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Plasma Processing of Artificial Bones and Bone Regenerative Medicine
人工骨プラズマ処理と骨再生医療

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In orthopedic surgeries, autologous bone grafting has been the golden standard for the treatment of bone defects. However, recent advances in synthetic calcium phosphate ceramics have altered the picture and ceramic bone substitutes have become as a standard along with autografts. Interconnected porous ceramics is also useful as a scaffold for bone tissue engineering. However, surface property of hydroxyapatite ceramics is hydrophilic. Recently, we found that plasma treatment was able to improve the hydrophilicity. Our preliminary data suggest that plasma treatment may facilitate bone regeneration in the pores and improve the integration of ceramics to host bone.

1. Bone Defect Treatment and Ceramics Bone Substitutes

In orthopedic surgeries, autologous bone grafting has been the golden standard for the treatment of bone defects. However, it has several disadvantages, such as the high incidence of complications related to the harvesting procedures. To avoid these disadvantages, various bone substitutes have been developed in Japan, and among them, porous ceramics made of calcium phosphate, hydroxyapatite or beta tri-calcium phosphate have been considered to be the most promising biomaterials.

Together with Covalent Material Co., and National Institute for Materials Science, we developed a fully interconnected porous HA ceramics, IP-CHA or “NEOBONE”[1]. This material has an excellent macro-porous structure with highly interconnected spherical pores (porosity: 75%) and appropriate compression strength (about 10 MPa) for surgical handling. Biocompatibility of IP-CHA as a bone substitute evaluated in a rabbit model of bone defect repair was much better than early porous ceramics products with less porosity and less interconnections suggesting that the interconnected porous structure facilitated the bone tissue ingrowth in the material and improved the integration to host bone. Until now, several products of interconnected porous ceramics including IP-CHA are clinically available as bone substitutes and they are found to be clinically very useful[2].

2. Bone Tissue Engineering Using Porous Ceramics as a Scaffold

Using these bone substitutes as a scaffold, we have been working on bone regeneration study. The main principle of current tissue engineering strategy for bone is a combination of biocompatible porous scaffold and bone-inducing molecules such as bone morphogenetic protein (BMP) or bone producing cells such as osteoblasts. First, we combined osteoblastic cells differentiated from bone marrow mesenchymal cells with IP-CHA discs as scaffold and found vigorous bone forming activity in rat subcutaneous ectopic bone formation model and rabbit forearm bone defect model[3]. We also tested BMP/IP-CHA composite for bone formation in several in vivo models including canine and the results were satisfactory. Furthermore, we recently investigated the safety and usefulness of osteoblastic cell/IP-CHA composite as a clinical research for the treatment of bone defects after the removal of bone tumors and found good results.

3. Plasma Surface Treatment Improved Hydrophilicity of HA

As discussed previously, interconnected porous

HA ceramics are useful material as a bone substitute and also as a scaffold material for bone tissue engineering. However, the surface properties of HA ceramics is basically hydrophobic and may not be an ideal surface for cell/tissue attachment or for supporting bone regeneration as a scaffold. Our recent study revealed that treatment of HA by plasmas significantly improved its hydrophilicity[4]. IP-CHA discs (ϕ5mm x h2mm) were placed in the discharge chamber and plasma treatment at a sub-atmospheric pressure was applied. As shown in Fig. 1, after plasma treatment, water infiltrated easily into the pores, indicating plasma treatment improved the hydrophilicity of inner pore surface.

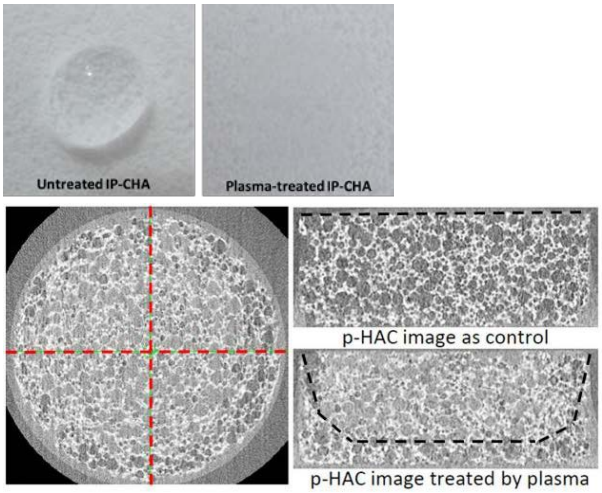

Fig. 1. Gross appearance (top) and micro-CT images (bottom) of IP-CHA discs treated with plasma for 3 minutes and 10 minutes, respectively. A drop of water containing an X-ray contrast agent was placed on the top surface of each disc and subjected to micro-CT analysis. The white color indicates the location of hydroxyapatite (HA), gray the location of water (i.e., water filled pores), and dark the location of empty space (unfilled pores). It is seen that, with plasma treatment, most pores are filled with added water, indicating the increase of hydrophilicity of inner pore surfaces.

4. Plasma Surface Treatment Enhanced the Integration of HA Bone Substitute *in vivo*

We have also examined the effect of plasma surface treatment on the biocompatibility of IP-CHA in two different in vivo bone defect models, rabbit femoral condyle intramedullary bone defect model and rat calvaria full thickness bone defect model. In both in vivo models, we found a tendency of better biocompatibility in plasma-treated group, with a significant difference in newly formed bone volume in the pores analyzed by micro-CT at 4 weeks after implantation (Fig. 2).

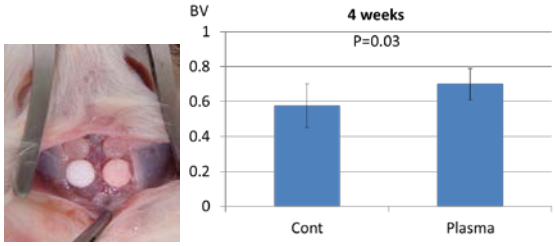

Fig. 2. Rat calvaria defect model. Full thickness calvaria bone defects were created by trephine and IP-CHA discs were placed in the defect. Micro-CT analyses were performed at 4 and 8 weeks after implantation. Plasma treatment significantly improved the bone volume in the IP-CHA pores at 4weeks.

Although our data are preliminary, they suggest that plasma treatment improves the hydrophilicity of porous HA ceramics and in consequence, may enhance the bone regeneration in the pores and IP-CHA integration to host bone. Surface property alteration may also affect the performance of HA ceramics as a scaffold for bone tissue engineering by various ways, for instance by changing the attachment of bone forming cells or by modifying the cytokine release kinetics in cytokine/IP-CHA composite system.

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