Apatite Deposition on Several Dental Biodegradable Materials in Simulated Body Fluid

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SYNOPSIS
In the present study, we aimed to investigate the bone adaptability of commercially available biodegradable materials. Four natural polymer biodegradable materials, Avitene, Sponzel, Terdermis, Koken Tissue Guide and one synthetic polymer material, GC membrane, were immersed into Hanks' balanced salt solution (HBSS) without organic species with pH=7.4 for 14 days. HBSS was used as a simulated body fluid (SBF), and SBF immersion experiment was performed as preliminary experiment of in vivo animal experiment. Avitene and Sponzel have been dissolved in HBSS. Apatite formation was detected on Terdermis, Koken Tissue Guide and GC membrane. Micro X-ray diffraction measurement revealed the formation of hydroxyapatite on three biodegradable materials after HBSS immersion. We can make clear the biocompatibility of different biodegradable materials using simple HBSS immersion experiment.

Key words: biomaterials, hydroxyapatite, biocompatibility, simulated body fluid

INTRODUCTION
Recently, some dental biomaterials have been introduced for periodontitis treatment, implant treatment, or hemostatic materials etc. The biocompatibility and the stability of the dental biomaterials were the predominant factor for clinical use. Biological experiments such as cell culture or animal experiment was preformed to assess the biocompatibility or stability of biomaterials.

On the other hand, some preliminary experiments were performed before biological experiments for screening the materials. One of the valuable methods is the immersion experiment in simulated body fluid (SBF). Kokubo and his colleague published numerous studies related with SBF immersion experiment for evaluate bone-bonding ability of biomaterials. They developed their own SBF, which ion concentration and composition was almost equal to human blood plasma component¹, and found the apatite formation on biomaterials after the immersion in SBF²-⁸.
Hanawa et al.\(^9,10\) also found the apatite formation on titanium surface after the immersion in Hanks’ balanced salt solution (HBSS) without organic species with pH=7.4. HBSS was used as SBF and the efficacy of HBSS as SBF was reported. Hayakawa et al.\(^11\) reported that apatite deposition on phosphorylated bonding agent and Takahashi et al.\(^12\) demonstrated the apatite formation on a thin-apatite coated titanium surface after the immersion in HBSS.

Some biodegradable materials such as GC membrane (GC, Tokyo, Japan) were used in dental clinics. To our knowledge, there have been no previous reports on the comparison of their biocompatibility. For the first step of the research for evaluating the basic properties of commercially available biodegradable materials, we aimed to investigate their bone adaptability. Some commercially available biodegradable materials were immersed into HBSS and the formation of apatite precipitation on materials was observed using scanning electron microscopy and x-ray diffraction technique.

### MATERIALS AND METHODS

Five kinds of biodegradable materials were used in this study as shown in Table 1. Some were made from natural polymers and the other was from synthetic polymers. They were shaped with the square form (1cm x 1cm).

They were immersed in 20ml HBSS without organic species, which was introduced by Hanawa et al.\(^9,10\) for 14 days in sealed polystyrene bottles at 37 °C. HBSS was prepared in our laboratory, and the ion concentrations of HBSS (mmol/L) without organic species are summarized in Table 2. The solutions and bottle were exchanged every day to expose the specimens to fresh solutions. Immediately after immersion, the specimens were again washed by deionized water to remove HBSS that they did not take up. The specimens were then immediately dried in a desiccator.

#### Table 1 Materials used in the present study

<table>
<thead>
<tr>
<th>Name</th>
<th>Main components</th>
<th>Lot number</th>
<th>manufacture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avitene</td>
<td>Collagen from dermis of cows</td>
<td>WBQND14</td>
<td>Davol Inc. Woburn, MA, USA</td>
</tr>
<tr>
<td>Sponzel</td>
<td>Crude collage from animal’s bone, skin, ligament, tendon</td>
<td>W025Y01</td>
<td>Astellas Pharm Inc. Tokyo, Japan</td>
</tr>
<tr>
<td>Teldemis</td>
<td>Atelocollagen from dermis of cows</td>
<td>050901</td>
<td>Terumo Corp. Tokyo, Japan</td>
</tr>
<tr>
<td>Koken Tissue Guide</td>
<td>Atelocollagen collagen and tendon</td>
<td>05110A</td>
<td>Koken Co., Ltd. Tokyo, Japan</td>
</tr>
<tr>
<td>GC membrane</td>
<td>Poly(lactic acid) acid-glycolic</td>
<td>0106221</td>
<td>GC Corp. Tokyo, Japan</td>
</tr>
</tbody>
</table>

#### Table 2 Ion concentration in Hanks’ balanced salt solution without organic species

<table>
<thead>
<tr>
<th>Ion</th>
<th>Concentration (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na(^+)</td>
<td>142</td>
</tr>
<tr>
<td>K(^+)</td>
<td>5.81</td>
</tr>
<tr>
<td>Mg(^{2+})</td>
<td>0.811</td>
</tr>
<tr>
<td>Ca(^{2+})</td>
<td>1.26</td>
</tr>
<tr>
<td>Cl(^-)</td>
<td>145</td>
</tr>
<tr>
<td>HPO(_4^{2-})</td>
<td>0.778</td>
</tr>
<tr>
<td>SO(_4^{2-})</td>
<td>0.811</td>
</tr>
<tr>
<td>HCO(_3^{-})</td>
<td>4.17</td>
</tr>
</tbody>
</table>
The crystallographic structure of the precipitates on degradable materials after HBSS immersion was analyzed by means of micro-X-ray diffraction (micro-XRD, θ-2θ diffractometer, XRD, Rigaku, RINT 2000, Tokyo), which had an X-ray source of CuKα, and a power of 50kV x 200 mA. The morphology of the precipitates on degradable materials after HBSS immersion was observed by Field-emission scanning electron microscope (FE-SEM, JEOL, JSM-6340F, Tokyo) at an accelerating voltage of 5 kV. The specimens were coated with platinum before FE-SEM observation.

RESULTS

The formation of white precipitates was observed on three kinds of materials, Terdermis, Koken Tissue Guide and GC membrane. But other two materials, Avitene and Sponzel, have been dissolved in HBSS after 14 days.

Micro-XRD spectra showed hydroxypatite formation on three kinds of materials after the immersion in HBSS as shown in Figs. 1-3. Three materials, i.e. Terdermis, Koken Tissue Guide and GC membrane, produced the same micro-XRD patterns. Major peaks in the spectra could be assigned as 002, 210, 211, 222 of peaks of hydroxyapatite.

Figs. 4-6 shows the FE-SEM pictures of the surface of degradable materials after the immersion in HBSS. SEM observation revealed that the surface of three kinds of materials (Terdermis, Koken Tissue Guide and GC membrane) was completely covered with hydroxyapatite globules. Surface appearance of hydroxyapatite globules on Terdermis was different from those on Koken Tissue Guide and GC membrane. The globules formation was less clear. Each globule on Koken Tissue Guide or GC membrane was composed of a group of numerous thin-film form flakes uniting and/or clustering together (Figs. 5, 6).
DISCUSSION

For the evaluation of in vitro biocompatibility, many studies have reported the formation of an apatite layer after materials are immersed in simulated body fluid. It is reported that in vivo bioactivity (osteconductivity) of biomaterials such as ceramics is precisely mirrored by their in vitro apatite-forming ability in an SBF. In the present study, HBSS was employed as simulated body fluid. Tanimoto et al. reported that in vivo bone formation of sintered tricalcium sheets confirmed the results of the in vitro HBSS immersion experiment, namely more apatite formation in HBSS corresponded with better bone formation.

In the present study, five kinds of biodegradable materials were used. Avitene and Sponzel were dissolved in HBSS immersion. Both were mainly used as hemostatic materials after tooth extraction, and made from collagen. The present in vitro study suggested that both Avitene and Sponzel will degrade during 14 days after implantation into extraction socket.

Apatite formation was observed on Terdermis, Koken Tissue Guide and GC membrane. Terdermis was made from atelocollagen and Koken Tissue Guide was made from atelocollagen and tendon collagen. Terdermis is a coating material used to rebuild the dermis element, and also used the repair of the mucous membrane in the oral mouth. Koken Tissue Guide was used to periodontal repair following reconstructive surgery. Both collagen materials did not degraded during HBSS immersion. This was due to the difference of collagen used in Avitene or Sponzel.

GC membrane was made from the synthetic macro-molecule, i.e. poly (lactic acid-glycolic acid), and was use for periodontal repair following reconstructive surgery. The feature of degradation of synthetic polymer material such as
GC membrane is not enzymatic degradation, and is hydrolyzed with non-enzyme. Therefore, it is possible to control the degradation of synthetic polymer materials to some degree by adjusting the molecular mass of the materials\textsuperscript{15}.

XRD measurements revealed that hydroxyapatite deposited on biomaterials by soaking in HBSS had a C axis (002) distribution when compared with XRD of apatite film deposited on titanium\textsuperscript{16}. Asada et al\textsuperscript{17} and Yen et al\textsuperscript{18} reported that apatite deposited by soaking in HBSS was tendency for the (002) distribution. The same result was obtained in this study.

It has already been reported that spongy collagen induced calcification of the bone under the culture condition\textsuperscript{19}. The surface chemistry between Ter-dermis, Koken Tissue Guide and GC membrane is presumed to be different each other. Hanawa suggested that calcium ion first adsorbed ion onto titanium surface covered with hydroxyl groups and then phosphate ion adsorbed\textsuperscript{9}. Thus apatite layer was formed by repetition of this process. More detailed study for surface analysis of present biodegradable materials should be needed for the elucidation of apatite formation mechanism.

At any event, we can make clear the biocompatibility of different biodegradable materials using simple HBSS immersion experiment. In vivo animal experiment will be needed to conclude the final biocompatibility of present biodegradable materials. However, Kokubo et al\textsuperscript{19} insisted that the examination of apatite formation on a material in SBF is useful for predicting the in vivo bone bioactivity and the number of animals and the duration of animal experiments can be reduced remarkably by using SBF immersion experiment.

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