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Studies on preparation of surfactant-assisted elliptical hydroxyapatite nanoparticles and their protein-interactive ability

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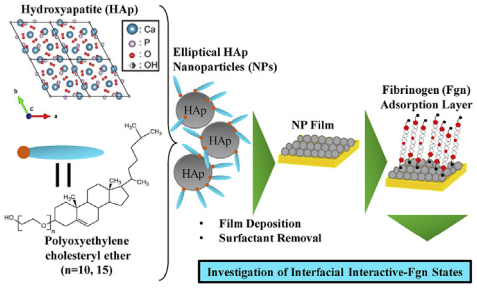
HIGHLIGHTS

- Elliptical hydroxyapatite nanoparticles (HAp NPs) were synthesized.
- Effect of poly (oxyethylene)cholesteryl ether on the NP shapes was investigated.
- Binding affinity and conformational change of fibrinogen (Fgn) were investigated.
- High affinity of Fgn on the HAp NPs was demonstrated.
- Ther temperature at 37 °C caused the preferential adsorption of Fgn for HAp.

GRAPHICAL ABSTRACT



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ABSTRACT

Addition of poly (oxyethylene)cholesteryl ether in the synthesis promoted the formation of the hydroxyapatite nanoparticles (HAp NPs) with the elliptical morphologies. The adsorption mechanism, interfacial interactions and conformation of the adsorbed fibrinogen (Fgn) on the HAp NP films were investigated. The Fgn adsorption behavior demonstrated the higher affinity of Fgn with the HAp NP films. At the lower temperature at 22 °C, Fgn was preferentially adsorbed on the gold though hydrophobic interactions with the larger Fgn adsorption amount. At the higher temperature at 37 °C or at the higher phosphate ion concentrations caused the structural changes of the adsorbed Fgn. Moreover, the temperature increment in PBS increased the Fgn adsorption amount with the rearrangement into the end-on orientation of the Fgn. Accordingly, HAp induced the electrostatic force and hydrogen bonding to be the stable Fgn-HAp NP film, minimizing the interactions among the water molecules. The HAp NP films with the preferentially elliptical crystalline shapes induced the Fgn steric conformation structures, and the secondary structural changes of the adsorbed Fgn by the increment of temperature were also supported by the Fourier transform infrared spectroscopy (FTIR) deconvolution results.

1. Introduction

Hydroxyapatite [$(Ca_{10}(PO_4)_6(OH)_2)$, HAp] is a non-toxic, bioactive and osteoconductive material, highly valuable for biomedical applications due to the compatibility with the human body because of the similarity to the composition of human hard tissues [1–4]. HAp has been widely used as a bone-substitute material in orthopedic and dental applications, and for soft tissue repairs [5–7], and is an attractive material for drug/protein/gene loading and delivery systems [8–10], antimicrobial agents [11–13], filler or packing for high performance liquid chromatography [14,15], sensors [16], cell targeting, fluorescence labeling and diagnosis material [17,18]. It has been known that the physicochemical properties in the biomedical fields mainly depend on the appropriate stoichiometry, shape, morphology and size of the HAp

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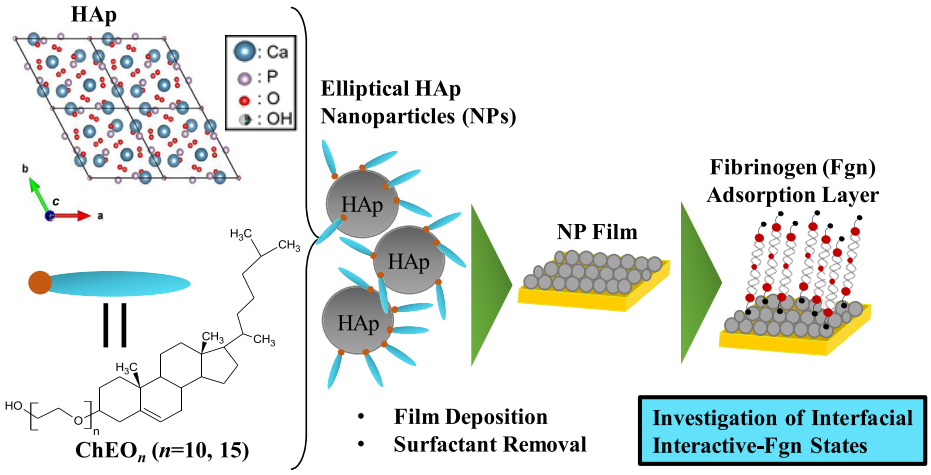

Scheme 1. Illustration of the investigation of the Fgn-interactive states on the HAp NP films of this study.

particles. The previous studies have reported that the HAp nanoparticles (HAp NPs) exhibited the enhanced resorbability and higher bioactivity as compared with the case of microparticles [19–21]. Therefore, the bio-interactive behavior of the HAp NPs in the body depends on their nanostructure and shape. Accordingly, the shape of the HAp NPs is thought to be important characteristic future.

It has been reported that HAp with the anisotropic and irregular shape often causes the inflammatory reactions in soft tissues and the bone formation was slower as compared with the spherical/ellipsoidal cases, suggesting that the use of rounded and smooth particles is preferable for filling tissue defects [13,22,23]. The spherical nanosized-HAp particles can contribute to the cell migration and extracellular matrix (ECM) growth through the vacancies that were formed between the particles [24,25]. Another advantage of the spherical/ellipsoidal particles is that they bear no risk for irritation or damage of mucosal layers of the cells [26,27]. The spherical/ellipsoidal microsized-HAp particles have been synthetized by a micro-emulsion method with the use of the surfactants [20,28]. Among the surfactants, polyethylene glycol, triethanolamine (TEA), ethylenediamine tetra-acetic acid (EDTA), sodium dodecyl sulfate (SDS), sodium dodecylbenzene sulfonate (SDBS), cetyltrimethylammonium bromide (CTAB) and (2-ethylhexyl) sulfosuccinate (AOT) have been the most commonly used. The disadvantage is that this method uses a large amount of oil and surfactant, and these phases cannot be recycled. The spherical/ellipsoidal microsized-HAp particles were synthesized in water-in-oil microemulsions consisting of cyclohexane as the oil phase, and a mixed poly (oxyethylene) nonyl phenol ether as the surfactant [28–30]. In contrast, the aqueous phase is the major phase in the emulsion, which can be formed by a small amount of oil and the surfactant phases [31]. Accordingly, this emerging method has the advantage of synthesizing the spherical/ellipsoidal microsized-HAp particles with less amount of oil and surfactant.

When the biomaterials are implanted in the human body, the proteins in the biological solution are adsorbed immediately on the surfaces [32,33]. During the interactions on the biomaterials at the biological environment, the proteins adsorbed on the surfaces determine the biocompatibility. The adsorbed proteins play an important role in the cell adhesion, grow and proliferation [32–34]. Quite a number of studies have clarified the factors involved in the adsorption layer (adlayer) of the proteins on the surface. Several factors such as charge, domain size, curvature, topography and wettability of the biomaterials affect the protein adsorption. The changes in the underlying the physicochemical properties could cause an alteration in the protein adlayer and affect directly the conformation and orientation of the protein, and the cell adhesion [35]. The cell adhesion is dominantly influenced by the fibrous glycoproteins of ECM, which are fibrinogen (Fgn), collagen, elastin, fibronectin (Fn), laminin, vitronectin, thrombospondins, and tenascins [32,37]. Fgn is the major plasma glycoprotein coagulation factor. This structural glycoprotein facilitates the adhesion, spreading and aggregation of the cells, which are important properties in the hemostasis process [38–40]. The adsorption amount and state of Fgn on the biomaterials such as the HAp NPs are an important parameter for improving the cell adhesion [41,42]. In addition, gold nanoparticles (AuNPs) has been used in the biomedical applications, including delivery vehicles for drugs and genes, imaging contrast agent and diagnostic sensors [43]. Diverse techniques have been used to obtain the information on the adsorption rate and the amount of adsorbed Fgn to determine the surface-bonding interactions and structures. Some useful techniques are the circular dichroism (CD), grazing angle Fourier transform infrared spectroscopy (GA-FTIR), fluorescence and electron spin resonance with spin labeling, quartz crystal microbalance with dissipation (QCM-D), atomic force microscopy (AFM) and surface plasmon resonance (SPR) [44–46]. Although the Fgn adsorption has been studied extensively focusing on the adsorption amount of Fgn, the protein–biomaterial interactions have not been completely understood yet. Thus, the investigation of the adsorption state of the Fgn on the biocmaterials is crucial for designing the biocompatibility.

In this study, the synthesis of the controlled elliptical HAp NPs in the presence of non-ionic surfactants [poly (oxyethylene) cholesteryl ethers, (ChEO*n*, *n* = 10, 15)] (Scheme 1) were investigated. The SPR studies on the Fgn adsorption on the HAp NP films were performed to clarify the bonding affinity and conformational changes with the adsorption. The secondary structures of the Fgn on the HAp NP films were also determined using the FT-IR spectral separation technique based on the deconvolution in the amide I band.

2. Experimental section

2.1. Synthesis of elliptical HAp NPs

Dipotassium hydrogen phosphate (K_2HPO_4, 99.0 wt%), calcium chloride dihydrate ($CaCl_2{\cdot}2H_2O$, 99.0 wt%), 20 vol% of tetramethylammonium hydroxide aqueous solution (TMAOH: $(CH_3)_4NOH$) and ethanol (C_2H_5OH, 99.5 wt%) as special grade chemicals were purchased from Wako Chemical Co., Ltd. The poly (oxyethylene)cholesteryl ether (ChEO*n* (n = 10 or 15): $C_{27}H_{45}(OCH_2CH_2)_n$) as special grade chemical was purchased from Nihon emulsion Co., Ltd. as shown in the chemical structure of Fig. S1, **ESM**. All the reagents of the present study were used without further purification.

In this study, five samples of the HAp NPs were synthesized.