Change in the morphology of hydroxyapatite nanocrystals in the presence of bioaffinitive polymeric species under the application of electrical field

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Abstract

Application of the external electrical field during the precipitation of hydroxyapatite (HAP) nanoparticles in the presence of bioaffinitive polymeric species, individual crystallites as well as their coherent agglomerates increases their anisotropy with the increasing aspect ratio of the crystallites, $L_c/L_a$ or of aggregated particles, $d_c/d_a$. The tendency was quite similar when we change the polymeric species between gelatin (GLT) and sodium salt of hyaluronic acid (HYA), although the extent of change is larger for GLT as compared to HYA, presumably due to stronger polymer–HAP interaction in case of HYA. External electrical field often causes severe agglomeration to fairly isotropic particles with substantial loss of the HAP crystallinity. This might be attributed to the strong ionic interaction between and COO$^-$ group of HYA, which is not the case with GLT.

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1. Introduction

In the light of increasing demands for bone substitutes and related biomaterials, a majority of the research studies aim at preparing artificial bones with lightweight, high strength and toughness. At the same time, there are different concepts of bone substitutes, i.e. to repair with minimum torture of the patients to use fluids, e.g. by injection [1].

In a series of studies carried out in the authors’ laboratory with respect to the nanocomposites comprising hydroxyapatite nanoparticles and silk fibroin (SF) [2], preparation and properties of nanocomposite fluids centered our interests. Strong affinity of the matrix polymeric species to the biological tissue, i.e. bioaffinity, is required for the present nanocomposite fluids. Out of a number of factors to improve the morphology of HAP nanoparticles and their chemical interaction with SF, we have also applied external electrical field during the preparation of the composites.

As for the application of the external electrical field to HAP ceramics, one of the directions is poling treatment to improve the bioaffinity, mainly by using cell cultivation methods [3,4].

Electrical stimuli are known to promote formation of hard and soft tissues as well [5]. It is also important to mention that electrical field is actually exerted in the animal body in the course of bone growth [6].

We actually examined preliminarily the effects of external electrical field on the system comprising HAP and GLT [7]. What we have observed were among others: (1) increase in the crystallinity and anisotropy of HAP nanoparticles, and (2) enhanced chemical interaction between HAP and GLT.

We here try to extend the above preliminary study by using HYA, which is closer to the actual biological soft tissues than GLT. We also compare the role of GLT and HYA, in order to elucidate, whether and to what extent the results obtained in our previous study could be extended and generalized.

2. Experimental

An aqueous solution of $\text{H}_3\text{PO}_4$ (Taisei Chemicals, purity, 85.15%) was added into an aqueous suspension of Ca(OH)$_2$ (Wako, purity, 97.7%) including GLT or HYA for 30 min. The mixtures were left standing for 60 min at room temperature under stirring with or without electric field. The electrical field (EF) was kept constant at 430 V/cm. We chose the condition, 10 Hz AC, based on our previous study with GLT [7]. Sample names with the concentrations of HAP in the nanocomposites are summarized in Table 1.
Crystallographical properties were determined by X-ray diffractometry (XRD) with Rietveld refinement [8]. Morphological properties were observed by transmission electron microscopy (TEM). Chemical interaction between HAP and HYA was examined by FT-IR. Dielectric constant was also obtained as a measure of possible change in the polarization of the products.

3. Results and discussion

3.1. Crystallographical properties

Table 2 shows the size of crystallites and their agglomerates. All the crystalline products were phase pure HAP. By applying EF, aspect ratios were increased. These coincided with the size of TEM images, as displayed in Fig. 1. In the system HAP–GLT, the size of aggregate became smaller by applying EF [7], contrary to the present HAP–HYA system.

Crystallinity and lattice distortions of samples are also given in Table 2. The crystallinity was defined relative to the well-crystallized, commercially available HAP (Mitsui Toatsu Chemicals). The lattice distortion was obtained from XRD after Rietveld analysis. In the HAp-GLT nanocomposite, the crystallinity was increased and the lattice distortion was decreased by applying EF, while in the HAP–HYA system, the crystallinity was decreased and the lattice distortion was increased.

It is also possible that the morphology of HAP in the nanocomposites is dominated by the molecular weight or chain length of the coexisting polymeric species. Note that the average chain lengths of GLT and HYA are ca. 30 and 1600 nm, so that the number of the HAP particles per polymer molecule is much larger in the case of HYA than GLT.

3.2. Particulate morphology

Fig. 1 shows TEM images of HAP in the nanocomposite with HYA. The size and aspect ratio ($d_c/d_a$) of the particles are also shown in Table 2. What we observed under TEM are the anisotropically agglomerated HAP particles. By applying EF, the aspect ratio was increased from 5.36 to 7.52, i.e. by 40% for YH10 and YH10E.

3.3. Interaction between HAP and HYA

FT-IR spectra of HAP–HYA composites after deconvolution are shown in Fig. 2. The peaks from amide I, amide II and carboxyl group in HYA were observed. By compounding with HAP, amide I peak at 1671 cm$^{-1}$ shifted to lower wave-number at around 1662–1658 cm$^{-1}$, amide II and carboxyl group peaks at 1549 and 1610 cm$^{-1}$ moved to higher wavenumber at 1562 and 1621–1625 cm$^{-1}$. This is explained by assuming the formation of coordination bonds between Ca$^{2+}$ of HAP and C=O and carboxyl groups of HYA.

### Table 1

| Sample names | HYA–HAP nanocomposite | HYA concentration, 0.05 wt% |
|--------------|------------------------|----------------------------|
| GH           | GLT–HAP nanocomposite  | GLT concentration, 1 wt%   |
| – 10         | HAP concentration, 1.0 wt% |
| – 25         | HAP concentration, 2.5 wt% |
| – E          | Without electric field |
| –           | With electric field     |

### Table 2

| Sample | Crystalline particle | Aggregated particle | Crystallinity (%) | LD (a) (%) | LD (c) (%) |
|--------|----------------------|---------------------|-------------------|------------|------------|
|        | $L_a$ (nm) | $L_c$ (nm) | $L_c/L_a$ | $d_a$ (nm) | $d_c$ (nm) | $d_c/d_a$ | $L_a$ | $L_c$ | $L_c/L_a$ | $d_a$ | $d_c$ | $d_c/d_a$ |
| GH25   | 6.8     | 14      | 2.1      | 34.7 | 155 | 4.67 | 51.9 | 0.71 | 0.97 |
| GH25E  | 4.7     | 15      | 3.2      | 30.6 | 190 | 6.21 | 56.4 | 0.66 | 0.83 |
| YH10   | 5.8     | 17      | 2.9      | 42.0 | 225 | 5.36 | 63.4 | 1.3  | 1.3  |
| YH10E  | 5.5     | 18      | 3.3      | 29.4 | 221 | 7.52 | 57.2 | 0.70 | 0.71 |
| YH25   | 6.1     | 18      | 3.0      | 29.5 | 243 | 8.24 | 80.0 | 0.87 | 0.56 |
| YH25E  | 6.4     | 21      | 3.3      | 35.1 | 285 | 8.12 | 72.6 | 1.03 | 0.66 |

LD, lattice distortion

Fig. 1. Primary aggregate image of HAP–HYA with or without electric field (TEM).

Fig. 2. IR spectra of HAP–HYA with or without electric field.

150015501600165017001750 Transmittance[a.u]
Wavenumber[cm$^{-1}$]

Amide I
COO$^-$
Amide II

1671
1662
1659
1610
1549
1562
1621
1625
3.4. Polarization

Dielectric constants of HAP–HYA composite sol were measured. The values at 1 MHz are summarized in Table 3. In the composites with low HAP concentration (YH10, YH10E), change was insignificant. While in the composites with high HAP concentration (YH25, YH25E), the dielectric constant of HAP–HYA was increased by ca. 40% by applying EF, i.e. to the similar extent to the aspect ratio. Thus, polarization of HAP–HYA composite is predominated by HAP, as in the case of the composite system with GLT [7]. Polarization mechanism of HAP in the case of poling the sintered HAP is known to be the self-organization of the polaron H\(^+\)–O\(^2–\) as a consequence of the successive proton migration into the proton vacancy around O\(^2–\) [9]. The possibility that the polarization of OH\(^–\) parallel to the c-axis is promoted was suggested in the HAP-GLT composite [7]. If polarization was promoted in the crystalline as described above, electrical charge may be located at the end of c-axis. This enhances anisotropic growth to increase the aspect ratio. Apparent parallelism of the increase of dielectric constant and aspect ratio is, then, quite reasonable.

4. Conclusions

By applying external electrical field during the precipitation of hydroxyapatite nanoparticles in the presence of HYA, individual crystallites as well as their coherent agglomerates increase their anisotropy with the increasing aspect ratio of the crystallites, \(L_c/L_a\), or of aggregated particles, \(d_c/d_a\). The increase in the aspect ratio parallels that of dielectric constant. The tendency was quite similar when we change the polymeric species to GLT, although the extent of the change is larger for GLT than for HYA, presumably due to stronger interaction between the HYA and HAP.

External electrical field often causes severe agglomeration with substantial loss of the HAP crystallinity. This might be attributed to the strong ionic interaction between and COO\(^–\) of HYA, which is not the case with GLT. Possible role of the molecular weight was also suspected.

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References

[1] J.F. Mano, R.A. Sousa, L.F. Boesel, N.M. Nevesand, R.L. Reis, Bioinert, biodegradable and injectable polymeric matrix composites for hard tissue replacement: state of the art and recent developments, Compos. Sci. Technol. 64 (2004) 789–817.
[2] L. Wang, R. Nemoto, M. Senna, Changes in microstructure and physico-chemical properties of hydroxyapatite–silk fibroin nanocomposite with varying silk fibroin content, J. Eur. Ceram. Soc. 24 (2004) 2707–2715.
[3] S. Nakamura, M. Nakamura, T. Kobayashi, Y. Sekijima, S. Kasugai, K. Yamashita, Osseous cell response to electrostatic stimulations of poled hydroxyapatite ceramics in canine diaphyses, Bioceramics 16 (2004) 849–852.
[4] M. Ueshima, S. Nakamura, M. Ohgaki, K. Yamashita, Electrovectorial effect of polarized hydroxyapatite on quasi-epitaxial growth at nano-interfaces, Solid State Ionics 151 (2002) 29–34.
[5] H. Ito, Mechanical stress for the bone and soft tissues, J. Nippon Med. Sch. 69 (2002) 146–148.
[6] F.B. Sachse, C.D. Werner, Development of a human body model for numerical calculation of electrical fields, Comput. Med. Imaging Graph. 24 (2000) 165–171.
[7] N. Shiba, M. Senna, Effects of electric field on the nuclei-growth processes of hydroxyapatite for bioaffinitive nanocomposites, Trans. Mater. Res. Soc. Jpn 29 (2004) 3431–3434.
[8] F. Izumi, T. Ikeda, A Rietveld analysis program RIETAN-98 and its applications to zeolites, Mater. Sci. Forum 198 (2000) 321–332.
[9] S. Nakamura, H. Takeda, K. Yamashita, Proton transports polarization and depolarization of hydroxyapatite ceramics, J. Appl. Phys. 89 (2001) 5390–5391.

| Dielectric constants of HAP–HYA with or without electric field |
|---------------------------------|-----------------|-----------------|-----------------|
| YH10 | YH10E | YH25 | YH25E |
| 94.2 | 93.5 | 87.2 | 130 |