Effect of Surface Properties of ABC (Apatite-Biologically Active Glass Composite) on Bending Strength

Masaaki MOCHIDA, Takamitsu FUJIU and Makoto OGINO
(Biomedical Engineering R & D Center, Nippon Kogaku K. K., 1773, Asamizodai, Sagamihara-shi 228)

ABC（アパタイト-生体活性ガラス複合体）の表面の性質が弯曲強度に及ぼす影響

すみ田昌昭・藤生尚光・荻野 誠
（日本光学工業（株）医用材料事業推進室）

Apatite-biologically active glass composite (ABC) was synthesized and its microstructure, bioactivity and bending strength were studied. The existence of fluorapatite (FAp), Na2Ca2Si3O9 and the remainder glass was confirmed by XRD and SEM. From in vitro experiment, rapid formation of stable FAp film in 7 days was observed by IRRS, SEM and EPMA. Bending strength of ABC increased slightly with treatment time in trisaminomethane buffer solution to 30 days while under similar condition the strength of sintered hydroxyapatite (HAp) decreased gently. The increase of bending strength of ABC was closely related with the surface ion migration to form FAp. Radical crack tip blunting due to ion migration and/or stress releasing around tip was considered to be the main reason. From in vivo experiment, the bone bonding strength of ABC was 27 MPa, higher than that of sintered HAp. The bone bonding strength, in this case, is equivalent to the fracture shear strength between bone and material after implantation in rabbit femur during 8 weeks. Furthermore, just the same as the result of in vitro experiment, FAp film formation on the surface of ABC was also observed in vivo, which suggested the possibility of slight increase of bending strength in living body. For clinical application as bone replacement or tooth implant, possible advantage of ABC regarding bending strength is expected to be (1) strength increase and (2) strength recovery after introduction of a new crack, during implantation in living body.

Key-words: Hydroxyapatite, Fluorapatite, Biologically active glass, Composite, In vitro experiment, In vivo experiment, Surface reaction, Bone bonding

1. Introduction

Biomaterials are classified into two main groups: the first consists bioinert materials while the second includes bioactive implant material. Bioinert material does not have so strong connective interaction with bone tissue as bioactive material. Bioactive material makes tight chemical bonding with bone.\(^{13}\) Biologically active glass (BAG) is well known for its high bone bonding strength.\(^ {1-3}\) On the other hand, it has disadvantage about its mechanical strength, as glass is a brittle material. Hydroxyapatite (HAp) is the most popular as bioactive material. However, as we reported,\(^ {5}\) its value of bone bonding strength is almost 70 % of BAG (4S S 5, see Table 1). The mechanical strength of sintered HAp is not high enough for some applications, either.\(^ {5}\) As to the clinical application, these bioactive materials are expected to apply for dental implant or bone tissue replacement where the load is moderate. Furthermore, when these bioactive materials are combined with metals or polymers, the materials are considered to possess high mechanical and bone bonding strength.

The mechanical stability of bioactive material in the body fluid is one of the significant problems in clinical applications.

For the purpose of possessing both high bone bonding strength and high mechanical strength, we developed a bioactive material ABC (Apatite-BAG composite) by sintering the mixture of HAp and BAG. ABC had both good bending and bone bonding strength.\(^ {6,7}\) In the present study, we attempted to clarify the micro-

<table>
<thead>
<tr>
<th>Table 1. Compositions of BAG (mol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{SiO}_2 )</td>
</tr>
<tr>
<td>( \text{Na}_2\text{O} )</td>
</tr>
<tr>
<td>( \text{CaO} )</td>
</tr>
<tr>
<td>( \text{CaF}_2 )</td>
</tr>
<tr>
<td>( \text{P}_2\text{O}_5 )</td>
</tr>
</tbody>
</table>
structure, bioactivity of ABC and investigated the effect of surface reactions on bending strength. The microstructure, the results of in vitro and in vivo experiments and the improvement of strength after in vitro experiment will be discussed comparing with sintered HA.

2. Experimental procedures

2.1 Synthesis of ABC

The chemical compositions of BAG are shown in Table 1. Using reagent grade chemicals, 45SF1/4 was melted in a platinum crucible at 1350°C. After pouring into mold, the glass was pulverized into grains under 200 mesh (smaller than 75 μm). HAp was prepared by the reaction of Ca(OH)₂ and H₃PO₄ in aqueous solution. The powders of BAG and HAp were mixed in 1 : 1 weight ratio, and re-pulverized with ethanol into 3 μm in mean particle diameter. After dried at 100°C, the mixed powder was pressed into a rectangular parallelepiped with a pressure of 100 MPa. The compact was heated at 960°C for 1 h, and annealed at a rate of 100°C/h to room temperature.

2.2 Structure analysis

Phase composition of the sintered body was determined by X-ray powder diffraction method (XRD). The microstructure of ABC was examined by scanning electron microscope (SEM) on the specimens after treatment in water at 100°C for 10 min.

2.3 Strength measurement

The pieces of the sintered ABC and HAp (Central Glass Co., Ltd.) materials were cut into the prisms of 4 × 4 × 10 mm, polished into #1000 finish by sand paper, and bending strength was measured using three-point bending test at a cross-head speed of 0.5 mm/min in air atmosphere. Bending strength of ABC, HAp and also some specimens immediately after in vitro experiment (without drying its surface) was measured in three-point bending test. For each test, the number of tested specimens was 16.

2.4 In vitro experiment

The specimens of ABC and HAp materials were soaked in trisaminomethane buffer solution (TBS) at 37°C. The pH was always adjusted 7.3. The ratio of surface area of each specimen to volume of the solution was kept at a constant value of 0.20 cm⁻² in each case. Respectively after 7 and 30 days of treatments described above, the surface of individual specimens was measured using IRRS technique. Then all tested specimens were cut perpendicular to the soaked surface and new-cut surfaces were analyzed by SEM and electron probe microanalysis (EPMA). The aim of this experiment was to observe the element distribution near the surfaces. The concentration of ions leached in TBS after in vitro experiment was measured using atomic absorption analysis (AAA).

2.5 In vivo experiment

After 8 weeks of implantation in rabbit femurs, the fracture shear strength between bone and ABC was measured by push out test. For the specimen implanted in canine mandible during 6 months, SEM and EPMA was also employed to observe bonding state and element distribution between bone and ABC.

3. The results and discussion

3.1 Phase analysis of ABC by XRD

X-ray diffraction patterns of ABC, sintered HAp, sintered FAp, crystallized 45S5 and crystallized 45SF1/4 in the range of 2θ =31-35° are shown in Fig. 1. XRD analysis of 45S5 and 45SF1/4 revealed the presence of crystalline phase which was identified as Na₂Ca₂Si₃O₉ (NCS). From Fig. 1, we can find that ABC is composed of FAp and crystallized 45S, although the starting materials for ABC are HAp and 45SF1/4. This result showed that F⁻ ion migrated from 45SF1/4 to HAp, and the glass phase crystallized. For ABC, 45SF1/4 contained F⁻ ion almost equimolecular with OH⁻ ion of HAp. And

![Fig. 1. XRD patterns of (a) crystallized 45SF1/4 (heat treated at 1000°C), (b) crystallized 45S5 (1200°C), (c) sintered FAp (1200°C), (d) sintered HAp (1200°C) and (e) ABC (960°C).](image-url)
if the whole F⁻ ion migrated from 45SF1/4 to HAp during sintering, the remainder glass would not contain F⁻ ion and its chemical composition would be close to 45S5.

The result of lattice parameter measurement (Table 2) seems to support the hypothesis about F⁻ ion migration. The dimension of a₀ axis of Ap presence in ABC was almost equal with analogous parameter of FAp.8)

3.2 The microstructure of ABC

SEM examination of etched surface of ABC, shown in Fig. 2 revealed the presence of Ap crystals about 500-1000 Å in dimensions. The gray circles about 0.1-0.5 μm are the cross section of NCS.

3.3 The properties of surface film generated after in vitro experiment

Figure 3 shows the IRRS of ABC before treatment and after 30 days of treatment in TBS. All the vibrations about HAp were attributed, and the characteristics of the Ap generated on the BAG were already discussed in previous paper in detail.4) Before treatment, the internal stretching vibrations (around 1000 cm⁻¹) and two bending vibrations (530, 600 cm⁻¹) of the PO₄³⁻ ion, which was contained in Ap structure, were observed. The stretching vibrations (920 cm⁻¹) and two bending vibrations (450, 510 cm⁻¹) of Si-O-R originated from crystallized BAG.

After 30 days of treatment, the vibrations of crystallized BAG disappeared, which indicates that the measured surface was fully covered with Ap film. Afterwards, the existence of CO₃²⁻ (1400 cm⁻¹) was also assigned. According to this
result, it is possible to suppose that the composition of Ap crystals in surface film was similar to Ap present in bone which can explain high bone bonding strength of ABC material.

ABC was proved to have the same kind of surface behavior as BAG, namely formation of Ap film after in vitro experiment.

Figure 4 shows the SEM photograph and line analysis of EPMA for the cross section of ABC treated during 7 days (168 h). Within 10-20 μm deep from the surface, Ca, P and F elements were mainly detected although very small amount of Si and Na elements were also detected. It was already proved from IRRS that the surface film consisted of Ap crystals. The results of SEM and EPMA confirmed this fact, furthermore the existence of F⁻ ions in the surface film revealed that the surface Ap film was made of FAp. X-ray diffraction pattern of Ap crystals from the thin surface film showed the peaks corresponded with that of FAp.

No difference in the thickness of FAp film was found in the case of samples treated 7 and 30 days. We supposed the termination of increase of FAp film thickness was caused by inhibition of ion diffusion (Na⁺, Ca²⁺, PO₄³⁻, H₃O⁺ etc.) through the FAp film generated. Figure 5 shows change of concentration of Na⁺ and Ca²⁺ ions in TBS as a function of treatment time. For ABC, concentration of ions reached almost constant values in 1 week. The FAp film reduced the ion leaching rate as its thickness increased. From SEM, EPMA and AAA examinations, we found that the thickness of FAp film on ABC surface after in vitro experiment reached constant value (10-20 μm) and the leaching of ions through the film almost terminated in 7 days.

On the contrary, sintered HAp was stable against in vitro experiment and particular surface change was not observed after treatment using IRRS, SEM (Fig. 6) and AAA (Fig. 5).

3.4 Increase of bending strength after in vitro experiment

Figure 7 shows the values of bending strength of ABC and sintered HAp as the function of soaking time. The measurement of strength during 0 day of treatment (non-treatment) was performed in the air atmosphere. However, the measurements of strength during 7 and 30 days (168 and 720 h) of treatments were performed immediately after in vitro treatment without drying the surfaces of specimens, namely the temperature was about 37°C and relative humidity was 100%. It is well known that the wet condition makes the strength of oxide ceramics lower.

For ABC, slight increase of bending strength was observed after 30 days of treatment in spite of experimental conditions in which the bending strength was expected to decrease. The bending strength of HAp decreased gently. Decreasing of bending strength of sintered HAp after in vitro experiment was probably caused by the slow crack growth of flaws with treatment time.

The mechanism of increase of bending strength
of ABC is discussed based on the theory applied for the similar phenomenon for glasses. Increase of mechanical strength for abraded or indented rods after treatment in water or annealing were reported. Two main models to explain strength increase have been presented. One is crack tip (Fig. 11) blunting and the other is release of residual tensile stress.

As we discussed in 3.3, ABC formed FAp film after in vitro experiment. It was considered that such a surface chemical reaction would change the shape and stress of surface flaws, which had significant influence on mechanical properties of brittle materials. The amount of ions used for FAp formation was estimated. Similar to the case of BAG, the whole atoms of surface FAp film were supplied from bulk of ABC. According to rough calculation, the amount of ions supplied from bulk surface layer of ABC to form FAp film was almost 10 wt% of the layer whose thickness was 50 μm. This dynamic surface modification are considered to affect the surface flaws originated during machining. In this way, the amount of ion migration around crack is considered to be more than that of the glasses, such as silica glass or soda lime glass which are generally used for the strength increase explanations. Thus crack tip is considered to blunt at high velocity and the stress around crack is considered to be removed with in vitro experiment. With the aim of direct observation of an area around crack in the case of in vitro experiment, the following study was employed.

Figure 8 shows the optical micrographs of ABC and HAp materials to compare the appearance of the area around indentation cracks. Vickers indenter was used to introduce cracks at a load of 9.8 N for 15 s in the air atmosphere. Figures 8 (a) and (b) are the photographs of ABC just after indentation and after in vitro experiments during 3 days. Figures 8 (c) and (d) are the photographs of HAp in analogous conditions. The appearance of the area around crack of HAp did not change after in vitro treatment. For ABC, however, the crack is beginning to be covered by FAp crystals. From this result, it is presumed that vigorous formation of FAp crystals may also occur in the area around crack tip, which could make sharp crack tip dull, release residual stress around tip and possibly shorten crack length. Thus the tip blunting and stress releasing around tip by FAp must have

![Fig. 8. The optical micrographs of the areas around indentation cracks. (a) ABC before in vitro experiment, (b) ABC after 3 days in TBS, (c) sintered HAp before experiment, (d) sintered HAp after 3 days in TBS.](image-url)
Table 3. The values of bone bonding strength (MPa).

<table>
<thead>
<tr>
<th>Bonding strength</th>
<th>Mean value</th>
<th>Standard deviation</th>
<th>Number of specimen</th>
<th>t value</th>
<th>Level of significance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>45S5[^4]</td>
<td>32</td>
<td>5.9</td>
<td>10</td>
<td>1.5</td>
<td>16</td>
</tr>
<tr>
<td>ABC</td>
<td>27</td>
<td>8.4</td>
<td>12</td>
<td>1.0</td>
<td>33</td>
</tr>
<tr>
<td>HAp[^4]</td>
<td>23</td>
<td>7.6</td>
<td>6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 9. SEM image and EPMA area profile of elements for the cross section of the interface of bone and ABC after 6 month implantation in canine mandible.
significant effect for strength increase.

3.5 Interfacial FAp layer between bone and ABC

The values of bone bonding strength by push out test are listed in Table 3. The value of ABC locates between 45S54 and HAp.

Figure 9 shows the SEM and area analysis of EMPA of the cross section of the interface between ABC and bone implanted in canine mandible during 6 months. The element distribution is shown in Fig. 10. From Fig. 9, FAp formation on the surface of ABC was confirmed because only Ca, P and F elements were distributed at the interface layer whose thickness was about 20 μm. Thus, for ABC, we confirmed that the results acquired from in vitro experiment can be directly applied to in vivo experiment concerning surface chemical reactions.

3.6 The possible advantages of ABC about mechanical strength for clinical applications

From the data presented above, we can expect two advantages regarding mechanical strength of ABC when it is implanted in living body. They are (1) stability of strength during implantation caused by crack tip blunting or crack tip stress release and (2) possibility of rapid recovery of mechanical strength after introduction of new cracks.

Increasing of bending strength after in vitro experiment may guarantee the mechanical strength with high reliability after implantation. Along with the formation of FAp film on the surface of ABC during implantation, surface flaws introduced during machining are expected to be modulated to make the bending strength of ABC stable, in spite of the initial deviation of bending strength by each flaw. Further investigation about the strength change after long period, a couple of years, is required.

As we discussed in 3.3, ABC formed stable FAp film on its surface in 1 week. After the formation of FAp film, ion leaching almost stopped. Supposing accidental introduction of fresh crack during implantation of ABC in living body, if the crack is long and sharp enough, it may decrease the mechanical strength of ABC at that instant (Fig. 11(a)). However the rupture of FAp film will induce ion leaching until new FAp film will be formed (Fig. 11(b)), consequently the mechanical strength may increase again. In this way, ABC is supposed to inhibit the decrease of mechanical strength by the new flaws introduced after implantation. Further investigations are in progress.

4. Conclusions

(1) ABC generated stable FAp film on its surface and its bending strength reached constant value after in vitro experiments. As the formation of FAp was also confirmed in canine mandible, the results of in vitro experiment are expected to be applied for in vivo experiment.

(2) The ion migration accompanied by the FAp film formation on ABC affected the surface flaws and resulted in the increase of bending strength after in vitro experiment.

References