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Microwave processed nanocrystalline hydroxyapatite: Simultaneous enhancement of mechanical and biological properties

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Abstract

Despite excellent bioactivity of hydroxyapatite (HA) ceramics, poor mechanical strength has limited its applications primarily to coatings and other non-load bearing areas as bone grafts. Using synthesized HA nanopowder, dense compacts with grain sizes in nanometers to micrometers were processed via microwave sintering between 1000 and 1150 °C for 20 minutes. Here we demonstrate that mechanical properties, such as compressive strength, hardness and indentation fracture toughness of HA compacts increased with a decrease in grain size. HA with 168± 86 nm grain size showed the highest compressive strength of 395±42 MPa, hardness of 8.4±0.4 GPa and indentation fracture toughness of 1.9 ±0.2 MPam^{1/2}. To study the *in vitro* biological properties, HA compacts with grain size between 168 nm and 1.16 µm were assessed for *in vitro* bone cell-materials interactions with human osteoblast cell line. Vinculin protein expression for cell attachment and bone cell proliferation using MTT assay showed surfaces with finer grains provided better bone cell-materials interactions than coarse grained samples. Our results indicate simultaneous improvements in mechanical and biological properties in microwave sintered HA compacts with nanoscale grain size.

Keywords

Microwave sintering; Hydroxyapatite; Bioactivity / *in vitro* biocompatibility; compressive strength / mechanical properties; Processing

1.0 Introduction

Ideal biomaterial for grafting purposes requires excellent biocompatibility and tissue integration ability *in vivo*. Hydroxyapatite (HA, Ca₁₀(PO₄)₆(OH)₂), the main mineral component of bone and teeth, is among the leading biomaterials satisfying these requirements. However, poor mechanical properties of pure HA limited its use in any load-bearing applications [1–4]. Moreover, HA tends to transform to tricalcium phosphate (TCP, Ca₃(PO₄)₂) phase during sintering, particularly above 1200 °C. For conventional sintering higher temperature is necessary to attain high density parts with superior mechanical

properties. Since both mechanical strength and bioactivity of HA depend strongly upon phase purity and its microstructure, presence of TCP in HA can further influence both mechanical and biological properties of HA.

Different approaches such as addition of dopants or making composites have been investigated in order to improve mechanical properties of HA ceramics [5–8]. Use of nanograin ceramics can also improve sinterability due to higher surface area, and resultant nanoscale grains can enhance mechanical reliability via reducing the critical flaw size. The high volume fraction of grain boundaries in nanograin compacts can increase fracture toughness as well as other mechanical properties [9–12]. However, it is difficult to maintain nano-grains in the final sintered body using conventional sintering due to rapid grain growth typically seen in nanopowders. Microwave sintering is found to be quite effective in maintaining nano-grains in ceramics due to very high heating rates that can be applied [13,14]. Microwave heating is also very effective in producing compacts with reduced manufacturing costs due to energy savings and shorter processing times [15–17]. Volumetric heating through microwave ensures uniform heating and with almost no thermal gradient, which allows higher heating rates and shorter processing time. For oxide ceramics, microwave heating is being used since the last two decades [18–20] but microwave sintering of phosphate ceramics is relatively a newer proposition [12,21–22]. Microwave sintering of calcium hydroxyapatite was first reported by Fang et al. [21] showing that microwave sintering ensures high density, better microstructure, higher strength for HA with relatively shorter processing time compared to conventionally processed samples.

In this research, microwave processing was successfully applied to densify HA compacts to achieve superior chemical homogeneity and microstructural uniformity. Fabrication of fully dense HA compact is difficult especially because most HA nanopowders have needle-shape morphology that hinder densification. Moreover, to obtain a HA with grain size near 100 nm, grain growth should be suppressed during densification by using a lower sintering temperature [23]. We have synthesized low aspect ratio HA nanopowders [24–26], and the compacts were sintered using a fully automated 3 kW and 2.45 GHz commercial microwave oven at different temperatures to achieve varying grain sizes. Compressive strength, hardness and indentation fracture toughness of microwave sintered HA compacts were evaluated as a function of grain size. HA compacts were also assessed for *in vitro* bone cell-materials interactions for cell attachment and proliferation using human osteoblast cell line.

2.0 Materials and Methods

2.1 Synthesis and characterization of HA nanopowders

HA nanopowders were synthesized using a standard method established in our laboratory [24]. 5 M aqueous solution of Ca^{2+} -ion was prepared by dissolving 0.01 moles (2.362 g) of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ in 2 ml distilled water. 0.006 moles (0.686 g) of phosphoric acid (H_3PO_4) (85.7%) was added to the system to maintain Ca to P molar ratio 1.67 to 1. Organic phase was prepared by addition of 10 vol% surfactant (NP12) in cyclohexane with vigorous stirring. HA nanopowder was synthesized at aqueous to organic ratio (A/O) of 1:15 by mixing aqueous and organic phase in this proportion. The pH of the medium was adjusted to 9 with drop-wise addition of NH_4OH to initiate reaction between $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ and H_3PO_4 to form HA nano-powders. All reactions were aged for 24h at room temperature to grow non-agglomerated HA nanopowders with high crystallinity. After aging, the emulsion was evaporated on the hot plate at 150 °C followed by complete drying at 450 °C. Dry precursor powder was calcined at 650 °C for 4h to get carbon free crystalline HA nanopowder [24].

2.2 Consolidation of HA compacts

As synthesized HA nanopowders were ball milled for 6h and then freeze dried. A suspension of freeze dried HA nanopowders were prepared by dispersing the powders in deionized water with the addition of ammonium polymethacrylate (NH₄PMA) solution (Darvan C, R T Vanderbilt, Norwalk, CT, USA) using mechanical stirring. The amount of NH₄PMA used in suspension is expressed with respect to dry weight of the powder, equivalent to the wt./wt. basis of the HA nanopowders. The suspension was dried in an oven at 150 °C and used for the preparation of HA compacts. Dried powders were pressed into pellets with approximately 12.5 mm in diameter and 2.2 mm in height using uniaxial pressing at 50 MPa, followed by cold isostatic pressing at 345 MPa for 10 min. Cylindrical HA compacts for uniaxial compression testing with approximately 5.5 mm in diameter and 10 mm in height were also prepared using the same technique.

2.3 Sintering and characterization of HA compacts

Initially green HA compacts were calcined in a muffle furnace at 600 °C for 2h to burn out the surfactant. HA compacts were then sintered in a 2.45 GHz 3 KW microwave furnace [MW-L0316V, LongTech Co., Ltd, ChangSha, HuNan, P. R. China] at 1000, 1100 and 1150 °C for 20 minutes. Samples were placed on a silicon carbide plate. They were surrounded by a hollow silicon carbide cylinder, which was used as the susceptor to enhance microwave heating through efficient coupling. The entire assembly was covered with ceramic fiber insulation. Sample temperature was measured continuously with the help of an optical pyrometer from the top with an accuracy of ± 1 °C. The operating power of the microwave system was optimized through a number of trial runs. Initially the power was set at 2000W and then after reaching 800 °C, it was increased to 2700 W, and finally when the desired temperature was attained, the power was adjusted to maintain a constant temperature over the entire soaking time. HA compacts sintered at different temperatures were polished with 0.01 mm diamond paste and thermally etched at 800 °C for 30 min in a muffle furnace to reveal its microstructure.

Sintered compacts were characterized for bulk density, phase composition, particle size, microstructural analysis, microhardness, indentation fracture toughness and compressive strength. The bulk density of the green and sintered compacts was measured from the sample dimension and mass of the compacts. The constituent phases of sintered HA compacts were determined at room temperature using a Philips fully automated x-ray diffractometer with Cu-K α radiation and a Ni- filter. The diffractometer was operated at 35 kV and 30 mA over the 2θ range of 20 to 60 degrees at a step size of 0.02 degree and a count time of 0.5 s/ step. A dynamic light scattering technique (NICOMPTM 380, Santa Barbara, CA, USA) was used to determine the particle size distribution of the synthesized HA. Fourier transform infrared (FTIR) spectra of the synthesized nano HA powders were obtained using an ATR-FTIR spectrophotometer (Nicolet 6700 FTIR, Madison, WI, USA) in the 400–4000 cm⁻¹ wave number range. Microstructure was characterized using a field emission SEM (FEI Corporation, OR). HA grain sizes were determined from the SEM images via a linear intercept method.

Contact angles using DI water and cell media on the HA compacts were measured using the sessile drop method on a face contact angle equipped with a stereomicroscope and a camera [Model VCA Optima, AST products, Billerica, MA, USA]. A 0.5–1.0 μ l droplet of distilled water and McCoy's 5A solution at pH 7.4 (cell culture medium) was suspended from the tip of the microliter syringe. The syringe tip was advanced toward the disk surface until the droplets made contact with the disk surface. The images were captured using the camera and the contact angle between the drop and the substrate was measured from the image.

Microhardness measurements were done using a Vicker's diamond indenter (LECO Corporation, St. Joseph, MI) using 500 g load with polished HA samples. Indentation fracture toughness was calculated using the equation – $K_{IC} = 0.016 \times (E/H)^{1/2} \times P/C^{3/2}$, where E is Young's modulus of the sample, H is microhardness in GPa, P is the applied load, C is the half of the crack length, and K_{IC} is the indentation fracture toughness [27]. We have used an E value of 40 GPa based on our samples compressive test data. Compressive tests were measured using a screw driven universal testing machine with a cross head speed of 0.002 mm/sec. Three compression test samples were used for each data point.

2.4 *In vitro* bone cell-materials interactions

Bone cell-materials interactions were studied using a human osteoblast bone (HOB) cell line. Cells were derived from an osteoblastic precursor cell line (OPC1) established from human fetal bone tissue. Triplicate samples per group were evaluated for all experiments. Cells were cultured on HA disk for a maximum incubation period of 7 days. Each sample was sterilized by autoclaving at 121 °C for 20 min prior to the cell culture experiment. Following this, cells were seeded onto the sample surfaces, placed in a 24-well plate. Cells were seeded at a density of 1×10^4 /well. Then 1 ml of McCoy's 5A medium (with L-glutamine, without phenol red and sodium bicarbonate) supplemented with 5% fetal calf serum (FCS), and 5% bovine calf serum (BCS) was added to each well. Cells were maintained at 37°C under an atmosphere of 5% CO₂ and 95% air. The culture media was changed every other day.

After culturing the cells on HA discs for a pre-specified number of days, the cells were fixed in 4% paraformaldehyde in 0.1M phosphate buffer that were kept for 24h at 4 °C for future use. Those samples were permeabilized 0.5% Triton X 100 in 0.1 M PBS for 10 minutes and blocked with TBST/BSA (tris-buffered saline with 1% bovine serum albumin, 250 mM NaCl, pH 8.3) for 1h at room temperature. Primary antibody against vinculin (Sigma-Aldrich, St. Louis, MO) was added at a 1:100 dilution and incubated at room temperature overnight. Vinculin was used to study osteoblast cell attachment onto HA compacts. The following day, samples were rinsed with TBST/BSA three times for 10 minutes each. The secondary antibody, Oregon green goat anti-mouse (GAM) (Molecular Probes, Eugene, OR), was added at 1:100 dilution and incubated at room temperature for one hour. After 2×5 min washing with TBST/BSA followed by 5 min washing with PBS, samples were then mounted on coverslips with Vectashield Mounting Medium (Vector Labs, Burlingame, CA) with propidium iodide (PI) and observed in confocal scanning laser microscopy (BioRad 1024 RMC, Hercules, CA, USA). Specific absorption of vinculin is identified by the expression of green fluorescence and nuclei counterstained with a chemical dye propidium iodide (PI) present in the mounting medium expressed as red fluorescence. For green fluorescence excitation Ar ion (488nm, 30 mW) laser was used at 50% of maximum output at full power and for red fluorescence excitation He-Ne (543 nm, 1.2 mW) laser was used at full output and full power. A combination of a band-pass filter transmitting 505–570 nm and a long pass filter at 560 nm was applied to obtain fluorescence images. Since the test samples were opaque, the confocal pinholes were opened up to 560 μm to pass more fluorescence light to improve fluorescence image quality. Presence of higher amount of green fluorescence represents higher amount of vinculin expression by these cells.

The MTT assay (Sigma, St. Louis, MO) was performed to assess cell proliferation on sintered HA discs. The MTT solution of 5 mg/ml was prepared by dissolving MTT in PBS, and filter sterilized. The MTT was diluted (50 μl into 450 μl) in serum free, phenol red-free Dulbecco's Minimum Essential medium (DME). 500 μl diluted MTT solution was then added to each sample in 24-well plates. After 2h incubation, 500 μl of solubilization solution made up of 10% Triton X-100, 0.1 N HCl and isopropanol were added to dissolve the formazan crystals. 100 μl of the resulting supernatant was transferred into a 96-well plate, and read by

a plate reader at 570 nm. Data are presented as mean $\pm$ standard deviation. Statistical analysis was performed on contact angle and MTT assay results using student's t-test and a P value < 0.05 was considered significant.

3.0 Results

3.1 Characterization of HA nanopowders

Figure 1 shows the x-ray diffraction pattern of synthesized HA nanopowders showing phase pure crystalline HA according to JCPDS 09-0432. The FTIR spectra shows various bands from the respective phosphate and hydroxyl groups of HA as shown in Figure 2, which are in agreement with other reported results [28–30]. From FTIR spectra in Figure 2, it is evident that synthesized nanopowders contained phase pure HA. The bands at 1020 and 1085 cm^{-1} are assigned to the components of the triply degenerate ν_3 antisymmetric P–O stretching mode. P–O symmetric stretching mode is detected at 962 cm^{-1} . The bands at 599 and 563 cm^{-1} are attributed to components of the triply degenerate ν_4 O–P–O bending mode and the doubly degenerate ν_2 O–P–O bending mode was evident at 475 cm^{-1} . Vibrational and stretching mode of hydroxyl group is found at 631 and 3572 cm^{-1} respectively. These findings agree with that reported by S. Koutsopoulos, and others [28–30.]. Figure 3 shows the TEM micrograph of synthesized HA nanopowders. The aspect ratio of synthesized HA nanopowders was found to be 4.28 ± 0.59 . The average particle size from dynamic light scattering (DLS) measurement was found to be 52 nm. The size distribution of synthesized HA nanopowders was between 35 and 90 nm.

3.2 Densification Study

Freeze dried HA nanopowders synthesized using reverse micelle as a template system were consolidated and sintered in a microwave furnace to fabricate dense HA compacts. Green density of HA compacts without any dispersant was between 45 to 47% of theoretical density. Different amount of ammonium polymethacrylate (NH_4PMA) dispersant was used to improve the green density of HA compacts. Figure 4 shows that 6 wt% of NH_4PMA was found to be the most effective in improving green density from 45% up to 57 % of theoretical density. XRD results of HA pellets sintered in microwave furnace at 1000, 1100 and 1150 $^{\circ}\text{C}$ for 20 minutes contains phase pure HA as validated by JCPDS 09-0432 and shown in Figure 1. In all cases, 6 wt% of NH_4PMA was added and the sintered density was greater than 97% of theoretical density. Figure 5 shows the SEM micrographs of HA compacts sintered at 1000, 1100 and 1150 $^{\circ}\text{C}$ for 20 min. With an increase in sintering temperature, the average grain size of sintered HA compacts increased from 168 nm to 1.16 μm .

3.3 Mechanical properties

The bulk densities of HA compacts sintered at 1000, 1100, and 1150 $^{\circ}\text{C}$ were $97.7 \pm 0.5\%$, $98.1 \pm 0.7\%$, and $97.6 \pm 0.5\%$ of theoretical density, respectively. With a change in sintering temperature from 1000 to 1150 $^{\circ}\text{C}$, the grain size of sintered HA compacts increased while the density remained almost the same. Mechanical properties and grain size of HA compacts are summarized in Table 1. The compressive strength of HA compacts increased with a decrease in grain size. HA compacts with an average grain size of 168 nm showed an average compressive strength of 395 ± 42 MPa. With an increase in grain size, microhardness of sintered HA compacts also decreased. The average microhardness value of HA compacts with an average grain size of 168 nm was 8.4 ± 0.4 GPa, whereas the hardness for grain size of 1.16 μm was 6.3 ± 0.5 GPa. Indentation fracture toughness for HA compacts of 168 nm grain size was 1.9 ± 0.2 $\text{MPa m}^{1/2}$, which is similar to reported fracture toughness of human cortical bone [31].

3.4. Contact Angles

Contact angles on sintered HA surfaces were measured using cell media and distilled water. The contact angle results are shown in Figure 6. It was noticed that the contact angle decreased with a decrease of grain size in HA improving the wettability of the surface due to higher amount of grain boundary areas. Contact angle of water increased from 28° to 43° when the grain size increased from 168 nm to 1.16 μm.

3.5 Bone cell materials interactions

Immunocytochemistry and confocal microscopy—The formation of vinculin-positive focal adhesion plaques increased gradually with cell culture time on all three HA compacts as shown in Figure 7 and Figure 8. After 1 and 5 days of cell culture period, the highest fluorescence staining of vinculin occurred on HA substrate having 168 nm grains as shown in Figure 7a and Figure 8a, while the 1.16 micron grained HA substrate showed the lowest amount of vinculin expression, as shown in Figure 7c and Figure 8c.

MTT assay—MTT assay was used to determine osteoblast cell proliferation on surfaces of HA compacts. Figure 9 shows a comparison of cell densities on microwave processed HA compacts after 1, 5 and 7 days. Cell proliferation was clearly evident over the duration of experiment. The number of osteoblast cells on HA compact with finer grain size was the highest in all day points of cell culture experiments. With an increase in grain size, osteoblast cell proliferation on HA compacts decreased.

4.0. Discussion

During synthesis of HA nanopowder using reverse micelle based template system, addition of NP12 surfactant forms small polar cores by organizing polar head groups away from non polar organic solvents. When aqueous solutions of $\text{Ca}(\text{NO}_3)_2$ and H_3PO_4 are mixed with the organic phase, water goes into the small polar cores forming microreactors. The ratio of Ca^{2+} and PO_4^{3-} ions in aqueous solution is maintained at 1.67:1 to synthesize stoichiometric HA. Addition of NH_4OH in the medium increased OH^- concentration, which helps in precipitation and formation of HA ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$). The overall reaction can be written as:

For processing highly dense HA compacts with ultrafine microstructure, it is important to prepare compacts with high green density. High surface area of synthesized HA nanopowders results in high frictional resistance during consolidation leading to poor green density. To increase green density we have added dispersant. The NH_4PMA is an anionic polyelectrolyte and can dissolve in aqueous solution, producing negatively charged carboxyl groups as shown in equation 1, which can be easily adsorbed on the positive entities (H^+ , Ca^{2+}) present at HA surface.

NH_4PMA effectively disperses HA nanopowders through an electrostatic mechanism and produced agglomerate free HA nanopowders with reduced frictional resistance during consolidation. Optimized amount of NH_4PMA is essential to maximize its dispersing action. Lower amount of NH_4PMA keeps HA nanopowders agglomerated and excess NH_4PMA leads to bridging flocculation. 6 wt% NH_4PMA is found to be the most effective in

improving green density of HA nanocompacts. Due to this increase in green density, more than 97% sintered density can be achieved in all samples. Our sintered density numbers are 7% higher than the other reported densities of sintered HA using microwave furnace [14] at 1000°C using similar conditions but without any dispersant.

Solid state sintering, is a diffusion-controlled process with different modes e.g., volume diffusion, grain boundary diffusion, and surface diffusion, and in ionic-covalent ceramics, the diffusing species are anions and cations. Microwaves, which are high frequency electromagnetic waves, are reported to interact with such ionic species and induce motion in them [32–33]. This induced motion tends to cause a departure from natural equilibrium of the system, and is resisted due to frictional, elastic and inertial forces. Owing to this resistance, the electric field associated with the microwave radiation is attenuated and caused volumetric heating of the material. Compared to conventional sintering, microwave sintering requires less time due to volumetric heating. This short heating time renders further grain growth unlike the conventional sintering. Another advantage of microwave sintering is the energy efficiency compared to conventional sintering. Calcium phosphate ceramics such as HA used in the present study, being ionic-covalent in nature, are also expected to be microwave-sensitive. Moreover, the presence of hydroxyl group in HA-structure has contributed further to microwave heating and densification.

In the present study, uniaxial pressing followed by CIP'ing ensures intimate particle to particle contact in green compacts. HA nanopowders are sintered to almost full density within 20 minutes at a minimum temperature of 1000 °C, maintaining a final average grain size of 168 nm in the sintered body. Grain size increases without decreasing densification, while the compacts are sintered at higher temperature, keeping the hold time constant at 20 min. HA samples sintered at 1150 °C for 20 min shows an average grain size of 1.16 micron, almost seven times greater than the samples sintered at 1000 °C for 20 min.

Fracture toughness of 1.9 ± 0.2 , 1.5 ± 0.3 , and 1.2 ± 0.2 MPam^{1/2} is obtained for 168 ± 86 nm, 520 ± 92 nm, and 1.16 ± 0.17 µm grain size, respectively. The fracture toughness values of conventionally sintered HA reported in literature is between ~0.6 to ~1 MPam^{1/2} [4,34–35], while a maximum value of 1.45 MPam^{1/2} was reported for microwave sintered HA [14]. The increase in fracture toughness due to microwave sintering is generally attributed to better sinterability and less grain growth than conventional sintering. Our maximum fracture toughness value of 1.9 ± 0.2 MPam^{1/2} is 31% greater than other reported number for HA under similar sintering condition [14] is due to an increased sintered density that we have achieved using dispersant. With a decrease in grain size of HA compacts, the relative volume of grain boundaries increase, which increases the resistance to indentation due to a large amount of stored energy. Similarly, the increase in indentation fracture toughness of HA compacts with a decrease in grain size can be attributed to increased resistance to crack propagation due to the presence of large number of grain boundaries in fine grained HA compared to coarse grain size HA compacts [4,10–11]. The reduced flaw size with a decrease in grain size is also responsible for an increase in the compressive strength. Figure 10 graphically shows the increase in compressive strength due to a decrease in average grain size. Using curve fitting, it is found that the compressive strength (σ) is inversely proportional to grain diameter (d) via the relationship –

$$\sigma = k/d^x \quad (3)$$

where k is the strengthening coefficient and both k and x are material specific. Our results do not show a classical Hall-Petch relationship as reported by others [14]. Clearly in HA, strengthening effect due to decreasing grain size is less prominent than what would be

predicted by the Hall-Petch equation. This behavior is presumably due to significantly higher interfacial areas as well as presence of more defects at the grain boundaries in HA compacts.

In vitro cell materials interactions aim at evaluating variations in early stages of bone cell attachment and proliferation due to grain size effects. Therefore, we have looked at vinculin protein expression for cell adhesion, and MTT assay for cell proliferation. Decreasing the grain size decreases the contact angle of both the water and cell media on HA compacts. Grain boundaries increase with the decrease in grain size. Lower contact angle is a result of higher surface energy due to more grain boundaries, which means better wettability, a key to cell-materials interactions [36–37]. Smaller grains show better cell-materials interactions due to favorable surface properties for bone cell attachment and growth. The formation of focal adhesion plaques is a prerequisite process for the development of signaling transduction in cell attachment, and is one of the important indicators for cell activity on the substrates. Vinculin aids in the assemblage of focal contacts by cross-linking and recruiting other proteins to form adhesive plaques. Vinculin also acts as an adhesion molecule between the cells and the substratum. It is mostly located at points of focal adhesion plaque, therefore the presence of vinculin represents formation of focal adhesion plaque. As cells attach to one another and to the substratum, adhesive proteins interact with and form bonds to adhesion receptors within the cellular membrane. Antibody bound to vinculin expressed green fluorescence and nuclei stained with propidium iodide (PI) in the mounting medium expressed red fluorescence. Clearly smaller grain size samples show better bone cell proliferation as well. From vinculin expression and cell proliferation data, it is clear that smaller grain size influences surface properties in which surface energy increases with decreasing grain size. Higher surface energy positively influence bone cell attachment and growth significantly even if there is no difference in composition.

Before closing, we would like to mention that we have shown simultaneous improvements in mechanical and *in vitro* biological properties of sintered HA compacts with grain size ranging from nano-meters to sub-micrometers to micrometers using microwave sintering. This conclusion is supported by phase analysis, compressive strength, microhardness, indentation fracture toughness and human osteoblast cell - material interaction studies.

5.0 Conclusions

Dense nanostructured HA compacts with average grain size between 168 ± 86 nm and 1.16 ± 17 μ m were processed using microwave sintering for 20 min at temperatures between 1000 and 1150 °C. Nanostructured HA with 168 ± 86 nm grain size showed highest compressive strength of 395 ± 42 MPa and indentation fracture toughness of 1.9 ± 0.2 MPa m^{1/2}. The same powder was used to process HA compacts with grain size 520 ± 92 nm and 1160 ± 170 nm. The decrease in strength due to increase in grain size was less prominent in HA than what would be predicted by the classical Hall-Petch equation. Microwave sintered HA compacts were also assessed for *in vitro* bone cell materials interaction using vinculin expression studies for adhesion and MTT assay for proliferation with human osteoblast cell line. An increase in bone cell adhesion and proliferation with decreasing grain size can be seen from the results, which is a direct consequence of better wettability in finer grained samples due to the presence of higher number of grain boundaries.

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Figure 1. X-ray diffraction of HA compacts, processed with 6 wt% dispersant, and then sintered at different temperature in microwave furnace for 20 minutes. Peaks were identified using JCPDS 09-0432.

Figure 2.
FTIR spectra of synthesized HA nanopowder.

Figure 3. Particle size distribution and transmission electron microscopy (TEM) image of synthesized HA nanopowder.

Figure 4.
Variation in green density of HA compacts as a function of change in weight per cent of dispersant.

Figure 5. Microstructure of HA compacts sintered for 20 minutes in microwave furnace at (a) 1000 °C, (b) 1100 °C and (c) 1150 °C.

Figure 6. Variation in contact angle of water and cell media on the surfaces of different grain size (a) $0.168 \pm 0.086 \mu\text{m}$, (b) $0.52 \pm 0.074 \mu\text{m}$ (c) $1.1 \pm 0.128 \mu\text{m}$ of HA compacts. * means = $P < 0.05$ based on statistical analysis using student's t-test.

Figure 7.
Confocal micrographs of vinculin expression in human osteoblast cells cultured on HA compacts with different grain size (a) $0.168 \pm 0.086 \mu\text{m}$, (b) $0.52 \pm 0.074 \mu\text{m}$ (c) $1.1 \pm 0.128 \mu\text{m}$ after day 1.

Figure 8. Confocal micrographs of vinculin expression in human osteoblast cells cultured on HA compacts with different grain size (a) $0.168 \pm 0.086 \mu\text{m}$, (b) $0.52 \pm 0.074 \mu\text{m}$ (c) $1.1 \pm 0.128 \mu\text{m}$ after day 5.

Figure 9.

MTT assay of human osteoblast cells cultured on HA compacts with different grain size after 1, 5 and 11 days.

Figure 10.

Variation in uniaxial compression strength as a function of grain size. The curve fitting shows a relationship as $\sigma = k / d^{0.18}$ with $k = 1002.2$ and $R^2 = 0.99$.

Table 1

Mechanical properties of HA compacts with variation in grain size

Sintering Cycle (° C/ minute)	Grain Size (μm)	Compressive strength (MPa)	Microhardness (GPa)	Indentation Fracture toughness (MPa $\text{m}^{1/2}$)
1000/20	0.168 $\pm$ 0.086	395 $\pm$ 42	8.4 $\pm$ 0.4	1.9 $\pm$ 0.2
1100/20	0.52 $\pm$ 0.092	328 $\pm$ 58	7.3 $\pm$ 0.3	1.5 $\pm$ 0.3
1150/20	1.16 $\pm$ 0.17	278 $\pm$ 35	6.3 $\pm$ 0.5	1.2 $\pm$ 0.2