Iranian Journal of Pharmaceutical Sciences
Winter 2011: 8(1): 335-342
ijps.sums.ac.ir

Original Article

Effect of the Synthesis Parameters on the Properties of Biphasic Ca(OH) -HA Nanopowders for Tissue Engineering Applications

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Nanocrystalline hydroxyapatite was precipitated from calcium hydroxide and phosphoric acid. Effects of precipitation temperature and different calcium to phosphate ratios (Ca/P) on the obtained powders were investigated. Characterization of the powders was performed using XRD and FTIR spectra, scanning electron microscopy, and transmission electron microscopy. Increase in precipitation temperature increases the size and crystallinity of obtained crystals. Samples with Ca/P ratios more than stoichiometric ratio (1.67) had calcium hydroxide in their structures which is suitable for dental applications. Lower precipitation temperatures lead to lower crystallinity which is ideal for dental applications due to an increase in calcium hydroxide release.

Keywords: Ceramics; Chemical synthesis; Electron microscopy; Nanostructures; X-ray diffraction.
Received: July 25, 2011; *Accepted:* September 26, 2011

1. Introduction

Repair of injured or defective tissues especially hard tissues has always been a great challenge in biomedical engineering [1]. Using autografts is the most ideal solution but has some problems such as: insufficient amount, pain, infection, etc. Allografts and xenografts are the next alternatives but also with problems such as: limitation of availability, high cost, and immunogenicity problems. Therefore, a need for synthetic biomaterials has emerged [2, 3]. Biomaterials used for hard-tissue repair should: 1) provide the injured site with needed mechanical strength; 2) contribute to the tissue regeneration and stimulate osteogenesis; 3) not be toxic [4]. Various biomaterials such as biodegradable polymers, cell-polymer scaffolds, ceramics and, bioactive glasses have been used in this area [4-8]. Ceramic biomaterials due to structural and chemical resemblance to bone mineral phases, non-toxicity, and great biocompatibility are good candidates for bone repair [9-17]. Among them, calcium phosphate ceramics with different success in orthopedics and dentistry have been used [9]. Hydroxyapatite (HA) and tricalcium phosphate (TCP) are the most researched calcium phosphate biomaterials. The onset of using HA in orthopedic and dental applications goes back to 1970 [12, 13, 18] and many researches in using HA in composites with polymers ,biomolecules and

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Table 1. Samples synthesized by precipitation process in different temperatures and phases appearing in the obtained powders structure.

Sample code	Ca/P	Temperature	Appearing phases
A	1.9	22 °C	HA , $Ca(OH)_2$, $Ca_3(PO_4)_2$
B		42 °C	HA , $Ca(OH)_2$, $Ca_3(PO_4)_2$
C		70 °C	HA , $Ca(OH)_2$, $Ca_3(PO_4)_2$
D		95 °C	HA , $Ca(OH)_2$, $Ca_3(PO_4)_2$
E	1.8	22 °C	HA , $Ca(OH)_2$, $Ca_3(PO_4)_2$
F		42 °C	HA , $Ca(OH)_2$, $Ca_3(PO_4)_2$
G		70 °C	HA , $Ca(OH)_2$, $Ca_3(PO_4)_2$
H		95 °C	HA , $Ca(OH)_2$, $Ca_3(PO_4)_2$
I	1. 67	22 °C	HA
J		42 °C	HA
K		70 °C	HA
L		95 °C	HA

other ceramics have been carried out. HA can also be used for surface coating of orthopedic and dental metal implants which promotes osseointegration process and reduces metal ion release [19]. In addition, HA has been used in molecular biology as biological chromatography support in protein and DNA isolation and has been used for fraction and purification of a wide variety of biological molecules [20].

Sixty to 70 percent of the bone inorganic portion is consisted of HA. This fact makes HA biomaterials to cause much less inflammation and immunogenic reactions at implanted site. Osteoconductivity is another important property of HA which makes it ideal for bone repair. Therefore, tendency to continue research on HA as a biomaterial for hard-tissue repair is growing [10, 11, 18, 21, 22].

Stoichiometric HA is has calcium to phosphorus ratio (Ca/P) of 1.67. Any deviation from this ratio leads to formation of other phases in addition to HA. HA can be prepared using ceramic processing methods from fine powders and precursors at high temperature. There are various ceramic processing methods to produce synthetic HA precursors such as: precipitation, sol-gel, hydrothermal processing, etc [20, 22, 23].

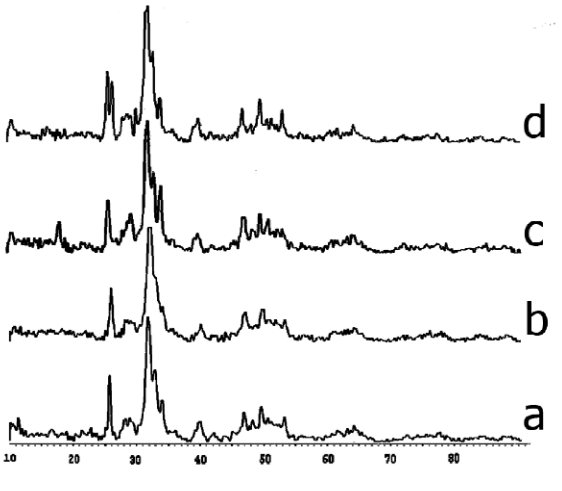

Figure 1. XRD spectra of the samples with stoichiometric Ca/P ratio at different precipitation temperatures (a=22 °C, b=42 °C, c=70 °C, d=95 °C).

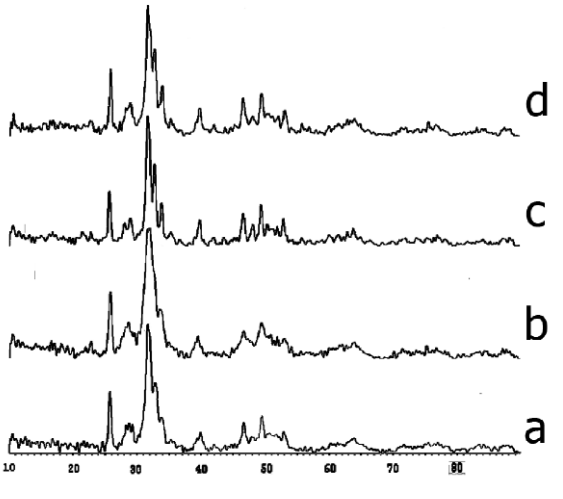

Figure 2. XRD spectra of the samples with Ca/P ratio of 1.8 at different precipitation temperatures (a=22 °C, b=42 °C, c=70 °C, d=95 °C).

Table 2. Particle size calculated using the relationship Debye–Scherrer

Sample code	A	B	C	D	E	F	G	H	I	J	K	L
Ca/P	1.9	1.9	1.9	1.9	1.8	1.8	1.8	1.8	1.67	1.67	1.67	1.67
T (°C)	22	42	70	95	22	42	70	95	22	42	70	95
Particle size (nm)	48	56	65	79	48	59	68	78	48	59	69	79

There are many works on precipitation of HA or its precursor phases from solutions containing Ca^{2+} and PO_4^{3-}, and acid-base reaction at constant composition. However, these methods need highly controlled parameters of the starting materials, PH and temperature of the solutions prepared to obtain HA monophase [24]. Here, HA has been directly precipitated from phosphoric acid and calcium hydroxide solutions. We are going to investigate the appropriate synthetic HA with desired composition and properties for use in dental composites and cements.

2. Materials and methods

Samples were prepared by aqueous precipitation reaction under different experimental conditions. Suspension of 0.5 M calcium hydroxide was prepared using calcium hydroxide powder (Merck). The suspensions were degassed, vigorously stirred and brought to the temperature range of 22-95 °C for two h. A 0.3 M phosphoric acid (Merck) solution was added drop by drop to the calcium hydroxide suspension at the same temperature ranges and kept for an hour. The pH of the mixture was controlled almost constant (between 10 and 11) by the addition of 1 mole of ammonium hydroxide (Merck) at the end of the precipitation process. The obtained mixture was stirred by magnetic stirrer for 2 h at 200 rpm and aged for 24 h at room temperature and centrifuged to complete precipitation process. The precipitate was dried at 100 °C during 12 h. The experiment was carried out with different ratios of calcium hydroxide and phosphoric acid to obtain desired Ca/P ratios.

Properties of powders such as morphology were characterized using XRD, FTIR spectroscopy, TEM and SEM. X-ray diffractometry was carried out using a Philips PW3710 diffractometer with X-ray wavelength of 1.54 Angestroms. FTIR spectroscopy was performed using a BRUKER VECTOR 33 spectrometer with IR range of 400-4000 cm^{-1} and 2 cm^{-1} resolution. Transmission electron microscopy was performed by a Philips CM200 FEG transmission electron microscope and scanning electron microscopy by a AIS2100-Koream, Seron technology.

3. Results and discussions

XRD spectra of the samples with Ca/P ratio of 1.67 shows the appearance of HA crystals amount and crystallinity of which increases by increase of temperature from 22 °C to 95 °C, as seen in Figure 1. The high width of HA spectra peaks indicates that the synthesized powders are nanocrystalline. XRD spectra for the samples with Ca/P ratio

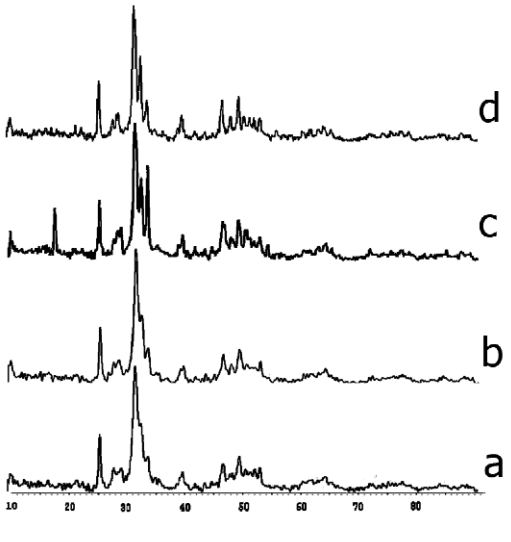

Figure 3. XRD spectra of the samples with Ca/P ratio of 1.9 at different precipitation temperatures (a=22 °C, b=42 °C, c=70 °C, d=95 °C).

Table 3. percent of calcium hydroxide phase measurements for different samples at different temperatures

Sample code	A	B	C	D	E	F	G	H	I	J	K	L
Ca/P	1.9	1.9	1.9	1.9	1.8	1.8	1.8	1.8	1.67	1.67	1.67	1.67
T (°C)	22	42	70	95	22	42	70	95	22	42	70	95
Percent of calcium hydroxide phase	30	30	30	30	15	15	15	15	10	10	10	10

of 1.8, 1.9 indicates the appearance of calcium hydroxide and calcium phosphate crystals in addition to HA, as seen in Figures 2 and 3. It shows that the amount of calcium hydroxide in samples with Ca/P ratios is more than the samples with stoichiometric ratio Ca/P=1.67.

The mean crystallite size (D) of hydroxyapatite and calcium hydroxide phases was calculated from the XRD line broadening using the Scherrer equation [8]:

$$D=[\frac{0.89\lambda}{B\cos\theta}] \quad (1)$$

where λ is the wavelength (CuKα), B the full width at half-maximum of the HA or calcium hydroxide and θ the diffraction angle.

Increasing the Ca/P gradually increases the amount of phase calcium hydroxide phase crystalinity and particle size increases with increasing temperature.

Hydroxyapatite is achieved at high temperatures (95 °C) shows the thermal stability and ripening does not occur. The maturation of the sediment increases with decreasing temperat. Hydroxyapatite obtained at low temperature (22 °C) has low crystalinity and appears to be a applicable ceramic biomaterial. Morphology and size of HA crystals play an important role in the sintering of bio-ceramics. Excellent mechanical properties are obtained when the hydroxyapatite is stoichiometry and crystals are small and uniform.

It is clear that by increasing calcium hydroxide concentration, the solution pH value increases. Since the antibacterial strength of cement is related directly to the pH, therefore, by measuring pH the behavior of the cement can be analyzed. The measurements taken after the synthesis of nano-powder and this process lasted for two h.

FTIR spectra of the samples with Ca/P ratios of 1.67, 1.8 and 1.9 are shown in Figure 4. FTIR characterization of the samples indicates the formation of HA. The amount of calcium hydroxide increases by an increase in Ca/P ratio as seen in the Figure 4.

The morphology of the precipitated crystals is shown in Figure 5. TEM micrograph of the sample A in Figure 5 shows that most of the crystals have a needle-like form with an average length of 80 nm and width of 10 nm.

SEM photos show the morphology of samples D, H, and L in Figure 6. Needle-like forms of the samples can be seen in agglomerated particles in SEM micrographs.

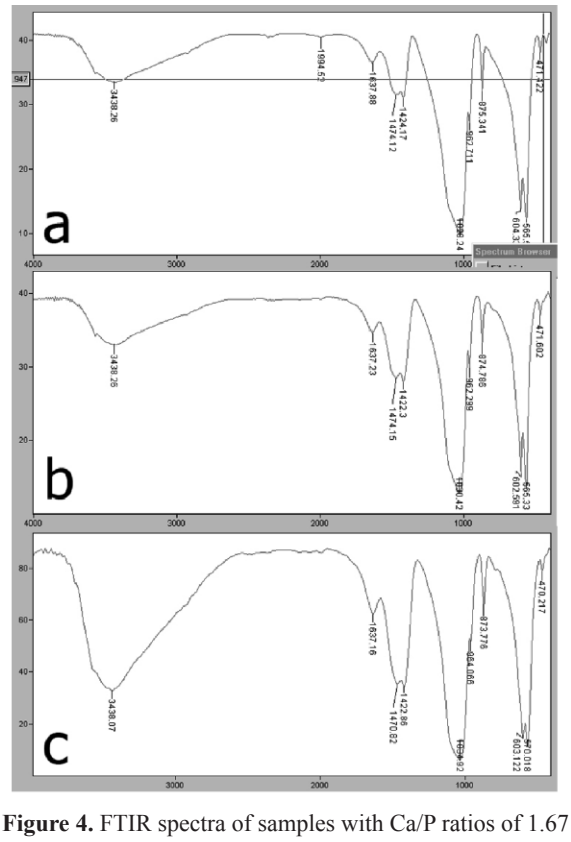

Figure 4. FTIR spectra of samples with Ca/P ratios of 1.67 (a) , 1.8 (b), and 1.9 (c) and precipitation temperature of 95 °C.

Table 4. PH measured for different samples for two hours

Sample code	A	B	C	D	E	F	G	H	I	J	K	L
Ca/P	1.9	1.9	1.9	1.9	1.8	1.8	1.8	1.8	1.67	1.67	1.67	1.67
T (°C)	22	42	70	95	22	42	70	95	22	42	70	95
Percent of calcium hydroxide phase	11	11	11	11	9	9	9	9	8	8	8	8

The form of precipitated crystals makes them ideal for using in dental composites by enhancing strength. With increase in the precipitation process temperature, the size of the precipitated crystals increases. Therefore, samples with lower temperature during precipitation process are more ideal for dental applications due to finer crystal size and lower crystallinity which increases the amount of calcium hydroxide release. Besides, at lower temperatures, the amount of calcium hydroxide is higher which is appropriate for dental applications.

The presence of calcium hydroxide in the samples with Ca/P ratios more than the stoichiometric amount, makes them ideal for dental applications. In fact, this leads to an increase in the amount of free calcium hydroxide and its dissolution in the environment which increases pH and reduces bacteria growth. Calcium hydroxide for its anti-bacterial characteristics, enhancing enzymes and growth factors release, and increasing the rate of drug release will be ideal for use in dental composites and cements [25, 26].

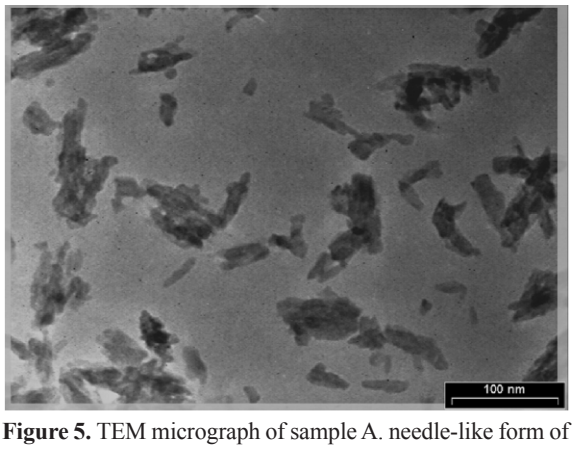

Figure 5. TEM micrograph of sample A. needle-like form of the crystals is shown.

4. Conclusion

It was shown that precipitation from solution is an easy and inexpensive route for HA synthesis. The morphology of the crystals

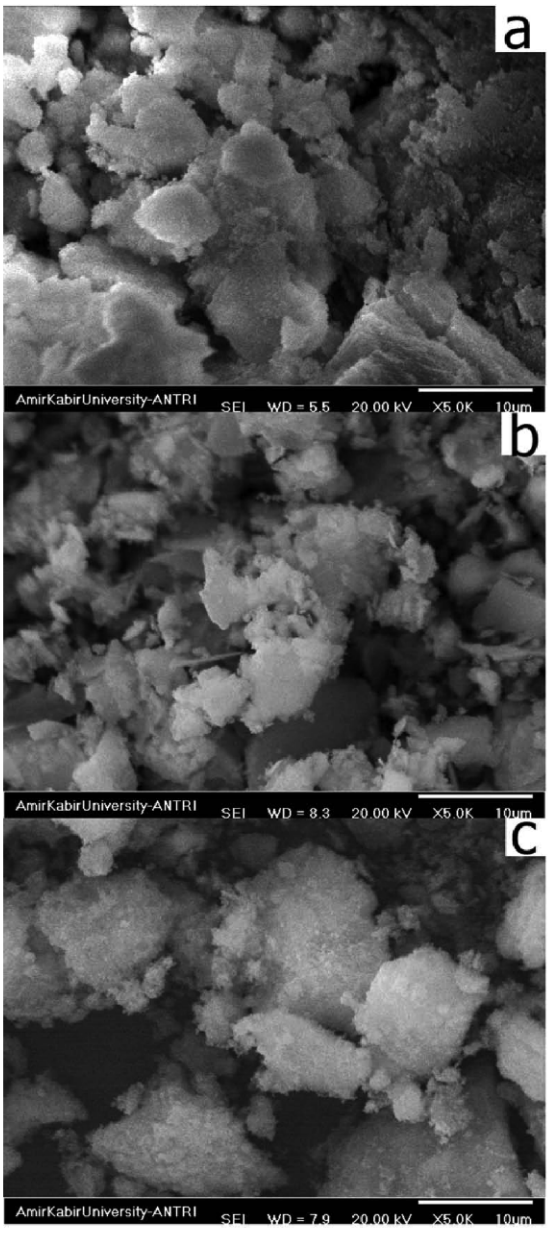

Figure 6. SEM micrographs of samples D, H, and L. samples with Ca/P ratios of 1.67 (a) , 1.8 (b), and 1.9 (c) and precipitation temperature of 95 °C.

obtained was needle-like which contributes to enhancing the mechanical properties of the composites or cements produced from these samples, used for dental applications. According to the results, in the samples synthesized with Ca/P ratios more than the stoichiometric ratio Ca/P=1.67, there will be some amounts of calcium hydroxide. The presence of calcium hydroxide leads to an increase in amount of free calcium hydroxide and its dissolution in the environment which increases pH and reduces bacteria growth. Calcium hydroxide because of its anti-bacterial characteristics, enhancing enzymes and growth factors release, and increasing the rate of drug release will be ideal for use in dental composites and cements. In addition, samples with lower precipitation process temperature have finer crystal size and further calcium hydroxide in their structure which makes them appropriate for dental applications. Lower crystallinity occurring at lower temperatures is more ideal for dental applications due to an increase in calcium phosphate release. In our future work, cements produced from the samples characterized in this work will be investigated for dental applications.

Reference

[1] Ma PX, Zhang R, Xiao G, Franceschi R.. Engineering new bone tissue in vitro on highly porous poly(alpha-hydroxyl acids)/hydroxyapatite composite scaffolds. *J Biomed Mater Res* 2001; 54: 284-93.

[2] Saikia KC, Bhattacharya TD, Bhuyan SK, Talukdar DJ, Saikia SP, Jitesh P. Calcium phosphate ceramics as bone graft substitutes in filling bone tumor defects. *Indian J Orthop* 2008; 42: 169-72.

[3] Sopyan I, Mel M, Ramesh S, Khalid KA. Porous hydroxyapatite for artificial bone applications. *Sci Technol Advanced Mater* 2007; 8: 116-23.

[4] Yaszemski MJ, Payne RG, Hayes WC, Langer R, Mikos AG. Evolution of bone transplantation: molecular, cellular and tissue strategies to engineer human bone. *Biomaterials* 1996; 17: 175-85.

[5] Miyamoto S, Takaoka K. TOPIC OF THE ARTICLE. *Ann Chir Gynaecol Suppl* 1993; 207: 69-75.

[6] Thomson RC, Yaszemski MJ, Powers JM, Mikos AG. Fabrication of biodegradable polymer scaffolds to engineer trabecular bone. *J Biomater Sci Polym Ed* 1995; 7: 23-38.

[7] Thomson RC, Yaszemski MJ, Powers JM, Mikos AG. In: Biomaterials for drug and cell delivery; A.G. Mikos, R. Murphy, H. Bernstein, and N.A. Peppas (eds.), Materials Research Society, *Pittsburgh*, 1994, pp. 33-40.

[8] Thomson RC, Wake MC, Yaszemski MJ, Mikos AG. In: Advances in Polymer Science. NA. Perppas, RS. *Langer*, (eds.), 1995, pp. 245-74.

[9] Oonishi H, Oomamiuda K. In: Handbook of Biomaterials Properties; J. Black and G. Hastings (eds.), Champman & Hall, London, 1998, pp. 407-17.

[10] Holmes RE. TOPIC OF THE ARTICLE. *Plast Reconstr Surg* 1979; 63: 626-33.

[11] Jarcho M, Kay JF, Gumaer KI, Doremus RH, Drobeck HP. Tissue, cellular and subcellular events at a bone-ceramic hydroxylapatite interface. *J Bioeng* 1977; 1 : 79-92.

[12] Kent JN, Quinn JH, Zide MF, Guerra LR, Boyne PJ. Alveolar ridge augmentation using nonresorbable hydroxylapatite with or without autogenous cancellous bone. *Oral Maxillofac Surg* 1983; 41: 629-42.

[13] Kent JN. TOPIC OF THE ARTICLE. *Dent Clin Morth Am* 1986; 30: 231-57.

[14] Morita S, Furuya K, Ishihara K, Nakabayashi N. Performance of adhesive bone cement containing hydroxyapatite particles. *Biomaterials* 1998; 19: 1601-6.

[15] Yap AU, Pek YS, Kumar RA, Cheang P, Khor KA. Experimental studies on a new bioactive material: HAIonomer cements. *Biomaterials* 2002; 23: 955-62.

[16] Clèries L, Fernández-Pradas JM, Sardin G, Morenza JL. Dissolution behaviour of calcium phosphate coatings obtained by laser ablation. *Biomaterials* 1999; 19: 1483-7.

[17] Hsu FY, Chueh SC, Wang YJ. Microspheres of hydroxyapatite/reconstituted collagen as supports for osteoblast cell growth. *Biomaterials* 1999; 20: 1931-6.

[18] Petit R, d'Orthopédie C. The use of hydroxyapatite in orthopaedic surgery: A ten-year review *European J Orthopaedic Surgery Traumatol* 1999; 9: 71-4.

[19] Lazi S, Zec S, Miljevi N, Milonji S. TOPIC OF THE ARTICLE. *Thermochim Acta* 2001; 374: 13-22.

[20] Afshar A, Ghorbani M, Ehsani N, Saeri MR, Sorrell CC. TOPIC OF THE ARTICLE. *Mater*

Des 2003; 24: 197-202.
[21] Koshino T, Murase T, Takagi T, Saito T. TOPIC OF THE ARTICLE. *Biomaterials* 2001; 22: 1579-82.
[22] Thamaraiselvi TV, Prabakaran K, Rajeswari S. Synthesis of Hydroxyapatite that Mimic Bone Minerology. *Trends Biomater Artif Organs* 2006; 19: 81-3.
[23] Saeri MR, Afshar A, Ghorbani M, Ehsani N, Sorrell CC. TOPIC OF THE ARTICLE. Mater Lett 2003; 57: 4064-9.
[24] Kim W, Saito F. Sonochemical synthesis of hydroxyapatite from H3PO4 solution with Ca(OH)2. *Ultrason Sonochem* 2001; 8: 85-8.
[25] Graham L, Cooper PR, Cassidy N, Nor JE, Sloan AJ, Smith AJ. The effect of calcium hydroxide on solubilisation of bio-active dentine matrix components. *Biomaterials* 2006; 27: 2865-73.
[26] Taichi I, Yoshihiro S, Hidetaka K, Yasuhiko A, Satoru Y. TOPIC OF THE ARTICLE. *Nippon Koku Inpuranto Gakkaishi* 2001; 14: 557-62.