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Original Research Paper

Preparation of gold/hydroxyapatite hybrids using natural fish scale template and their effective albumin interactions

Yadong Chai, Masami Nishikawa, Motohiro Tagaya *

Department of Materials Science and Technology, Nagaoka University of Technology, Kamitomioka 1603-1, Nagaoka, Niigata 940-2188, Japan

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ABSTRACT

The gold (Au) nanoparticles (NPs) with the diameter of 15–40 nm were successfully synthesized in the hierarchical hydroxyapatite (HAp) nanostructures of natural fish scale templates, which were carried out by the Au^{3+} ion chemisorption, reduction and calcination processes to form the AuNPs/HAp hybrids. The AuNPs size as well as the surface plasmon resonance (SPR) absorption maximum was preserved with the hybridization process. Moreover, the AuNPs/HAp hybrid nanostructures exhibited preferential protein adsorption behavior at the biological bovine serum albumin (Ab) concentration regions that correspond to be 1.5 μM in the cell culture medium and 15.1 μM in human blood, and the Ab adsorption equilibrium constant of AuNPs/HAp hybrid was higher than that of the HAp alone. The SPR absorption maxima of the Ab-adsorbed AuNPs/HAp fish scales were red-shifted as compared with those of the AuNPs/HAp fish scales. Therefore, we synthesized the AuNPs using the fish scale template to exhibit the preferential protein adsorption, which will be a great significance to research the AuNPs/HAp hybrid functions.

1. Introduction

It is known that there are various inorganic structures formed by biomineralization in organisms. In particular, the biocompatible inorganic material of hydroxyapatite (HAp, $Ca_{10}(PO_4)_6(OH)_2$) has widely been researched in the biomimetics such as human bone, teeth, and fish scales [1–3]. HAp has great value and significance because of its biocompatibility and has been exploited in the biomedical fields such as bone repair and tissue engineering materials, and protein adsorbents [4–7]. The highly-biocompatible surface properties can be enhanced by the inorganic/organic hybrid interfaces. Thus, the physicochemical properties such as morphologies, crystalline, and nano/micro-structures should be controlled by the hybrid states (see Scheme 1).

The scales of teleost fish are useful hybrids containing HAp and type I collagen [8–14]. The structures of fish scale can be divided into three regions: the outer limiting layer, external osseous layer, and internal fibrillary plate [8]. The outer limiting layer is composed of the random meshwork of acidic glycoproteins [9]. The first to be mineralized is the external layer where the needlelike HAp crystals hybridized with randomly oriented collagen fibrils, and then the internal layer and outer limiting layer occurred [10,11]. In particular, the internal layer is composed of a multilayer lamellar structure. In each lamellar, highly-ordered collagen fibrils with the diameter of 60–100 nm that are closely packed and oriented in one direction, and the HAp crystals parallel to collagen fibrils are observed [12,13]. Moreover, the collagen fibrils are arranged perpendicular to each other between adjacent lamellar forming a plywood-like structure [14]. Thus, it is important to use the hierarchical nano/micro HAp structures in fish scales as the template for supporting various functional materials.

The gold nanoparticles (AuNPs) are emblematic example of biomedical nanomaterials and have been investigated for the applications such as drug delivery vehicles [15], thermotherapy [16], and biosensors [17]. The multiple qualities of AuNPs, which are utilized in readily surface modification, controlled biocompatibility, and surface plasmon resonance (SPR), is affected by the particles size, shape, inter-distance, and environmental refractive index [18]. The investigation of the hybrid properties of HAp and AuNPs is meaningful for developing novel biomaterials. Thus, the HAps/AuNP hybrids have been reported for the biomedical applications such as bone tissue repair and regeneration [19], enhance the blood compatibility [20], and immunosensing [21]. It is important to find the novel AuNPs/HAp hybrid structures and their functions. However, the synthetic HAp particles are often precipitated sepa-

* Corresponding author.
E-mail address: tagaya@mst.nagaokaut.ac.jp (M. Tagaya).

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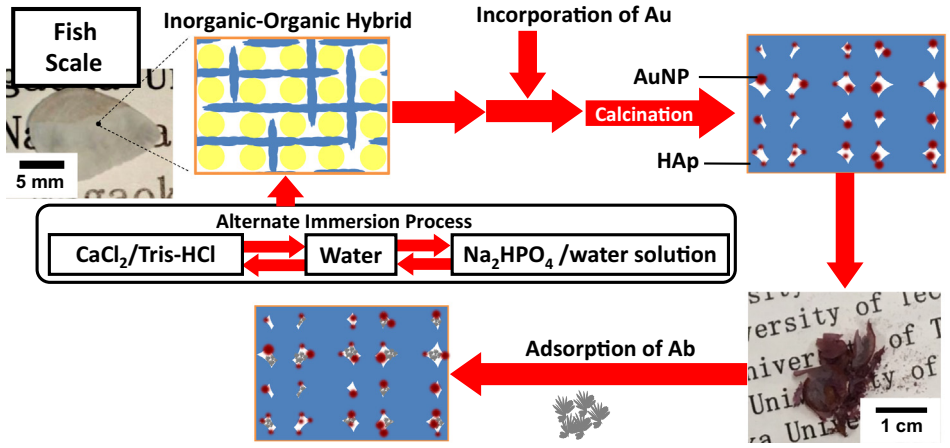

Scheme 1. Illustration of preparation processes of the AuNP-incorporated porous HAp derived from fish scale and subsequent Ab adsorption.

rately and then assembled with AuNP in the hybrid preparation process.

In this study, we carried out the Au^{3+} chemisorption and subsequent reduction by a reducing agent to address the AuNPs incorporation into the hierarchical nano/micro structures of the fish scale HAp. Moreover, the serum protein adsorption ability into the AuNPs/HAp hybrid structures was evaluated. From the application perspectives, the widespread potential of the natural scale-templated AuNPs/HAp hybrids is anticipated.

2. Experimental procedure

2.1. Materials and preparation

Based on the "alternate soaking method" [22], HAp was densely formed inside the fish scale. In details, the dry Tilaipa fish scales (obtained from Japan Tuna Bait Co., Ltd.) were transferred in 20 mL of H_2O (pH = 7.4) adjusted with 1 N of NaOH (Wako Chemical Co., Ltd) and immersed for 12 h. There are three processes under the temperature of 37 °C as follows; the fish scales were washed with 30 mL of H_2O for 10 s and allowed to statically stand for 50 s ("process 1"), and then immersed in 20 mL of Na_2HPO_4 (Wako Chemical Co., Ltd) aqueous solution (120 mM, pH = 9.2) for 5 min ("prosess 2"). The fish scales were washed with 30 mL of H_2O for 10 s and allowed to stand for 50 s, and was immersed in 20 mL of the aqueous solution (pH = 7.4) containing $CaCl_2$ (200 mM; Wako Chemical Co., Ltd) and tris(hydroxymethl)aminomethane (Tris; 100 mM, Wako Chemical Co., Ltd) for 5 min ("process 3"). We repeated "process 1–3" with 5 cycles, and the fish scales were dried at 60 °C for 12 h. The fish scale was donated as 5cy.

For the precipitation of Au, 0.1 g of 5cy was immersed in 30 mL of the aqueous solution containing hydrogen tetrachloroaurate(III) tetrahydrate ($HAuCl_4{\cdot}4H_2O$; Wako Chemical Co., Ltd.) with the different concentrations of (0.14, 0.28 and 0.56 mM) at room temperature for 2 h, and then was heated at 70 °C and 10 mL of the aqueous solution of trisodium citrate (4.47 mM, Wako Chemical Co., Ltd.) as a reducing agent was added and stirred for 30 min. The fish scales were dried at 60 °C for 12 h and calcined at 250 °C for 3 h and at 550 °C for 4 h. Here, the synthetic AuNPs dispersion liquids derived from 0.14, 0.28 and 0.56 mM of the $HAuCl_4{\cdot}4H_2O$ aqueous solutions were abbreviated as Au**1**/Water, Au**2**/Water, Au**3**/Water, respectively, and the Au-incorporated fish scales immersed in the $HAuCl_4{\cdot}4H_2O$ aqueous solutions were abbreviated as 5cyAu**1**, 5cyAu**2**, 5cyAu**3**.

The supernatant liquids after the reduction reaction were optically measured by the change in the concentration of Au in the solution to obtain the incorporated Au amounts on the fish scales, which was determined by the absorbance changes at 526 nm in UV–visible absorption spectra. The calibration curve from the AuNPs dispersion liquids was shown in the ESM, Fig. S1. The AuNPs dispersion liquids was prepared by adding 10 mL of the aqueous solution of trisodium citrate in 30 mL of the aqueous solution containing $HAuCl_4{\cdot}4H_2O$ with the different concentrations of 0.14, 0.28, 0.42 and 0.56 mM, which was the same procedure mentioned above. The correlation coefficient was 0.99243.

2.2. Protein adsorption

We experimented the protein adsorption on 5cy and 5cyAu fish scales. Bovine serum albumin (Ab; Wako Chemical Co., Ltd) was dispersed in phosphate buffer saline (PBS; DS Pharma Biomedical Co., Ltd) with the ions (K^+: 4.15 mM, Na^+: 153 mM, HPO_4^{2+}: 9.57 mM, Cl^-: 139.57 mM) to prepare Ab/PBS liquid with the Ab concentrations of 1.51 and 15.08 μM. 40 mg of the fish scale was immersed in 5 mL of Ab/PBS at room temperature for 0.5, 1, 2, 3 and 4 h. The adsorption amount per unit surface area with the immersion time was plotted.

The adsorption isotherms of Ab on 5cy and 5cyAu fish scales were also measured. 40 mg of the 5cy and 5cyAu3 were immersed in 5 mL of the Ab/PBS liquid with the Ab concentrations of 1.96, 7.49, 15.32, 22.57, 30.40 μM and 1.84, 7.46, 15.19, 22.75, 30.17, 45.67, 61.13 μM at room temperature for 4 h. The adsorption amount per unit surface area with the equilibrium concentration was plotted.

In these experiments, the supernatant liquids were optically measured by the change in the concentration of Ab in the PBS before and after the reaction to obtain the Ab adsorption amounts on the fish scales, which was determined by the absorbance changes in PBS at 278 nm in UV–visible absorption spectra. The calibration curve of the Ab dispersion PBS liquids with the Ab concentrations of 1.51, 7.54, 15.08, 22.63 and 30.17 μM was measured and shown in the ESM, Fig. S2. The correlation coefficient was 0.99998. The adsorption amount at the equilibrium state (W) was calculated by the Eq. (1) based on the adsorption isotherms. On the basis of the Langmuir adsorption isotherm formula, the equation of state for the one-component adsorption can be represented as follows:

$$C/W = 1/(K_{eq} \cdot W_{max}) + (1/W_{max})C \quad (1)$$

C, K_{eq} and W_{max} are the Ab concentration in the equilibrium state, the adsorption equilibrium constant and the maximum adsorption amount, respectively. The K_{eq} and W_{max} were determined from the slope of a C/W versus C plot. The Ab adsorption based on the

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