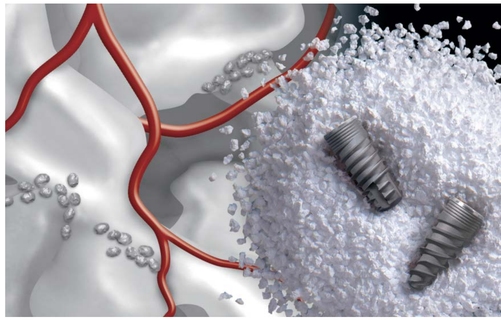
P194, March 17 12:40-12:50

Effectiveness of Carbonate Apatite Bone Graft Substitute by 1, 3, 4-wall bone defect model of Beagle dog



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GC Corporation

Introduction

Although an autograft is a gold standard of bone grafting, there is some limitation. Xenograft is also used but risk for infection potentially and we focused on chemically synthetic bone graft material. Focusing on bone composition, bone is a carbonate apatite containing carbonate ions, and it is known that carbonate ions play an important role in bone metabolism. As a result of extensive research, we developed a carbonate apatite bone graft substitute (Cytrans Granule, CO_3Ap, launched in Japan 2018) and a chemically synthetic resorbable membrane (Cytrans ElaShield, PBM, launched in Japan 2020).

In this study, we evaluated the effect of CO_3Ap and PBM on bone regeneration in vivo.

Composition of Bone

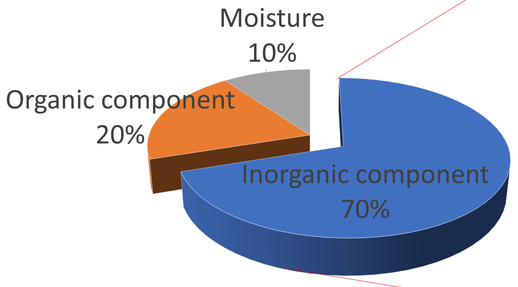


Figure 1. Composition of Bone

Inorganic composition of each material

Component	Contents（wt%）			
	Bone	HAp	β-TCP	Cytrans® Granules
PO_4^{3-}	46.6	56.8	61.2	48.0
Ca^{2+}	34.8	39.8	38.8	34.0
CO_3^{2-}	7.4	—	—	12.0
Na^+	0.9	—	—	4.15
Mg^{2+}	0.72	—	—	—
Cl^-	0.13	—	—	—
K^+	0.03	—	—	—
F^-	0.03	—	—	—

Figure 2. Cytrans® Granules
S(0.3-0.6 mm), M(0.6-1.0 mm), L(1.0-2.0 mm)

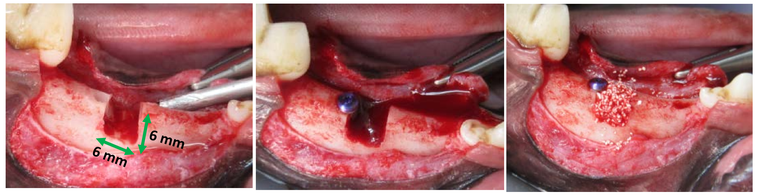
Figure 3. Cytrans® ElaShield
(Poly L-lactide- ε -caprolactone, PBM)

Method

The animal study protocol was approved by Institutional Animal Care and Use Committee of Hamri Co., Ltd., which has been approved by AAALAC International.

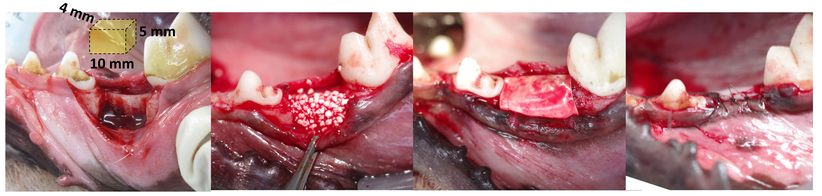
Study A:

After extraction of the bilateral mandibular anterior molars of beagle dog, it healed for 8 weeks. An 1-wall peri-implant bone defect (6×6 mm) was prepared and ϕ3.0 mm dental implants were placed in distal side of the defect. The defect was filled with CO_3Ap(Cytrans Granules, GC). Sham group was set as a control. After a healing period of 12 weeks, μCT and histological evaluation were performed. For histological evaluation, the newly formed bone area was measured by using microscopic images of undecalcified section (Villanueva Goldner stain) and Image J (NIH)



Study B:

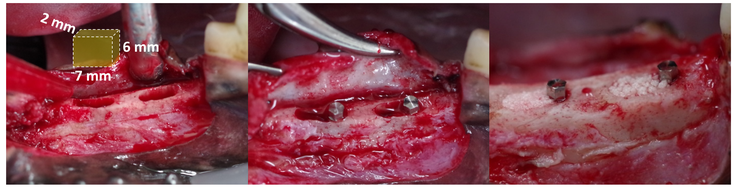
A buccal dehiscence type 3-wall bone defect (10×4×5 mm) was prepared on the mandibular premolars. The defect was filled with CO_3Ap, and it was covered by PBM. A without-membrane group and sham group were set as a control. After 12 weeks of healing, then micro-CT and histological evaluation were performed. 3D reconstruction was performed using CT images. The volume of newly formed bone was then calculated using CT analysis software (VG Studio Max).



Study C:

Box-shaped defects (7×2×6 mm) were prepared, and ϕ2.2 mm implants were placed in distal side of the defects. The defect was filled with CO_3Ap. Freeze-dried bone allograft (FDBA), deproteinized bovine bone mineral (DBBM), Hydroxy apatite (HAp) and β-Tricalcium phosphate (TCP) were set as controls.

After healing period of 12 weeks, the samples along with the surrounding tissue were carefully harvested from the mandibular bone. Subsequently, Villanueva Goldner stain was applied to all sections and observed using a light microscope.



Results of Study A (1-wall)

In μ-CT analysis, the new bone volume was 139.0 mm^3 at CO_3Ap, which was higher than 133.3 mm^3 of the control. The new bone volume in the upper part of the defect was 12.0 mm^3 at CO_3Ap and 7.7 mm^3 at the control. CO_3Ap showed good regeneration especially in the upper part of the bone defect without loss of bone height. (Fig. 4-6)

As results of histological evaluation, new bone area was 46.3% in CO_3Ap group, which was higher than 38.5% in control group. (Fig. 7,8)

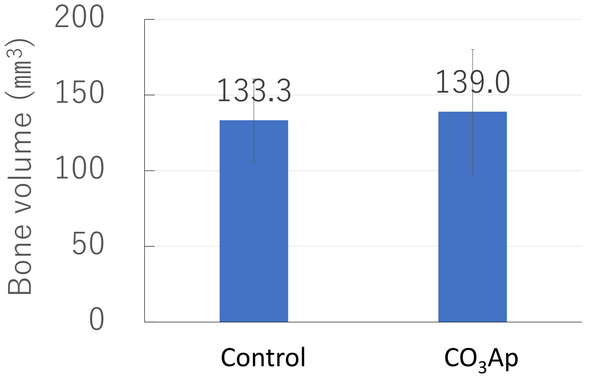


Fig 5. Bone volume using CT analysis

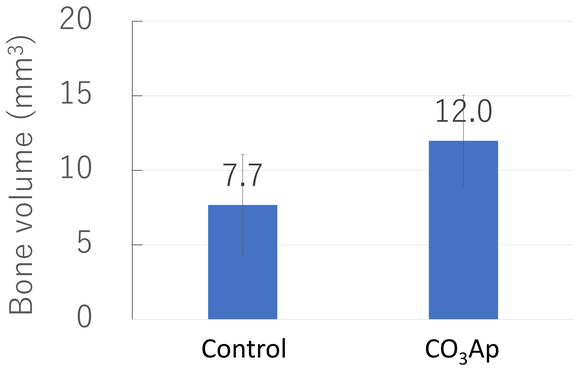


Fig 6. Bone volume in the upper part of defect using CT analysis

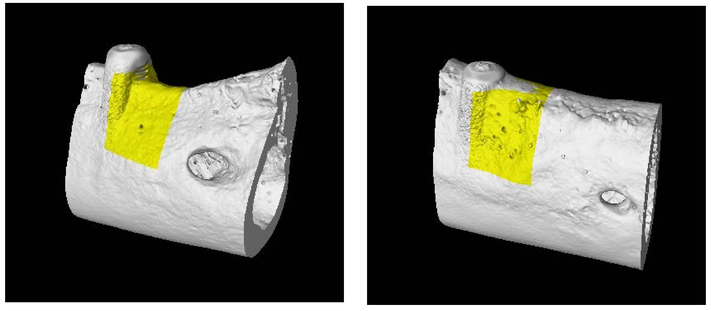


Fig 4. 3D CT image of mandibular bone (Left: Control, Right: CO3Ap, Yellow area: Defect area)

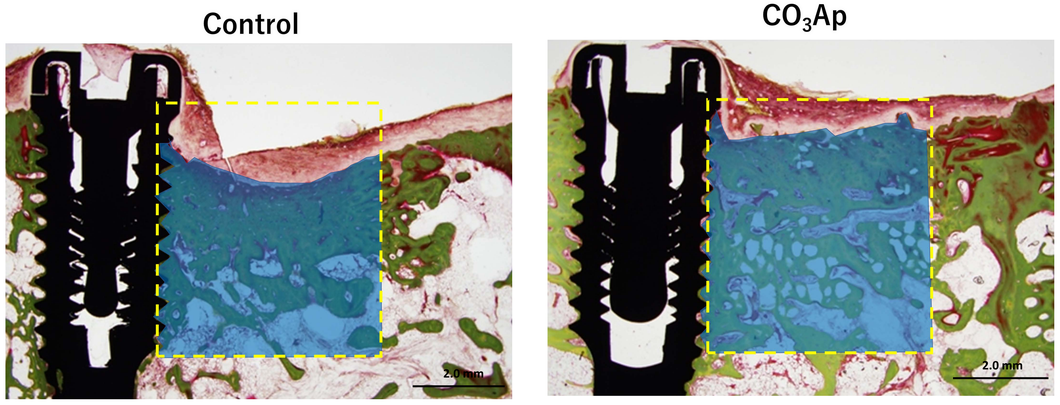


Fig 7. Histological image (Villanueva Goldner stain, Green; matured bone tissue, Red: osteoid)

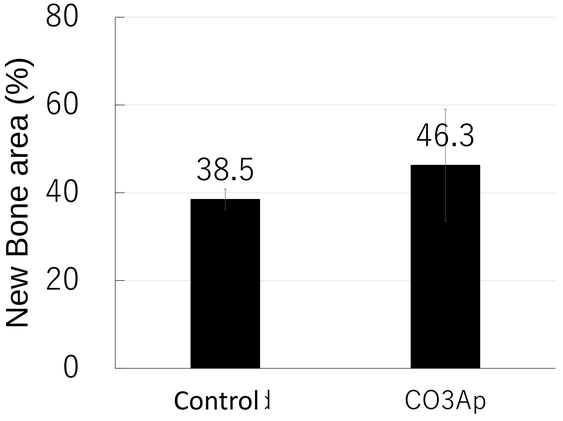


Fig 8. Quantitative histomorphometry analysis of the new bone area

Results of Study B, C

Study B（3-wall）:

Histological evaluation revealed that the calcified area on CT observation was bone tissue in all cases. Newly formed bone volume ratio was 93.9% in the PBM group, 82.3% in the only CO_3Ap group, and 55.1% in the sham group.

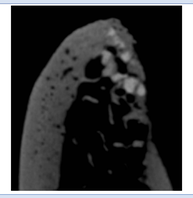
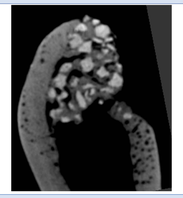
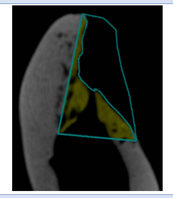
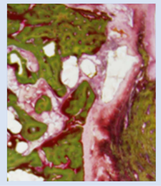
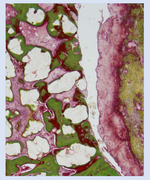
	PBM+CO_3Ap	CO_3Ap	Sham
Inflammation	No	No	No
Micro CT image			
Histological Image (Villanueva Goldner Stain, Green: matured bone, Red: Osteoid)			

Figure 9. CT and Histological image

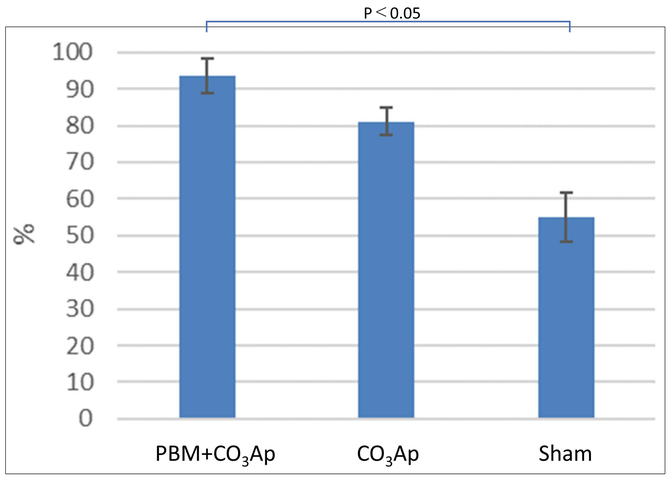


Figure 10. Newly formed bone volume by CT analysis

Study C (4-wall) :

As a result of histological evaluation, the descending order of bone augmented area was CO_3Ap, FDBA, DBBM, HAp and β-TCP. In FDBA, DBBM, HAp and β-TCP group, bone regeneration was insufficient at the upper part of the defect, however CO_3Ap group showed sufficient bone regeneration and maintained the bone height.

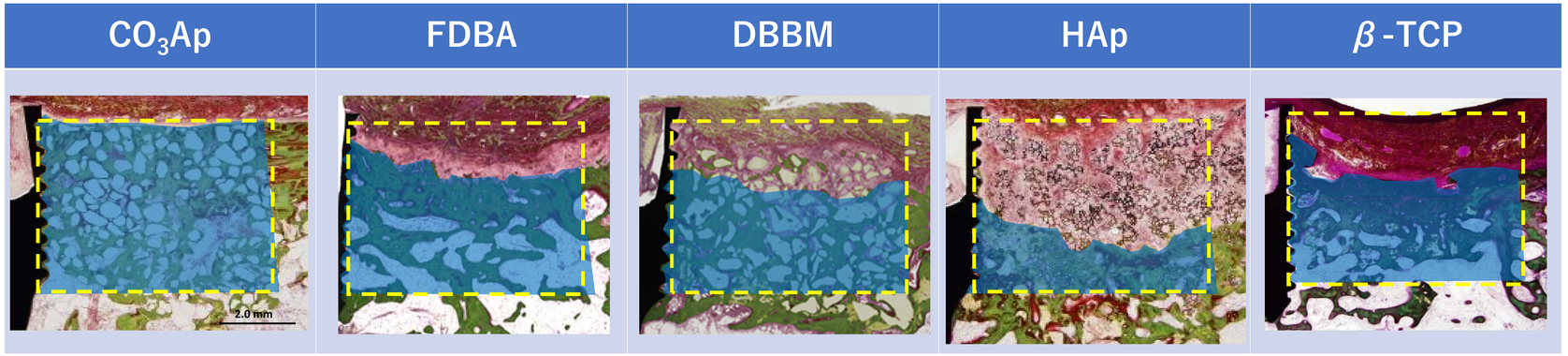


Figure 11. Histological image of each specimen (Osteoid : Magenta, Calcified bone : Yellow green)

Discussion

In this preliminary study, CO_3Ap showed the potential of being a superior and biocompatible bone graft substitute. CO_3Ap granules have been reported to be more susceptible to phagocytosis by osteoclasts than hydroxyapatite or β-tricalcium phosphate. It has also been reported that osteogenic differentiation of human bone marrow cells was accelerated with CO_3Ap compared to cell culture plates or HAp, suggesting that CO_3Ap facilitates osteogenic differentiation of bone marrow cells and osteoblasts[1].

Furthermore, it promoted bone formation effectively when combined with PBM which was able to make sufficient space for the period required for bone tissue regeneration.

PBM is consist of two layers, porous layer and solid layer. Porous layer is for retaining bloods and solid layer is for inhibiting soft tissue from invading to bone defects. The two-layer structure provides a barrier property, strength, and space for bone regeneration.

1) M Fujioka-Kobayashi et al, J Biomed Mater Res, 2022, 110, 1524-1532

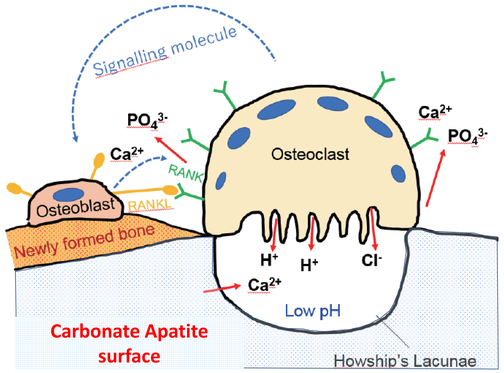
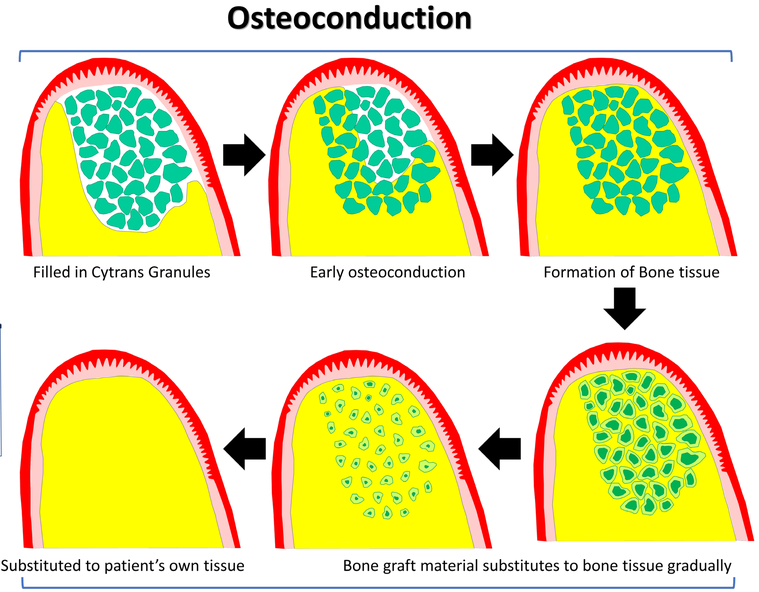


Fig 12. Mechanism of Bone remodeling



Conclusion

CO_3Ap is expected as clinically useful materials for bone graft treatment. Furthermore, it is thought that CO_3Ap can effectively regenerate bone by combining it with PBM.

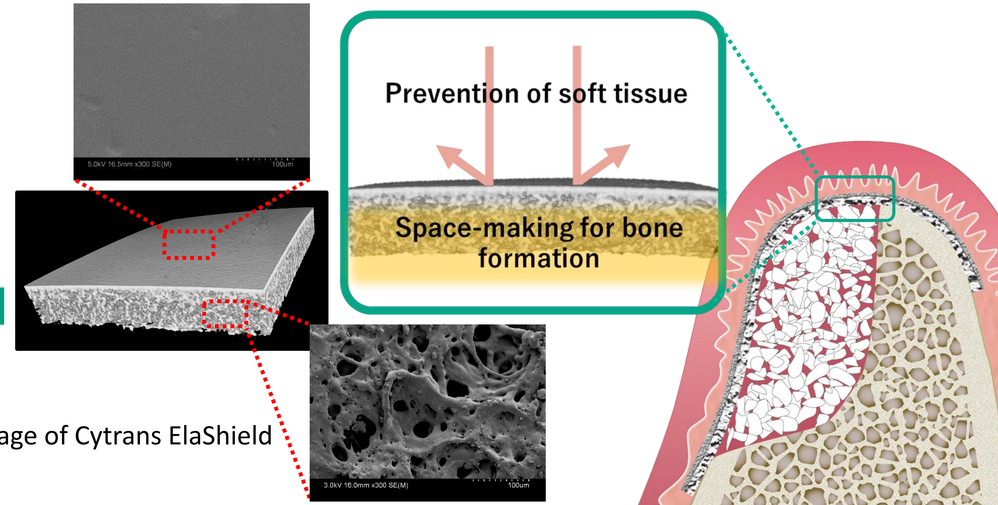


Fig 13. CT image of Cytrans ElaShield