



In Vivo Evaluation of Carbonate Apatite Bone Graft Material and Resorbable GBR Membrane

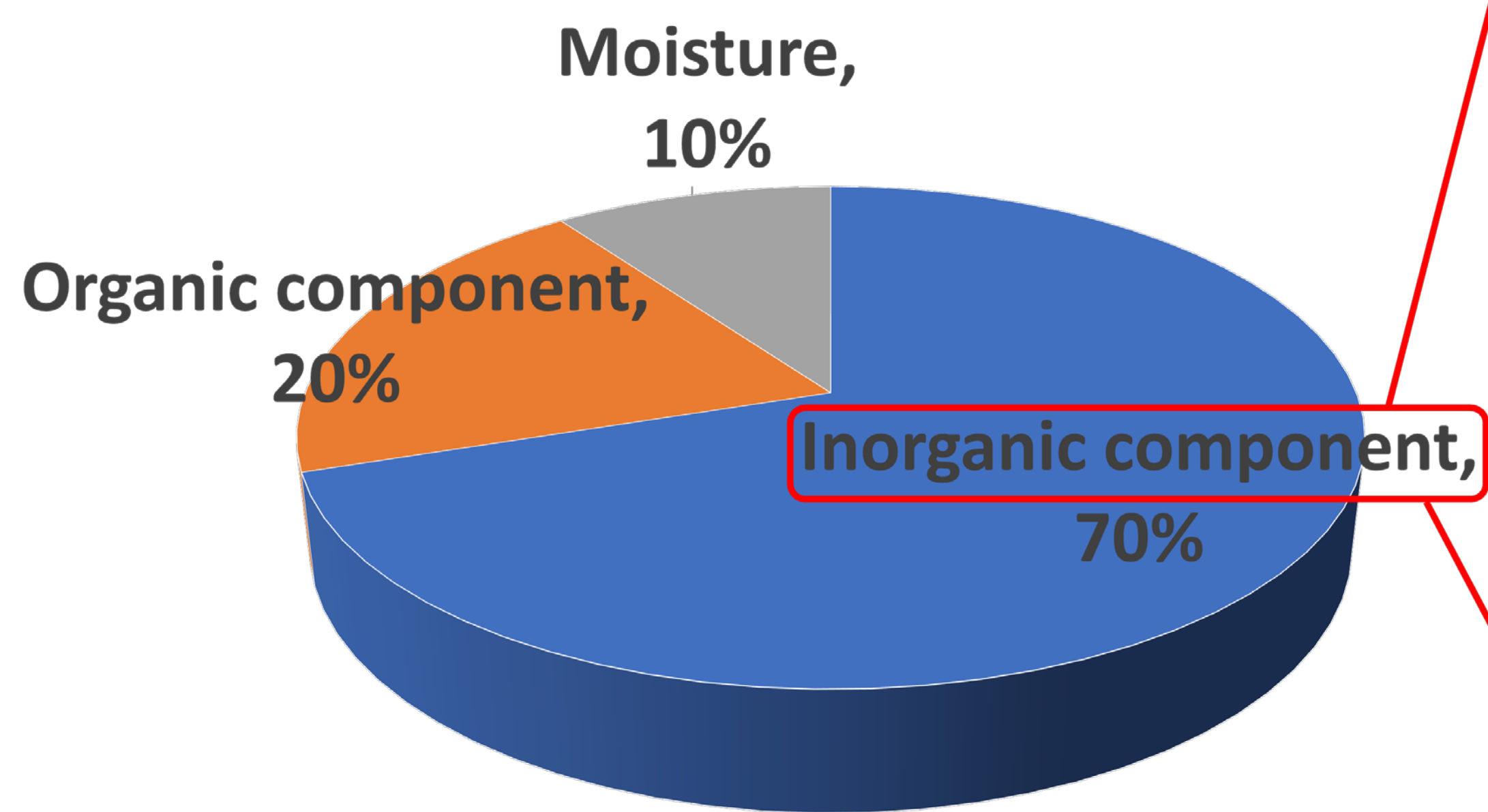
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INTRODUCTION

The inorganic component of human bone is the carbonated apatite containing 4-8wt% of carbonate ion which is known as a key element for bone remodeling. We have developed a synthetic carbonated apatite bone graft substitute (CO₃Ap, Cytrans® Granules) and a resorbable bilayer membrane made of synthetic poly (L-lactide-ε-caprolactone) (PBM, Cytrans® ElaShield).
In this study, we aimed to evaluate the effect of CO₃Ap and PBM on bone regeneration using canine.

Composition of Bone



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 1. Composition of Bone

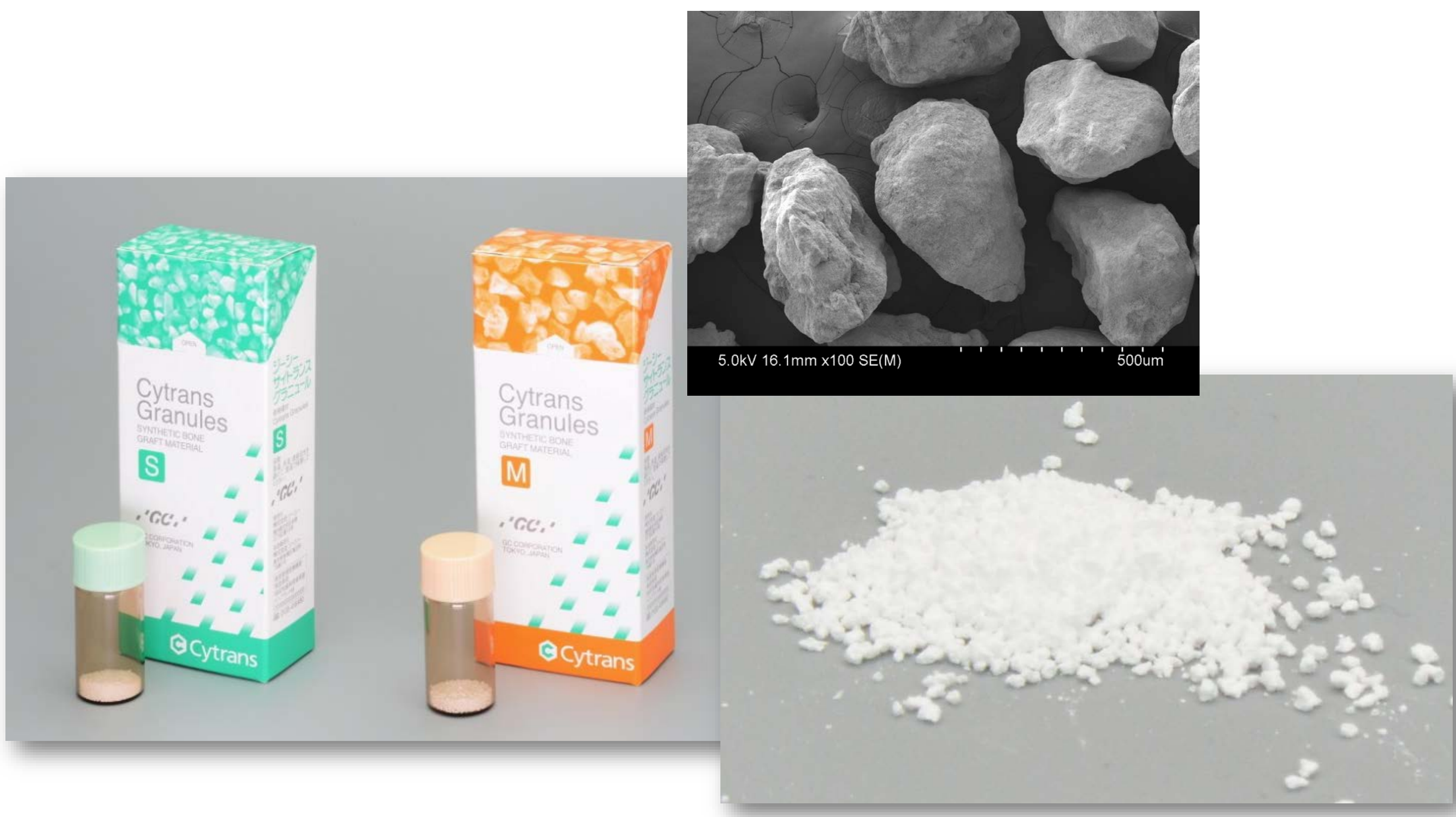


Figure 2. Cytrans® Granules
(Synthetic carbonated apatite bone graft substitute, S size : 0.3-0.6 µm, M size : 0.6-1 µm)

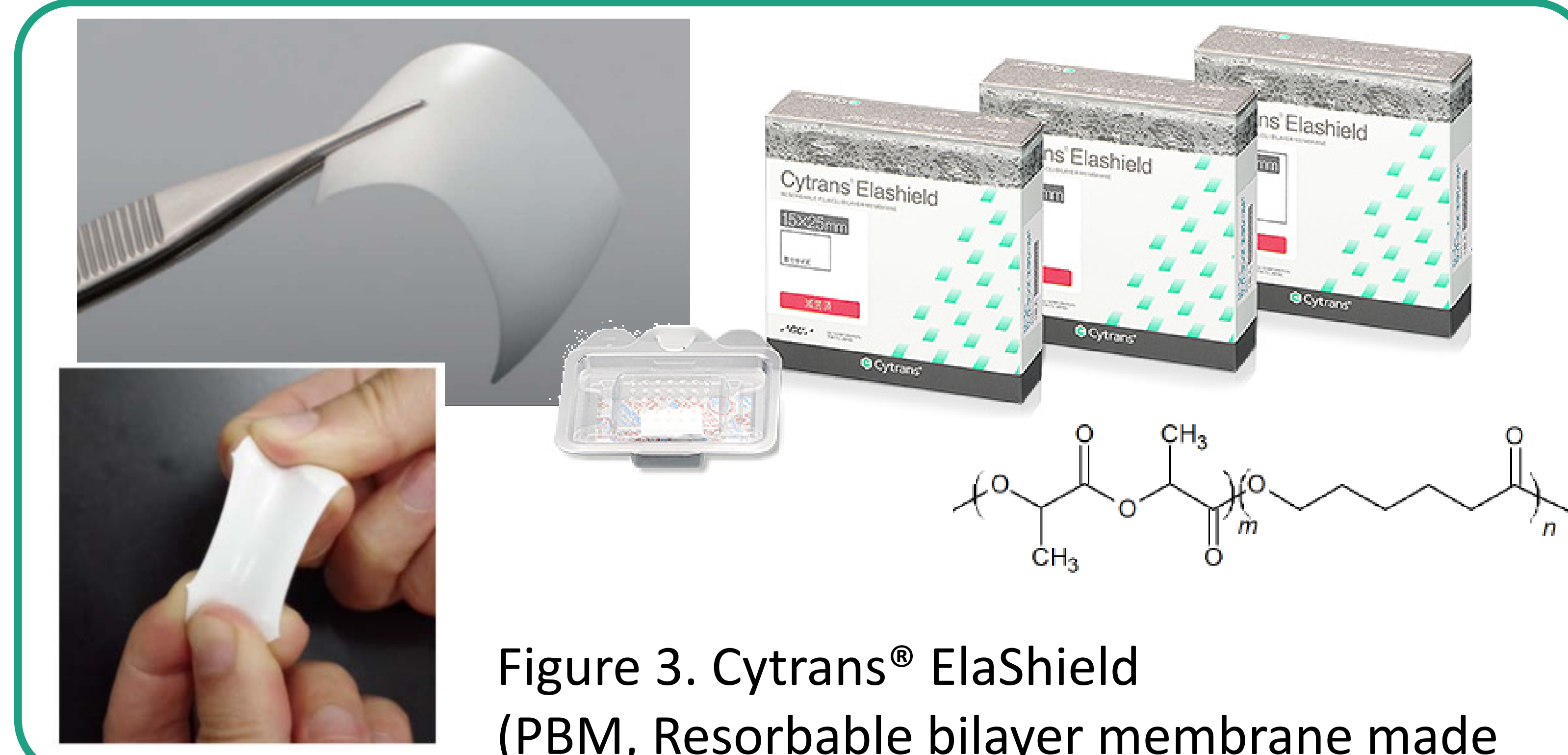


Figure 3. Cytrans® ElaShield
(PBM, Resorbable bilayer membrane made of synthetic poly L-lactide-ε-caprolactone)



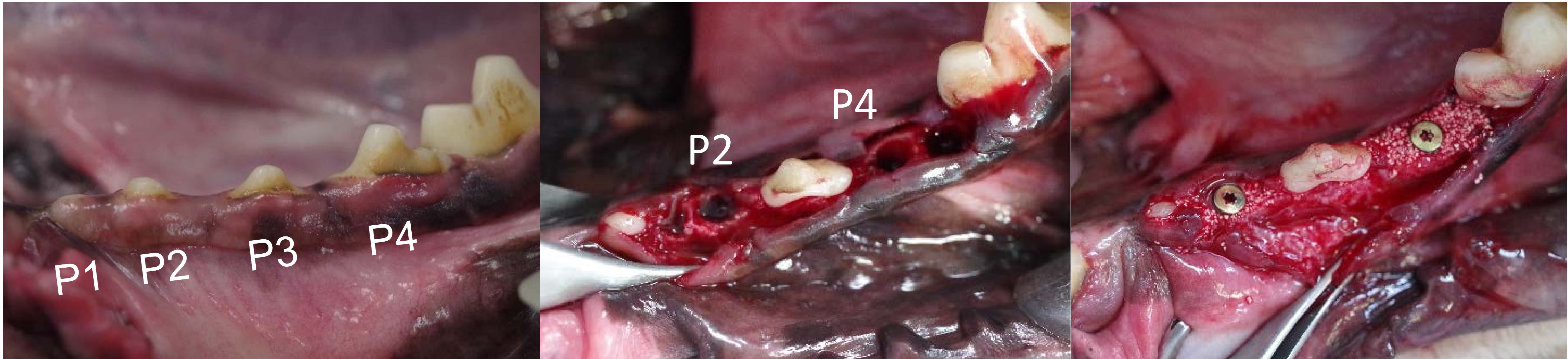
MATERIALS & METHODS

Animal experiments were performed at Hamri Co., Ltd., which has been approved by the AAALAC International.

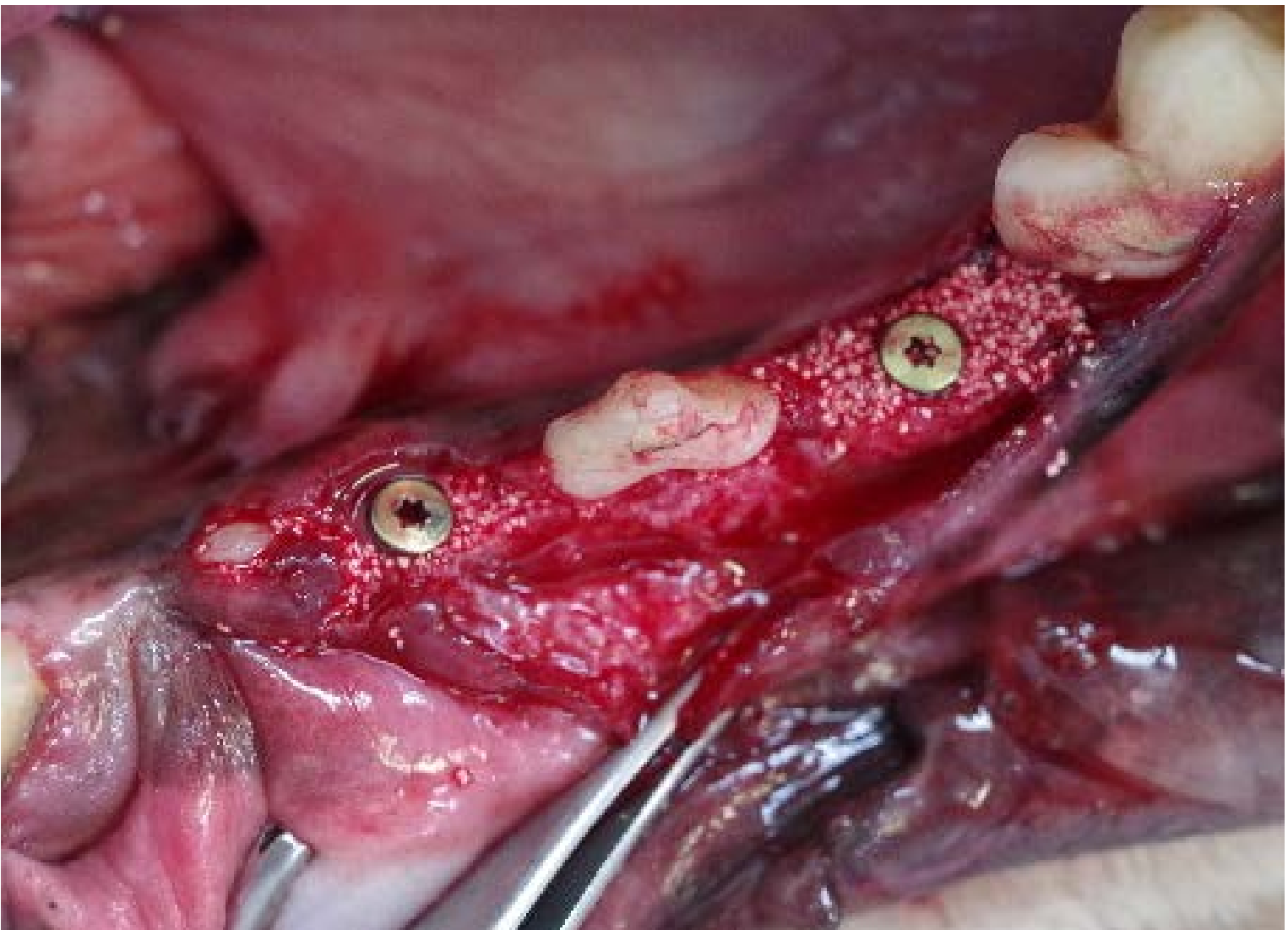
[Study A]

Second and fourth lower premolars were extracted bilaterally, and third one was preserved. ϕ 3.8 mm dental implants were placed in center of the distal and mesial sockets. The extraction sockets were randomly divided into CO₃Ap, β -TCP, HAp and sham group. After healing period of 12 weeks, histological evaluation were performed. The percentages of newly formed bone area (NFB) and bone-to-implant contact (BIC %) was evaluated using image analysis software (Image J). Epithelial downgrowth area was analyzed by using the microscopic images.

Group	Materials	Manufacture	Size	Composition
CO ₃ Ap	Cytrans [®] Granule	GC	0.3-0.6 mm	Carbonate Apatite
HAp	NEOBONE [®]	CoorsTek	0.25-1.0 mm	Hydroxy Apatite
β -TCP	Cerasorb [®] M	Curasan MG	0.15-0.5 mm	β -tricalcium phosphate
Sham	-	-	-	-



Extraction of P2 and P4

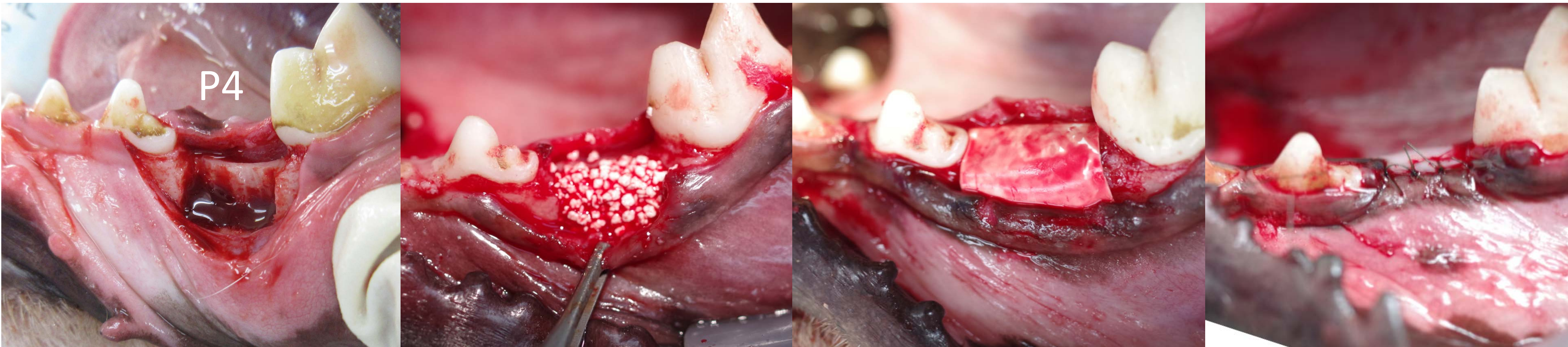


Filling of CO₃Ap

[Study B]

A buccal dehiscence type 3-walled bone defect (10 × 4 × 5 mm) was prepared on the mandibular fourth premolars of Beagle dog. The defect was filled with CO₃Ap, and covered by PBM. CO₃Ap group (without-membrane) and sham group were set as a comparative control. After 12 weeks of healing, then micro-CT and histological evaluation were performed. For the statistical significance test between each groups, multiple comparison tests were executed using statistical analysis software.

Group	Membrane	Bone Graft
PBM+CO ₃ Ap	Cytrans [®] ElaShield	Cytrans Granules, 0.6-1.0 mm
CO ₃ Ap	-	Cytrans Granules, 0.6-1.0 mm
Sham	-	-



Preparing of the defect

Filling of CO₃Ap

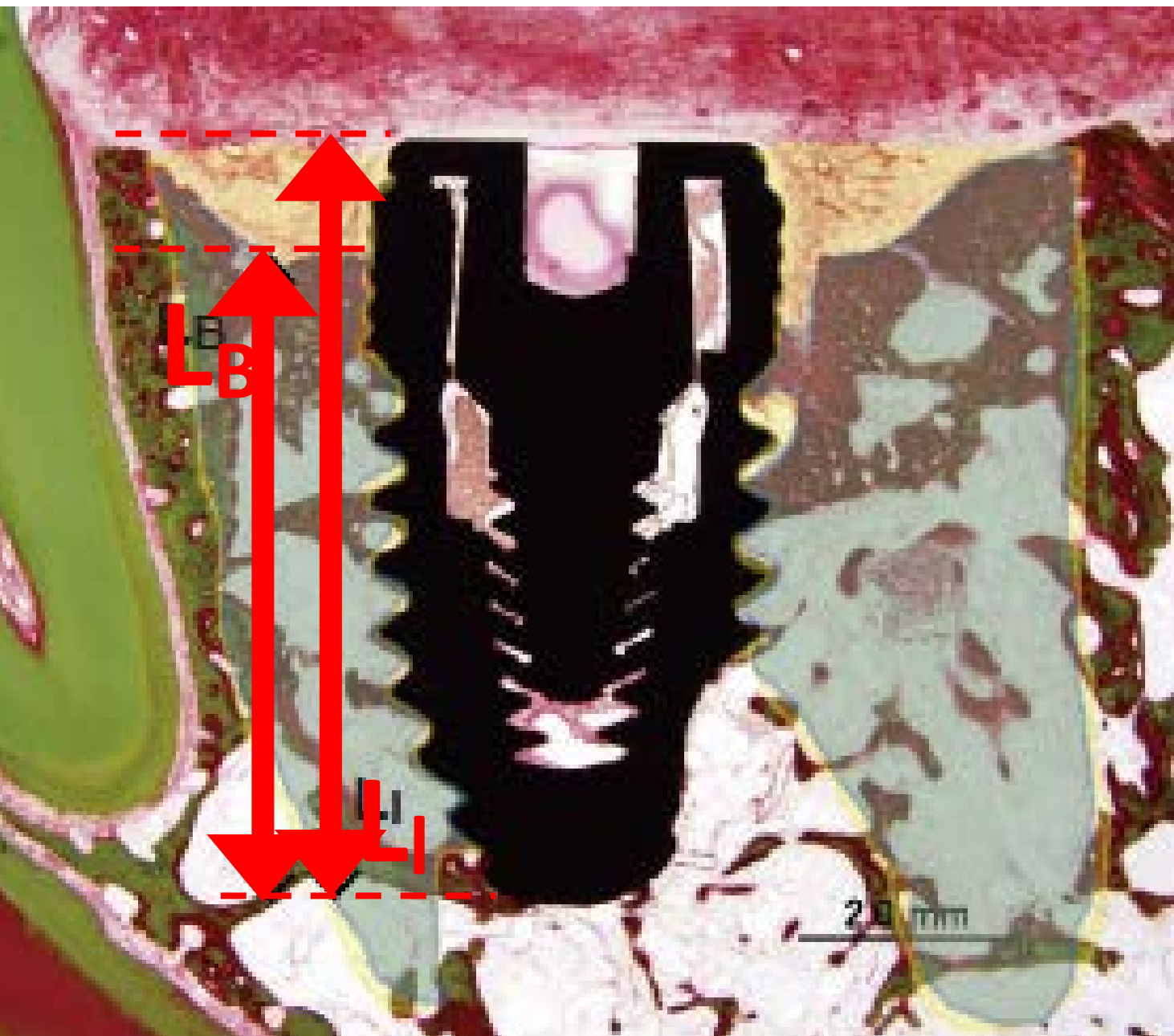
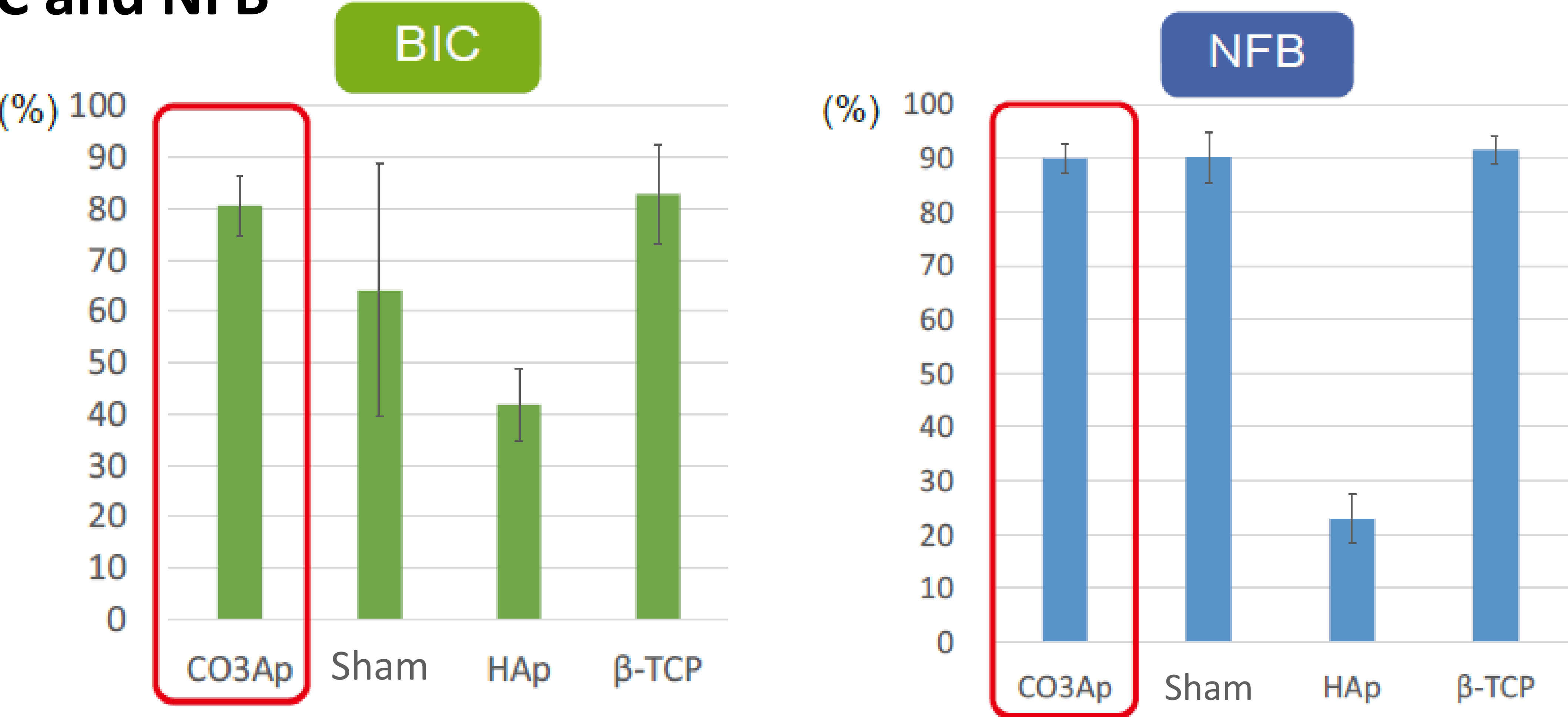
Covering of PBM

Suturing

RESULTS [Study A]

- From the results of histomorphometric evaluation, bone-to-implant contact (BIC) was 80.6% in the CO₃Ap group, 82.8% in the β-TCP group, 41.9% in the HAp group and 64.1% in the sham group. Newly formed bone area (NFB) was greater in CO₃Ap (90.0%), β-TCP (91.6%) and sham (90.2%) than in the HAp (23.0%).
- Epithelial downgrowth area was less in the CO₃Ap (0/4 site) than β-TCP (3/4 site), HAp (4/4 site) and sham (3/4 site) group.

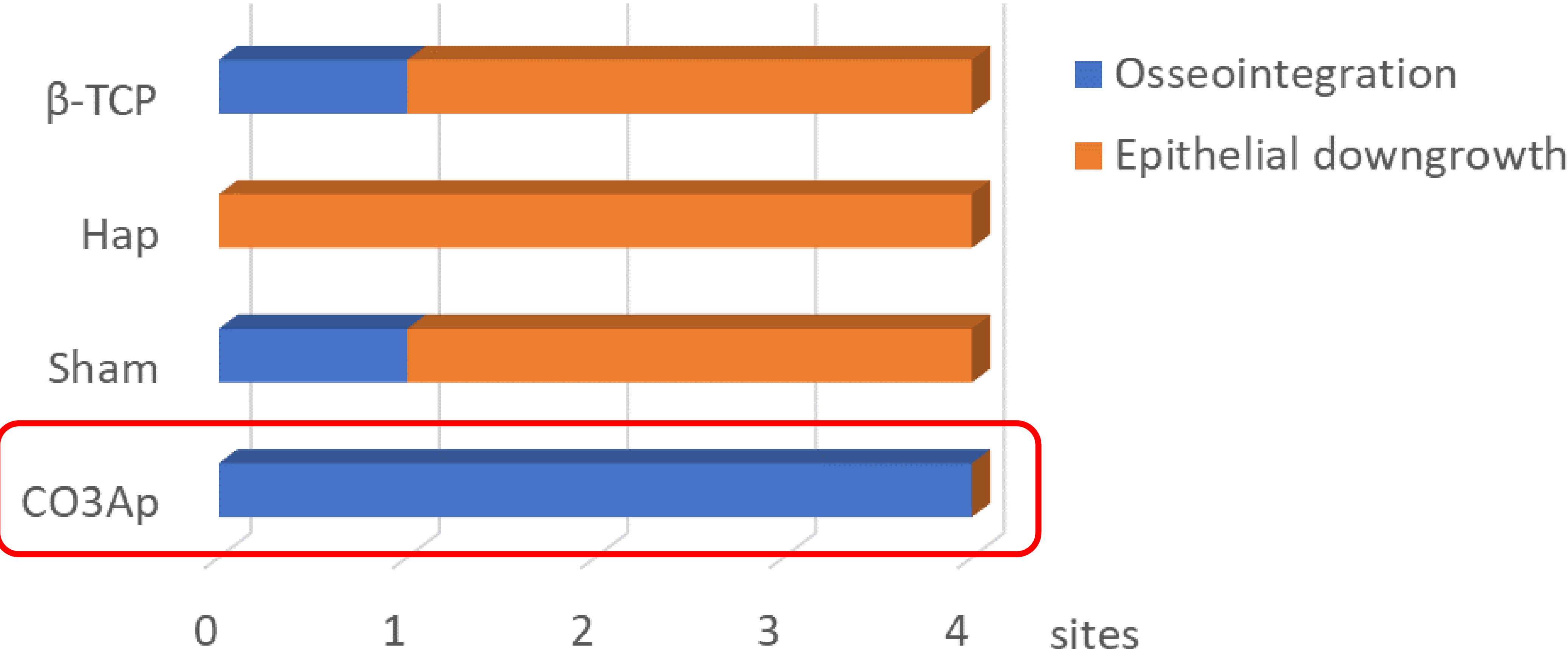
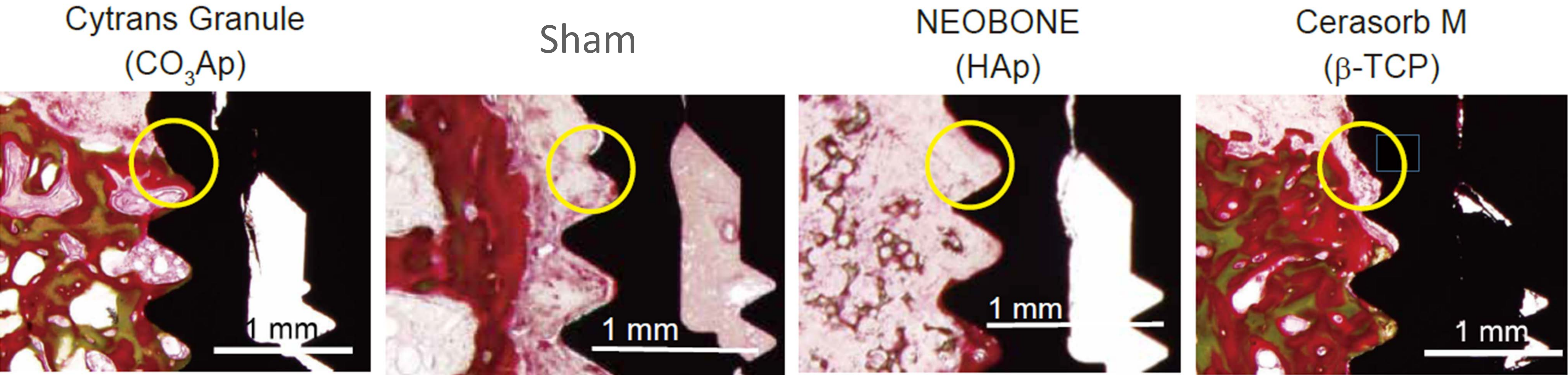
① BIC and NFB



$$BIC (\%) = \frac{LB}{LI} \times 100$$

$$NFB \text{ (Newly formed bone area ratio) } (\%) = \frac{\text{Area of newly formed bone}}{\text{Area of defect area}} \times 100$$

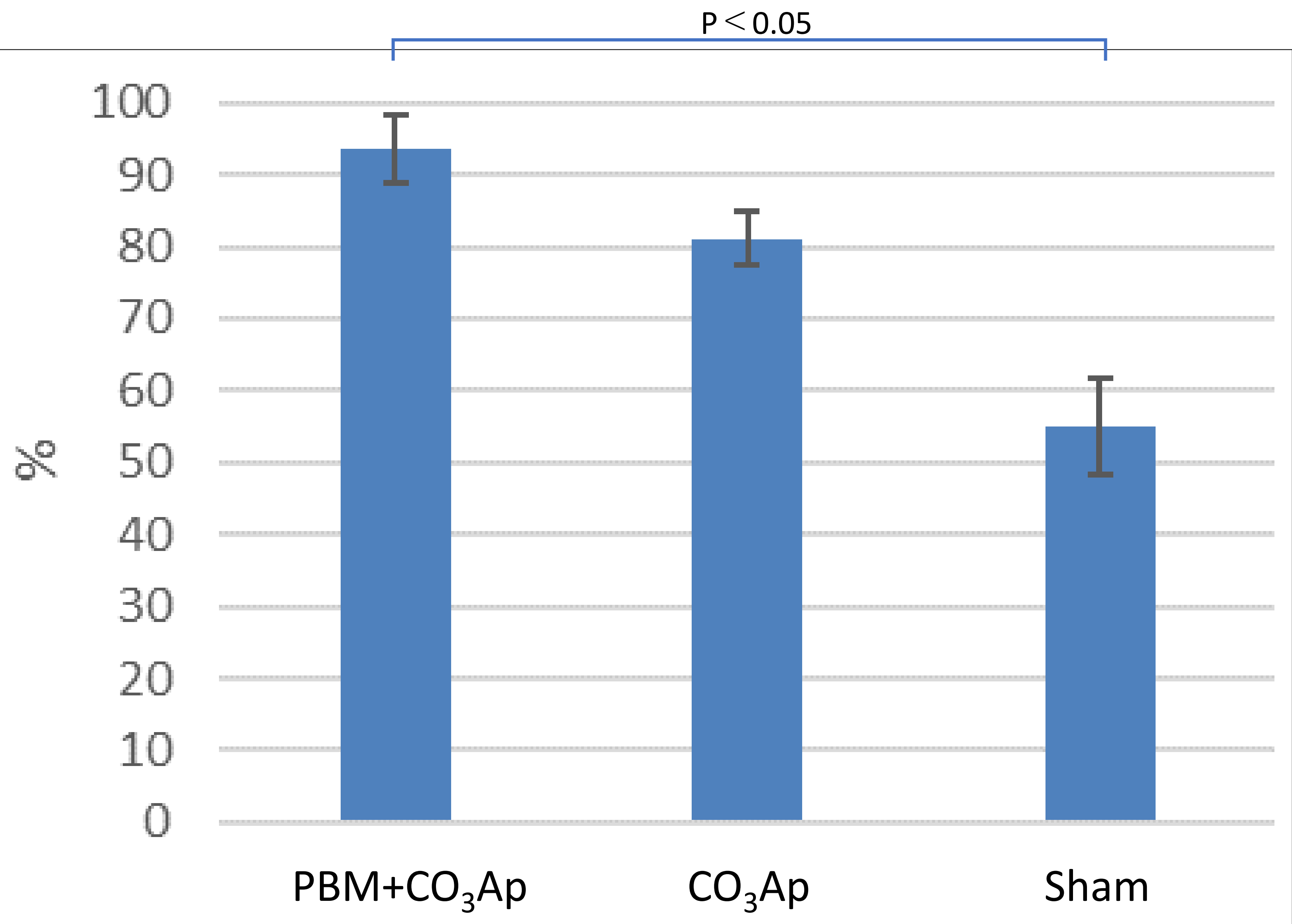
② Epithelial Downgrowth Area



RESULTS [Study B]

- No inflammation was observed in all groups, and no suspected symptoms for safety (wound dehiscence, membrane exposure, infection) were observed for 12-week healing period.
- Newly formed bone volume ratio was 93.9% in the PBM+CO₃Ap group, 82.3% in the CO₃Ap group, and 55.1% in the sham group. Histological evaluation revealed that the calcified area on CT observation was bone tissue in all cases, and in the PBM group, continuous tissue from surrounding bone to newly formed bone was observed in the defect.

Newly formed bone volume ratio

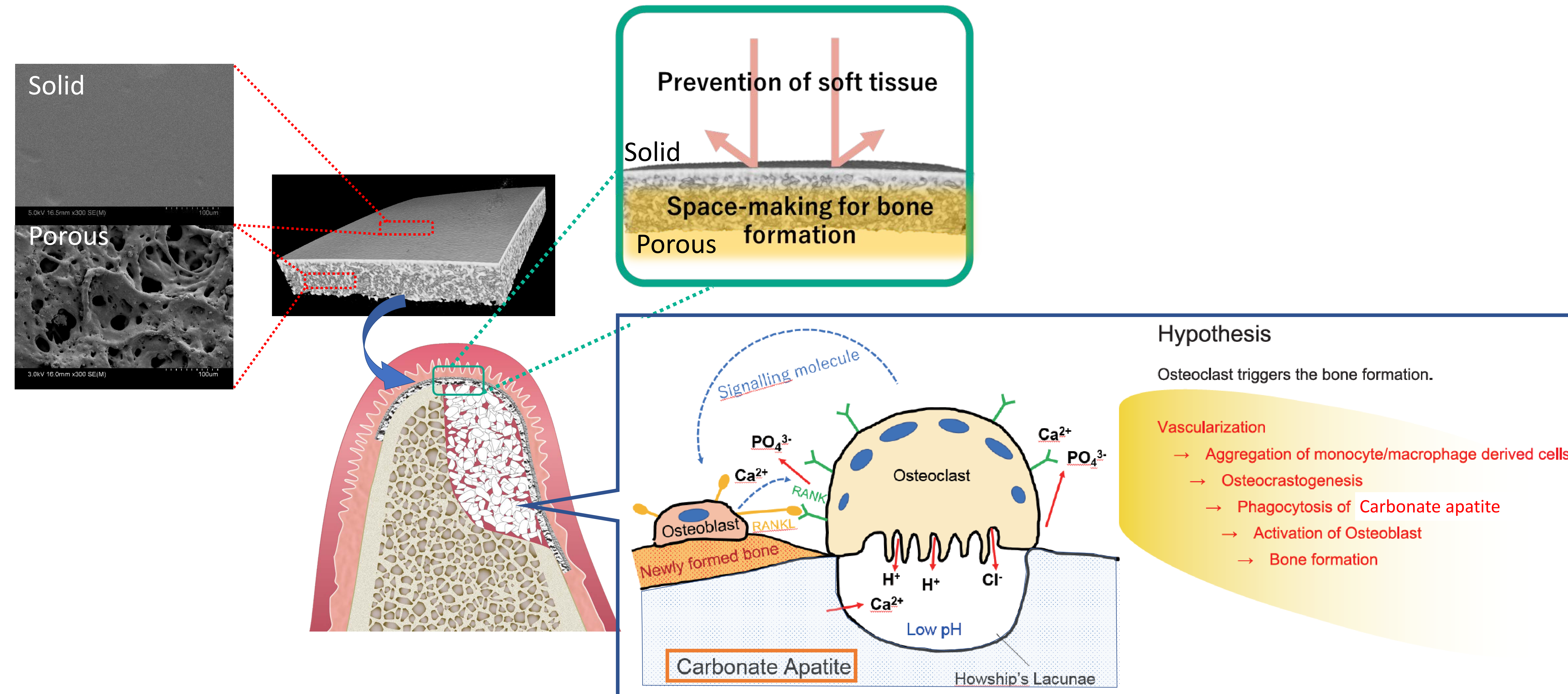


	PBM+CO ₃ Ap	CO ₃ Ap	Sham
Inflammation	No	No	No
Micro CT image			
Histological Image (Villanueva Goldner Stain, Green: matured bone, Red: Osteoid)			

DISCUSSION

In this preliminary study, CO₃Ap showed the potential of being a superior and biocompatible bone graft substitute. 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.



CONCLUSION

It is expected that CO₃Ap and PBM are clinically useful materials for bone graft treatment.