IN VITRO TESTING OF CALCIUM PHOSPHATE WHISKERS FORMED BY A MICROWAVE-ASSISTED MOLTEN SALT PROCESS

Sahil Jalota, Sarit B. Bhaduri, and A. Cuneyt Tas

School of Materials Science and Engineering, Clemson University,
Clemson, SC 29634, USA

ABSTRACT

Calcium phosphate (single-phase HA, single-phase TCP, and biphasic HA-TCP) nanowhiskers and/or powders were produced by using a microwave-assisted "combustion synthesis (auto ignition) /molten salt synthesis" hybrid route.

Aqueous solutions containing $NaNO_3$, $Ca(NO_3)_2 \cdot 4H_2O$ and KH_2PO_4 (with or without urea) were irradiated in a household MW oven for 5 minutes at 600 watts of power. The as-synthesized precursors were then simply stirred in water at RT for 1 hour to obtain the nanowhiskers or powders of the desired calcium phosphate (CaP) bioceramics. In vitro testing (cytotoxicity, cell viability, and total protein assay) of the whiskers was performed by using the mouse osteoblast cells (7F2). These bioactive and osteoconductive whiskers/fillers are designed for use in biopolymer-based bone substitute scaffolds.

INTRODUCTION

Calcium hydroxyapatite (HA: $Ca_{10}(PO_4)_6(OH)_2$), the major inorganic phase of bones, is a member of the "apatite" family. However, biological apatites (e.g., enamel, dentin, and bone), differ from pure and synthetically produced calcium hydroxyapatite in stoichiometry, composition, crystallinity, and also in other physical and mechanical properties [1].

Minor cations (e.g., Na^+, K^+, Mg^{2+}, Sr^{2+}, Ba^{2+}, Cu^{2+}, Zn^{2+}, or Fe^{2+}), and anions (e.g., HCO_3^-, HPO_4^{2-}, Cl^-, and F^-) are also associated with biological apatites.

These can be considered as ionic substituents in the apatite structure. The more resorbable ß-TCP phase (tricalcium phosphate, $Ca_3(PO_4)_2$), when present in synthetic bone substitute materials, helps improving the resorption and bioactivity of the implanted bioceramics, and increase their ability in taking part in the in vivo bone remodeling processes.

HA or TCP powders have generally been synthesized from aqueous solutions for use in bioceramic applications. It is known [2] that calcium hydroxyapatite is the least soluble and the most stable calcium phosphate phase in aqueous solutions at pH values higher than 4.2. HA has been synthesized either in neutral or highly alkaline media [3-6] to ensure the thermal stability of the formed phase after high-temperature (1100°-1300°C) sintering.

Synthesis of HA in neutral or slightly acidic media [7] is known to be a more complicated and difficult task. The synthesis of biphasic mixtures of HA and TCP has also been achieved by aqueous coprecipitation [8]. HA-TCP biphasic bioceramics, owing to the higher in vivo solubility of β-TCP, display increased bioactivity as compared to more stable pure HA samples [9].

This paper utilizes a microw aforementioned materials [10, combustion synthesis (CS) b purity a-alumina powders. W from "Combustion Synthesis" mostly solid reactants [13, 1 fuel in a $Ca(NO_3)_2 \cdot 4H_2O$ and ously used to synthesize calci acid and ethylene glycol, mi Pechini process [16] was also phate powders [17]. Howeve and Pechini-like process [17] 1200°C for prolonged perio (i.e., HA, TCP or biphasic). synthesis (MSS). Molten salt shown to form monodisperse ture of HA powders and $K_2S($ to remove the K_2SO_4 glass a munication reports the devel assisted, molten salt-comb manufacture nanowhiskers a bioceramics. The work is ar utilizes the useful features of

EXPERIMENTAL PROCE

Aqueous solutions containin trated HNO_3, and urea (all re pared in 10 mL of deionized Table I. The first chemical a one was urea. Small amoun purpose of dissolving back a following the addition of Ca each case shown in Table I. beakers were then placed on covered with an upside dow auto ignition-molten salt proc into a household microwave Boca Raton, FL) for only 5

Sample	$NaNO_3$	$Ca(NO_3)_2$
	(g)	(g)
HA	5.00	1.00
TCP	5.00	1.00
Biphasic	5.00	1.00

Table I
Sample Preparation for the

This paper utilizes a microwave-assisted "Auto Ignition" process to synthesize the aforementioned materials [10, 11]. The auto ignition synthesis has been referred to as combustion synthesis (CS) by Kingsley and Patil [12] for the preparation of high-purity a-alumina powders. We prefer to use the term auto ignition to distinguish it from "Combustion Synthesis" or "Self-Propagating High Temperature Synthesis" using mostly solid reactants [13, 14]. The process, which used urea (H_2NCONH_2) as the fuel in a $Ca(NO_3)_2 \cdot 4H_2O$ and $(NH_4)_2HPO_4$ system of starting chemicals, was previously used to synthesize calcium phosphate powders [15]. A variant procedure (citric acid and ethylene glycol, mixed with $Ca(NO_3)_2 \cdot 4H_2O$ and $NH_4H_2PO_4$) based on the Pechini process [16] was also shown to work well for the synthesis of calcium phosphate powders [17]. However, both the combustion synthesis (or auto ignition) [15] and Pechini-like process [17] required high-temperature calcination steps (at 1000° to 1200°C for prolonged periods) for the formation of the calcium phosphate phases (i.e., HA, TCP or biphasic). This is where the advantage can be taken of molten salt synthesis (MSS). Molten salt synthesis technique [18], which uses a molten flux, was shown to form monodispersed whiskers of HA upon heating and cooling of a mixture of HA powders and K_2SO_4 from 1100° to 1200° C, followed by washing in water to remove the K_2SO_4 glass and expose the HA whiskers [19, 20]. The present communication reports the development of an extremely simple and rapid microwave-assisted, molten salt-combustion (auto ignition) synthesis hybrid procedure to manufacture nanowhiskers and nanopowders of HA, TCP and HA-TCP biphasic bioceramics. The work is an example of our synergistic processing technique that utilizes the useful features of the aforementioned processes.

EXPERIMENTAL PROCEDURE

Aqueous solutions containing dissolved $NaNO_3$, $Ca(NO_3)_2 \cdot 4H_2O$, KH_2PO_4, concentrated HNO_3, and urea (all reagent-grade, Fisher Chemicals, Fairlawn, NJ) were prepared in 10 mL of deionized water in 30 mL-capacity PyrexÒ beakers, as shown in Table I. The first chemical added and dissolved in water was $NaNO_3$, while the last one was urea. Small amounts of concentrated nitric acid were added for the sole purpose of dissolving back any colloidal calcium phosphates that might have formed following the addition of Ca- and phosphate-salts into the $NaNO_3$ solution. Thus, for each case shown in Table I, clear-transparent solutions were obtained. Small sample beakers were then placed onto 10 × 10 × 1 cm alumina insulating fiberboards and covered with an upside down 250 mL-capacity glass beaker. To proceed with the auto ignition-molten salt process, the above-mentioned sample assemblies were placed into a household microwave (MW) oven (Sunbeam, max. power 600 W, 2.45 GHz, Boca Raton, FL) for only 5 minutes.

Sample	$NaNO_3$	$Ca(NO_3)_2 \cdot 4H_2O$	KH_2PO_4	15.69 M HNO_3	Urea	Ca/P
	(g)	(g)	(g)	(mL)	(g)	
HA	5.00	1.00	0.345	0.10	1.75	1.67
TCP	5.00	1.00	0.384	0.10	—	1.50
Biphasic	5.00	1.00	0.345	0.10	0.075	1.67

Table I
Sample Preparation for the HA, TCP, and HA-TCP Biphasic Whisker Samples

MW oven was always operated at its maximum power setting. At the end of 5 minutes of MW heating within a well-ventilated fume hood, the sample beakers were taken out and their contents were let to cool down to RT in ambient atmosphere over the next 10 to 15 minutes. The substance in the sample beaker was then scraped off with a spatula, and directly placed (without grinding) into 500 mL of deionized water at room temperature ($22 \pm 1°$ C) and vigorously stirred for 1 hour. Formed suspension was filtered by using a vacuum-assisted Buechner funnel, and washed with 2 L of water, prior to drying the powders at $80°$ C for 2 hours. Samples were characterized by powder XRD (XDS 2000, Scintag, Sunnyvale, CA), FTIR (Nicolet 550, Thermo-Nicolet, Woburn, MA), SEM (S-3500 and S-4700, Hitachi, Tokyo, Japan), FESEM (Hitachi S-4700), and ICP-AES (61E, Thermo Jarrell Ash, Woburn, MA) analysis. Mouse osteoblast cells (7F2, ATCC, Rockville, MD) were used for cell culture and attachment studies. Cells were first grown at $37°$ C and 5% CO_2 in alpha MEM, augmented by 10% FBS. The culture medium was changed every other day until the cells reached a confluence of 90-95%. Osteoblasts were seeded at a density of 10^5 cells/cm^2. Cell cytotoxicity measurements were carried out after 24 hours, cell viability assessment was performed after 72 hours and total protein amount were measured after 7 days. Adhesion of the cells was quantified 24 hours after seeding. Trypan blue was added and the cells were counted using an Olympus BX60 light microscope. Only cells that stain blue were deemed necrotic because of plasma membrane damage. For statistics, all experiments were performed in triplicate where $n=3$. Analysis of variance was performed using the Tukey-Kramer multiple comparisons test. Osteoblast morphology after attachment was further examined by using SEM, after sputter-coating the samples with platinum.

RESULTS AND DISCUSSION

Sample solutions placed into the MW oven first went through an initial stage (1 minute) of evaporation and boiling of water. Urea is known [21] to decompose into biuret (H_2N-CO-NH-CO-NH_2, i.e. $C_2H_5N_3O_2$), cyanuric acid (HCNO), and ammonia (NH_3), when it is heated to about $200°$ C. Biuret itself then decomposes when heated at temperatures>$300°$ C. Nitrate solutions, on the other hand, usually decompose at low temperatures with the evolution of gases, such as NO_2, NO and N_2O_5. Moreover, we used $NaNO_3$, which melts at $307°$ C [22] at a high concentration in our liquid samples. It would have been possible to lower this salt melting temperature further if we were using mixtures of $NaNO_3$ and KNO_3 [23-29]. All the samples glowed red in the MW oven between the 90 and 240 seconds of heating time. In high urea solutions, intermittent sparks were also observed within that time period. This is the evidence of occurrence of combustion in this hybrid process. In the solutions containing urea, an exothermic reaction occurs which generates high temperatures leading to deformation of the Pyrex® beakers. At the end of 5 minutes of MW irradiation, the recovered sample beakers only contained a liquid phase of slightly yellowish tint. $NaNO_3$ did not evaporate and it formed a molten salt. This liquid phase solidified at RT within the following 4-5 minutes, by forming visible dendritic crystals of $NaNO_3$ on its surface. Powder XRD data of all the unwashed samples (i.e., after 5 minutes of microwave heating, but without wash in water) only showed the presence of $NaNO_3$ as the crystalline phase, as seen in Figure 1(a). However, as expected, FTIR data of the unwashed precursors revealed the presence of characteristic phosphate IR bands (labeled in Figure 1(b), as given in detail elsewhere [15, 19].
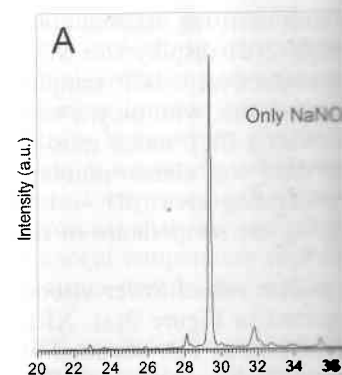
Fig. 1
(a) XRD and

Unwashed precursors were c
(20 to 700 mm). Following
dissolution and removal of r
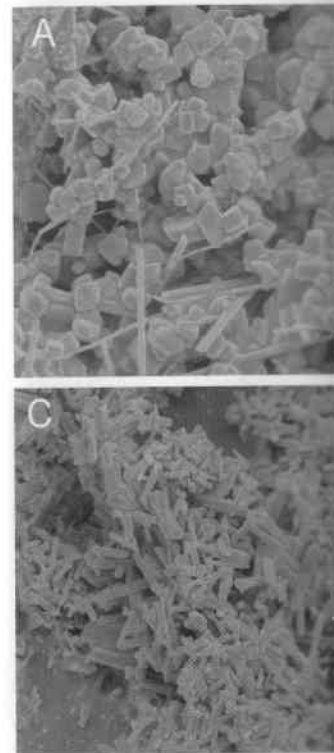raised to about 11.5-11.8 at
of the solutions at t=0 was
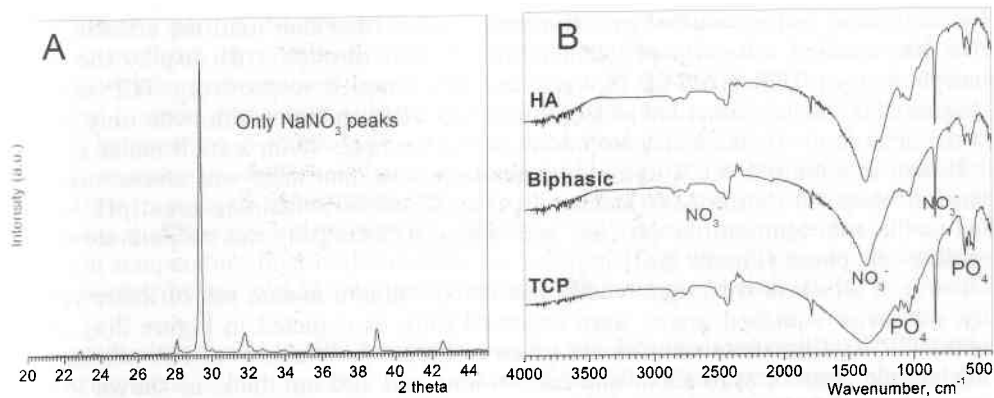
Fig. 2 SEM micrographs o

Fig. 1
(a) XRD and

(b) FTIR data (transmittance)
of MW-heated, unwashed CaP precursors

Unwashed precursors were collected from the reaction beakers as relatively big chunks (20 to 700 mm). Following washing in water, these chunks turned porous, upon the dissolution and removal of molten $NaNO_3$. pH values of the washing solutions in situ raised to about 11.5-11.8 at the end of 1 hour (t=60 min) of stirring at RT. pH value of the solutions at t=0 was around 10.
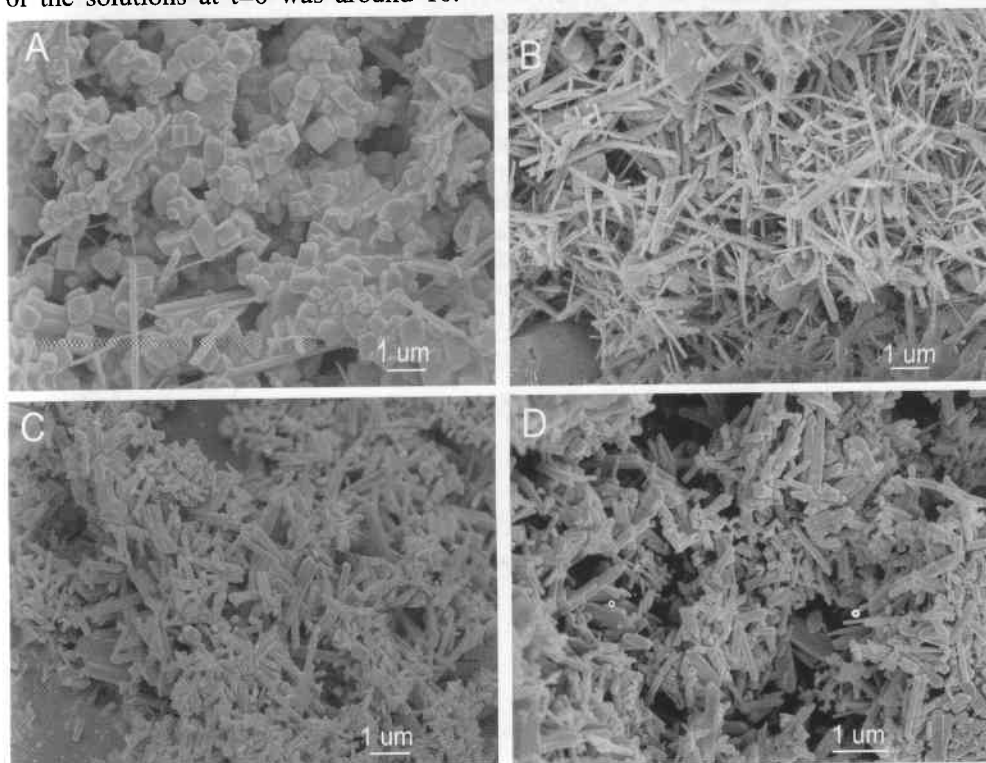

Fig. 2 SEM micrographs of (a) TCP, (b) HA-TCP biphasic, and (c) HA samples

193

Powders were further washed in a Buechner funnel filter unit until the effluent solution has reached a neutral pH value. Figures 2(a) through 2(d) display the SEM morphology of TCP, HA-TCP biphasic and HA samples, respectively. TCP samples (Figure 2(a)) mainly consisted of Whitlockite (β-TCP) rhombohedra, with only a very small amount of whiskers. The solutions of TCP samples (with a Ca/P molar ratio of 1.50 and solution pH $\approx$ 0.9) did not contain any urea, and there was almost no combustion observed during MW irradiation. The solutions containing urea (pH $\approx$ 1.5) led to the auto ignition-like process, and helped in increasing the temperature of the molten salt phase (Figure 2(b)).

Growth of whiskers was more readily facilitated within a molten salt of lower viscosity, otherwise equiaxed grains were observed [30], as depicted in Figure 2(a). XRD and FTIR data for these samples are given in Figure 3. Whiskers of HA obtained by this simple process were about one micron-long and 100 nm-thick, as shown in Figures 2(c) and 2(d).

The biphasic samples, Figure 2(b), contained around 60% TCP and 40% HA.

The volume percentage of the HA phase can easily be adjusted by varying the urea content in the starting solutions, as shown in Figure 4. ICP analyses of the HA, TCP and HA-TCP biphasic samples revealed that they contain around 1.5 wt% Na and 250 ppm K.

In the case of HA samples, such ionic substitutions surely causes a deviation from the perfect stoichiometry of $Ca_{10}(PO_4)_6(OH)_2$.

However, non-stoichiometry in an apatitic calcium phosphate sample is perfectly acceptable if the aim is to produce a synthetic biomaterial which can resorb in vivo, and readily take part in the bone-remodeling process [31]. Human bones contain a total of 1.3 wt% of cations like Na^+, K^+ and Mg^{2+} [1].

The process developed here yields bone-like calcium phosphate samples, as confirmed by the FTIR data of Figure 3(b).

Carbonate ion concentration in human bones amounts to a significant 5.8 wt% of the total weight [1]. It is also a well-known fact that either synthetic hydroxyapatites [32] or human bones [33] heated above 700°C readily lose their carbonate ions.
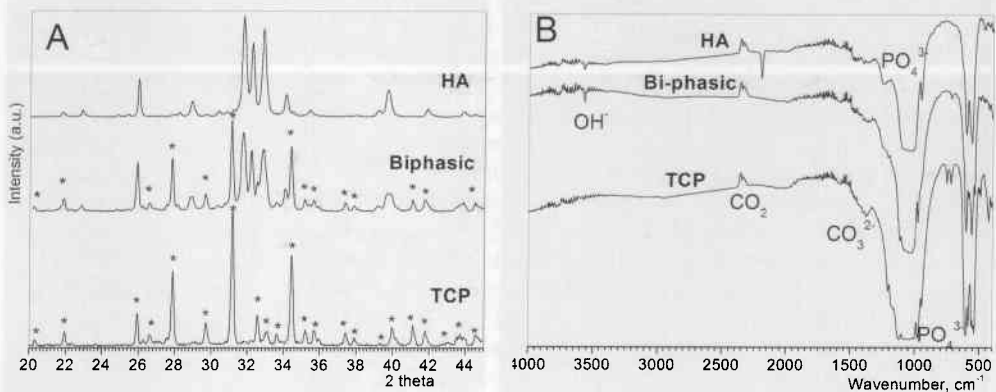
An important part of the pre were to perform the combu samples) under conventiona chamber furnace, temperatu This is because the reaction through a container wall, con from their precursors in so important that the particle g the local temperature distribu perature distribution would

MW irradiation should lead t solution system, achieving a the preparation of submicron this process by the molten N phate precipitates. Such prec the excess water. Due to the evidenced by the observatio ers), and these in situ proc bath. Embryonic particles of cleation and growth processe its melting point down to r preferred growth axes of the probably occurs during this n whisker-growth" process [3:

Solubility of the specific ca of temperature) has a sign The degree of superheating the resultant aspect ratio of growing out of the cooling r time at the peak temperatur follow-up studies, which we

Cell viability and protein histograms obtained with th tests using mouse osteoblas Figures 4 and 5, respectivel It is not abnormal to see i tests performed with osteob more basic and nano-textur surfaces would allow an incr of cells attached on them. Therefore, nanowhiskers of h were able to register the higl cell viability (Figure 4), in d son to rhombohedra of TCP Figure 2(a).

Fig. 3
(a) XRD (*: TCP peaks), and (b) FTIR data (transmittance) of washed CaP samples

An important part of the present work is the assistance of microwave heating. If we were to perform the combustion of our initial solutions (for the biphasic and HA samples) under conventional heating, for instance, in an electrical-resistance heated chamber furnace, temperature distribution of the solution would be inhomogeneous. This is because the reaction beakers would have been heated by thermal conduction through a container wall, convection and agitation. Since the synthesis of nanoparticles from their precursors in solutions consist of nucleation and particle growth, it is important that the particle growth is terminated [34]. Those processes partly rely on the local temperature distribution within the solution. Any inhomogeneity in the temperature distribution would then cause broad distributions in particle size.

MW irradiation should lead to homogeneous, i.e., molecular-level heating of the polar solution system, achieving automatic control of the above two processes required for the preparation of submicron-size whiskers or particles. The liquid phase formed during this process by the molten $NaNO_3$ dissolves all of the initially formed calcium phosphate precipitates. Such precipitates form during the initial boiling and evaporation of the excess water. Due to the presence of urea, an auto ignition process is initiated (as evidenced by the observation of intermittent sparks in the irradiated reaction beakers), and these *in situ* processes raise the extent of superheating in the molten-salt bath. Embryonic particles of calcium phosphate phases are formed through the nucleation and growth processes. As the molten ionic bath cools, while passing through its melting point down to room temperature, rapid crystallization occurs along the preferred growth axes of the ceramic phase. Calcium phosphate nanowhisker growth probably occurs during this rapid process, by a sequential "dissolution–crystallization—whisker-growth" process [35].

Solubility of the specific calcium phosphates in molten $NaNO_3$ (which is a function of temperature) has a significant role in the crystallization process to follow. The degree of superheating of the molten $NaNO_3$ must also have a strong effect on the resultant aspect ratio of the formed whiskers. The morphology of the whiskers growing out of the cooling molten salt bath could be strongly dependent on soaking time at the peak temperature, as well as the cooling rate. Such issues further warrant follow-up studies, which we plan to report in due time.

Cell viability and protein concentration histograms obtained with the cell culture tests using mouse osteoblasts are given in Figures 4 and 5, respectively.

It is not abnormal to see in cell culture tests performed with osteoblasts that the more basic and nano-textured substrate surfaces would allow an increased number of cells attached on them.

Therefore, nanowhiskers of hydroxyapatite were able to register the highest scores of cell viability (Figure 4), in direct comparison to rhombohedra of TCP as shown in Figure 2(a).
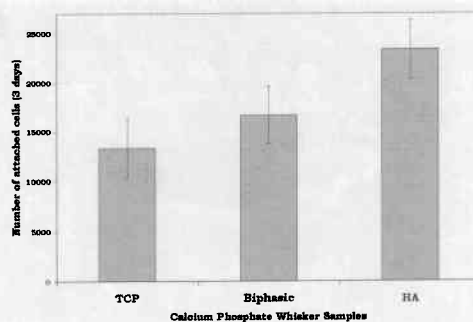

Fig. 4 - Cell viability

In terms of the results (Figure 5) of protein concentration tests performed with the osteoblasts, the variation observed between the different calcium phosphates of this study tended to be less subtle, however, still being in favor of the HA nanowhiskers.

The extent of osteoblast adhesion and proliferation on the samples of this study was monitored by making use of the SEM photomicrographs (Figure 6). The electron microscope was operated at 1 kV during image recording.
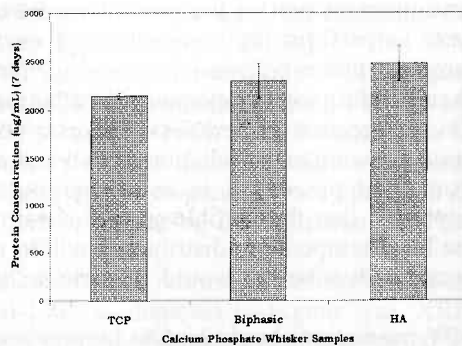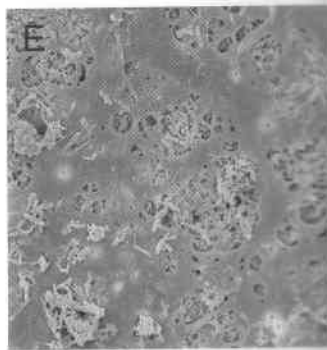
Fig. 5 - Protein concentration

Fig. 6 (e) & (f): Osteoblas

Although the cell density u
showed itself in the microg
hering very well to both wh
surfaces to the osteoblasts t

CONCLUSIONS

A new microwave-assiste
monodispersed nanowhiskea
cium phosphates. This new
tion) and molten salt syr
"Synergistic Processing." T
fuel in the combustion part c
of microwave (MW) heatin
watts, followed by 1 h of
sufficient to form nanowhi
aspect ratio of 10. Procedu
single phase HA, and biph
duced in this study were ch
protein concentrations were
adhered to the TCP, HA-T
MW-assisted molten salt s

REFERENCES

[1] L. L. Hench, *J. Am. C*

[2] M. Jarcho, C. H. Bolen
    J. Mater. Sci., **11**, 202

[3] M. Asada, Y. Miura, A.
    3202-5 (1988).

[4] C. P. A. T. Klein, J. l
    Groot, *Biomaterials*, **1**

[5] E. Ebrahimpour, M. Jo
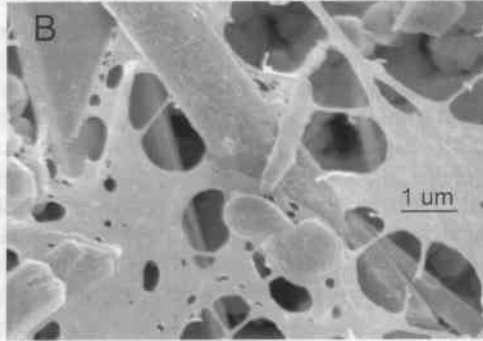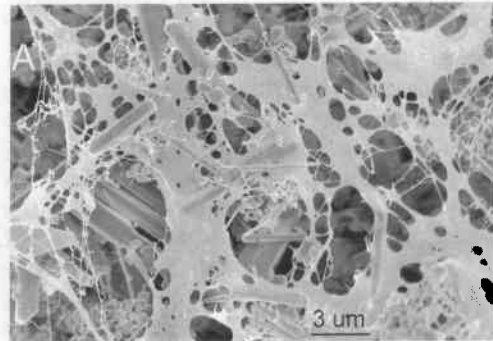   *Interf. Sci.*, **159**, 158-1

Fig. 6
(a) & (b): Osteoblast attachment on TCP whiskers at two different magnifications

TCP whiskers shown in Figure 6 were produced by using 0.075 g of urea in preparing the solutions used in MW irradiation (*see Table I*). This urea amount was then the same which was used in producing the biphasic samples. However, in synthesizing TCP whiskers the Ca/P molar ratio was fixed at 1.50. Adhesion of the osteoblasts on these whiskers, Fig. 6(b), was quite noticeable.
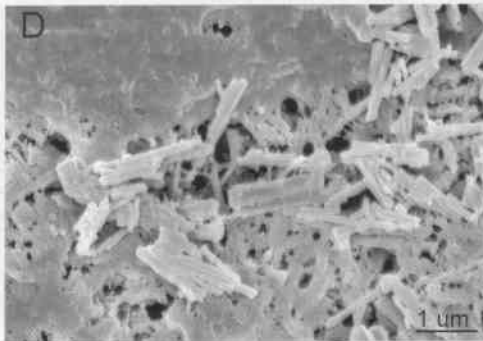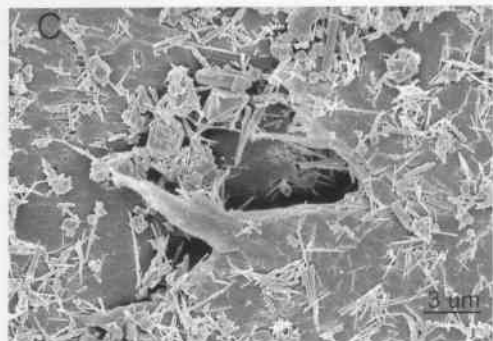
Fig. 6
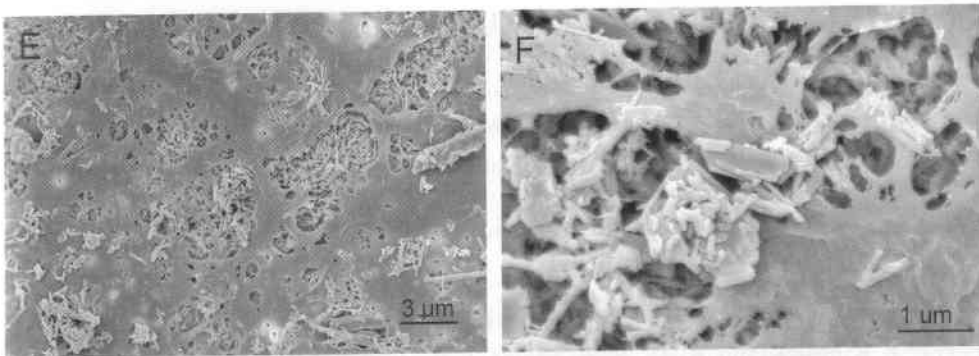*(c) & (d): Osteoblast attachment on biphasic HA- TCP whiskers*

Fig. 6 (e) & (f): Osteoblast attachment on HA whiskers

Although the cell density used in this study (10^5 cells/cm^2) was a bit high, and it showed itself in the micrographs of biphasic and HA samples, osteoblasts were adhering very well to both whiskers. Pellets of whisker bodies also provided nanorough surfaces to the osteoblasts to extend their filipodia and proliferate further.

CONCLUSIONS

A new microwave-assisted process has been developed for the synthesis of monodispersed nanowhiskers and nanopowders of Na- and carbonate-containing calcium phosphates. This new procedure is a hybrid of the urea-combustion (auto ignition) and molten salt synthesis techniques, and represents the philosophy of "Synergistic Processing." The fluxing agent used was NaNO$_3$. Urea was used as the fuel in the combustion part of this process. According to this synthesis route, 5 minutes of microwave (MW) heating of aqueous solutions in a household MW oven at 600 watts, followed by 1 h of washing of the irradiated samples in water at RT was sufficient to form nanowhiskers. 100 nm-thick HA nanowhiskers obtained had an aspect ratio of 10. Procedure was successfully shown to yield single phase TCP, single phase HA, and biphasic HA-TCP samples. Nanowhiskers or powders produced in this study were chemically similar to human bones. Osteoblast viability and protein concentrations were found to be the highest on the HA whiskers. Osteoblasts adhered to the TCP, HA-TCP biphasic and HA whiskers produced by the route of MW-assisted molten salt synthesis.

REFERENCES

[1] L. L. Hench, *J. Am. Ceram. Soc.*, **74**, 1478-1510 (1991).

[2] M. Jarcho, C. H. Bolen, M. B. Thomas, J. Bobock, J. F. Kay, and R. H. Doremus, *J. Mater. Sci.*, **11**, 2027-2035 (1976).

[3] M. Asada, Y. Miura, A. Osaka, K. Oukami, and S. Nakamura, *J. Mater. Sci.*, **23**, 3202-5 (1988).

[4] C. P. A. T. Klein, J. M. A. de Blieck-Hogerworst, J. G. C. Wolke, and K. de Groot, *Biomaterials*, **11**, 509-512 (1990).

[5] E. Ebrahimpour, M. Johnson, C. F. Richardson, and G. H. Nancollas, *J. Colloid Interf. Sci.*, **159**, 158-163 (1993).

[6] A. C. Tas, F. Korkusuz, M. Timucin, and N. Akkas, *J. Mater. Sci.-Mater. M.*, **8**, 91-96 (1997).

[7] D. Bayraktar and A. C. Tas, *J. Mater. Sci. Lett.*, **20**, 401-403 (2001).

[8] N. Kivrak and A. C. Tas, *J. Am. Ceram. Soc.*, **81**, 2245-2252 (1998).

[9] G. Daculsi, O. Laboux, O. Malard, and P. Weiss, *J. Mater. Sci.-Mater. M.*, **14**, 195-200 (2003).

[10] R. H. G. A. Kiminami, M. R. Morelli, D. C. Folz, and D. E. Clark, *Am. Ceram. Soc. Bull.*, **79**, 63-67 (2000).

[11] R. H. G. A. Kiminami, M. R. Morelli, D. C. Folz, and D. E. Clark, *Mater. Trans.*, **42**, 1661-1666 (2001).

[12] J. J. Kingsley and K. C. Patil, *Mater. Lett.*, **6**, 427-432 (1988).

[13] S. Bhaduri, S. B. Bhaduri, and K. A. Prisbrey, *J. Mater. Res.*, **14**, 3571-80 (1999).

[14] S. B. Bhaduri, S. Bhaduri, J. G. Huang, and W. R. Tinga, *Ceram. Eng. Sci. Proc.*, **20**, 227-234 (1999).

[15] A. C. Tas, *J. Eur. Ceram. Soc.*, **20**, 2389-2394 (2000).

[16] M. P. Pechini, US Patent No. 3330697, 1967.

[17] J. Pena and M. Vallet-Regi, *J. Eur. Ceram. Soc.*, **23**, 1687-1696 (2003).

[18] R. H. Arendt, *J. Solid State Chem.*, **8**, 339–347 (1973).

[19] A. C. Tas, *J. Am. Ceram. Soc.*, **84**, 295-300 (2001).

[20] A. C. Tas, *Powder Diffr.*, **16**, 102-106 (2001).

[21] H. Varma, K. G. Warrier, and A. D. Damodaran, *J. Am. Ceram. Soc.*, **73**, 3103-3105 (1990).

[22] D. R. Lide (Ed.), Handbook of Chemistry and Physics, p. 4-99, 72nd edition, 1992. CRC Press, Boca Raton, FL.

[23] P. Afanasiev and C. Geantet, *Mater. Chem. Phys.*, **41**, 18-27 (1995).

[24] L. Saadi, R. Moussa, A. Samdi, and A. Mosset, *J. Eur. Ceram. Soc.*, **19**, 517-520 (1999).

[25] F. Bondioli, A. B. Corradi, C. Leonelli, and T. Manfredini, *Mater. Res. Bull.*, **34**, 2159-2166 (1999).

[26] L. Vayssieres, N. Beermann, S. E. Lindquist, and A. Hagfeldt, *Chem. Mater.*, **13**, 233-5 (2001).

[27] A. B. Beleke, M. Mizuhata, and S. Deki, *Phys. Chem. Chem. Phys.*, **5**, 2089-2095 (2003).

[28] M. Park, C. L. Choi, W. T. Lim, M. C. Kim, J. Choi, and N. H. Heo," *Micropor. Mesopor. Mat.*, **37**, 81-89 (2000).

[29] P. Afanasiev, *Chem. Mater.*, **13**, 459-461 (2001).

[30] K. H. Yoon, Y. S. Cho, and D. H. Kang, *J. Mater. Sci.*, **33**, 2977–2984 (1998).

[31] D. Knaack, M.E.P. Goad, M. Aiolova, C, Rey, A. Tofighi, P. Chakravarthy, and D.D. Lee, *J. Biomed. Mater. Res.*, **43**, 399-409 (1998).

[32] A. C. Tas, *Biomaterials*, **21**, 1429-1438 (2000).

[33] K. D. Rogers and P. Daniels, *Biomaterials*, **23**, 2577-2585 (2002).

[34] H. B. Yin, T. Yamamoto, Y. Wada, and S. Yanagida, *Mater. Chem. Phys.*, **83**, 66-70 (2004).

[35] S. Jalota, A. C. Tas, and S. B. Bhaduri, *J. Mater. Res.*, **19**, 1876-1881 (2004).

COMPARISON OF
THREE DIFFE

Sahil Jalota

School of Materials

ABSTRACT

Coating of titanium-based
calcium phosphate (CaP) in
body fluids (SBF) have the
immersed titanium alloys w
M NaOH-etched surfaces of
three different SBF solution
in this comparative study w
tional SBF or c-SBF), ii) 27
27 mM HCO$_3^-$ HEPES-NaO
HCO$_3^-$) was quite slow in fc
37° C, whereas Tas-SBF of
ples. Cell viability, protein c
Ti6Al4V strips by using m

INTRODUCTION

SBF solutions are able [1-3]
ceramics or polymers (with
tions, in close resemblance
Salt Solution (HBSS) [5]. w
blood plasma. EBSS, whick
should be considered as a c
has a Ca/P ratio of 1.62. E
cal saline first developed by
solutions are also able to s
low Ca/P ratio.
For mimicking the ion con
relatively low Ca^{2+} and HP
[8]. pH values of SBF solu
buffers, such as TRIS (tris-l
(2-hydroxyethyl)-1-piper
The buffering agent TRIS p
stance, was reported [11] u
Ca^{2+}, which further reduces
time calcium phosphate coa
yet been reported for HEPE
4.2 mM (equal to that of HB
was first popularized by Ko
variant of HBSS, whose Ca