Introduction
The ideal bone substitute is a material that can be completely replaced by newly formed bone. Hydroxyapatite has been widely used as a bone substitute because of its excellent biocompatibility and osteoconductive ability. The clinical application of hydroxyapatite may be limited, because it does not seem to be resorbed appreciably. In order to improve these disadvantages, we have successfully developed sintered carbonate apatite, which resembles bone apatite in its chemical properties and is resorbed. The purpose of this study is to evaluate whether the sintered carbonate apatite has the abilities of osteoconduction and resorption in vivo.

Methods
Carbonate apatite and hydroxyapatite were synthesized in a manner described previously (1), by mixing calcium nitrate solution and disodium hydrogen phosphate solution containing, respectively, 6 and 0 mol disodium carbonate at 100 °C. No phases other than apatite were detected by X-ray diffraction and infrared spectroscopy. The carbonate apatite had a carbonate content of about 12 wt%. Carbonate apatite and hydroxyapatite particles were placed in cylindrical molds made of paper. The sintered carbonate apatite and sintered hydroxyapatite were produced by heating the samples for 2 hours at 750 °C and 1200 °C, respectively. The concentration of carbonate of the sintered samples was approximately 6 wt%, which was quite close to the amount of carbonate in bone apatite. The size of the cylindrical porous material was 4 mm in diameter and 4 mm in height. The porosity was approximately 20% and the mean pore size was 175µm. When examined by scanning electron microscopy, the sintered carbonate apatite appeared to be composed of grains, ranging in size from 0.3 to 1.0 µm (crystal size 0.01 µm), whereas the sintered hydroxyapatite had about ten times larger grains (crystal size 0.1 µm).

Twenty mature female Japanese white rabbits underwent surgery on both knees under intravenous pentobarbital sodium anesthesia. Bone defect was generated in the distal epiphysis of femur. The size of the defect was 4 mm in diameter and 4 mm in depth. The defect in one knee was filled tightly with the sterile sintered carbonate apatite (CA), while the defect in the other was filled tightly with the sterile sintered hydroxyapatite (HA) or left empty as a control. The rabbits were killed 4 to 24 weeks postoperatively by an overdose of pentobarbital sodium. The number of animals killed at weeks 4, 12, and 24 were 4, 8, and 8, respectively. The distal femur was removed and the defects with or without the implant were evaluated by micro-focus computed tomography (MFX-CT), which has a spatial resolution of the order of 10 µm before sectioning for histology. For undecalcified histological evaluation, the samples were embedded in methylmethacrylate. Sections 7 µm in thickness were prepared and stained with toluidine-blue. For decalcified histological evaluation, the samples were decalcified in ethylene diamine tetraacetic acid, and embedded in paraffin. Sections 5 µm in thickness were prepared and stained with hematoxylin and eosin. We carried out quantitative analysis of the porosity of the materials (total pore area / total area) and bone ingrowth into the materials using a computer-assisted system (NIH image) from the data of MFX-CT. The quantity of regenerated bone into the pores of the materials was expressed as a percentage (total bone area / total pore area). Statistical analyses were performed using ANOVA and post hoc tests. The level of significance was p<0.05.

Results
No implant was lost as a result of infection. Bone growth into and around the materials increased with time. Newly formed bone was put in direct contact with both CA and HA. The implants were mostly surrounded by lamellar bone after 12 and 24 weeks. None of the specimens showed obvious inflammatory reaction in the groups of CA and HA. The porosity of CA increased significantly with time. This finding showed the resorption of CA (Fig. 1). However, the porosity of HA did not increase. Bone ingrowth into CA and HA increased from 4 to 12 weeks significantly (Fig.2). In the controls, the defects were filled with bone and fibrous tissue, but no complete repair with bone tissue was obtained even after 24 weeks.

Discussion
To synthesize sintered stoichiometric hydroxyapatite, we have to heat the material to over 1100 °C. Heating over such a high temperature increases the crystallinity dramatically, reducing its solubility in acid solutions. In contrast, β-tricalcium phosphate is more resorbable than hydroxyapatite. Because β-tricalcium phosphate is a material foreign to living tissue, it is likely to be phagocytosed by macrophages, evoking a local inflammatory response (2). For the use of scaffold of bone, it would be desirable to develop more soluble sintered apatite. The presence of carbonate in the apatite lattice makes the sintering temperature decrease and the crystallinity lower. The stability of the apatite structure decreases and reactivity toward acid dissolution increases (3). And it would contribute to resorption in bony tissue. In conclusion, sintered carbonate apatite seems to have successful osteoconductive and resorbable abilities in this rabbit experiment, and would be useful as a biodegradable bone substitute.

References

**Department of Dental Materials and Technology, Asahi University Faculty of Dentistry, Motosu, Gifu, Japan.

48th Annual Meeting of the Orthopaedic Research Society
Poster No: 0733