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Sol-gel synthesis and apatite-formation ability of nanostructure merwinite ($Ca_3MgSi_2O_8$) as a novel bioceramic

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The need to improve synthetic materials for human bone replacement is significant. Nanostructured bioceramics are expected to have better bioactivity than microcrystals. Merwinite ($Ca_3MgSi_2O_8$) ceramic is a new bioceramic with good biocompatibility. The aim of this study was the preparation, characterization and evaluation of the bioactivity of nanostructured merwinite. Nanostructured merwinite was synthesized by a sol-gel process. Evaluation of the bioactivity was preformed by immersing the nanostructured merwinite in a simulated body fluid (SBF) and the formation of apatite on the surface of the immersed nanostructured merwinite was investigated. The results showed that hydroxyapatite (HAp) was formed after soaking for 7 days. Osteoblast viability and proliferation was measured by a cell proliferation kit I (MTT). Our study indicated that nanostructured merwinite possessed an apatite-formation ability, show a good bioactivity and might be used for preparation of new biomaterials.

Key words: Merwinite, Sol-gel synthesis, Bioceramics, Simulated body fluid, Hydroxyapatite.

Introduction

Previous research has indicated that CaO and SiO_2 are mandatory components for biocermaics to possess bone-like apatite-formation ability and bioactivity for bone implant materials [1-4]. For example, an in vivo study on diopside ($CaMgSi_2O_6$) ascertained this material is bioactive [4-7]. Merwinite is a ceramic that includes ions such as Si-, Ca- and Mg-. Therefore, it is reasonable to assume that merwinite may be a bioactive material. It has been shown that sintered sol-gel-derived merwinite powder compacts can induce bone-like apatite formation after soaking in SBF for 10 days [8]. A preliminary study suggests that merwinite might be a potential bioactive implant material [9]. Merwinite is a mineral with a monoclinic structure, a density of 3.15-3.33 g/cm^3 and a melting temperature of > 1450 ºC.

In this communication, we report the chemical synthesis of nanostructured merwinite powders using a sol-gel method, an evaluation of the apatite-formation ability of nanostructured merwinite by soaking the powders in a simulated body fluid (SBF) and the biocompatibility of nanostructured merwinite.

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Experimental Procedure

Nanostructured merwinite was prepared by a sol-gel process using tetraethyl orthosilicate ($(C_2H_5O)_4Si$, TEOS), magnesium nitrate hexahydrate ($Mg(NO_3)_2{\cdot}6H_2O$) and calcium nitrate tetrahydrate ($Ca(NO_3)_2{\cdot}4H_2O$) as raw materials. Briefly, the TEOS was mixed with water and 0.1 M HNO_3 and hydrolyzed for 30 minutes under stirring. Then, the $Mg(NO_3)_2{\cdot}6H_2O$ and $Ca(NO_3)_2{\cdot}4H_2O$ were added into the mixture (molar ratio: TEOS : $Mg(NO_3)_2{\cdot}6H_2O$: $Ca(NO_3)_2{\cdot}4H_2O$ = 1 : 1.44 : 0.85), and reactants were stirred for 5 h at room temperature. After the reaction, the solution was maintained at 60 ºC for 1 day and dried at 120 ºC for 2 days to obtain a dry gel. The dried gel was heated for 24 h in an electrical box furnace at 700 ºC using a heating rate 5ºK·minute^{-1} to eliminate residual nitrates. Then, the powders were calcined at 1300 ºC in an electrical box furnace for 2 h in air. Calcined powders were analyzed by X-ray diffraction (Philips PW 3710 diffractometer.) with a monochromated CuKa radiation, and the microstructure of calcined powders was observed by scanning electron microscopy (SEM; Philips XL 30). For the evaluation of the apatite-formation ability, the nanostructured merwinite was soaked in SBF solution at pH 7.25 for 7 days at a solid/liquid ratio of 1.5 mg/ml. The SBF solution was prepared according to the procedure described by Kokubo [10]. After soaking for 1, 3, 5 and 7 days, the samples were filtrated, rinsed with distilled water,

dried at 60 °C and characterized by fourier transform infrared spectroscopy (FTIR) and SEM.

SaSo-2 Osteoblast cells were used for biocompatibility. The cells were cultured in polystyrene plates enriched with Dulbecco's Modified Eagle Medium (DMEM), (Gibco BRL), supplemented with 10% fetal bovine serum, 100 IU/ml penicillin (Sigma) and 100 mg/ml streptomycin (Sigma), and incubated at 37 °C in a humid atmosphere with 5% CO_2. When the cells reached confluence, culture media was replaced by a media containing DMEM and nanostructured merwinite extracts and incubated for 72 h under the same conditions. Negative (Ultra High molecular weight Poly Ethylene) and positive (copper) controls were used. After 72 h, the cells were observed under an optical microscope (Olympus IX71).

Results and Discussion

Fig. 1 show the XRD pattern of the merwinite powder prepared by a sol-gel method and calcined at 1300 °C. The Bragg peaks clearly show the typical pattern of merwinite (JCPDS No. 25-0161). The crystallite size of merwinite at this temperature is about 30 nm according to the XRD line-broadening technique and using the Scherrer equation.

Fig. 2 show SEM micrographs of nanostructured merwinite before and after soaking in SBF solution for 7 days. The surface of the nanostructured merwinite ceramic was smooth before soaking (Fig. 2(a)). In contrast, after soaking for 7 days, a layer of spherical particles of bone-like apatite crystals fully covered the surface of the nanostructured merwinite ceramic (Fig. 2(b)).

Fig. 3 show the FTIR spectra of the samples after soaking in SBF for different periods. The characteristic bands exhibited in the samples that were immersed in SBF for 1, 3, 5 and 7 days spectra are assigned here: (a) the band observed at 3455 cm^{-1} was due to the stretching mode of the hydrogen-bonded OH^- ions; (b) the band at 1680 cm^{-1} arises from intercalated H_2O; (c) the band at 1050 cm^{-1} arises from ν_1 PO_4, the bands at 895 arises from ν_3 PO_4, the bands at 613 cm^{-1} and 541 cm^{-1} arise from ν_4 PO_4 and finally the band at 450 cm^{-1} arises from ν_2 PO_4. Therefore, according to the above results, an apatite layer was formed on the surface of samples in SBF solution.

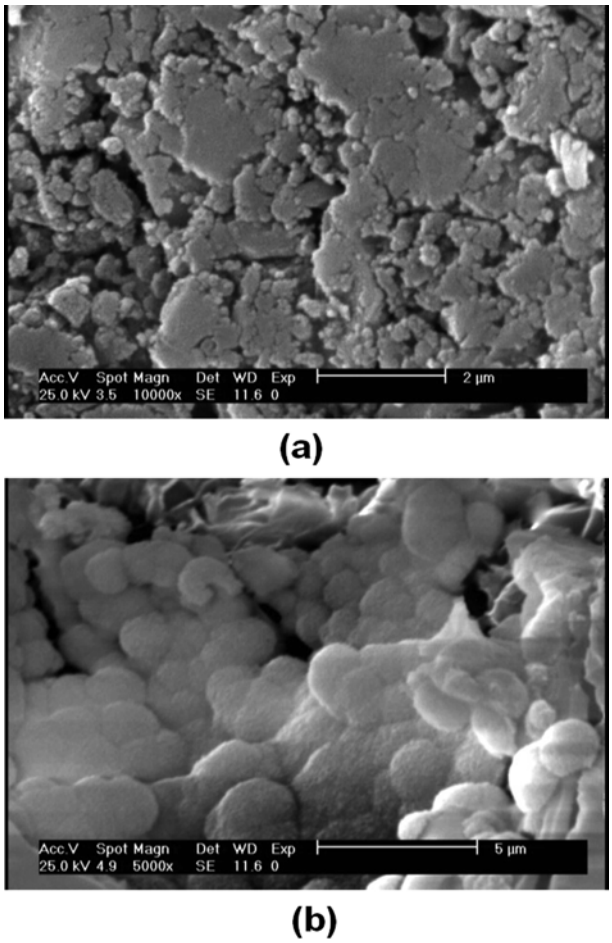

Fig. 2. (a) SEM micrographs of the synthesized merwinite powder before (a) and after (b) soaking in SBF solution for 7 days.

Fig. 4 show 72 h-incubated SaSO-2 osteoblast cells with a supernatant extracted merwinite after immersion in the media for the purpose of studying the effect of extracted merwinite in the media on the morphological changes. The cells displayed a spindle-shaped morphology and formed a monolayer. Osteoblast viability and proliferation were measured by a cell proliferation kit I (MTT). The

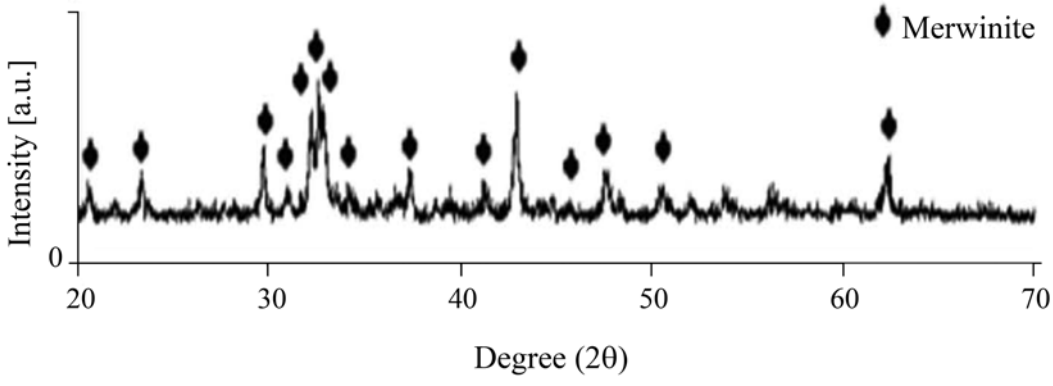

Fig. 1. XRD pattern of the synthesized merwinite.

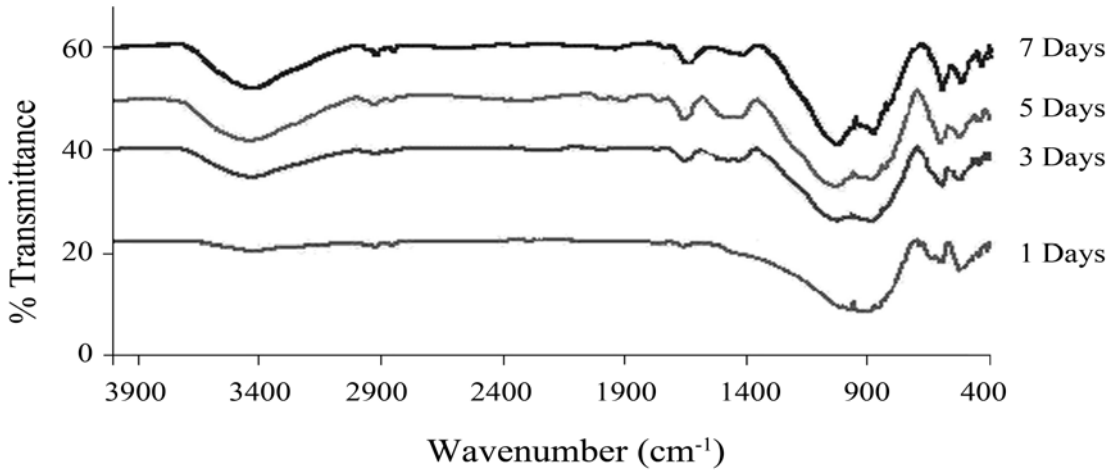

Fig. 3. FTIR spectra of the synthesized merwinite after soaking in SBF for different periods.

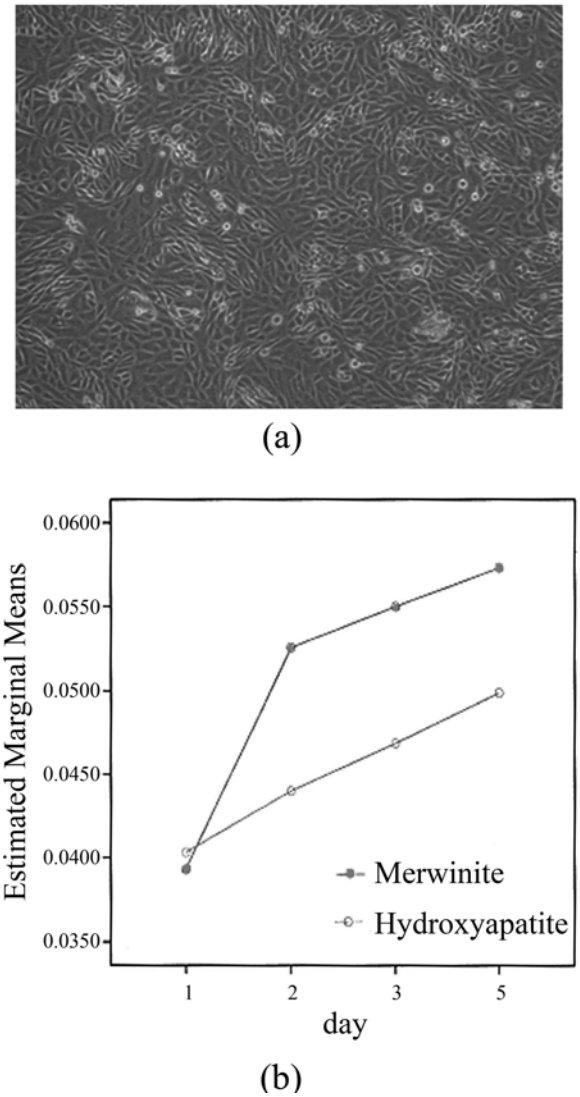

Fig. 4. (a) Optical microscope image of the SaSO-2 osteoblast cells after an incubation period of 72 h and (b) Osteoblast proliferation, as measured by the MTT assay, following 1, 2, 3 and 5 days in culture with a nanostructured merwinite extract and HA extract were collected after 24, 48 and 72 h.

repeated measure tests showed that the cell proliferation for the nanostructured merwinite extract was significantly different from the HA extract. A stronger trend in cellular viability and proliferation was observed for osteoblasts exposed to specific merwinite extract solutions for different days. (Wilks' Lambda = 0.039, P value = 0.000)

It is important to point out the crystalline lattice of two bioceramics, merwinite and akermanite ($Ca_2MgSi_2O_7$), is approximately different. The elemental composition of the above-mentioned materials is similar meaning that merwinite has one more calcium and oxygen in its chemical formula as compared with akermanite. Our results showed that merwinite possessed an apatite-formation ability. It is worth mentioning that according to the above explanations, it may suggested that a compositional similarity is an important factor to determine an ability of apatite deposition on ceramics, but further work needs to be done to confirm this assumption.

Conclusions

In summary, nanostructured merwinite was successfully synthesized via a sol-gel route. The *in vitro* study showed that nanostructured merwinite could induce hydroxyapatite formation on their surface after 7 days of soaking in SBF. Eventually, our study suggests that nanostructured merwinite ceramic holds the potential for use as a bioactive material.

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