Development of nitrogen-doped hydroxyapatite ceramics

Nao Kaneko, Yuhei Suzuki, Ryo Umeda, Ryota Namiki, Chihiro Izawa, Tomoko Ikeda Fukazawa, Michiyo Honda, Takahiro Takei, Tomoaki Watanabe and Mamoru Aizawa

Abstract

Hydroxyapatite (Ca$_{10}$(PO$_4$_3)(OH)$_2$; HAp) is widely used as a biomaterial due to its high biocompatibility. However, biological apatite present in bone and teeth contains various ions in the HAp crystal structure. Thus, biological apatite has many strains and defects, which may impart high osteoconductivity to apatite. To clarify the effects of the strain and/or defects in the HAp crystal structure on the bioactivity, nitrogen-doped (N-doped) HAp ceramics were fabricated by heating pure HAp ceramics. This N-doped method is well-known as a technique to modify the chemical structure at the surface. Some properties of the N-doped HAp ceramics were examined for optimization of the heating temperature. N-doped HAp ceramics fabricated by heating at 850°C in an NH$_3$ atmosphere had N$_2$O molecules in the crystal structure. Therefore, the N-doped HAp ceramics fabricated by the N-doping method have a rare molecule-substitution structure. In conclusion, we have developed the N-doped HAp ceramics which have a rare molecule-substitution structure.

1. Introduction

Hydroxyapatite (Ca$_{10}$(PO$_4$_3)(OH)$_2$; HAp) is widely used as a biomaterial in clinical applications due to its high biocompatibility and osteoconductivity. However, stoichiometric HAp is inferior with respect to bone formation in auto-grafting. As one of the methods to enhance the osteoconductivity of HAp, its chemical composition should be altered to achieve a molecular composition similar to biological apatite. It is also well-known that the HAp lattice can easily substitute cationic and anionic ions. Biological apatite in bone and teeth is substituted with various ions in the crystal structure. Thus, HAp crystals have much strain and many defects, and thus have more efficient bioactivity than pure HAp ceramics [1,2]. In previous reports, various ions, such as Si [3], Sr [4], Zn [5], Cu [6], Mg [7], and B [8], in bone mineral were substituted into the HAp structure.

In our previous study focused on cation doping, Yokota et al. [9,10] fabricated sodium- or potassium-substituted HAp. They have also successfully fabricated bone HAp ceramics, including bone minerals with six types of ions; Na$^+$, K$^+$, Mg$^{2+}$, F$^-$, Cl$^-$ and CO$_3^{2-}$. The presence of defects was confirmed, and biological evaluation in vitro and in vivo showed that these defects may promote early-stage bone formation [11–13].

As described above, a number of studies have reported the effects of cations in biological apatite [3–11]. An artificially obtained material may have promoted bioactivity by the effect of substituted ions; for example, magnesium ions promote bone formation [14]. On the other hand, there aren’t any reports of anion doping into HAp crystal. Thus, we have focused on anion doping to induce defects in HAp crystals.

Also, nitrogen-doping method is generally used to modify the surface of a material in the field of photocatalysis [15]. N-doping methods exchange oxygen in a metallic oxide for nitrogen. This method has been conducted using various processes, such as heating in an NH$_3$ atmosphere, which is widely used [15]. In this study, we attempted to apply the nitrogen doping method to HAp. It is expected that oxygen in HAp is replaced with nitrogen by the nitrogen-doping. Also, stains and defects in the HAp crystal structure caused by nitrogen doping can be expected to improve bioactivity.

In this study, the N-doped ceramics were fabricated under various heating temperatures during nitrogen doping, and examined some properties of the resulting ceramics. Moreover, the chemical species induced by N-doping method were determined using the N-doped HAp ceramics fabricated by the optimized temperature.

2. Experimental section

2.1. Fabrication of N-doped HAp ceramics

Commercially-available HAp powder (HAp-100, Taihei Chemical Industrial Co., Ltd.) was used. The HAp powder (1.0 g) was uniaxially compressed at 100 MPa to...
form pellets 20 mm in diameter and 11.5 mm thick. Pure HAp ceramics was fabricated by firing the compacts at 1200°C for 5 h in an air atmosphere. The surface of the pure HAp ceramics was polished to fix the surface roughness, and the ceramic pellets were then ultrasonically washed with ethanol and dried. The resulting ceramics are referred to as Pure HAp ceramics.

N-doping of Pure HAp ceramics was conducted at 750, 850 and 950°C to determine the most suitable heating temperature. The N-doped HAp ceramics were prepared by heating Pure HAp ceramics in an NH₃ atmosphere at 750, 850 and 950°C for 5 h. The resulting ceramics are referred to as N-doped (X °C) HAp ceramics, where X denotes the heating temperature.

As a control, Non-doped HAp ceramics without nitrogen species were also prepared by heating pure HAp ceramics in an air atmosphere at 750, 850 and 950°C for 5 h. The resulting ceramics are referred to as Non-doped (X °C) HAp ceramics, where X denotes the heating temperature.

### 2.2. Material properties of the N-doped HAp ceramics

Surface roughness of the resulting ceramics was measured by surface roughness measuring device (SURFTEST SV-3100, Mitutoyo Corporation, Japan). The relative density was calculated as the bulk density divided by theoretical density of HAp (3.16 g/cm³).

The crystalline phases of the resulting ceramics were identified using X-ray diffraction (XRD; Ultima IV, Rigaku Co., Japan) with Cu-Kα radiation. XRD data were collected under the following conditions: 2θ range of 10-50°, sampling width of 0.02°, and a scan rate of 1°/min. Crystalline phases were identified using the International Center for Diffraction Data Powder Diffraction File (ICDD-PDF) for HAp (#09-0432). The chemical state and depth profile of the N-doped HAp ceramics were examined using X-ray photoelectron spectroscopy (XPS; JPS-9000SX, JEOL, Japan) with Mg-Kα radiation. An Ar ion gun was used to obtain elemental depth profiles. The etching rate was 15 nm/min from 1 to 20 min [16]. Infrared absorption spectra of the ceramics were measured using attenuated total reflection (ATR) with a Fourier transform infrared spectrophotometer (FT-IR; IR Prestige-21, Shimadzu Co., Japan), in the range of 400–4000 cm⁻¹.

Surface zeta-potential was determined using a zeta-potential analyzer (ELSZ-2, Otsuka Electronics Co., Japan). Polystyrene latex particles coated with hydroxypropyl-cellulose were used as the monitor particles, which were diluted 200 times in 10 mmol·dm⁻³ NaCl aqueous solution. The measurement was performed using a laser Doppler method (based on electroosmosis profile estimation) at 37°C and pH 7.0 with a quartz solid-sample cell unit. The surface wettability was evaluated using a static contact angle meter (Simage03, Excimer Inc., Japan) by dropping ultrapure water onto the specimen surface and determining the surface wettability using the ATAN 1/2θ method. The contact angle was calculated using Eq. (1), where h is the height and r is the base radius of the droplet.

$$\theta = 2\tan^{-1}(h/r)$$  (1)

### 2.3. Determination of chemical species of nitrogen in N-doped HAp ceramics

N-doped and Non-doped HAp powders were prepared instead of bulk ceramics to clarify the chemical state of nitrogen in the N-doped HAp ceramics. The specific surface area of the powder is larger than that of bulk ceramics; therefore, the N-doped and Non-doped HAp powders were prepared as a model. Pure HAp powder was prepared by heating commercially-available HAp powder (HAp-100; Taihei Chemical Industrial Co., Ltd.) at 1200°C for 5 h in an air-flow atmosphere. N-doped HAp powder was prepared by heating this pure HAp powder in an NH₃ atmosphere at 850°C for 5 h; the resulting powder is referred to as N-doped (850°C) HAp powder. As a control, Non-doped HAp powder without nitrogen species was also prepared by heating pure HAp powder in an air atmosphere at 850°C for 5 h, the resulting powder is referred to as Non-doped (850 °C) HAp powder, where the X denotes the heating temperature.

Crystalline phases and lattice constants of the resulting powders were identified by the XRD technique. XRD data were collected under the following conditions: 2θ range of 10–50°, sampling width of 0.02°, and a scan rate of 0.1°/min. Crystalline phases were identified using the ICDD-PDF for HAp (#09-0432). FT-IR spectra of the specimens were obtained in the region of 400–4000 cm⁻¹ with a spectral resolution of 4 cm⁻¹ using specimens pellets prepared with KBr. The chemical species of nitrogen in the N-doped HAp powder were determined by thermogravimetry mass spectrometry (TG-MS; Thermo Mass, Rigaku, Japan). TG-MS analysis was performed at a heating rate of 10°C/min under a He atmosphere (300 cm³/min). The amount of sample is 50 mg and the Pt sample pan was used in this work.

### 3. Results and discussion

#### 3.1. Fabrication of N-doped HAp ceramics and their properties

The surface of the pure HAp ceramics was polished to fix the surface roughness. The values, mean ± SD, of the roughness Ra of ceramics were 1.443 ± 0.085 and 0.092 ± 0.033 μm before polishing and after polishing, respectively. Also, the surface roughness
of the Non-doped (850°C), N-doped (750°C), N-doped (850°C) and N-doped (950°C) HAp ceramics were 0.085 ± 0.013 μm, 0.071 ± 0.020 μm, 0.090 ± 0.023 μm and 0.088 ± 0.007 μm, respectively. Therefore, the surface roughness was not affected by nitrogen and non-nitrogen dopings, and the surface roughness was less than 0.1 μm among all of the examined specimens. Therefore, the influence of the surface roughness on the cells was eliminated by unifying the surface roughness to less than 0.1 μm.

The relative density of Pure HAp ceramics was 94.56 ± 0.66%. The relative density of the Non-doped (850°C), N-doped (750°C), N-doped (850°C) and N-doped (950°C) HAp ceramics were 95.61 ± 0.94%, 94.57 ± 1.44%, 93.91 ± 2.17% and 93.95 ± 2.25%, respectively. Therefore, after nitrogen and non-nitrogen dopings, the relative density of all specimens was on the order of 95%, which was not significantly different from the relative density of Pure HAp ceramics.

Figure 1 shows XRD patterns of the Pure HAp ceramics, the Non-doped HAp ceramics, and the N-doped HAp ceramics. The XRD patterns of all specimens indicated that the crystalline phase was a single phase of HAp, that is the crystalline phase of the N-doped HAp ceramics maintained a single phase of HAp after N-doping.

The chemical state of nitrogen at the surface of the N-doped HAp ceramics was analyzed using XPS, and the spectra are shown in Figure 2(a). The XPS spectra of all specimens showed that the peaks of O (23 and 531 eV), Ca (45, 347, 351, and 440 eV) and P (130 and 188 eV) could be assigned to typical HAp. Furthermore, in only N-doped, a trace peak of N (398 eV) was observed; therefore, the surface of the N-doped HAp ceramics contained nitrogen after N-doping. With a comparison of the specimens prepared at different heating temperatures revealed the nitrogen peaks of the N-doped (750°C) and N-doped (950°C) HAp ceramics were weak; however, that of the N-doped (850°C) HAp ceramics was the strongest among the examined specimens. Consequently, we reported that the heating temperature of 850°C was best for N-doping.

A depth profile of the N-doped (850°C) HAp ceramics was analyzed (Figure 2(b)). The etching rate of HAp was 15 nm/min. The peak of nitrogen became gradually weaker with the etching time and was detected until 10 min of etching. As a result, nitrogen was determined to be present until ca. 150 nm below the surface of the N-doped (850°C) HAp ceramics. The XPS peak for calcium was weak in the vicinity of the surface; however, it increased with a decrease in the nitrogen peak. The N-doped HAp ceramics incorporated nitrogen chemical species by N-doping; therefore, the HAp crystal structure was also assumed to have incorporated strain. This was considered to be the reason that the XPS peak for calcium was weak in the vicinity of surface. However, the peak for nitrogen disappeared and the intensity of the calcium peak became constant. As a result, nitrogen may be efficiently incorporated into HAp crystal at 850°C, and it is suggested that the HAp crystal structure has strain due to the presence of the doped nitrogen. Figure 3 showed typical absorptions for HAp. The bands around 3400–3800 cm⁻¹, especially at 3567 cm⁻¹, were attributed

![Figure 1](image_url). XRD patterns of HAp ceramics: (a) pure HAp, (b) Non-doped HAp (850°C), (c) N-doped HAp (750°C), (d) N-doped HAp (850°C) and (e) N-doped HAp (950°C).
The peak at 962 cm$^{-1}$ was attributed to $v_1$ PO$_4^{3-}$, those at 431 and 458 cm$^{-1}$ to $v_2$ PO$_4^{3-}$, those at 1046, 1058 and 1096 cm$^{-1}$ to $v_3$ PO$_4^{3-}$, and those at 568 and 602 cm$^{-1}$ to $v_4$ PO$_4^{3-}$ [12]. The peak at 2010 cm$^{-1}$ was only observed in the only N-doped HAp ceramics; this absorption resembles the N-N stretching vibration of N$_2$O. Therefore, this result shows that N-doped HAp ceramics contained nitrogen by N-doping.

Figure 4 shows results of the contact angle for each specimen. The contact angle of the Pure HAp ceramics was 54.45°. The contact angles of the N-doped (750°C), N-doped (850°C) and N-doped (950°C) HAp ceramics were 55.09°, 72.99° and 90.02°, respectively; therefore, the surface became more hydrophobic with an increase in the heating temperature. The contact angle of polystyrene plates used as a cell culture substratum was 75.39°, which is similar to that of N-doped (850°C) HAp. Therefore, it is considered that the N-doped (850°C) HAp ceramics would make a good cell culture substratum [18].

The surface zeta-potentials of the ceramics were measured at pH 7.0, and the results are shown in Figure 5. The zeta-potentials of the Pure HAp and N-doped (850°C) HAp ceramics were $-16.24$ and $-20.08$ mV, respectively. However, the zeta-potentials for the N-doped (750°C), N-doped (850°C) and N-doped (950°C) HAp ceramics were shifted to the more negative side, with values of $-23.23$, $-25.09$ and $-24.11$ mV, respectively. Compared with the Pure HAp ceramics, the potential energy of the N-doped HAp ceramics was negatively shifted. From the XPS results, the peak for calcium when the heating temperature was 850°C became weak in the vicinity of the surface. These results show that the amounts of calcium at the surface of the N-doped HAp ceramics decreased compared with that for the Pure HAp and Non-doped HAp ceramics. The surface potential of the N-doped HAp ceramics may have more negatively charged due to the changes of the surface charge by N-doping. Thian et al. have reported that a material surface with a negative charge promotes bone formation [19]. Therefore, the charge of the surface produced by N-doping may be effective for the promotion of bone formation.

### 3.2. Characterization of N-doped HAp powder

The chemical state of nitrogen in the N-doped HAp ceramics was examined using the N-doped HAp powder as a model. Two sample powders, i) N-doped (850°C) HAp powder and ii) Non-doped (850°C) HAp powder, were used. Figure 6 shows XRD patterns of the prepared powders. The crystalline phases of both the prepared
powders were identified to be HAp. Table 1 gives the lattice constants of the prepared HAp powders. There was almost no difference in the a-axis and c-axis lattice constants of the Pure HAp and Non-doped HAp powders. However, the lattice constant of the N-doped HAp powder was lower than that of the Pure HAp and Non-doped HAp powder. The change in the lattice constant after N-doping indicated a chemical species may be induced in the HAp crystal by N-doping.

FT-IR spectra of the prepared powders were measured to determine the functional groups in the N-doped HAp powder. There was almost no difference in the a-axis and c-axis lattice constants of the Pure HAp and Non-doped HAp powders. However, the lattice constant of the N-doped HAp powder was lower than that of the Pure HAp and Non-doped HAp powder. The change in the lattice constant after N-doping indicated a chemical species may be induced in the HAp crystal by N-doping.

FT-IR spectra of the prepared powders were measured to determine the functional groups in the N-doped HAp powder and the results are shown in Figure 7. The FT-IR spectra showed that the absorptions of OH\(^{-}\) and PO\(_4^{3-}\) groups could be assigned to typical HAp. A strong sharp band at 3567 cm\(^{-1}\) is assigned to OH\(^{-}\) ions in the crystal lattice. The bands at 1,096,962 cm\(^{-1}\) and 602–431 cm\(^{-1}\) were assigned to the stretching and deformation vibration of PO\(_4^{3-}\) ions, respectively [17]. Furthermore, the absorption at 2010 cm\(^{-1}\) was observed in only the N-doped HAp powder, as with the N-doped HAp ceramics; the band at 2010 cm\(^{-1}\) resembles the N-N stretching vibration of N\(_2\)O.

Figure 8(a and b) shows mass spectrometry analysis of the gas released from the Non-doped (850°C) and N-doped (850°C) HAp powders, respectively. The m/z = 44 signal was observed in both specimens at 400°C or lower, which may be due to the presence of CO\(_2\) adsorbed on the surface of specimens. A comparison of the N-doped and Non-doped HAp powders (Figure 8(b)) revealed the release of gas with m/z = 44 was detected above 850°C from only the N-doped (850°C) HAp powder. This released gas is considered to originate from a chemical species in the HAp crystal structure. In
addition, the signal of $m/z = 30$ was also observed above 850°C in only the N-doped HAp (850°C) powder, which corresponds to NO molecules. NO molecules may be formed by the decomposition of $N_2O$ molecules. According to NIST Standard Reference Database Number 69, the highest intensity of mass spectrum of $N_2O$ is $m/z = 44$, following $m/z = 30, 14, 28$ and 16. The

| Sample powder       | Lattice parameters | Lattice volume |
|---------------------|--------------------|----------------|
|                     | $a$-axis (nm)      | $c$-axis (nm)  | $nm^3$ |
| Pure HAp            | 0.94183(7)         | 0.6881(8)      | 1.580  |
| Non-doped (850°C)   | 0.94158(7)         | 0.68798(7)     | 1.579  |
| N-doped (850°C)     | 0.94069(14)        | 0.6873(15)     | 1.575  |

Figure 6. XRD patterns of HAp powders: (a) pure HAp powder, (b) Non-doped HAp (850°C) powder and (c) N-doped HAp (850°C) powder.

Figure 7. FT-IR spectra of HAp powders: (a) pure HAp powder, (b) Non-doped HAp powder and (c) N-doped HAp (850°C) powder.
gases with $m/z = 44$ and $m/z = 30$ were detected in only the N-doped HAp powder, which indicates that N$_2$O molecules may be incorporated into the HAp crystals by N-doping. This result is consistent with the FT-IR assignment to the N-N stretching vibration. On the basis of these results, it is considered that N$_2$O molecules are incorporated into the HAp crystal structure by the N-doping method.

A limited number of studies have reported on molecular-trapping into HAp crystals. For instance, Ito et al. [20] reported that the BO$_2$ group of boron apatite was trapped in defects of OH sites in HAp crystals. The BO$_2$ molecule has a linear triatomic structure and the B-O bond length is 0.1277 nm. It is considered that the CO$_3^{2-}$ groups in carbonate apatite are substituted with the OH or PO$_4^{3-}$ ions. The CO$_2$ molecule has a linear triatomic structure and the C-O bond length is 0.11600 nm. Therefore, the bond length of the CO$_2$ molecule is close to that of BO$_2$ molecule, and the chemical structures are similar. It has thus been determined that CO$_2$ molecules can be substituted to OH sites of HAp crystals. In the present study, we suggested that the N$_2$O molecule also has a linear triatomic structure. The N-O bond length is 0.120 nm and the N-N bond length is 0.112 nm. Thus, the N$_2$O molecule has a similar structure to both CO$_2$ and BO$_2$ molecules. N$_2$O molecules could thus be incorporated into the HAp crystal structure. In this study, although we aimed to substitute anions into the HAp crystal by anion doping, it was determined that the present N-doped HAp ceramics incorporate N$_2$O molecules.

Figure 8. Mass spectrometry analysis of the gas released from (A) Non-doped (850°C) powder and (B) N-doped (850°C) HAp powders, respectively. (C) A comparison of the released gas of $m/z = 44$ and $m/z = 30$ from the (a) Non-doped (850°C) and (b) N-doped (850°C) HAp powders.
Therefore, the N-doped HAp ceramics fabricated by the N-doping method have a rare molecule-substitution structure.

4. Conclusion

N-doped HAp ceramics were fabricated by the N-doping method, and some material properties were examined. XPS spectra indicated that the amount of nitrogen in the N-doped HAp ceramics heated in an NH3 atmosphere at 850°C was the highest among the examined specimens. Nitrogen was also found to be present from the surface of the resulting ceramics to a depth of 150 nm in the N-doped (850°C) HAp ceramics. In addition, the N-doped HAp ceramics had strain due to the incorporation of chemical species. TG-MS measurements indicated that N2O molecules are incorporated into the HAp crystal structure by N-doping. In conclusion, it was clarified that the N-doped HAp ceramics have strain and defects due to the incorporation of N2O molecules. It should be noted that N-doped HAp ceramics have a rare molecule-substitution structure. In near future, we will report the in vitro assays for the adhesion, proliferation and osteogenic differentiation ability of bone marrow stromal cells cultured on the present N-doped HAp ceramics.

Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID
Tomoko Ikeda Fukazawa http://orcid.org/0000-0002-5265-4706

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