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INVESTIGATION ON BIOLOGICAL PROPERTIES OF DENTAL IMPLANT BY Ce-TZP/Al_2O_3/HA BIO-NANO-COMPOSITES

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Early years, bio-nano-composites materials are interesting in advanced engineering applications fields. In order to, biological properties of the 10Ce-TZP/Al_2O_3 nano-composite was evaluated by the addition of HA nanopowder for medical applications. In this research, a bio-nano-composite consisting of CeO2- stabilized zirconia (Ce-TZP), Al_2O_3 and Hydroxyapatite (HA) powders proposed to use for medical application .The first step, nano-powders of Ce-TZP and Al_2O_3, were blended by fast milling machine. Then, nano-powder of HA was added to the alumina - zirconia (ZA) mixture from 10 to 40 vol%, and homogenized again. In final step, mixed powder was cold pressed and sintered at1600 °C for 120 min. Crystal phase of the sintered samples were characterized by X-ray diffraction(XRD),the density and bending strength were measured and cellular response test was performed with osteoblastic cell-lines by MG63 cell-lines and the morphology of the proliferated cells was observed with FESEM too.
From the strength and cellular response evaluations of samples ,specimens with 30 vol% HA showed the best result, also XRD patterns confirmed this results .

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Keywords: Biocompatibility, Nanocomposite, cellular response, Osteoblast

1. Introduction

Hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$), Zirconia (ZrO_2) and Alumina (Al_2O_3) are known as important implant materials, [1-3]. HA has often been considered as a candidate for use in load –bearing applications due to bonds and promotes the new bone formation necessary for implant osseointegration to bone directly. However, its poor mechanical properties limit its application to load-bearing parts, [4-11].

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Alumina and zirconia have excellent biocompatibility. The main advantages of Al_2O_3 is its high hardness and wear resistance, while ZrO_2 exhibits higher strength and fracture toughness and the composite consisting of the two has higher ductility and fracture toughness than each constituent, [12]. Some interest has been paid to composites comprising of hydroxyapatite and another phase of high mechanical properties and bio-inertness, such as alumina and zirconia, [13]. Cerium oxide or Ceria is used to stabiles the tetragonal polycrystalline structure of zirconia at room temperature, the product called Ceria-partially stabilized zirconia (Ce-TZP) [15]. Ce-TZP/Al_2O_3 is a composite in which Ce-TZP particles are added to Al_2O_3 matrix as the second phase to modify the fracture behavior of composite, Because, it has high fracture toughness [14]. In additional, Ce-TZP/Al_2O_3 nano-composites could be superior to Y-TZP in clinical use [16,17]. The biocompatibility of zirconia-alumina (ZA) nano-composites in load-bearing applications such as dental/orthopedic implants was significantly enhanced by the addition of bioactive HA [4].

In this work, the effect of HA is studied on biological properties of Ce-TZP/Al_2O_3. In order to, bio-nano-composites were fabricated by mechanical milling of pre-synthesized nano-scaled constituents, and cold pressing and sintering Phase analysis; strength and biological properties of produced composites were evaluated by different characterization methods.

2. Experimental

For this study, we used of Al_2O_3, HA and ZrO_2 nanopowders synthesized by sol-gel, chemical precipitation and hydrothermal methods (with particle sizes 112,9,12 nm respectively). The process Schematic is given in figure 1.

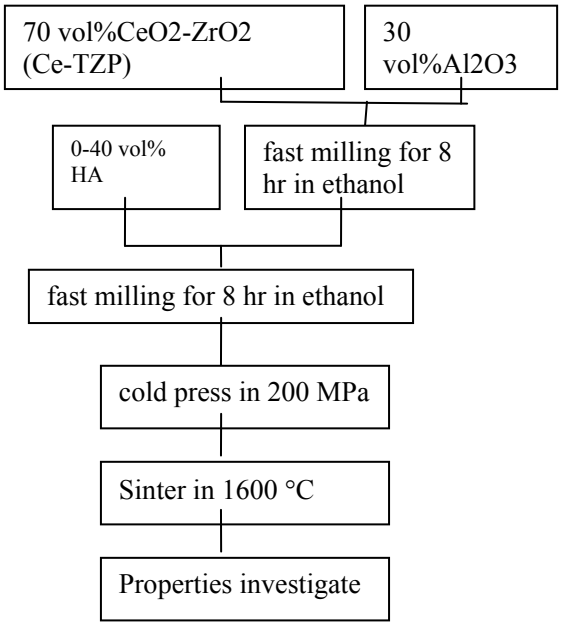

Fig.1. The schemac of the process

As shown ZA nano -composite powders were prepared by fast milling machine (sharifi co. iran) of 70 vol% 10Ce-TZP and 30 vol% Al_2O_3 powder in ethanol for 8 hr. Obtained powder was mixed with HA (up to 40vol%) in ethanol by fast milling machine again for 8 hr. Mixed slurry was dehydrated, crushed and sieved, and uniaxial cold pressed at a pressure of 200 MPa by universal test machine (Zwick roll-Z100). Cold pressed samples were sintered at 1600 °C for 2 hr by electrical furnace (Vecstar). The density of the specimens was measured by the Archimedes method [ASTM B311-08]. Crystalline phase analysis was performed by XRD (Bruker model D8). The bending strength was measured by means of three- point bend test with universal test machine (Zwick roll-Z100) [ASTM C1674-08].

In order to investigate the proliferation behavior on the ZA–HA composites, the samples used for cell tests were prepared from disc specimens with 10mm diameter that after polishing both sides with diamond slurries down to 1 mm, the specimens were washed and sterilized at 121°C for 20 minutes.

The samples were placed in a 24 well tissue culture plate and seeded with 25 µl of the $3x10^4$ cells/cm^2 MG63 (National Cell Bank of Iran-no NCBI C…) cell density suspension. Cells were allowed to adhere for 2 hours then flooded with 1 ml of Dulbecco's modified Eagle's medium (DMEM, Gibco-USA), 2mM L-glutamine (Gibco), 100 IU/ml of penicillin and 100 µg/ml of streptomycin (Gibco) supplemented with 10% fetal bovine serum (FBS, Gibco-USA) and placed back into the incubator. The fabricated specimens, including pure HA as a positive control, were placed into a 24-well plate, which was followed by plating on all the discs.

The cells were cultured for 7 days in a humidified incubator under an atmosphere of 5% CO2 at 37°C. After detaching the cells from the specimens with a trypsin-EDTA solution (0.25% trypsin, 0.05% EDTA-Gibco) and staining with 0.4% trypan blue, the living cells were counted using a hemocytometer (Paul Marienfeld GmbH & Co-Germany). Each set of tests was performed in triplicate.

For observation the morphology of the proliferated cells with FESEM (Hitachi s-4160) cells were cultured for 5 days as above then fixation with glutaraldehyde(2.5%),dehydration with graded ethanol (50%,60%,70%,80%,90%and 100%).

The results were expressed as means with standard errors. The data were then analyzed by one-way ANOVA, followed by the post hoc paired Student's two-tailed t-test. The significance levels were notified significant (*, if $p< 0.05$) and highly significant (**, if $p<0.01$) in the tables.

3. Results

Fig. 2 shows the densities of the ZA nano-composites containing various amounts of HA. Compared to the theoretical density, which was calculated by the rule of mixture, the density of the specimens with a low HA content almost matched with the theoretical value. However, as the amounts of HA were increased, the discrepancy between the measured and theoretical densities increased.

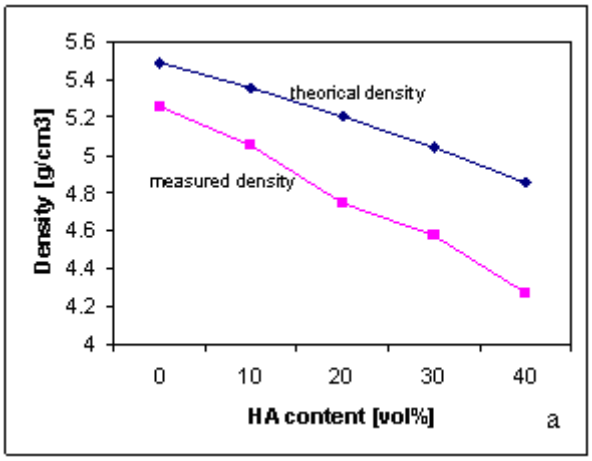

Fig. 2. Schematic of density: ZA-HA nano-composites as a function of the HA content, bulk density g/cm3.

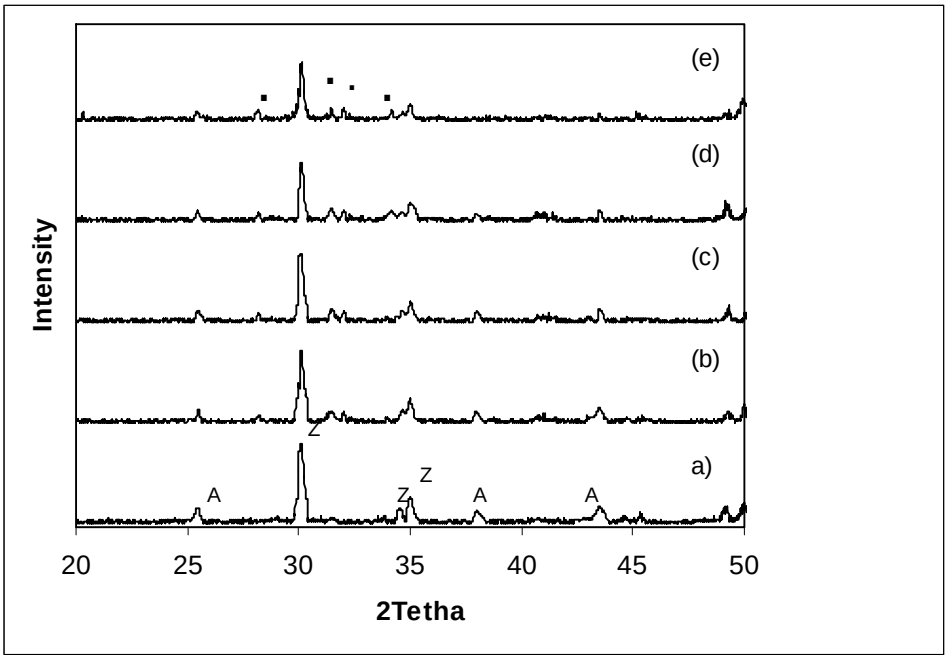

Fig.3. XRD patterns of nano-composites, a: without HA and with b: 10, c: 20, d: 30 and e: 40 vol% HA. .: TCP, .: HA, A: Alumina, and Z: Zirconia.

Fig 3, illustrates the XRD patterns of different composite materials. When no HA was added, only peaks corresponding to ZrO_2 and Al_2O_3 are present, as shown in fig. (3-a). With addition of 10, 20, 30 and 40 vol% HA (fig. (3-b-c-d-e), respectively), as well as the peaks corresponding to HA, TCP peaks, were also detected, that TCP component was originated from the reaction between HA and ZrO_2. The bending strengths of the nanocomposites are shown in fig 4, as the HA content was increased up to 40vol% the strength decreased of 850 to 137 MPa.

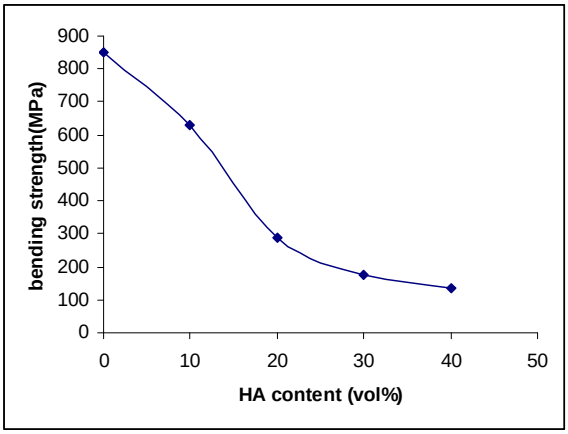

Fig .4. Schematic of bending strength: ZA-HA nano-composites as a function of the HA content.

Biocompatibility tests are in progress. Fig5 illustrates Cell proliferation results on the specimens after 7 days culture. The number of cells was significantly increased by the addition of 10% HA to the ZA (ANOVA, $p<0.05$). The number of cells increased steadily with further addition of HA (ANOVA, $p<0.001$). When 40% HA was added to the ZA, the proliferation rate on that specimen became comparable to that on the pure HA means of wells±S.D.

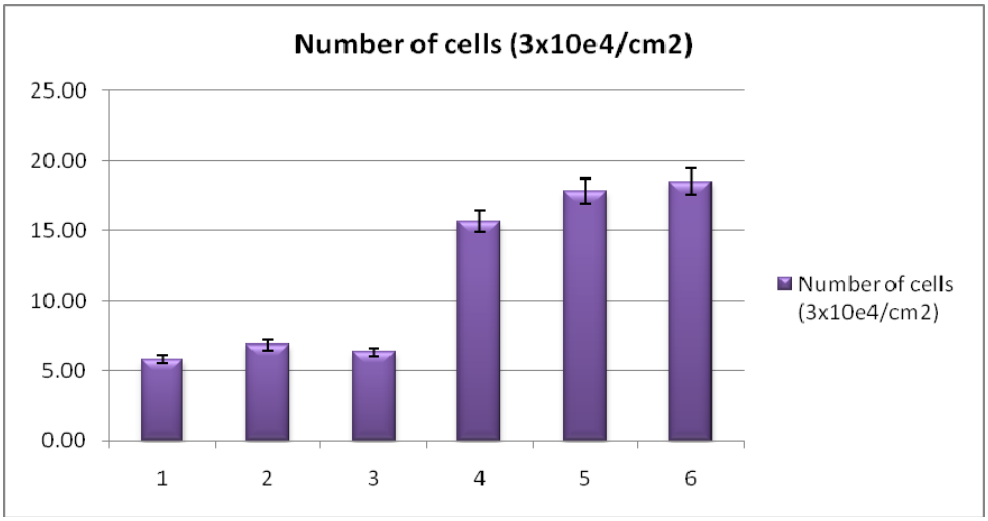

Fig.5. Cell proliferation results on the specimens after 7 days culture. 1:ZA,2:10HA,3:20HA,4:30HA,5:40HA,6: HA

Comparison	Mean Difference	q	P value
Column A vs Column F	-12.667	20.121 ***	P<0.001
Column A vs Column E	-12.000	19.062 ***	P<0.001
Column A vs Column D	-9.833	15.620 ***	P<0.001
Column A vs Column C	-1.167	1.853 ns	P>0.05
Column A vs Column B	-1.000	--- ns	P>0.05
Column B vs Column F	-11.667	18.533 ***	P<0.001
Column B vs Column E	-11.000	17.474 ***	P<0.001
Column B vs Column D	-8.833	14.032 ***	P<0.001
Column B vs Column C	-0.1667	--- ns	P>0.05
Column C vs Column F	-11.500	18.268 ***	P<0.001
Column C vs Column E	-10.833	17.209 ***	P<0.001
Column C vs Column D	-8.667	13.767 ***	P<0.001
Column D vs Column F	-2.833	4.501 **	P<0.01
Column D vs Column E	-2.167	3.442 *	P<0.05
Column E vs Column F	-0.6667	1.059 ns	P>0.05

Fig. 6 shows morphology of the proliferated cells on the specimens. As was observed the specimens with30vol%HA show most of the cell proliferation.

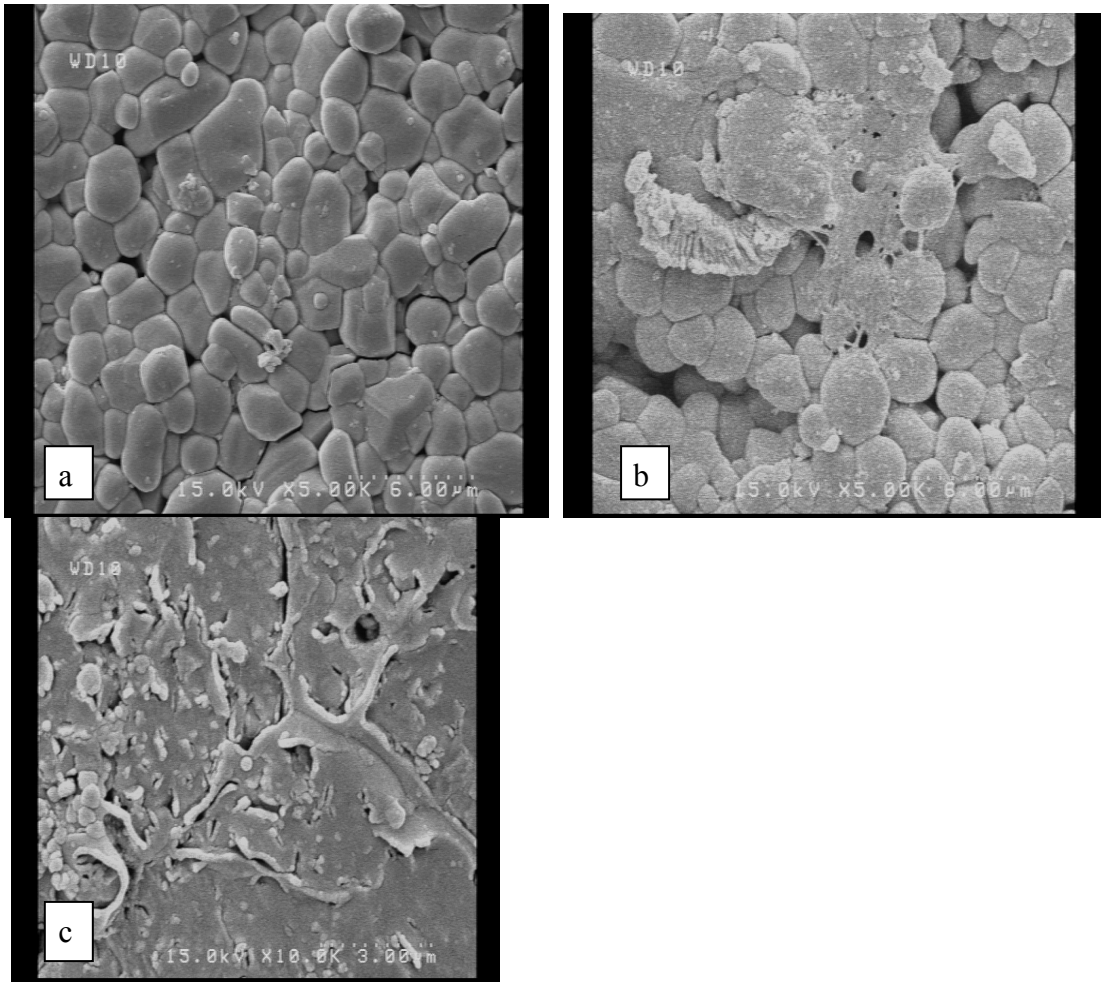

Fig. 6. Morphology of the proliferated cells on (a) pure ZA, (b) 30 vol%HA/ZA, (d) pure HA

4. Discussion

As a bio-inert implant material, ZA nano-composite is considered to have sufficient strength. However, its biocompatibility such as satisfactory osseointegration and faster bone regeneration has to be improved by the addition of calcium phosphates such as HA, and TCP, [1,4]. TCP component was originated from the reaction between HA and ZrO_2. Both HA and TCP are formed of calcium and phosphate component, which are essential to satisfactory osseointegration and faster bone regeneration to be achieved, [4,7].Just as shown in the XRD patterns, intensities of the HA and TCP peaks increased steadily with increasing HA content.

5. Conclusions

Hydroxyapatite (HA)-zirconia-alumina (ZA) bio-nano-composites were produced by mechanical blending of separately synthesized nano-scaled powder. Composite materials were characterized by phase analysis and cellular response test. From mechanical and biological properties evaluation of the 10Ce-TZP/Al2O3/HA bio nano composites, specimens with 30 vol% of HA, was found to be the optimal composition for biological applications.

Acknowledgments

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