

## Effects of electric field on the nuclei-growth processes of hydroxyapatite for bioaffinitive nanocomposites

Naoko Shiba and Mamoru Senna

Keio University, 3-14-1 Hiyoshi, Yokohama 223-8522, Japan

Fax: 81-45-564-0950, e-mail: [senna@applic.keio.ac.jp](mailto:senna@applic.keio.ac.jp)

DC and AC electric field (EF) at 600V/cm was applied during preparation of bio-affinitive nano-composites comprising hydroxyapatite (HAp) nanoparticles and gelatin (GLT). Changes in the crystallinity and polarization behavior by applying EF were discussed in terms of X-ray diffraction data refined by Rietveld analysis and Cole-Cole plots from dielectric measurement, respectively. From the former analysis we confirmed the decrease of crystallite size and the increase of aspect ratio of HAp-GLT composite by applying low frequency, particularly DC. When low frequency was applied during preparation of HAp-GLT composites, contributions to the dielectric properties appeared in two components and activated the interaction between HAp and GLT. These results enable us to characterize chemical interaction in the present nanocomposites and accordingly, to optimize the new preparation method for nanocomposites under EF in conjunction with the chemical and electrical properties of the nanocomposite products.

Key words: bio-affinitive nano-composites, hydroxyapatite, gelatin, electric field

### 1. INTRODUCTION

Substitutes of hard and soft tissues in the human body attract interests in conjunction with regeneration treatment. Electric stimulation may stimulate osteogenesis or osteoanagenesis [2], in favor of the treatment technique against complex fracture or anosteoplasia. However, studies on the junctions in both tissues, e.g., those between teeth and gingivae are scarce, and there are no example of reports of regeneration treatment using the composite sol. We focus in this study on the compounding of materials to fill the space between hard and soft tissues, i.e., hydroxyapatite  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$  (HAp) - gelatin (GLT) nano-composite sol. We also examine the effects and the mechanism of polarization with HAp and particular emphasis on the morphology of HAp nanoparticles and their interaction with GLT.

### 2. EXPERIMENTAL

GLT powders were added in water and swelled for 10min at room temperature. Then the samples were put into a water bath at 50°C to complete dissolution for 20min. Preparation of the composite sol were started by adding 2.6wt% aqueous solution of  $\text{H}_3\text{PO}_4$  (Taisei Chemicals, purity, 85.15%) into 2.75wt% aqueous suspension of  $\text{Ca}(\text{OH})_2$  (Wako, purity, 97.7%) with 7.0wt% GLT while keeping the initial Ca/P molar ratio constant at 1.67, i.e., the stoichiometry of HAp. After standing for 30min at room temperature under vigorous stirring, ac or dc electric field of 600V/cm were applied for 1h. The mixture without GLT was also prepared. The composite sol was vacuum-dried at room temperature for 24h for further characterization. Sample names are summarized in Table 1.

X-ray diffractometry (XRD), dielectric constant measurement, and transmission electron

Table 1 Sample names

GLT	G-
HAp-GLT composite	HG-
Non-electric field	E0
DC electric field	DC
AC electric field(10Hz)	AC10
AC electric field(25Hz)	AC25
AC electric field(50Hz)	AC50
AC electric field(500Hz)	AC500
AC electric field(1000Hz)	AC1000

microscope (TEM) were used as analytical tools. Rietveld analysis of the collected XRD data was performed with RIETAN-2000 [3].

### 3. RESULTS AND DISCUSSION

Fig.1 shows dielectric constants of HAp-GLT composite sol. Polarization is promoted by compounding. The  $\Delta\epsilon'$  value of HG-E0 is more than twice and a half as large as those of unitary system, of either HAp or GLT. Polarization is promoted by applying EF, particularly DC. The  $\Delta\epsilon'$  value of HG-DC is more than four times as large as that of HG-E0.

Fig.2 shows Cole-Cole plots of HAp-GLT

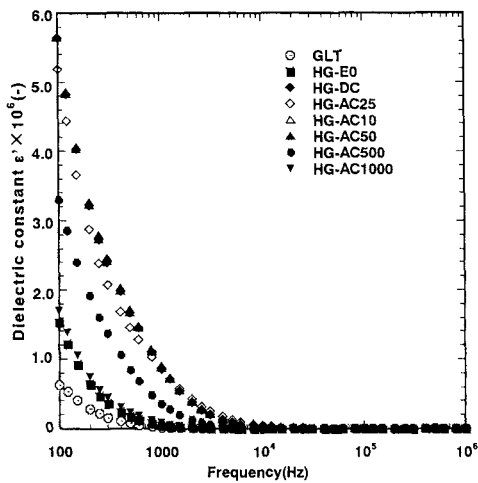


Fig.1 Dielectric constant of HAp-GLT with or without electric field

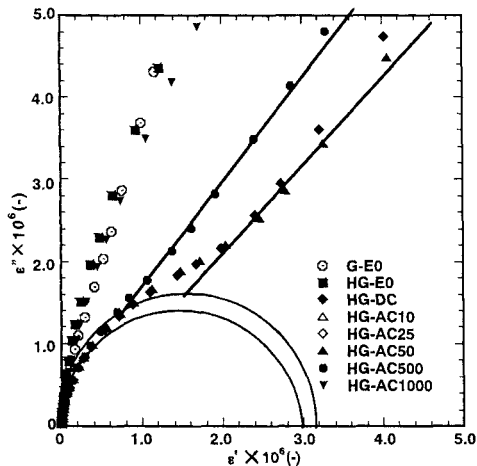


Fig.2 Cole-Cole plots of HAp-GLT with or without electric field

composite sol. A hemi-circular element is observed only for HG-DC, -AC10, -AC25, -AC50, while almost linear relation is exhibited for all the other samples. The former indicates two distinct polarization components, HAp and GLT. The smooth junction between the two components is presumably due to the chemical interaction between these polar species.

Fig.3 shows FT-IR spectra of the samples and Fig.4 shows the relationship between the shift of amide I and amide II,  $\Delta\epsilon''$  and the EF frequency. Amide I and Amide II peaks at  $1639$  and  $1531\text{cm}^{-1}$  moved to higher frequency at  $1658$  and  $1551\text{cm}^{-1}$  by compounding HAp and GLT and lower frequency at  $1652\sim 1654$  and  $1550\text{cm}^{-1}$  by applying EF. These peaks of HG under applied EF brings closer to the peaks of HG-E0 with increasing the EF frequency and Amide I peak of HG-DC moved to the lowest frequency from  $1658\text{cm}^{-1}$  to  $1652\text{cm}^{-1}$ . From the former, the polarization species cannot follow up to the speed

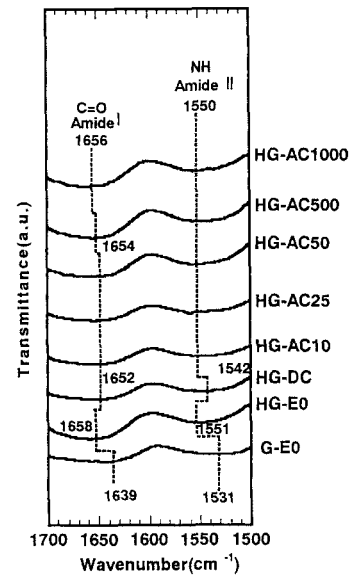


Fig.3 FT-IR spectra of GLT and HAp-GLT

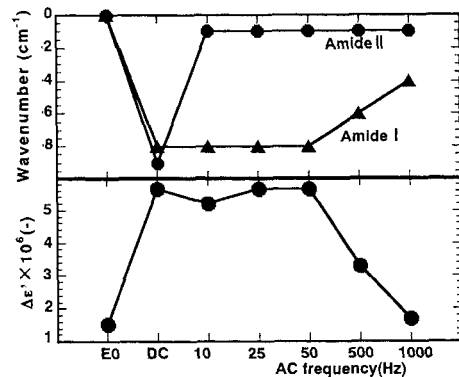


Fig.4 The relationship between FT-IR shift,  $\Delta\epsilon''$  and frequency of applied electric field

of changing positive and negative poles as the frequency becomes higher. The latter is explained by assuming that  $\text{Ca}^{2+}$  of HAp coordinates C=O of GLT [4]. This, in turn, decreases the polarizability of  $\text{O}^{2-}$ . Therefore, the change of Amide I shift is similar to that of  $\Delta\epsilon''$ .

Peak separation was performed as shown in Fig. 5, on the XRD diffraction profile to evaluate the change of the crystallinity of HAp by the EF treatment.  $\text{CaF}_2$  (Kanto, Guaranteed grade) was used as an internal standard and the crystallinity was defined relative to the well-crystallized HAp (Mitsui Toatsu Chemicals). Fig.6 shows the crystallite size of the samples obtained from XRD after Rietveld refinement. All the crystalline products were phase pure HAp.

The crystallinity of HAp was increased from 33% to about 45% by the EF treatment, particularly by DC. The decrease in the crystallite size and the aspect ratio was the most remarkable for low frequency. The relative crystallite number

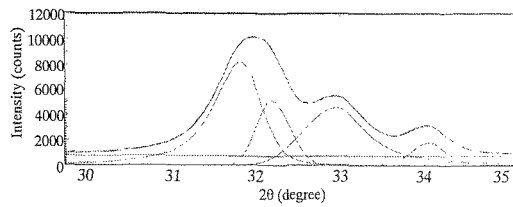


Fig.5 Peak separation of XRD

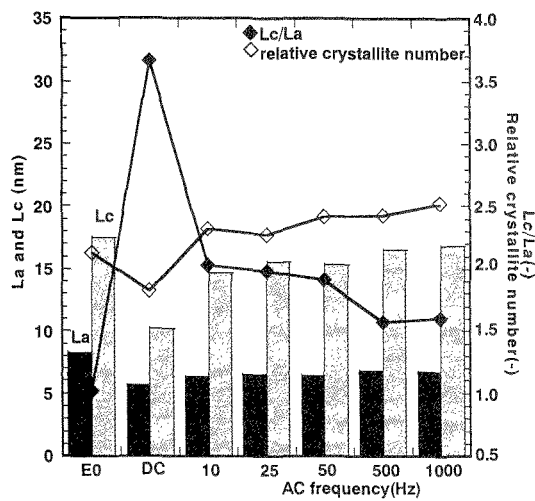
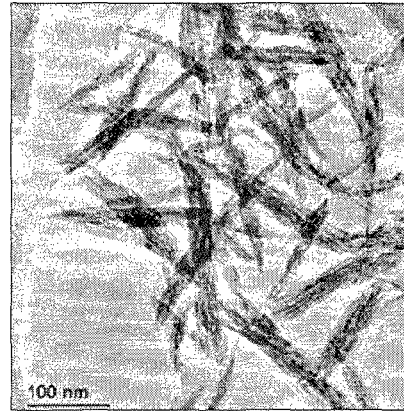


Fig.6 The relationship between crystallite size, relative crystallite number and frequency of applied electric field

was increased by EF, particularly by DC. The increased nucleation probability by DC can be expected. Increase in the polarity of C=O of the main chains of GLT favors the interaction with  $\text{Ca}^{2+}$  of HAp [4], triggering the nucleation of HAp.

Fig.7 shows TEM images of HAp-GLT. Fig.8 shows the size and aspect ratio (c-axis/ a-axis) of the aggregates. The aggregates are needlelike with their aspect ratio between 5.2 and 7.4. The aggregate aspect ratio and the average size were increased by EF application. The increase was the most remarkable for HG-DC.

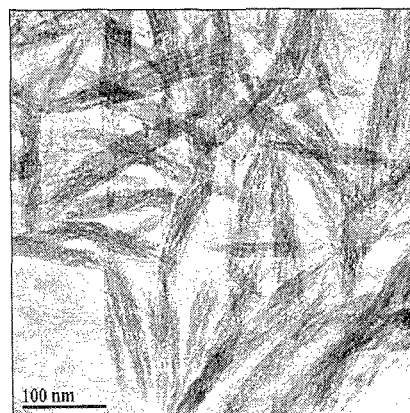
Polarization mechanism of HAp in the case of poling the sintered HAp is known to be the self-organization of the polaron  $\text{H}^+ - \text{O}^{2-}$  as a consequence of the successive proton migration into the proton vacancy around  $\text{O}^{2-}$  [5]. There is no guarantee whether a similar mechanism works in the present case. In case of DC where positive and negative poles are fixed, the polarization of OH<sup>-</sup> parallel to the c-axis is promoted and the crystallites can be connected through the OH<sup>-</sup> column. The growth of the crystallites is promoted in this manner in the c-axis direction for the aggregated crystallites to become needlelike. Based on the hypothesis of proton polarization, we reasonably expect the significant role of associated hydrogen bonds. Larger value



HG-E0

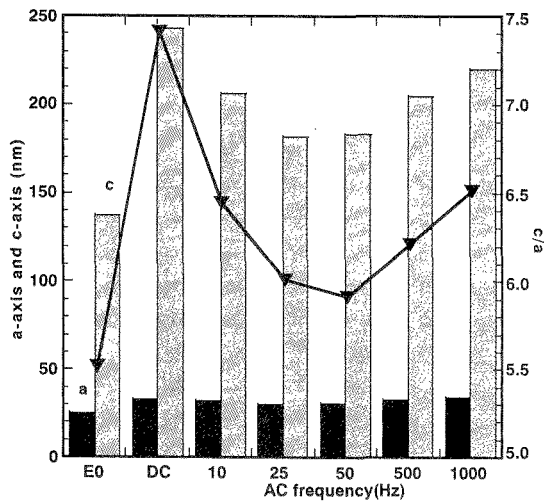


HG-DC



HG-AC1000

Fig.7 TEM images of HAp-GLT with or without electric field



**Fig.8** The relationship between the size of the aggregates and frequency of applied electric field

of  $\Delta\epsilon'$  for the sample HG-DC is ascribed to the longer OH – OH bond length [6]. This may explain the formation of aggregates with higher aspect ratio for the sample HG-DC.

From the foregoing, the author concludes that the EF application during HG nanocomposites preparation promotes polarization of composites, elevates the interaction between HAp and GLT and hence, controls the morphology of composites. These favor applications in the regeneration treatment.

#### 4. REFERENCES

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