Preparation and Characterization of Magnetic Carbonate Apatite/Chitosan/Alginate Composite Scaffold

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Abstract. Treatment for bone cancer has begun to be experimented with ferrimagnetic for magnetic induction hyperthermia. On the other hand, composites of bioceramics and biopolymer have been studied for scaffold as these materials resemble the structure of bone. The current study investigated the magnetization of calcium aluminum ferrite magnetic (CaAl4Fe8O19) incorporated in carbonate apatite, alginate and chitosan, that serves as a scaffold. CaAl4Fe8O19 powder were synthesized using calcium nitrate, aluminium nitrate and ferrous chloride using the sol-gel method. Combining the carbonate apatite/chitosan/alginate composite and CaAl4Fe8O19 using the freeze-dry method has produced carbonate apatite/alginate/chitosan/CaAl4Fe8O19 composite scaffolds. The CaAl4Fe8O19 powder and the scaffolds were observed using SEM (scanning electron microscope) and their magnetization were measured using VSM (vibrating sample magnetometer). It was shown that the scaffold is a composite structure of CaAl4Fe8O19 particles, having diameter ranging from 0.5 to 2 µm, embedded in the pore walls of the carbonate apatite/alginate/chitosan matrix. The saturation magnetization Ms and remanence magnetization Mr of the CaAl4Fe8O19 particles were 20 and 2.0 emu/g, whereas, those of the scaffold were 4.3 and 2.0 emu/gr. The addition of the carbonate apatite/alginate/chitosan composite into CaAl4Fe8O19 decreased the fraction and/or magnetic of the CaAl4Fe8O19 particles.

Introduction

Cancer treatments with hyperthermia method have used superparamagnetic nanoparticle-based ferrite optimized with magnetic resonance imaging (MRI). In the method of hyperthermia, superparamagnetic nanoparticles that are in the vicinity of external alternating magnetic field generate heat due to the occurrence of magnetic hysteresis loss, thus destroying cancer cells [1]. The generated heat, 41-45°C, heat up the bone cancer, altering cancer cell metabolic conditions resulting in vascular occlusion causing stasis and hemorrhage in cancer cells due to decreased of cancer cells pH [2]. Iwasaki [3] showed that magnetite/hydroxyapatite composite materials have good characteristics of hyperthermia, providing magnetic activity to the personal needs of the patient.

Magnetism-induced nanoparticles in scaffolds are currently gaining interest for their potential applications in and tissue engineering. Wu [4] showed that the magnetic mesoporous bioactive glass
scaffolds can enhance mitochondrial activity of mesenchymal stem cells, showing that the material has the potential for cancer therapy together with tissue engineering by bone regeneration. Bone regeneration by tissue engineering using scaffolds has been used to manage bone problems. Natural bone comprise of an inorganic/organic composite mainly collagen fibers and nano-structured hydroxyapatite. Carbonate apatite has been one of the excellent candidates for bone regeneration, because of its similarity to chemical composition of natural bone [5]. In the last decade, the composite of carbonate apatite incorporated in biopolymers has attracted attention. Besides, the selected natural polysaccharides, alginate and chitosan are kinds promising candidate. Alginate is the name of a family of linear copolymers extracted from brown algae, containing mannuronic acid and guluronic acid in an alternating structure. Chitosan, a deacetylated derivative of chitin, is a biodegradable cationic biopolymers. Carbonate apatite/alginate/chitosan composite for scaffold materials possessed better flexibility, degradability and biocompatibility. The scaffolds will act as a guiding and stimulating matrix for the cells growth [6-8].

Magnetic scaffolds has been proposed for bone cancer treatment and bone regeneration engineering. The scaffolds will act as a guiding and stimulating matrix for the cells, providing magnetic activity, to the patient needs. [9] Designing of the magnetic particles into formulations of scaffolds constitutes one of the research areas in cancer treatment. Superparamagnetic iron oxide nanoparticles has been synthesized from Fe$_2$O$_3$, Fe$_3$O$_4$, or mixed of Fe$_2$O$_3$ and Fe$_3$O$_4$ particles [10-12]. Recently, a very promising candidate of magnetite calcium aluminium ferrite (CaAl$_4$Fe$_8$O$_{19}$) have been experimented, as it is Ca-based, [13] which would be biocompatible to bone. The current study, therefore, investigated the magnetization of CaAl$_4$Fe$_8$O$_{19}$ incorporated in carbonate apatite/alginate/chitosan composite.

**Experimental Methods**

**Synthesis of the carbonate apatite powder.**

Synthesis of the carbonate apatite powder was carried out by employing three precursors, e.g. calcium nitrate, diammonium hydrogen phosphate solution and calcium carbonate using the sol-gel method. In brief, solutions of calcium nitrate and diammonium hydrogen phosphate were mixed using a magnetic stirrer and continued with pH adjustments. Then, calcium carbonate was added to the mixture, which allowed the the mixture to produce a milky white solution. Next was the centrifugation process. Supernatant were separated from the sediment by a centrifuge machine. The sediments were placed on a petri dish, aged for 1 week at room temperature and dried. The dried powder were then calcined for 2 hours at 700ºC, to produce carbonate apatite powder.

**Synthesis of calcium aluminate ferrite (CaAl$_4$Fe$_8$O$_{19}$).** Magnetic particles were synthesized using calcium nitrate, aluminum nitrate and ferrous chloride precursors. All precursors were mixed using a magnetic stirrer until dissolved. pH adjustment was done by adding NaOH 1M up to the pH of 7. With a centrifugation process, supernatant was separated from the sediment. The sediment, seen as black powder, was vacuum-dried and calcined in a nitrogen furnace at 750ºC for 2 hours, to produce CaAl$_4$Fe$_8$O$_{19}$ powder.

**Synthesis of carbonate apatite/alginate/chitosan/ CaAl$_4$Fe$_8$O$_{19}$ composite scaffolds.** First, solutions of carbonate apatite, chitosan, and alginate were mixed. Then, CaAl$_4$Fe$_8$O$_{19}$ powder was combined with the mixture and stirred using a homogenizer. Finally, CaCl$_2$ crosslinker was added with a continuous stirring. The produced gel solution were frozen at -40ºC for 24 hours and 0.1-0.2 torr using a freeze-dryer (Biotron Clean Vac, Korea), to produce carbonate apatite/alginate/chitosan/CaAl$_4$Fe$_8$O$_{19}$ composite scaffolds.

**Characterizations.** Morphology of CaAl$_4$Fe$_8$O$_{19}$ particles and the carbonate apatite/alginate/chitosan/CaAl$_4$Fe$_8$O$_{19}$ composite scaffolds were observed by SEM (JEOL, JSM-6360 LA, Japan). Prior to SEM observation, the scaffolds were coated with Au/Pd alloy.

Magnetic hysteresis were measured by VSM (Oxford Tipe 1.2H). VSM measurements were performed under a maximum applied magnetic field of 1 T / 12 kOe (956 kA/m) at room
temperature in quasi-static conditions. The scaffolds were evaluated in terms of hysteresis curve and area saturation magnetization.

Results and discussion

The current study has produced CaAl<sub>4</sub>Fe<sub>8</sub>O<sub>19</sub> powder and carbonate apatite/chitosan/alginate/ CaAl<sub>4</sub>Fe<sub>8</sub>O<sub>19</sub> composite scaffolds, as shown in Fig. 1.

![Fig. 1. (a) CaAl<sub>4</sub>Fe<sub>8</sub>O<sub>19</sub> powder (black), (b) CaAl<sub>4</sub>Fe<sub>8</sub>O<sub>19</sub> particle response to an external magnetic field, (c) carbonate apatite powder (white), (d) carbonate apatite/chitosan/alginate/CaAl<sub>4</sub>Fe<sub>8</sub>O<sub>19</sub> composite scaffolds.](image)

It was seen in Fig.1 that the synthesized CaAl<sub>4</sub>Fe<sub>8</sub>O<sub>19</sub> powder was black (Fig. 1a). In Fig. 2b, the CaAl<sub>4</sub>Fe<sub>8</sub>O<sub>19</sub> powders demonstrated a response to an external magnetic field (permanent magnetic rods) in water suspension showing that the powders were accompanied with magnetic properties. The synthesized carbonate apatite powders were white, as were shown in Fig.1c. whereas, the carbonate apatite/chitosan/alginate/CaAl<sub>4</sub>Fe<sub>8</sub>O<sub>19</sub> composite scaffolds were in a cylindrical form having diameter of 15 mm and thickness of 10 mm (Fig.1d).

**Morphology.** SEM image of the CaAl<sub>4</sub>Fe<sub>8</sub>O<sub>19</sub> powders and the carbonate apatite/chitosan/alginate/ CaAl<sub>4</sub>Fe<sub>8</sub>O<sub>19</sub> composite scaffold were shown in Fig. 2.

![Fig. 2. SEM image of the (a) CaAl<sub>4</sub>Fe<sub>8</sub>O<sub>19</sub> particles and (b) carbonate apatite/chitosan/alginate/CaAl<sub>4</sub>Fe<sub>8</sub>O<sub>19</sub> composite scaffold.](image)

Based on Fig. 2(a), the CaAl<sub>4</sub>Fe<sub>8</sub>O<sub>19</sub> powders were seen as sperical particles with particle size ranging from 0.5 to 2 µm. In Fig 2(b) it can be seen that the carbonate apatite/chitosan/alginate/CaAl<sub>4</sub>Fe<sub>8</sub>O<sub>19</sub> composite scaffold possessed pores with diameter around 250 to 300 µm. In addition, the pore diameter was in compliance with the requirement of a good scaffold for
use in bone tissue engineering, which was 300-350 µm [6]. The pores were very well interconnected by pore walls. It was assumed that the pore walls contain carbonate apatite and CaAl₄Fe₈O₁₉ powders that were embedded in the mixture of chitosan and alginate matrix, thus constructing the composite structure of the scaffold.

**Magnetic Properties.** The CaAl₄Fe₈O₁₉ particles and the carbonate apatite/chitosan/alginate/ CaAl₄Fe₈O₁₉ composite scaffold showed hysteresis curves as in Fig. 3.

![Hysteresis curves of CaAl₄Fe₈O₁₉ particles and carbonate apatite/chitosan/alginate/ CaAl₄Fe₈O₁₉ composite scaffold.](image)

In Fig. 3, it was seen that the hysteresis curves consisted of saturation magnetization Ms, remanence magnetization Mr and coercivity field Hc. The saturation magnetization Ms is the state when the material was not able to absorb a stronger magnetic field such that an increase of magnetization force produces no significant change in magnetic flux density. The remanence magnetization Mr, is any magnetic induction that remains in a magnetic material after removal of an applied saturating magnetic field. Coercivity field is equal to the demagnetizing force required to reduce residual induction to zero in a magnetic field after magnetizing to saturation. The magnetic properties analyzed from both hysteresis curves were summarized in Table 1.

<table>
<thead>
<tr>
<th>No.</th>
<th>Sample</th>
<th>Coercivity, Hc [Oe]</th>
<th>Saturation Ms [emu/gr]</th>
<th>Remanence Mr [emu/gr]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CaAl₄Fe₈O₁₉</td>
<td>300</td>
<td>52.0</td>
<td>20.0</td>
</tr>
<tr>
<td>2</td>
<td>Carbonate apatite /chitosan / alginate / CaAl₄Fe₈O₁₉ composite scaffold</td>
<td>300</td>
<td>4.3</td>
<td>2.0</td>
</tr>
</tbody>
</table>

It was shown in Table 1 that the magnetic values occurred from the CaAl₄Fe₈O₁₉ particles were higher that those from the carbonate apatite/chitosan/alginate/ CaAl₄Fe₈O₁₉ composite scaffold. (magnetic scaffold). Incorporation of the carbonate apatite/chitosan/alginate composite into the CaAl₄Fe₈O₁₉ particles has altered the magnetic values of the magnetic scaffold. The saturation magnetization Ms and the remanence magnetization Mr of the CaAl₄Fe₈O₁₉ particles and the magnetic scaffold, decreased from 52.0 to 4.3 emu/g and from 20.0 to 2.0 emu/g, respectively. The reduction of both magnetic values seemed to be due to a reduced fraction of the CaAl₄Fe₈O₁₉ particles when mixed with the carbonate apatite/chitosan/alginate composite. When the fraction of
the CaAl₄Fe₈O₁₉ particles in the magnetic scaffold declined, the CaAl₄Fe₈O₁₉ particles could only produce slight magnetization (Table 1).

The carbonate apatite/chitosan/alginate/ CaAl₄Fe₈O₁₉ composite scaffold obtain from the current study when it is applied with MRI it may not be enough to produce hyperthermia. Efficient hyperthermia to generate local temperature would have occured from very fine superparamagnetic particles, for which they influences the magnetic properties of the material. Baldi, et al. [13] stated that the size of the magnetic particles would determine the effectiveness of cancer treatment with hyperthermia method. The magnetic values of CaAl₄Fe₈O₁₉ obtained from the current study were lower than those obtained from the work of Baldi, et al. [14], and Gil and Mano [9], that has produced superparamagnetic particles with particles size in nano-scale. Relatively large particle sizes possess low surface area. The relatively large partial size of CaAl₄Fe₈O₁₉ obtained from the current study seemed to have relatively low surface area, thus low magnetic values.

The current study has produced magnetic carbonate apatite/chitosan/alginate/ CaAl₄Fe₈O₁₉ composite scaffold, although with low magnetization. In line with the magnetization, the magnetic scaffold would not be readily used with MRI to produce hyperthermia. In order to obtain greater magnetization of the scaffolds, efforts need to synthesize CaAl₄Fe₈O₁₉ particles in nano-size scale. It is expected that the use of nano-size CaAl₄Fe₈O₁₉ in combination with carbonate apatite/chitosan/alginate/CaAl₄Fe₈O₁₉ composite would produce relatively high magnetic values of the carbonate apatite/chitosan/alginate composite scaffold.

**Conclusion**

The current study has synthesized CaAl₄Fe₈O₁₉ particles with particle sizes ranging from 0.5 to 2 µm and has prepared carbonate apatite/chitosan/alginate/CaAl₄Fe₈O₁₉ composite scaffolds with interconnected structure with pore diameter of 250-500 µm; the saturation magnetization Ms and remanence magnetization Mr were 52.0 and 20.0 emu/gr, respectively. The incorporation of the carbonate apatite/chitosan/alginate composite into the CaAl₄Fe₈O₁₉ powders has limited their magnetization. This has caused the saturation magnetization Ms and remanence magnetization Mr of the prepared scaffolds lowered to 4.3 and 2.0 emu/gr, respectively. The decline magnetic values of the carbonate apatite/chitosan/alginate/CaAl₄Fe₈O₁₉ composite scaffold was suggested to be due to the addition of carbonate apatite/chitosan/alginate/CaAl₄Fe₈O₁₉ composite, and consequently decreased the fraction and/or the magnetic values of CaAl₄Fe₈O₁₉.

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**References**


