Biocompatibility of Hydroxyapatite-Alumina and Hydroxyapatite-Zirconia Composites including Commercial Inert Glass (CIG) as a Ternary Component

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Abstract

Hydroxyapatite (HA), chemical formula Ca10(PO4)6(OH)2, is a very popular bioceramic for orthopedic and dental applications. Although HA has excellent biocompatibility, its inferior mechanical properties make it unsuitable for load-bearing implant applications. Therefore, HA should be strengthened by a secondary phase to produce a composite that possesses robust mechanical properties. The aim of this study was to compare the microstructural and mechanical properties and biocompatibility of HA-Al2O3 and HA-ZrO2 composites with the addition of 5 and 10 wt% commercial inert glass (CIG) independently and to determine from the studied composites the one with the most suitable composition for biomedical applications. The powders were pressed and then, the pellets were sintered between 1000 – 1300 °C for four hours. The thermodynamic analyses of the samples were performed by means of DTA followed by the thermodynamic analysis program FactSage. Microstructural characterizations were carried out using SEM + EDS and XRD, while hardness and compression tests were performed to measure the mechanical properties. The results showed that the compressive strength and the microhardness of HA-Al2O3 composites increased with rising CIG content and increasing sintering temperature. On the other hand, for HA-ZrO2 composites, increasing CIG content caused an elevation in hardness and a decrease in compressive strength values at 1300 °C. The biocompatibility tests (in vitro and in vivo) were performed on those composites that possessed the highest physical and mechanical properties. In conclusion, the optimum CIG content was determined to improve the mechanical properties and biocompatibility of the composites. The mechanical properties and biocompatibility of HA-Al2O3 composites have been found to be lower than those of HA-ZrO2 composites. In this study, the ideal composite was selected as HA-ZrO2-5 wt% (HZC5) sintered at 1200 °C.

Keywords: Hydroxyapatite, ceramics, composites, microstructure, mechanical properties, biocompatibility

I. Introduction

Calcium phosphate (CaP) ceramics are widely used to repair and reconstruct damaged parts of the human skeleton. Different types of CaP, such as hydroxyapatite (HA), tetra calcium phosphate (TTCP), tricalcium phosphate (TCP), dicalcium phosphate anhydrous (DCP), amorphous calcium phosphate (ACP) and biphasic calcium phosphate (BCP), are available for use as hard tissue substitutes. Hydroxyapatite (HA) (Ca10(PO4)6(OH)2) is the most promising material for bone replacement based on its chemical composition. It is similar to the mineral parts of bone and tooth and confers excellent biocompatibility and osteoconductivity properties. Additionally, HA materials do not exhibit any cytotoxic effects. However, the application of HA is limited for use as implant material because of its inferior mechanical properties. There have been many investigations aimed at improving the mechanical properties of HA, like preparing them as composite materials. Addition of second-phase ceramic materials (e.g. zirconia, titania or alumina) into the HA matrix for enhancing strength and toughness has been an interesting subject in recent years. Among them, alumina (Al2O3) has been widely recognized as a reinforcing agent based on its excellent wear resistance, high hardness and high abrasion resistance. The Al2O3 ceramic used in biomedical applications is α-Al2O3, known as corundum.

Zirconia (ZrO2) is also a well-known reinforcing agent as a result of its relatively high mechanical strength, toughness, wear resistance, corrosion resistance and biocompatibility. At ambient pressure, ZrO2 polymorphs occur in three forms; monoclinic (M), tetragonal (T), and cubic (C) depending on the temperature. As a result of phase transformation, pure and single-phase ZrO2 tends to fracture even at room temperature, leading to deterioration in mechanical properties and consequently making
it unsuitable for clinical applications. Therefore, ZrO2-based composites possess enhanced toughness following controlled phase transformation of stabilized ZrO2 doped with yttria (Y2O3) (Y-TZP) 16. Bioactive glasses and glass ceramics are biomaterials with osteoconductive and osteoinductive properties as well as being able to repair and replace diseased or damaged bone 8,17. The glass ceramics after sintering have superior mechanical properties, enhanced biocompatibility, bioactivity, and no toxicity, making them useful as a biomaterial in artificial bone and dental implants 18. These properties result from their progressive dissolution in physiological medium, where the release of calcium, phosphate, and sodium ions will undergo formation of an apatite layer that in turn creates a strong bond with the surrounding bone tissues 17,19.

There are a number of studies that have investigated HA-Al2O3, HA-ZrO2 and HA-CIG composites. Yelen et al. 20 looked at HA-Al2O3 biocomposite powders. In this study, Al2O3 was derived with the sol-gel method, then, mixed with HA powders. The gel mixture was heat-treated at 1300 °C for two hours. XRD results showed that the powders were composed of α-alumina and apatite-based phases, such as tricalcium phosphate and HA, forming a highly porous structure observed by SEM. Pujiyanto et al. 21 studied a porous HA-ZrO2 composite in which HA was synthesized from local gypsum by means of the microwave hydrothermal method. Different amounts of ZrO2 (0, 20, 30 and 40 wt%) were mixed with HA for six hours and green pellets obtained after pressing were then sintered at 1450 °C for two hours. It was then determined that the compressive strength of the porous HA-ZrO2 composite decreased when the ZrO2 percentage increased up to 40 wt%. This might be a consequence of enhanced porosity, cracked ZrO2 grain, reaction of ZrO2 with HA producing CaZrO3, β-TCP and α-TCP and cracking of the HA matrix because of the phase change of tetragonal ZrO2 into monoclinic ZrO2. Salman et al. 22 investigated the sintering effect on the mechanical properties of composites made of bovine hydroxyapatite (BHA) and commercial inert glass (CIG). In this study, HA was derived from calcined bovine and was doped with 5 and 10 wt% CIG. Composite materials were then obtained by sintering. Addition of glass components into the HA structure in small quantities is very popular for improving sinterability and thus improving the mechanical performance of HA biomaterials. The experimental results showed that an optimum amount of glass was sufficient for obtaining promising compression strength and microhardness values, indicating suitability for load-bearing biomedical applications.

The objective of this study was to compare the microstructural and mechanical properties and biocompatibility of HA-Al2O3 and HA-ZrO2 composites with the addition of 5 and 10 wt% commercial inert glass (CIG), separately.

II. Materials and Methods
(1) Materials
Commercial synthetic hydroxyapatite (Acros Org., BE) was used as the major component. Alumina (α-corundum) (Seranit Org, TR), zirconia (doped with 10 – 15 % Y2O3) (Alfa Aesar, DE) and commercial inert glass (CIG) were selected as additives.

Firstly, the 90 wt% HA-10 wt% Al2O3 and the 90 wt% HA-10 wt% ZrO2 powders were mixed separately for 24 h in a ball mill, after which the powders were remixed and ball-milled with 5 and 10 wt% CIG for 4 h. In this way, HA-Al2O3-5 wt% CIG, HA-Al2O3-10 wt% CIG (HAC5), HA-ZrO2-5 wt% CIG (HZC5), HA-ZrO2-10 wt% CIG (HZC10) composites were produced. The powders were pressed at 350 MPa to form cylindrical pellets (height: 11 mm; diameter: 11 mm) and then they were sintered at 1000, 1100, 1200, and 1300 °C for 4 h (+5 °C min⁻¹).

Table 1: Chemical analysis of the CIG.

<table>
<thead>
<tr>
<th>Oxide</th>
<th>wt%</th>
</tr>
</thead>
<tbody>
<tr>
<td>SiO2</td>
<td>68.80</td>
</tr>
<tr>
<td>Na2O</td>
<td>17.02</td>
</tr>
<tr>
<td>CaO</td>
<td>9.25</td>
</tr>
<tr>
<td>MgO</td>
<td>1.77</td>
</tr>
<tr>
<td>Fe2O3</td>
<td>0.084</td>
</tr>
<tr>
<td>TiO2</td>
<td>0.017</td>
</tr>
<tr>
<td>Al2O3</td>
<td>2.15</td>
</tr>
<tr>
<td>Cr2O3</td>
<td>0.012</td>
</tr>
<tr>
<td>CuO</td>
<td>0.0036</td>
</tr>
<tr>
<td>Other</td>
<td>Trace Amount</td>
</tr>
</tbody>
</table>

(2) Characterization techniques and mechanical tests
Density, Vickers microhardness, and compression strength tests were performed to determine the mechanical properties of the composites. The density of the samples was gauged with the Archimedes method. The hardness of the samples was measured with a Vickers microhardness testing system (HMV Shimadzu, JP), using 200 g load for 15 s and the compression tests were carried out using a Universal Testing machine (Shimadzu, JP) at a crosshead speed of 3 mm/min (sample diameter: 11 mm; sample height 11 mm). The powders were analyzed with Differential Thermal Analysis (DTA) (TA Instruments Q600, US) up to 1300 °C using the 10 °C/min heating rate in an air atmosphere. The thermodynamic analysis software FactSage 24 was used to determine stable phases and their physical state between transformation temperatures. X-ray diffraction (XRD) analysis was carried out using a Brucker D8-Advanced X-ray diffractometer (DE) with Cu Kα radiation. Scanning Electron Microscopy (SEM) (Jeol JSM-5910 LV – Low Vacuum Scanning, US) and Energy Dispersive Spectroscopy (EDS) (Oxford Inca Energy 200, UK) were used for microstructural characterization of the composites.
(3) Biocompatibility Tests

In order to investigate the biocompatibility behavior of the samples and whether they had robust mechanical properties, in vitro and in vivo tests were performed. In vitro tests were carried out in simulated body fluid (SBF), prepared according to the Kokubo prescription under controlled temperature (36.5 °C) and pH (7.4). Samples were removed from the solution after 1, 2, 3, and 4 weeks. The morphology of the intensive apatite layer on the surface and microstructure of the samples were examined with SEM. After in vitro and in vivo tests had been performed, the HZC5 composite sintered at 1200 °C showed the best performance.

For the case of in vivo tests, the cells were prepared according to the method of Maniatopoulos et al. Rat bone narrow stem cells were isolated from the femora of 5-week-old male Wistar rats and cultured in 75-cm² flasks in α-MEM (Sigma Aldrich, Tokyo, Japan) with 10 % fetal bovine serum (Invitrogen, Tokyo, Japan) at 37 °C in a humidified atmosphere consisting of 95 % air and 5 % CO₂. After 24 hours, fresh medium was replaced and the cells were allowed to grow until 80 % confluency. The media were replaced every two days. Cells were expanded in vitro until passage three and once confluent, the culture medium was aspirated and the flask was washed with sterile phosphate buffer saline (PBS), the cells being detached using 10 % Trypsin-EDTA (Invitrogen, Tokyo, Japan). The cells were centrifuged at 2000 rpm for 5 minutes and 1x10⁴ cells were seeded into each well in a 24-well plate in α-MEM (Sigma Aldrich, Tokyo, Japan) in accordance with the manufacturer's protocol. After 7, 10, and 14 days in incubation, 100 μL of AlamarBlue™ stain (Invitrogen, Tokyo, Japan) for 15 min at room temperature. The cells were then stained with Alizarin Red S solution (Sigma Aldrich, Tokyo, Japan) and incubated for 15 min at room temperature with gentle shaking. Following this, the Alizarin solution was aspirated and the cells were washed again thrice before observation under a light microscope. Calcium deposit nodules were observed based on their distinct red color. The quantification of the Alizarin Red S was based on destaining using 10 % cetylpyridinium chloride (CPC) in 10 mM sodium phosphate (Sigma Aldrich, Tokyo, Japan) for 15 min at room temperature. Each extract was diluted 10-fold in 10 % CPC solution; and the Alizarin Red S concentration was determined by means of absorbance measurement at 562 nm on a multi-plate reader as done previously.

Statistical analysis: All data were examined based on five different measurement values and repeated in triplicate. The results are presented as standard deviation. Statistical analysis was based on one-way analysis of variance (ANOVA) with Turkey’s post hoc test used for multiple comparisons (SPSS, IBM, USA) where a p-value < 0.05 was considered significant.

III. Results and Discussion

(1) Density results

Fig. 1 depicts the density data and Table 2 summarizes the porosity values of composites sintered at different temperatures.

As seen in Fig. 1, with increasing sintering temperature, the density of the composites increased while their porosity decreased. HAC10 composite has the lowest porosity and the highest density of the HAC composites. HZC5 composite has the lower porosity and the highest density of the HZC composites. When we compare both composites the highest density was obtained in the HZC5 composite sintered at 1300 °C and the lowest porosity was obtained in the HAC10 composite sintered at 1300 °C.
Table 2: The porosity values of composites sintered at different temperature.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>HAC 5 wt% CIG</th>
<th>HAC 10 wt% CIG</th>
<th>HZC 5 wt% CIG</th>
<th>HZC 10 wt% CIG</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>40.89 ± 1.51</td>
<td>37.96 ± 0.85</td>
<td>35.86 ± 0.66</td>
<td>42.48 ± 0.10</td>
</tr>
<tr>
<td>1100</td>
<td>40.03 ± 0.24</td>
<td>37.49 ± 0.83</td>
<td>31.67 ± 1.61</td>
<td>31.81 ± 0.08</td>
</tr>
<tr>
<td>1200</td>
<td>35.86 ± 0.66</td>
<td>31.58 ± 1.24</td>
<td>18.29 ± 3.56</td>
<td>22.71 ± 1.67</td>
</tr>
<tr>
<td>1300</td>
<td>19.10 ± 2.85</td>
<td>05.49 ± 0.94</td>
<td>08.44 ± 0.42</td>
<td>19.84 ± 0.86</td>
</tr>
</tbody>
</table>

Fig. 1: The density values of composites sintered at different temperatures.

(2) Thermodynamic analysis

Fig. 2 shows the DTA analysis of the HAC5 composites while Fig. 3 illustrates the corresponding Fact Sage thermodynamic equilibrium results in which the transformation of hydroxyapatite is intentionally separated to elucidate it. Similarly, Fig. 4 describes the DTA analysis whereas Fig. 5 portrays the corresponding equilibrium results of the HAC10 composites. It was clearly seen that the thermodynamic computation indicated that while the amount of hydroxyapatite (HA) \( \text{Ca}_{10}(\text{OH})_2(\text{PO}_4)_6 \) phase was decreasing, the whitlockite (W) \( \text{Ca}_3(\text{PO}_4)_2 \) phase started to form at 1100 °C thermodynamically for HA-Al\(_2\)O\(_3\) composites.

With rising temperature, almost all HA present in the structure was transformed to the W. Gehlenite phase \( (\text{CaAl}_2\text{SiO}_7) \) and liquid slag formed above 1200 °C. The endothermic peak temperatures were observed at 1100 – 1150 °C, 1150 – 1200 °C and 1200 – 1250 °C for HAC5 corresponding to W and Gehlenite phase formations (Fig. 2). Similar behavior occurred for HAC10 at 1100 – 1200 °C in Fig. 4, confirming with thermodynamic predictions that given in Fig. 3 and Fig. 5. HA transformation to W started nearly at 1125 °C and ended at 1225 °C observed in Fig. 3. The 5 wt.% weight change seen in Fig. 2 was the result of water evaporation. Equation 1 represents the transformation of HA.

\[
\text{Ca}_{10}(\text{OH})_2(\text{PO}_4)_6 \rightarrow 3\text{Ca}_3(\text{PO}_4)_2 + \text{CaO} + \text{H}_2\text{O} \quad (1)
\]
Fig. 4: The DTA analysis result of the HAC10 composite.

Fig. 5: The FactSage equilibrium diagram of the HAC10 composite.

Fig. 6: The DTA analysis result of the HZC5 composite.

Fig. 7: The FactSage equilibrium diagram of the HZC5 composite.

Fig. 8, Fig. 9 illustrate the equilibrium results and the DTA analysis of the HZC5 and HZC10 composites, respectively. The HA-W transformation of the HA-ZrO₂ composites look the same as the HA-Al₂O₃ composites. The transformation temperature is 1100 °C (Figs. 6 – 7) and HA was transformed to W completely as previous. At 1207 °C, ZrO₂ (Fig. 6) changed from a monoclinic to tetragonal structure. At the same temperature, calcium ions coming from HA diffused into the zirconia phase and formed the calcium zirconate phase (CZ) (CaZrO₃). In addition, slag formation occurred between 1000 – 1300 °C and the addition of CIG is the key to slag formation (Fig. 6). DTA analysis indicated that the endotherm was observed between 1150 – 1250 °C as a consequence of the crystal formation of CaZrO₃ and phase transformation of ZrO₂ according to Eq. 2 – 3 as predicted thermodynamically (Fig. 7).

\[
\text{ZrO}_2 \text{ (Monoclinic)} \rightarrow \text{ZrO}_2 \text{ (Tetragonal)} \quad (2)
\]
\[
\text{ZrO}_2 + \text{CaO} \rightarrow \text{CaZrO}_3 \quad (3)
\]

The results of HZC10 are slightly different. There was no depletion of ZrO₂ and no formation of CaZrO₃. The slag was the dominant phase based on the higher amount of CIG addition, which possibly lowered the melting tem-
perature by forming eutectics. The endothermic phase transformation of ZrO$_2$ occurred nearly at 1170 °C, lower than 5 wt% CIG added composites most probably due to missing CaZrO$_3$ phase formation (Fig. 9). The decomposition of HA also occurred faster than HZC5 possibly because of the higher amount of slag present in the system.

(3) XRD Analysis

Fig. 10 and Fig. 11 depict XRD patterns and corresponding phases obtained for HA-Al$_2$O$_3$ and HA-ZrO$_2$ composites sintered at 1000 °C and 1300 °C. The product phases of HA-Al$_2$O$_3$-CIG composites after sintering at 1000 °C are HA, Al$_2$O$_3$ and W whereas composites sintered at 1300 °C include HA, Al$_2$O$_3$, W and Ca$_2$Al$_2$SiO$_7$ and sodium calcium silicate (SCS) for 10 wt% CIG in addition. Conversely, for composites HA-ZrO$_2$ with 5 and 10 wt% CIG addition after sintering at 1000 °C, phases formed are HA, ZrO$_2$, W, calcium zirconate (CZ) whereas HZC5 composite sintered at 1300 °C included HA, ZrO$_2$, W, SCS. Also, HZC10 composite sintered at 1300 °C contained calcium silicate (CS) phase in addition to these phases.

(4) SEM-EDS analysis

Figs. 12a – 12f present the microstructure and corresponding EDS results of HAC5 and HAC10 sintered at 1000 °C and 1300 °C. When Fig. 12a is compared with Fig. 12c, grain growth is evident, and this occurred because of increasing sintering temperature. When Fig. 12a is compared with Fig. 12b, the porosity decreased and a more compact microstructure was obtained with increasing CIG content.

Fig. 8: The DTA analysis result of the HZC10 composite.

Fig. 9: The FactSage equilibrium diagram of the HZC10 composite.

Fig. 10: XRD diagrams of HAC5 and HAC10 (a) sintered at 1000 °C (b) sintered at 1300 °C.
Fig. 11: XRD diagrams of HZC5 and HZC10 (a) sintered at 1000 °C (b) sintered at 1300 °C.

Fig. 12: Microstructures and EDS analysis of HA-Al$_2$O$_3$ composites (a) HAC5 at 1000 °C, (b) HAC10 at 1000 °C, (c) HAC5 at 1300 °C, (d) HAC10 at 1300 °C, (e) EDS analysis results of hydroxyapatite phase, (f) EDS analysis results of gehlenite phase.
Fig. 13: Microstructures and EDS analysis of HA-ZrO$_2$ composites (a) HZC5 at 1000 °C, (b) HZC10 at 1000 °C, (c) HZC5 at 1300 °C, (d) HZC10 at 1300 °C, (e) EDS analysis of hydroxyapatite phase, (f) EDS analysis of zirconia phase (g) EDS analysis of glassy phase.

Fig. 14: The crack formation of HZC10 sintered at 1300 °C.
(5) Mechanical test results

Fig. 15 summarizes the compression strength and Vickers microhardness results of samples sintered at different temperatures. Mechanical properties of HAC composites increased with increasing CIG content. In the HAC10 composite, glassy phase (SCS) formed above 1200 °C, which caused an increase in hardness and strength values (129.36 HV, 53.82 MPa). For the HZC composites, the highest Vickers microhardness was obtained in HZC5 sintered at 1300 °C. The highest compression strength was measured again in HZC5 sintered at 1200 °C. For HZC composites, when sintering temperature increased, calcium ions diffusing from HA into the zirconia phase formed a calcium zirconate phase. Addition of excess CIG (glass powders) enhanced the HA decomposition to TCP (tricalcium phosphate), possibly the result of liquid slag formation as noticed in Fig. 6. Additionally, there has been a phase change from monoclinic to tetragonal zirconia at 1300 °C bringing a volume increase as expected and SCS occurred at this temperature. A dramatic reduction in the mechanical properties of the HZC10 composite (16.42 MPa; 39.66 HV) was observed because of the formation of CS glassy phase (Fig. 13). When HZC composites were compared with HAC composites, the microstructures and mechanical properties of the former were shown to be superior.

Fig. 15: Mechanical properties of HAC-HZC composites (a) Compression strength (b) Microhardness.

Fig. 16: SEM micrographs of the HAC10 samples sintered at 1200 °C after immersed in SBF in BSE mode at different duration; (a) 1, (b) 2, (c) 3, (d) 4 weeks.
(6) Biocompatibility test results

The in vitro bioactivity tests were performed on the composites exhibiting highest physical and mechanical properties. Fig. 16, Fig. 17, and Fig. 18 show, respectively, surface morphology of HAC10, HZC5 composite sintered at 1200 °C, and HZC5 composite sintered at 1300 °C after 1, 2, 3 and 4 weeks of immersion in SBF.

Starting from the first week, intensive apatite layer formation for all samples was determined. When the immersion time in SBF was increased, the size and quantity of the apatite layer increased, too, as expected. Surprisingly, the size and quantity of the apatite layer also increased with increasing sintering temperature. This is probably based on an excessively smooth morphology, well-arranged grains, and presence of the glassy phase enhancing apatite formation at higher sintering temperatures. At the end of 4 weeks, the samples’ surfaces were totally covered by the apatite layer. For the HZC5 composites sintered at 1200 °C, the existing apatite nucleation was observed only around ZrO2 grains in the first week. At the end of 3 weeks, the apatite accumulation also occurred on the ZrO2 grains. For HZC5 composites sintered at 1300 °C, the apatite nucleation was more abundant than the other two composites and ZrO2 grains were covered by the apatite layer starting from the first week. Of all the composites, the HZC5 sintered at 1300 °C showed the fastest apatite formation. Following the bioactivity tests, the samples were dried for characterization of the apatite layer formed during bioactivity tests. Many cracks were observed on the apatite layer after drying caused by thermal shock during rapid cooling process (Figs. 16, 17, and 18).

Fig. 19 illustrates the ALP activity of the HZC5 composite sintered at 1200 °C after 14 days of culture, whereas Fig. 20 shows the Alizarin Red S Staining after 7 days of culture. The growing bones need alkaline phosphates. Therefore, alkaline phosphate production is an indicator of biological activity. The sample displayed significantly higher levels of ALP secretion.

Fig. 21 shows the cell number of the HZC5 composite sintered at 1200 °C after the experiment. The cell number increased in a time-dependent manner. Positive cell production was initially detected at Day 7 and then followed by significant upregulation at Day 14.

Cell viability of the HZC5 composite sintered at 1200 °C was > 95 % for all. When the biocompatibility test results (in vitro and in vivo) were evaluated, it was concluded that samples were definitely biocompatible.
IV. Conclusions

The findings of this study are summarized as follows:

1. The highest density, the lowest porosity, the highest Vickers microhardness, and the highest compression strength values were obtained in HAC10 composites sintered at 1300 °C among HA-Al2O3 composites. The compression strength and microhardness of composites increased with increasing CIG content.

2. The highest density and hardness values were measured in the HZC5 composite at 1300 °C, while the highest compression strength was obtained with HZC5 composites at 1200 °C among all examined
composites. The compression strength and the microhardness of the composites decreased with increasing CIG content.
3. Sintering temperature played an important role in phase distribution, just as expected. With increasing sintering temperature, tricalcium phosphate (TCP), whtloktite (W) and glassy phases were formed from the decomposition of hydroxyapatite.
4. Grain growth occurred with increasing sintering temperature as expected for all composites. When the microstructural properties of HA-Al2O3 and HA-ZrO2 composites were compared, the HA-Al2O3 composite’s porosity was decreased and the microstructure became denser with increasing CIG content. For HA-ZrO2 composites, increased CIG content enhanced grain growth, and glassy phases were formed contributing to crack formation during cooling.
5. The composites having the best physical and mechanical properties also showed improved bioactive properties. Starting from the first week, intensive apatite layer formation on the surface was determined. Apatite nucleation was also observed on ZrO2 grains. At the end of the four weeks, the matrix was completely coated with an apatite layer.
6. The ALP activity level and cell number of the samples increased after 7 and 14 days of culture.
7. Optimum CIG content was provided based on the increase in the mechanical properties and bioactivities of the composites. The microstructure, mechanical properties and bioactivities of the HA-Al2O3 composites were found to be lower than those of the HA-ZrO2 composites. Of all the test results, the optimum microstructure, mechanical properties and biocompatibility were obtained for the HZC5 composite sintered at 1200 °C.
8. These results can be considered an indication for the potential use of these ternary composites for bone repair and/or bone replacements in orthopedics.

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