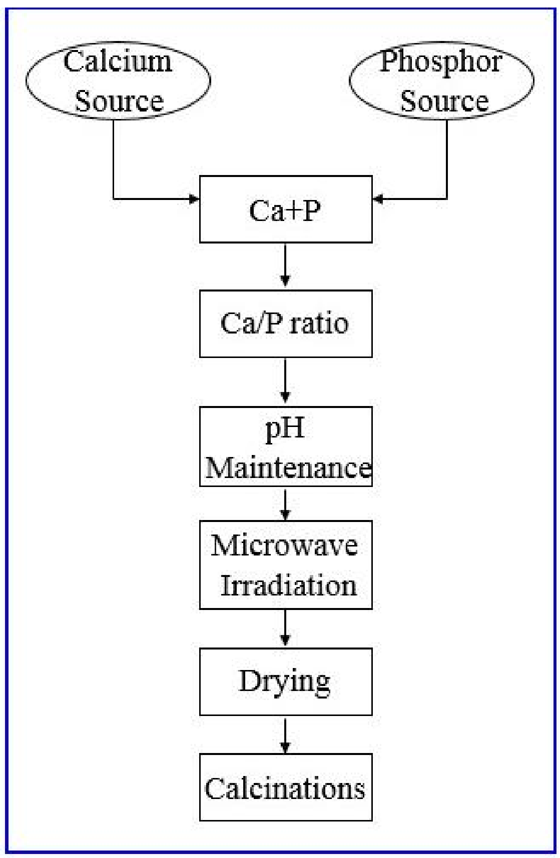

Fig. 21: Microwave Assisted Irradiation Method

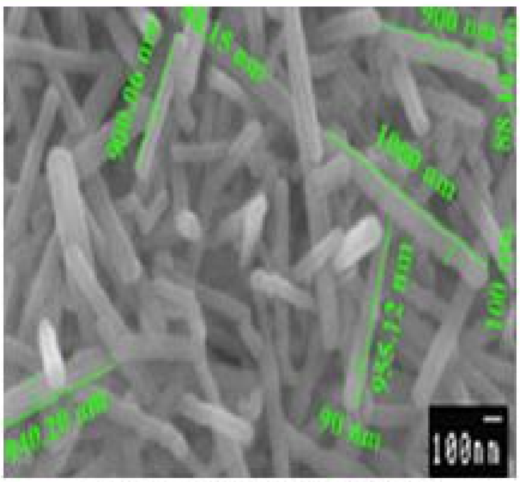

Fig 22 SEM Image by Gobi

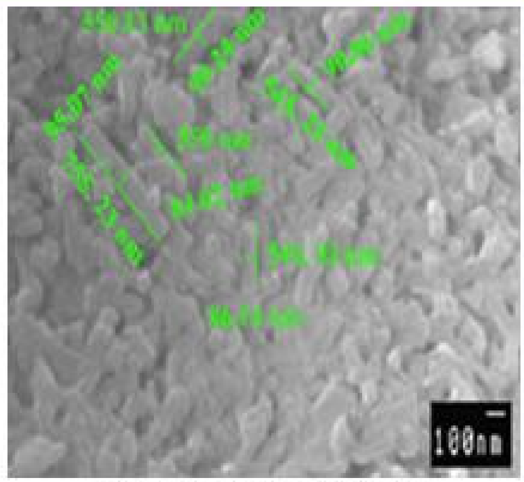

Fig 23 SEM Image by Gobi

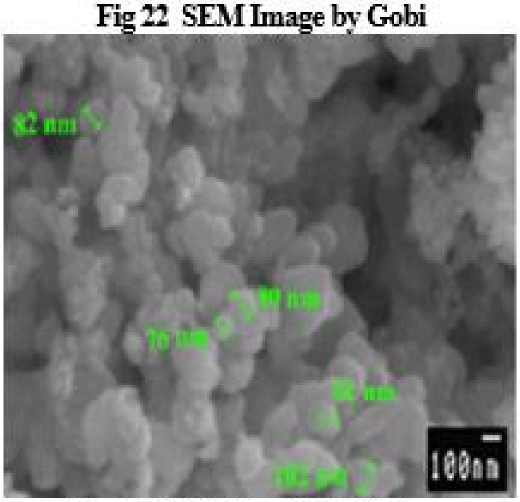

Fig 24 SEM Image by Gobi

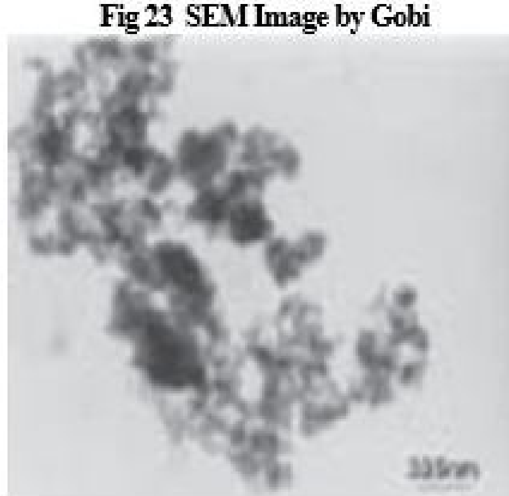

Fig 25 SEM Image by Siddharthan

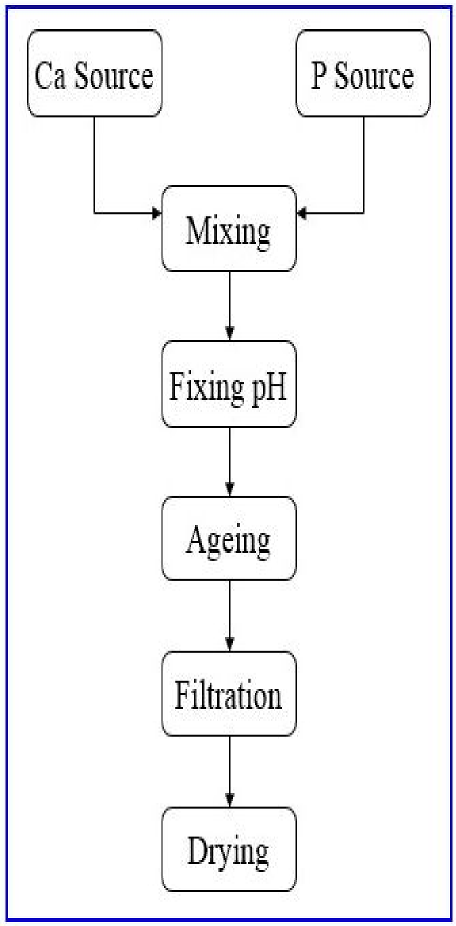

Fig. 26 : Sol Gel Method

Table 6. Sol Gel Method

S. No	Author Year	Precursor/ Ca/P ratio/ pH	Aging	Drying & Calcinations	Characterization	Remarks
1	Aldona et al 2006	Calcium acetyl acetonate Five different precursors/ 1.67	2 hrs	250 °C for 6hrs 400 °, 600°, 750°, 1000° C for 3hrs	XRD -The use of tri alkyl phosphate as starting material resulted in the higher amorphous side phase	Phase purity can be controlled by changing the nature of the precursor.
2	Anbalagan et al 2006	Calcium nitrate Tri methyl phosphate/ 1.67	16hrs	60 °C 300°to 900°C for 2hrs	XRD-Pure crystalline HAP Fig (27)	The degree of crystalliniy and the morphology of the HAP mainly depends on the processing parameters
3	K.P. Sanosh Et al 2009	Calcium nitrate Ortho phosphoric acid 1.67 10	24hrs	65 °C for 24 hrs 200 ° to 800 °C for 30 minutes	XRD- High purity product at low temperature obtained	The crystallinity, Ca/P ratio and particle size mainly depends on the calcinations temperature
4	Changesheng Liu et al 2001	Calcium Nitrate Diammonium hydrogen phosphate 1.67 10-11	-	-	- Fig(28)	Transformation of Octa calcium phosphate to calcium phosphate and amorphous calcium phosphate rapidly and then to calcium deficient HAP and HAP
5	Khelendra Agarwal (2011)	Calcium nitrate tetra hydrate Phosphoric pent oxide 1.67	-	80 °C for 20hrs 400 °C to 750 °C for 8 hrs	XRD- As the temp increases, peaks become more significant and increase in the crystallinity SEM- Agglomerated images Fig(29,30)	Powder produced are highly useful in bone replacement material

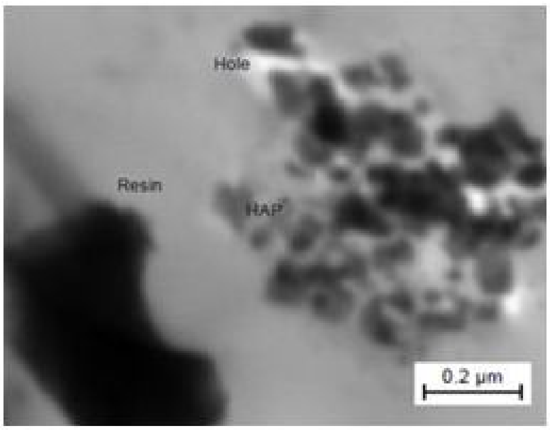

Fig 27 SEM Image by Anbalagan

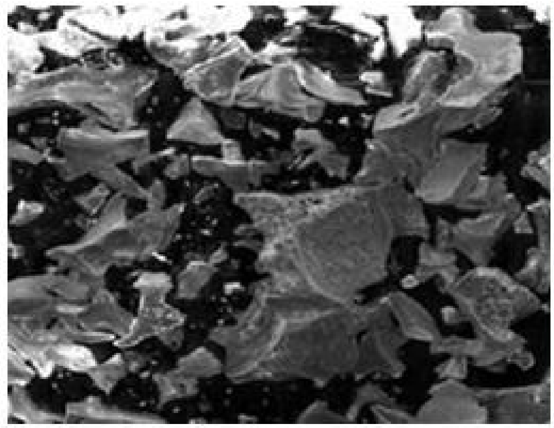

Fig 28 SEM Image by Agarwal

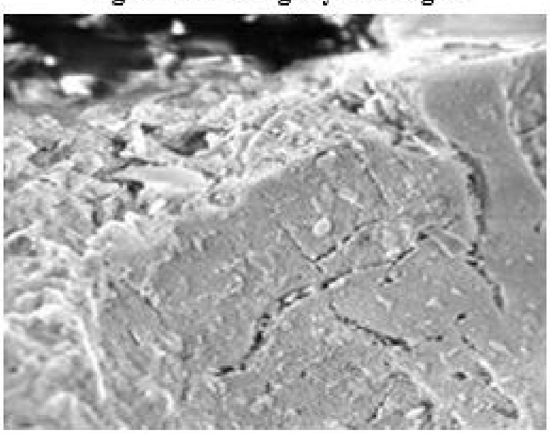

Fig 29 SEM Image by Agarwal

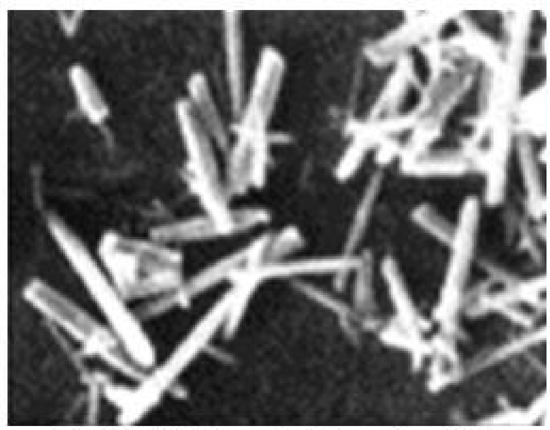

Fig 30 SEM Image by Changsheng Liu

3. CONCLUSION

In the present research article six different methods for the preparation of nano HAP have been described and the latest research in those methods in the last two decades have also been reviewed.

Of the methods listed above with their varying methodologies, the authors feel that the sol gel method is the simplest and easiest of the described methods to produce high purity, homogeneous nano HAP for subsequent usage in biomedical and orthopedic applications.

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