

***In vitro* Bioactivity of Copper-doped Akermanite Ceramic**

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Abstract

The aim of this paper is to study the effect of copper (Cu²⁺)-doped into the akermanite structure prepared via high-energy planetary ball milling route in order to enhance the biological response. In this work, the comparisons of physical and mechanical properties between pure and Cu-doped akermanite ceramics were made at the same sintering temperature 1200°C. According to the current results, Cu²⁺ promotes pore elimination of akermanite ceramic to a significant level (open porosity: < 5%), subsequently improved diametral tensile strength of modified samples to three- or four-folds with respect to pure samples. Additionally, apatite-forming ability of Cu²⁺-doped akermanite ceramic was assessed in stimulated body fluid (SBF) as well as the effects of ion release from substrate dissolution was evaluated. The results revealed that Cu²⁺ incorporation into akermanite structure induced the formation of bone-like globular Ca-P phase crystals. Copper dopant slowed down the rate of ion release of akermanite, thus decreased the chemical solubility and maintained a more stable pH environment while sustaining the ability to form apatite in physiological-like solution.

Keywords: akermanite, bioactivity, ball milling, copper, mechanical properties.

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Introduction

The Ca-Mg-Si-containing ceramics have been proved to be bioactive last ten years ago. Akermanite (AK; $\text{Ca}_2\text{MgSi}_2\text{O}_7$) has received significant attention owing to its excellent apatite mineralization by the release of soluble ionic products of Ca^{2+} , Mg^{2+} , and Si^{4+} [1]. The synergic effect of bioactive ions Mg^{2+} and Si^{4+} in akermanite could facilitate skeletal and vascular development activities under osteoporotic condition (i.e., a condition of low bone density with deteriorated bone microstructure [2]. The silica bonding in akermanite structure provides higher mechanical characteristics than CaP-based ceramics. Even though akermanite retains both good *in vitro* and *in vivo* biological responses, its low fracture toughness limited the use in load-bearing tissue regeneration [3] and the enhancement remains a clinical challenge.

Copper (Cu^{2+}) is an essential trace element for mammals due to its blood vessel formation (osteogenesis). Cu^{2+} showed high antimicrobial activity at the site of implantation [4]. A lack of copper significantly decreased the mechanical properties of bone, which apparently related to a decrease in bone collagen formation [5]. N. Kong reported that Cu^{2+} and Si^{4+} ions released from copper-doped calcium silicate stimulated the vascularization of human umbilical vein endothelial cells (HUVECs) [6]. Therefore, the investigations on the combined effects of Cu^{2+} and akermanite might be beneficial to enhance mechanical and biological properties for bone replacement materials.

In the present work, high-energy wet planetary ball milling was utilized as a processing technique due to its simplicity of experiment, low production cost and rapid synthesis of the product. An investigation on physico-mechanical properties and *in vitro* biological activities of pure and Cu^{2+} -doped akermanite ceramics was conducted.

Materials and Methods

Sample preparation

The pure and Cu^{2+} -doped akermanite powders are synthesized through high-energy wet planetary ball milling using calcium oxide (reagent grade, Aldrich), magnesium oxide (99%, Alfa Aesar), silicon dioxide (99%, Sigma-Aldrich), and copper (II) oxide (99%, Sigma-Aldrich). The reagents were ball milled with stoichiometric composition of $\text{Ca}_{2-x}\text{Cu}_x\text{MgSi}_2\text{O}_7$ (where $x = 0, 0.25, 0.45$) in a planetary ball mill (PM 100, Retsch) for 4 h at ambient conditions. The rotational speed of main disc was set at 500 rpm. The ball-to-powder weight ratio is 10:1. Deionized water was used as suspension media. The resultant slurry was oven dried at 100°C for 24 h, subsequently the dried cake was ground and sieved using a $200\ \mu\text{m}$ sieve. Following that, the resultant powders were uniaxially pressed in an evacuated stainless metal die at 150 MPa into 13 mm diameter discs. Subsequently, the pellets were sintered at 1200°C for 3 h in air atmosphere.

Material characterizations

To determine the active modes of vibration, Fourier transform infra-red radiation (FTIR) spectra were recorded in absorbance mode in the wavenumber range of $4000\text{--}400\ \text{cm}^{-1}$. Field emission scanning electron microscope (FESEM; Zeiss SupraTM Gemini 35 VP) was utilized for morphological analysis of akermanite sintered pellets. Open porosity of the pellets was determined in accordance with ASTM B962-17 [7] by applying Archimedes' relations. Diametral tensile strength (DTS) of the sintered bodies was evaluated in accordance with

ASTM D 3967–95a [8] using a universal testing machine (Instron 3369) with a loading rate of 0.5 mm/min.

In vitro bioactivity assessment

To understand the effect of Cu^{2+} -doping on akermanite, the sintered pellets were immersed in SBF buffered at pH 7.4 following Kokubo's protocol [9], then incubated at 36.5°C for 1, 7, 14 and 21 days. Afterwards, the dried samples were studied by FTIR and FESEM techniques. Ion concentration in the medium was analysed using an inductively coupled plasma optical emission spectrometer (ICP-OES; PerkinElmer Optima 7300 DV). The pH values were measured for each incubation period using a digital pH meter (Eutech Cyberscan pH 510).

Results and discussion

FTIR analysis

Figure 1 represents the resultant absorbance spectra of prepared samples sintered at 1200°C . The spectra displayed the Ca-O bending modes at 404 cm^{-1} , the O-Mg-O bending modes at 475 cm^{-1} , the Ca=O band at 586 cm^{-1} , the dual O-Si-O absorption bands at 641 and 683 cm^{-1} , the Si-O stretching modes at 852 , 934 and 972 cm^{-1} , the symmetric stretching Si-O-Si band at 1021 cm^{-1} , which are the typical functional groups of akermanite [10]. Partial incorporation of Cu^{2+} into akermanite structure did not change any functional groups, revealing structurally stable with dopant concentrations.

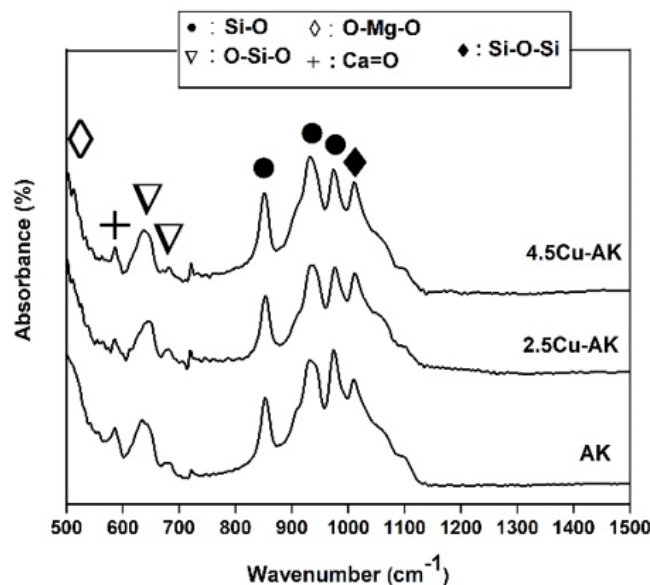


Figure 1. FTIR spectra of pure and Cu^{2+} -doped akermanite ceramics

Morphological and physico-mechanical evaluations

Figure 2 shows the grain growth effect of Cu^{2+} ion on akermanite. The grains are in irregular shaped geometry. A gradual development of grains was observed in terms of increasing Cu^{2+} additive. The pore elimination was effective after doping, developing stronger grain boundaries.

The open porosity was decreased from 38.13% to 4.01% and 3.89% with respect to dopant content, indicating improved sinterability. Subsequently, diametral tensile strength (DTS) of the doped ceramics had escalated from 12.39 MPa to 31.61 MPa and 28.84 MPa which is about three or four times that of pure ceramics sintered at 1200°C. The lower DTS value in AK was due to its high residual porosity. The reduced DTS in 4.5Cu-AK is likely to be due to the combination of crystalline and glassy phases that made the specimens to be more brittle. Therefore, our finding highlighted that the low value of open porosity was in turn a strong reason for high mechanical strength.

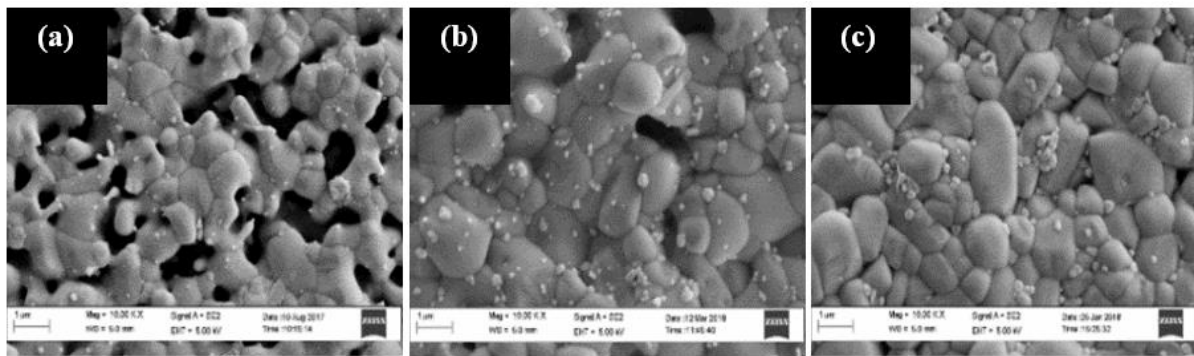


Figure 2. FESEM micrographs of outermost surfaces: (a) AK, (b) 2.5Cu-AK, and (c) 4.5Cu-AK ceramics at 1200°C

In vitro bioactivity test

FTIR analysis

The Ca-P phase on AK and Cu-AK substrates post-immersion in SBF was analyzed using FTIR technique (Figure 3 (a-c)). In AK ceramics, the sharp substrate peaks of Ca-O, O-Si-O and Si-O were significantly decreased and the new phosphate peaks started to appear just after 1-day incubation. The phosphate vibrations were identified at wavenumbers 468, 600, 960, and 1090 cm^{-1} [11], reflecting the highly crystalline apatite mineralization. The weak O-H stretching band was recognized around 630 cm^{-1} [12]. The substantial growth of more crystalline hydroxyapatite occurred after 7 days and continued until the end of third week, identifying soaking duration-dependent behaviour of akermanite.

Comparatively, a slight delay in apatite formation could be identified in the cases of Cu^{2+} -doped ceramics. A gradual decline in substrate peaks and a progressive increment of phosphate peak intensity of hydroxyapatite were observed until the end of second week. The characteristic phosphate peaks became sharper after longer third weeks' immersion, proclaiming an increase in crystallinity. This proved Cu^{2+} ion slowed down the initial surface degradation rate of akermanite, ascribing to smaller interplanar distance and more covalent bonding behaviour after Cu^{2+} substitution for Ca^{2+} .

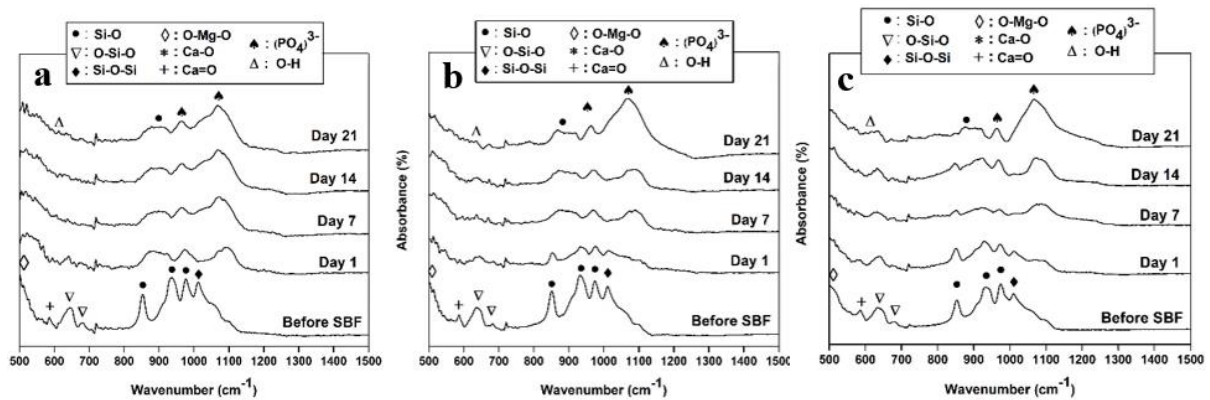


Figure 3. Time-dependent FTIR spectra: (a) AK, (b) 2.5Cu-AK, and (d) 4.5Cu-AK ceramics

Morphological analysis

Morphology of apatite layer accumulated on SBF-treated AK and Cu-AK substrates was visualized by FESEM technique (Figure 4 (a-d)). The surface of substrate was smooth before soaking in SBF (Figure 3). After exposed to SBF for 21 days, the surface became coarse in all cases. In AK, surface of substrate was covered with the nucleated spherical-like agglomerates. In comparison, in metal-doped counterparts, morphological change of apatite grew from loose precipitates to dense globules as a function of Cu^{2+} content, which ascribed to the higher crystalline phase inductivity, producing more compact apatite layer on the substrate. Under the effect of ion exchange between substrate and physiological-like solution, some micro-cracks appear which might be associated with the contraction of hydrated layer during drying process at room temperature in air atmosphere [13].

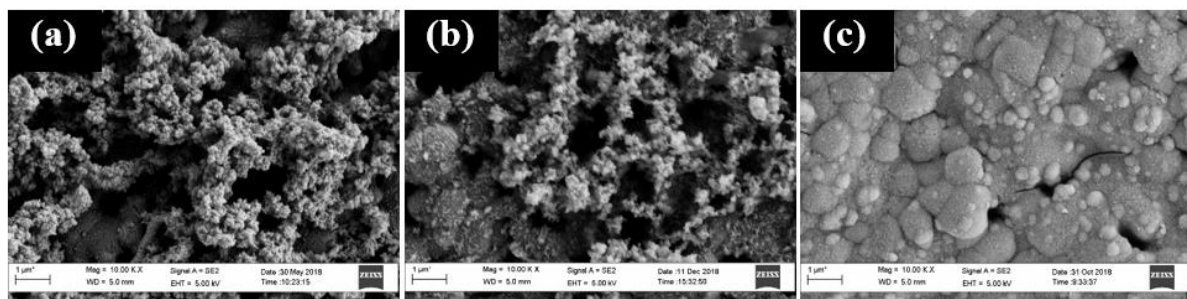


Figure 4. FESEM analysis of (a) AK, (b) 2.5Cu-AK, and (c) 4.5Cu-AK ceramics after 21 days in SBF (Magnification = 10,000 X, Scale bar = 1 μm)

Ion release and pH assessments

To understand the kinetic surface reactions of the ceramics, the quantitative examination of ion release was carried out at various time intervals (Figure 5 (a-d)). The profiles demonstrated that Ca^{2+} , Mg^{2+} , and Si^{4+} ions were leached from all akermanite samples, plus a minor amount of Cu^{2+} ions from modified counterparts. The released ions Ca^{2+} , Mg^{2+} and Si^{4+} had elevated rapidly within a first week, thereafter gradually increases until day 21. Ca^{2+} increased more quickly as compared to Mg, with the reason that the much greater release rate of Ca^{2+} ions from the host structure owing to the lower bond strength of Ca^{2+} than that of Mg^{2+} . Si^{4+} species progressively increased until day 21 with a quite slow dissolution rate, then

reached a nearly steady-state. Different from the release of Ca^{2+} , Mg^{2+} and Si^{4+} ions, the dissolution of P^{5+} ion showed a general downward trend, and reached nearly zero after one week, which indicated a high tendency of good apatite formation on akermanite surface.

On the other hand, the addition of Cu^{2+} decreased the overall ion release of ceramics, subsequently retarded the formation of apatite layer on the substrates. Mg^{2+} content in the solution showed higher concentration than Ca^{2+} and Si^{4+} , because of being less reactive. Concerning with the behaviour of Cu^{2+} , Cu^{2+} release rate was slow and remained in the same range during the whole time interval.

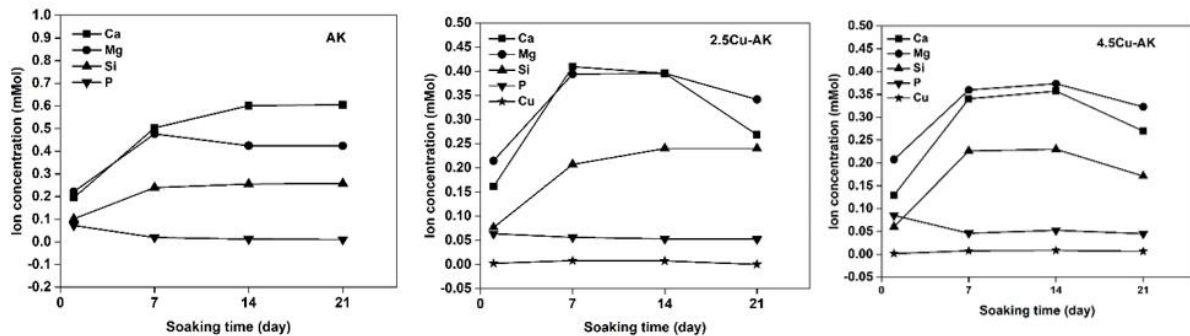


Figure 5. Ion release of substrates after soaking in SBF at different immersion periods

Solution pH greatly associated with the exchange rate of soluble ions (Figure 6). All compositions showed a progressive pH trend within first two weeks of immersion, indicating some alkaline products were releasing during degradation. However, within third week of immersion, the pH was on the decline with mineralization time which might be correlated with the consumption upon apatite formation on ceramics surface. Cu^{2+} addition significantly lowered the pH of medium which could be favourable for reducing the risk of cytotoxicity of ceramic as implant. The pH was maintained in the range 7.8 – 8.4, proving that the samples were chemically stable in SBF as well as non-acidic behaviour. These ion release and pH results support FTIR and FESEM findings mentioned earlier in this work.

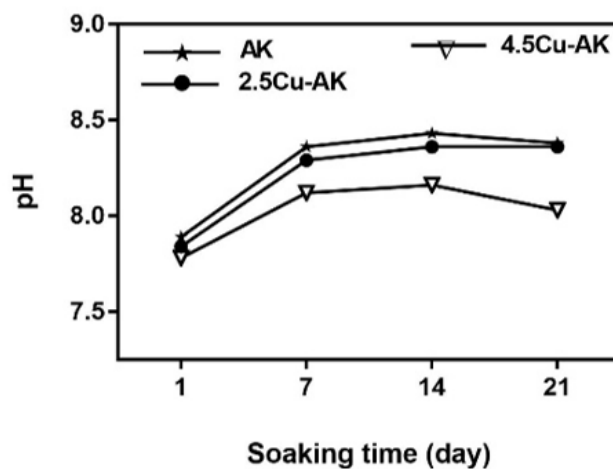


Figure 6. pH value versus immersion time of AK and Cu-AK ceramics

Conclusions

Pure and Cu²⁺-doped akermanite ceramics have been synthesized by high-energy planetary ball milling route with subsequent sintering at 1200°C for 3 h. According to the results, Cu²⁺ remarkably accelerated the sinterability of akermanite with significant reduction in porosity (<5%). Subsequently, this improved the mechanical strength of the ceramics. *In vitro* study reveals that the crystalline phase of apatite crystals increased with Cu²⁺ content. Cu²⁺ suppressed the degradation rate of substrates, thus more stable pH condition of the medium was sustained during immersion.

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Author Contributions

All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure of conflict of interest

The authors have no disclosures to declare.

Compliance with Ethical Standards

The work is compliant with ethical standards.

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