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Process and kinetics of bonelike apatite formation on sintered hydroxyapatite in a simulated body fluid

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Abstract

The surfaces of two hydroxyapatites (HA), which have been sintered at different temperatures of 800 and 1200 °C, was investigated as a function of soaking time in simulated body fluid (SBF) using transmission electron microscopy (TEM) attached with energy-dispersive spectrometry (EDX) and laser electrophoresis spectroscopy. The TEM–EDX indicated that after soaking in SBF, both the HAs form bonelike apatite by undergoing the same surface structural change, i.e., formations of a Ca-rich amorphous or nano-crystalline calcium phosphate (ACP) and a Ca-poor ACP, which eventually crystallized into bonelike apatite. Zeta potential characterized by the electrophoresis indicated that during exposure to SBF, the HA surfaces reveal negative surface charge, thereby interacting with the positive calcium ions in the fluid to form the Ca-rich ACP, which gains positive surface charge. The Ca-rich ACP on the HAs then interacts with the negative phosphate ions in the fluid to form the Ca-poor ACP, which stabilizes by being crystallized into bonelike apatite with a low solubility in the SBF. The exposure times for formations of these phases of the Ca-rich ACP, the Ca-poor ACP as well as the apatite were, however, all late on HA sintered at 1200 °C, compared with the HA sintered at 800 °C. This phenomenon was attributed to a lower initial negative surface charge of the HA sintered at 800 °C than of that one sintered at 1200 °C, owing to poverty in surface hydroxyl and phosphate groups which are responsible for the surface negativity of the HA. These indicate that sintered temperature of HA might influence not in terms of the process but in terms of the rate of formation of biologically active bonelike apatite on its surface, through which the HA integrates with living bone.

Keywords: Hydroxyapatite; Bioactivity; Apatite; Simulted body fluid (SBF); Surface potential

1. Introduction

Bioactive ceramics, e.g., Bioglass®, sintered hydroxyapatite, and glass-ceramic A-W, are biomaterials, which when implanted into bone defects, forms spontaneously a layer of biologically active bonelike apatite on their surfaces to induce chemical integration of bone tissue [1–4]. Owing to this prime biological property, the bioactive ceramics not only have been useful in bone repairs and replacements, but also are inspiring new bioactive materials [2,5,6]. For example, tough bioactive materials essential for load-bearing bone repairs have been developed by subjecting titanium metal and titanium alloys to simple NaOH and heat treatments, which produce a bioactive surface layer of sodium titanate in situ on the surfaces of the metals [5–7]. Soft bioactive materials have been developed by co-polymerizing bioactive silica or titania with polymers through sol–gel process [5,6,8]. Namely, a detailed insight into the mechanism bonelike apatite formation on bioactive ceramics is considered essential

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for developing bioactive materials with enhanced or novel physical, chemical and biological functions.

Concerning Bioglass®, glass-ceramic A-W and bioactive titanium metal, in vitro assessments using simulated body fluid (SBF) [9] have early suggested that they induce the bonelike apatite formation by forming specific functional groups, such as Si–OH and Ti–OH groups, on their surfaces in body environment [10–13]. More recently, these surface functional groups have been shown to reveal a negative charge to first interact with the positive calcium ions in the body fluid, forming calcium compound such as amorphous calcium silicate or calcium titanate [14–16]. These amorphous calcium compounds were assumed to reveal positive charge to combine the negative phosphate ions in the fluid, forming an amorphous calcium phosphate, which later crystallizes into bonelike apatite.

The sintered hydroxyapatite (HA) has been also documented to integrate with bond tissue by forming the bonelike apatite on its surface in bony defect [2,4], but the process of the apatite formation on the HA is not clear yet in the above context. In addition, Niwa et al. [17] early documented that the ability of bone integration of HA decreases as its sintered temperature increases. This suggests that the HA might reveal different kinetics of the bonelike apatite formation, and thereby different apatite-forming abilities, by the sintered temperature. Specifically, the HAs with different profiles of sintered temperature provide interesting model concerning the mechanism of bonelike apatite formation in both terms of process and kinetics. In this study, we adopted two HAs, which were sintered at different temperatures, to investigate their changes in surface composition, structure and potential as a function of soaking time in SBF. The process and kinetics of the bonelike apatite formation on the HAs were discussed in terms of changes in surface composition and structure, and rationalized in terms of change in surface potential.

2. Materials and methods

2.1. HA specimens and soaking in SBF

Starting materials were typical dense HA polycrystals (Mitsubishi Materials Co., Tokyo, Japan), which were sintered at different temperatures of 800 and 1200 °C. Raw HA powder, which has been single-phased by calcination at 800 °C, was compacted by cold one-directional and isostatic pressings, and sintered into polycrystal bulks at each temperature for 3 h. They were designated as HA-800 and HA-1200 by their sintered temperatures, and characterized by scanning electron microscopy (SEM: S-2500CS, Hitachi Co., Tokyo, Japan), powder X-ray diffraction (XRD: RINT-2500, Rigaku Co., Tokyo, Japan) and Fourier transformed infrared spectroscopy (FT-IR: Perkin-Elmer Optoelectronics Inc., CA, USA).

The polycrystal bulks HA-800 and HA-1200 were pulverized by dry ball milling in a high-purity zirconia pot, and sieve-screened into particles less than 5 μm in size. Each of the HA-800 and HA-1200 particles 50 mg in mass were immersed in 120 mL of an acellular SBF with pH (7.40) and ionic concentrations (Na^+ 142.0, K^+ 5.0, Mg^{2+} 1.5, Ca^{2+} 2.5, Cl^- 147.8, HCO_3^- 4.2, HPO_4^{2-} 1.0, SO_4^{2-} 0.5 mM) nearly equal to those in human blood plasma at 36.5 °C. The SBF was prepared by dissolving reagent-grade chemicals of NaCl, $NaHCO_3$, KCl, $K_2HPO_4 \cdot 3H_2O$, $MgCl_2 \cdot 6H_2O$, $CaCl_2$ and Na_2SO_4 (Nacalai Tesque Inc., Kyoto, Japan) into distilled water and buffering at pH 7.40 with tris(hydroxymethyl)aminomethane ($(CH_2OH)_3CNH_3$) and 1.0 M hydrochloric acid (Nacalai Tesque Inc., Kyoto, Japan) at 36.5 °C.

2.2. Surface analyses of HA specimens

After immersion and soaking in SBF for various periods, surface composition and structure of the HA particles were analyzed using TEM (JEM-2000FX, JEOL Co., Tokyo, Japan) attached with EDX (VOYAGER III, NORAN Instruments Inc., Middletown, WI, USA). For TEM-EDX analysis, the HA particles removed from the SBF were dispersed in ethanol and deposited onto poly(vinylformal) film supported by 200 mesh, 3 mm diameter nylon grid. In EDX analysis, the as-measured results were calibrated using references of extra pure reagents of tricalcium phosphate ($Ca_3(PO_4)_2$; Nacalai Tesque Inc., Kyoto, Japan) and hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$; Sigma Chemical Co. Ltd., St. Louis, MO, USA).

Surface potential of the HA after soaking in SBF was analyzed in terms of zeta potential, which was measured using a laser electrophoresis spectroscopy (Model ELS9000 K, Otsuka Electronics Co., Osaka, Japan). For electrophoresis, the HA particles removed from the SBF were immediately dispersed into fresh SBF filled in a high-purity silica glass cell. The HA particle weight to SBF volume ratio was kept the same with that for TEM specimen preparation to make the HA particles be floating in SBF during the potential measurement. The glass cell was immediately equipped into the electrophoresis system to measure zeta potential of the HA surface. This system adopts laser Doppler electrophoresis to measure electrophoretic mobility of HA particles. The zeta potential (ζ) is given by Smoluchowski equation,

$$\zeta = 4\pi\eta U/\varepsilon, \quad (1)$$

where U is the electrophoretic mobility of HA, η the viscosity of solution, i.e., 1 cp for water, and ε the