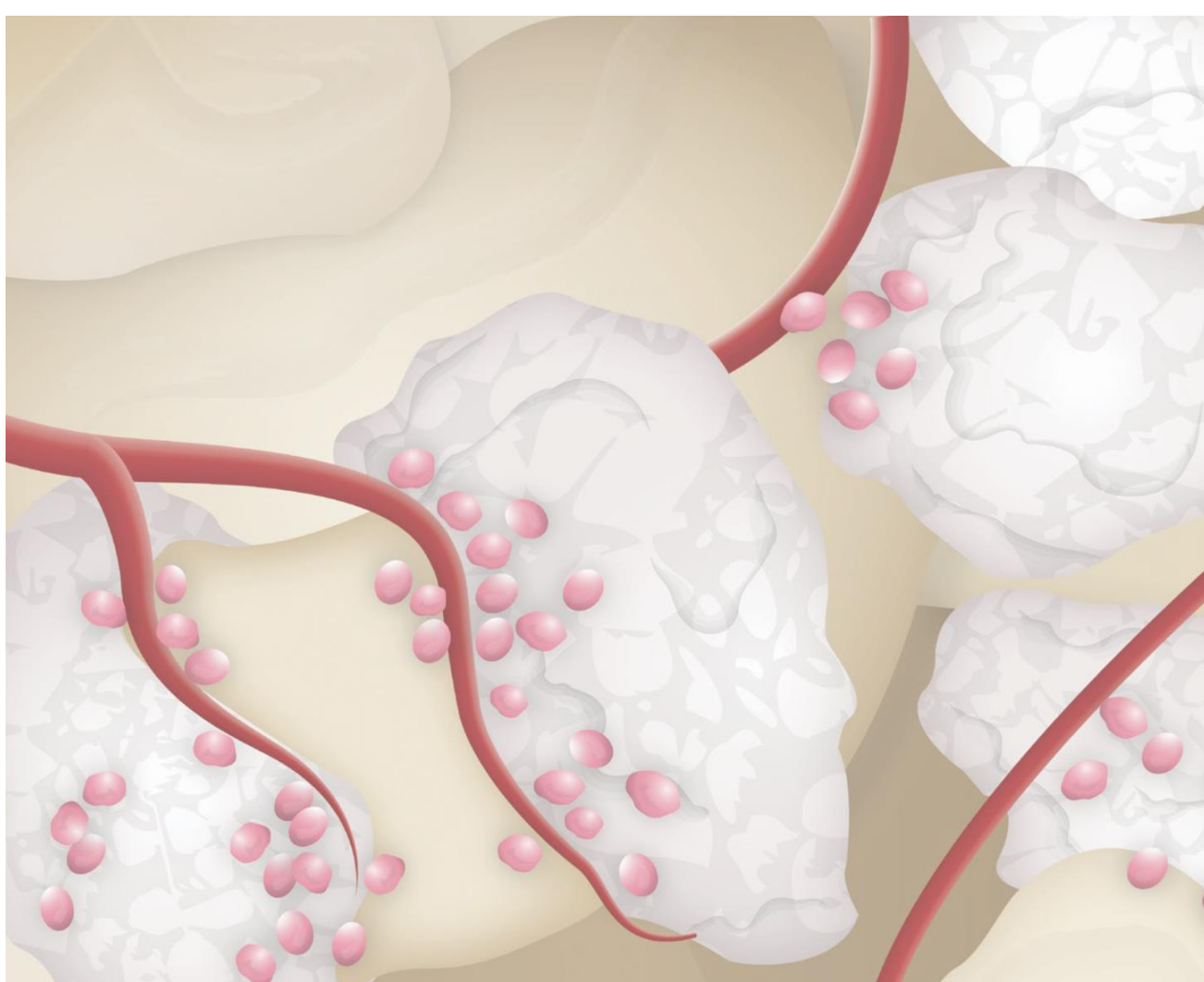




Effect of Carbonated Apatite on Bone Regeneration in Dog Buccal Dehiscence Type Defects

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INTRODUCTION

Bone graft material is an important alternative to autogenous bone graft. In 2018, GC launched a synthetic carbonated apatite (CO₃Ap, Cytrans Granules, GC Corporation) bone graft material based on CO₃Ap, an inorganic component of human bone. CO₃Ap has been widely used in clinical situations, however, scientific evidence is required to demonstrate its effectiveness in various treatment modalities. The aim of this study was to evaluate the effect of CO₃Ap on bone regeneration in dog 3-wall buccal dehiscence bone defect.

Table 1. Inorganic composition of bone and bone graft materials [1]

Component	Contents (wt%)			
	human bone	HAp (hydroxyapatite)	β-TCP (β-tricalcium phosphate)	CO ₃ Ap (Carbonated apatite)
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	-	-	-

[1] LeGeros RZ, *Monographs in Oral Sci*, 1991 15 1 201

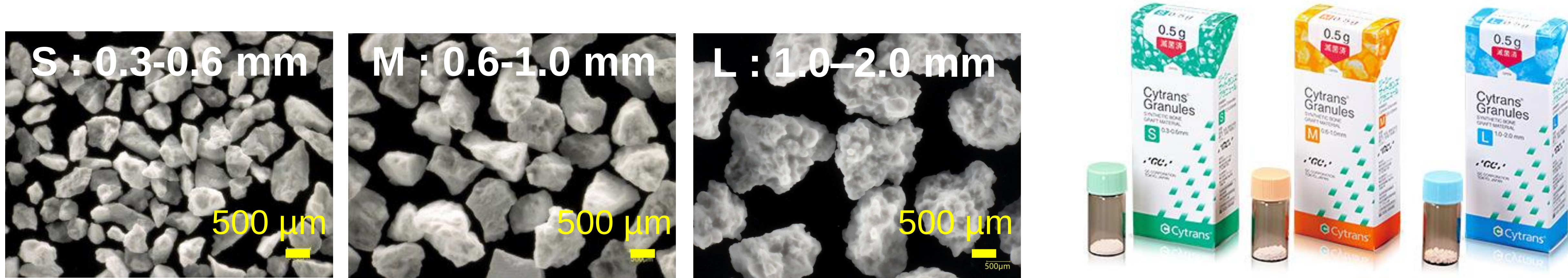


Figure 1. Carbonated apatite granules

MATERIAL&METHODS

Animal Study*

1. Lower premolars (P4) were extracted from 6 adult dogs.
2. After a healing period of 8 weeks, a 3-wall bone defect ($10 \times 4 \times 5$ mm) was prepared at the proximal section of molars with buccal dehiscence.
3. The defect was filled with CO₃Ap granules, and the surgical incision was closed without membrane. Unfilled defects group was set as a control.
4. After 12 weeks of implantation, micro computed tomography (μ CT) and histological evaluation were performed.

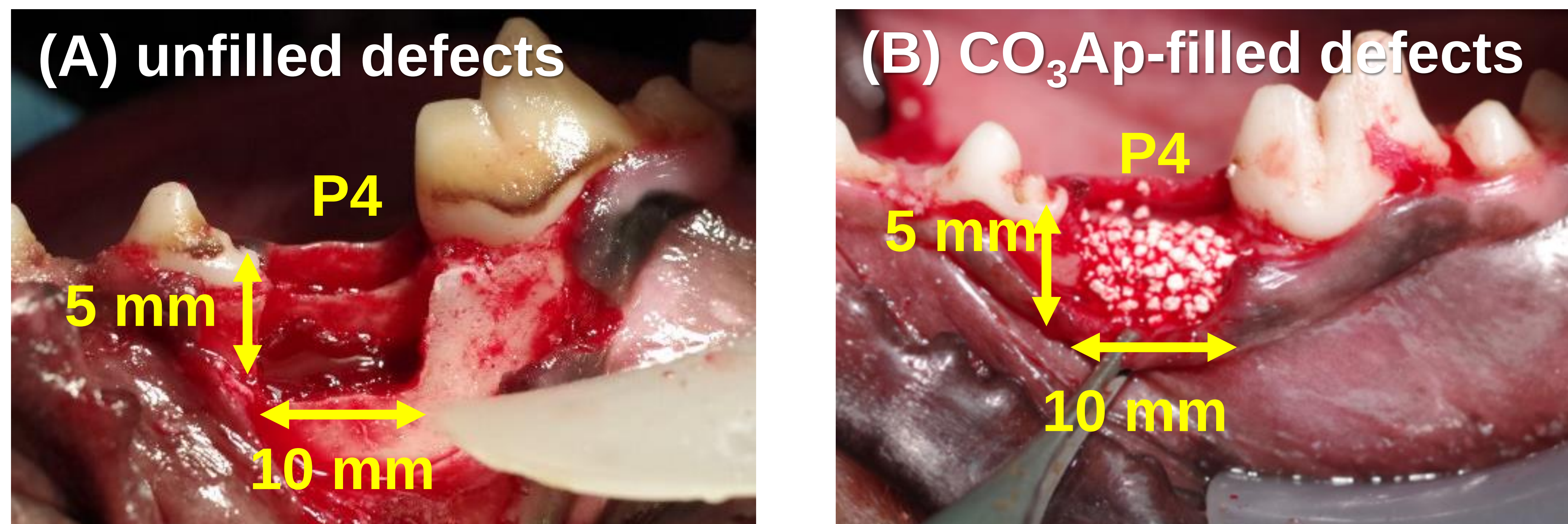


Figure 2. Images showing bone defects in animal study

Material

Animal: 6 male beagles

Test sample: CO₃Ap (particle size : 0.3–0.6mm)

Evaluation

1. μ CT images were taken by Inspexio SMX-100CT (SHIMADZU, Kyoto, Japan) and were analyzed by CT analysis software (VG studio) to calculate the ratio of newly formed bone, signified by highly radiopaque areas. Results were analyzed by Mann-Whitney U test.
2. Hematoxylin and Eosin staining (H&E staining) was applied to all tissue sections to evaluate the formation of new bone by microscope.
3. In H&E staining images, the granules surrounded by new bone were counted to calculate their percentage out of all granules.

*The experimental protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Hamri Co., Ltd. (Ibaraki, Japan); approval number IACUC No. 18-H049, 19-H097.

RESULTS

Clinical Findings

There were no signs of inflammation, wound dehiscence, leakage of CO₃Ap, or infection in both groups.

μCT analysis

In μCT analysis of control group, the new bone formation was observed in the bottom part of the defect. Alternatively, in the CO₃Ap group, the new bone formation was observed throughout the entire defect area. The volume percentage of new bone in CO₃Ap group was 81.1%, significantly higher than the 55.1% in the control group. (Mann-Whitney U test, $P < 0.05$)

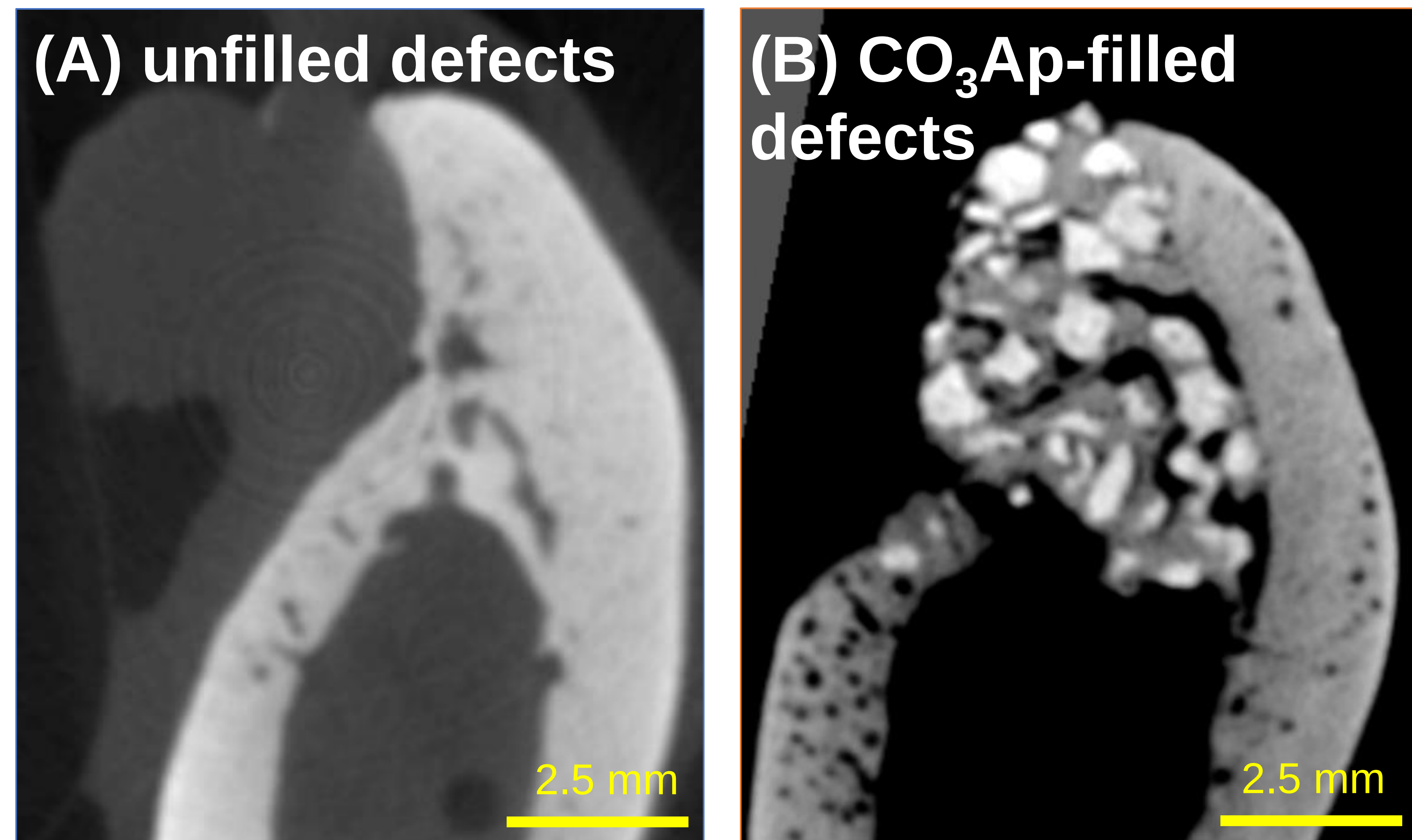


Figure 3. Representative μCT images of coronal sections showing (A) unfilled defects and (B) CO₃Ap-filled defects. Areas with high radiopacity in (B) represent newly formed bone.

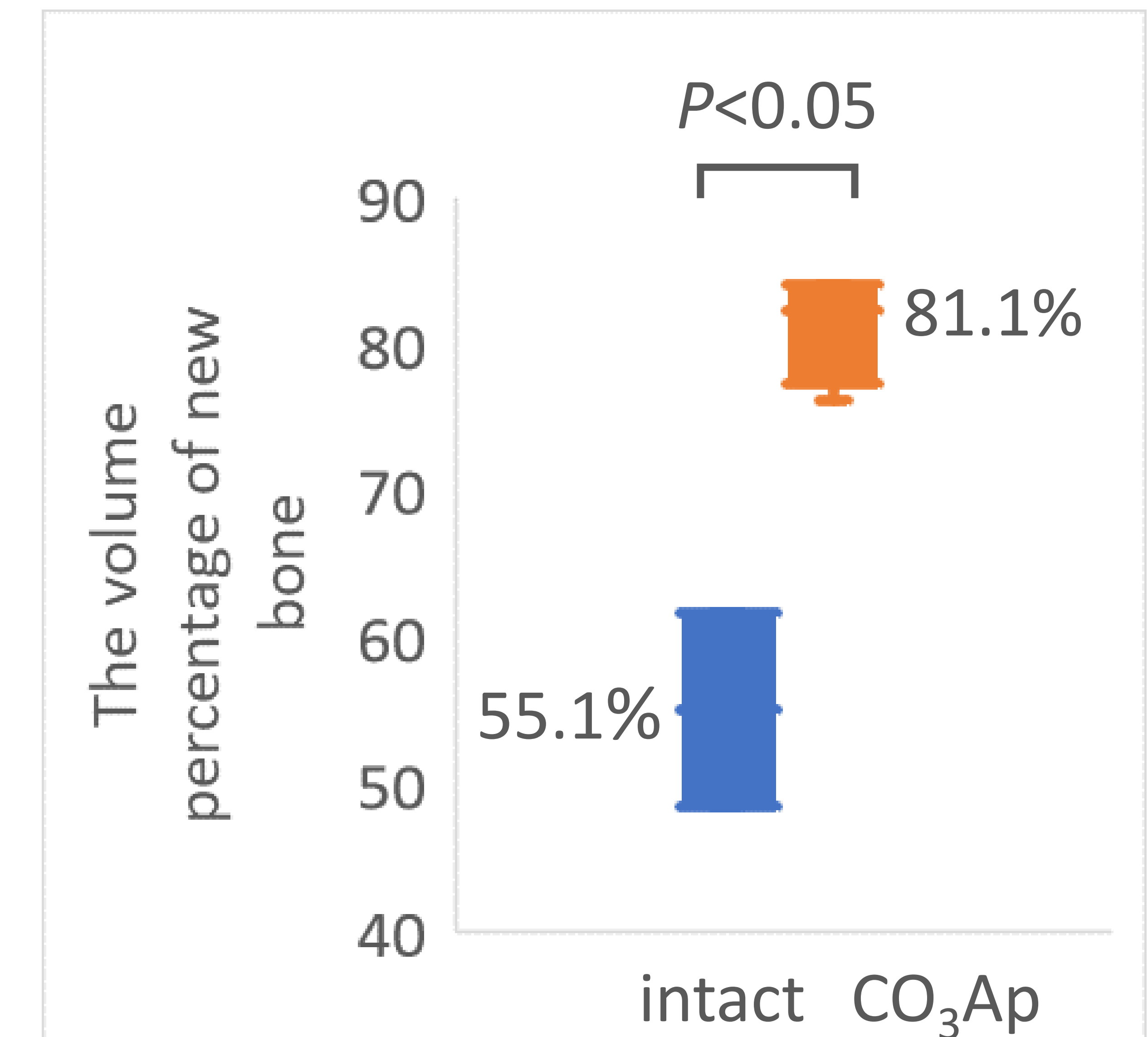


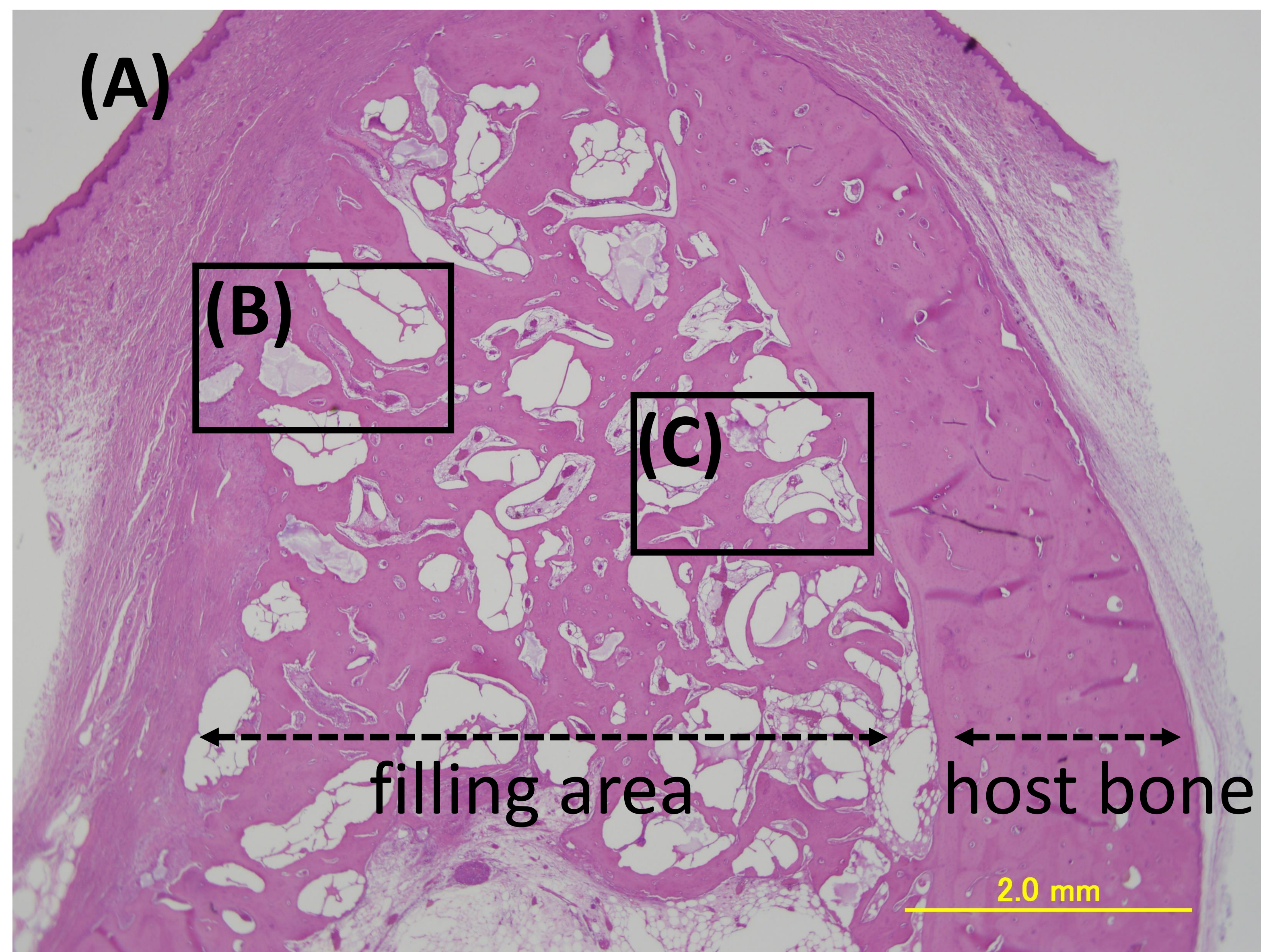
Figure 4. The volume percentage of new bone

RESULTS

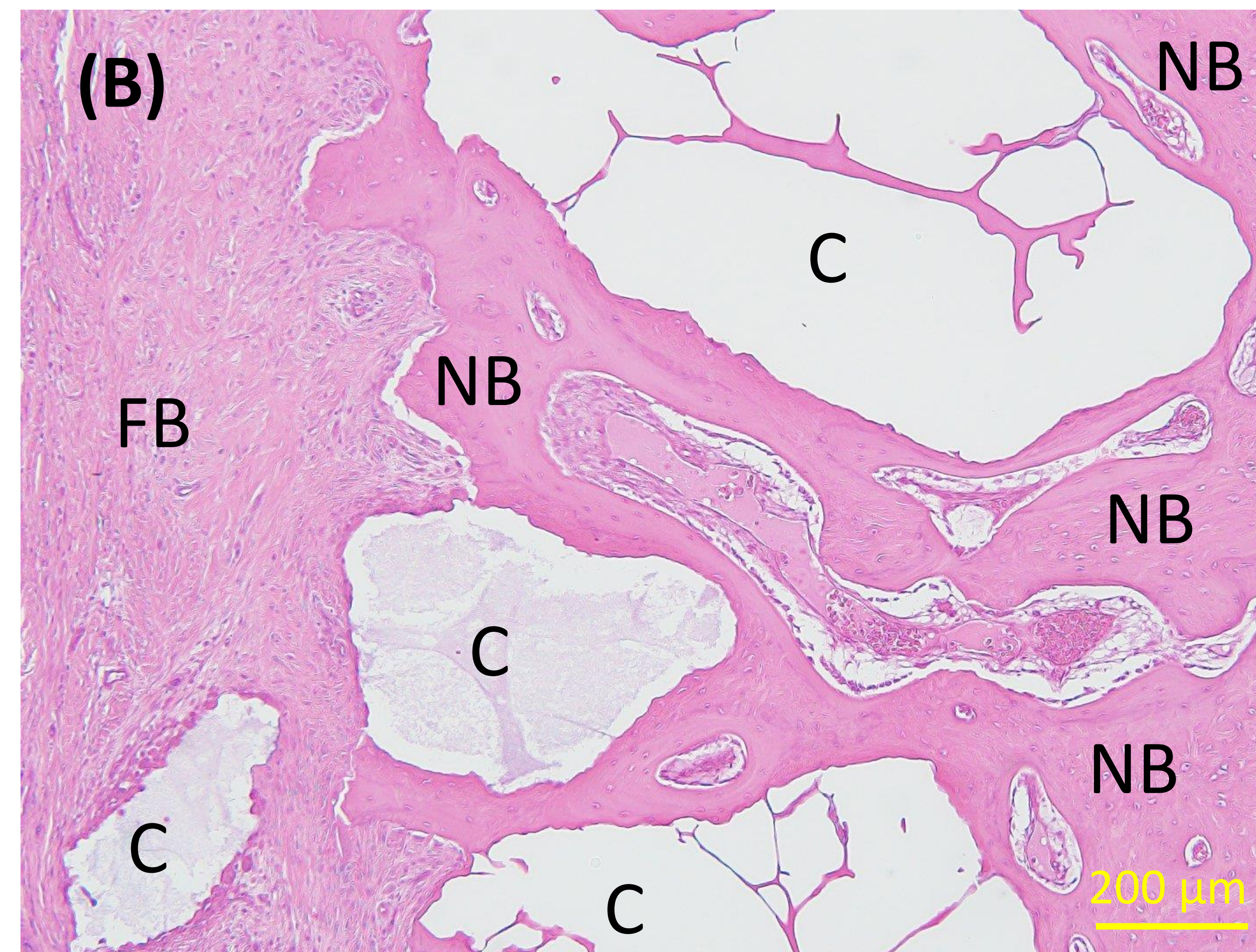
Histological Evaluation

Histological evaluation of CO₃Ap group revealed that new bone was generated around granules in most of the filling area, with a few invasions of soft tissue on the surface. The percentage of granules surrounded by new bone out of all granules is 92%. These results indicated that the composite tissue of CO₃Ap and new bone was generated in the defect area.

(A) Overview of coronal sections



(B) Enlarged view of outer layer part



(C) Enlarged view of inner part of bone defect

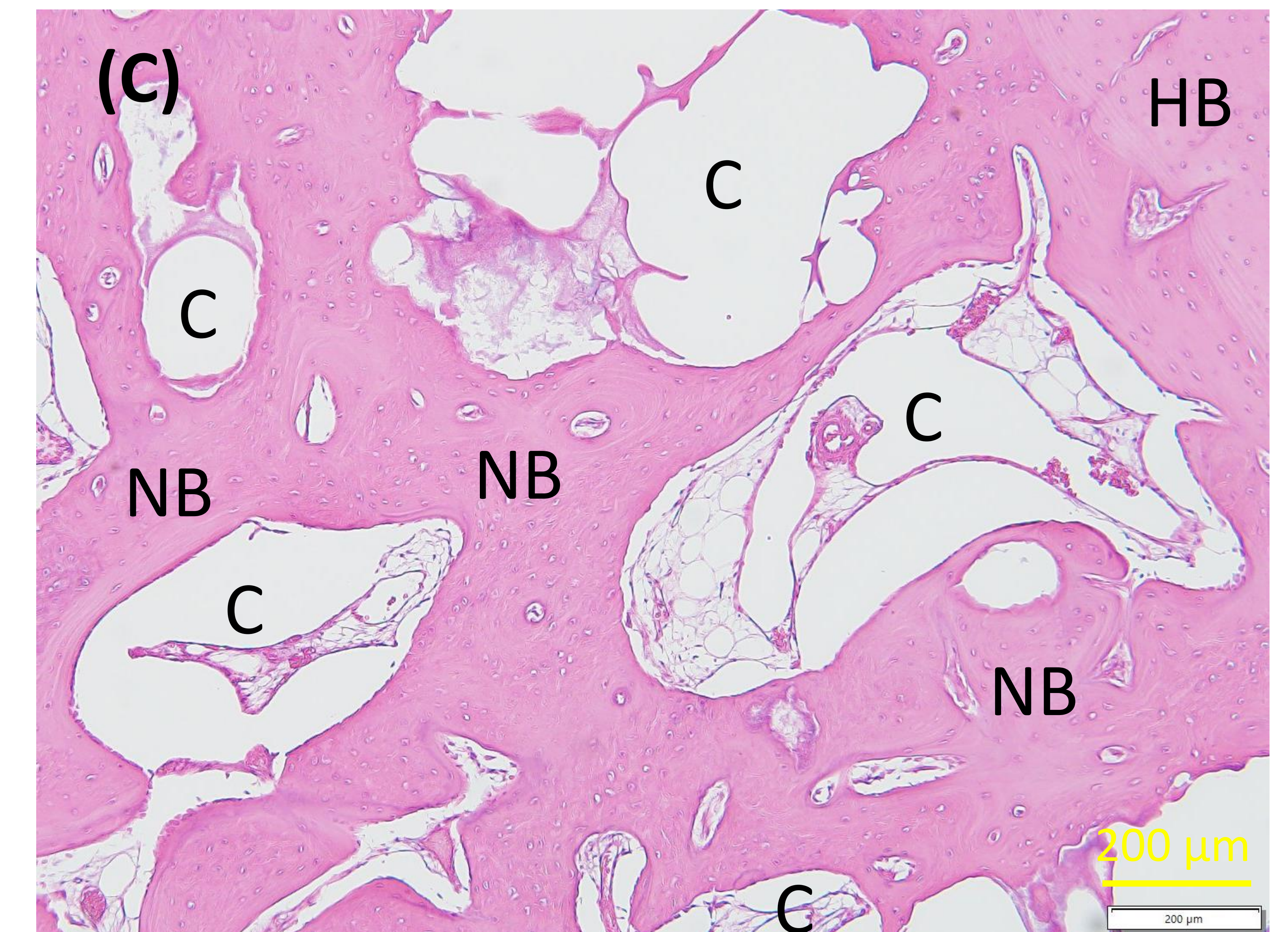


Figure 5. H&E staining images of CO₃Ap group

C: CO₃Ap, NB: new bone, HB: host bone, FB: fibrous tissue

DISCUSSION

In this study, results that showed CO₃Ap can be a remarkable choice as a bone graft substitute and scaffold for bone regeneration. CO₃Ap granules have been reported to activate phagocytosis by osteoclasts rather than deproteinized bovine bone material (DBBM), hydroxyapatite, or β -tricalcium phosphate [2,3]. It has also been reported that CO₃Ap has osteoblastic differentiation potential of human bone marrow cells earlier than culture plates or HAp, suggesting that CO₃Ap facilitates bone marrow cells and osteoblasts osteogenic differentiation [4]. In this study, the CO₃Ap group defects were filled with new bone while some granules also remained. The carbonated apatite granules remained in the composite tissue would be gradually resorbed and replaced by bone.

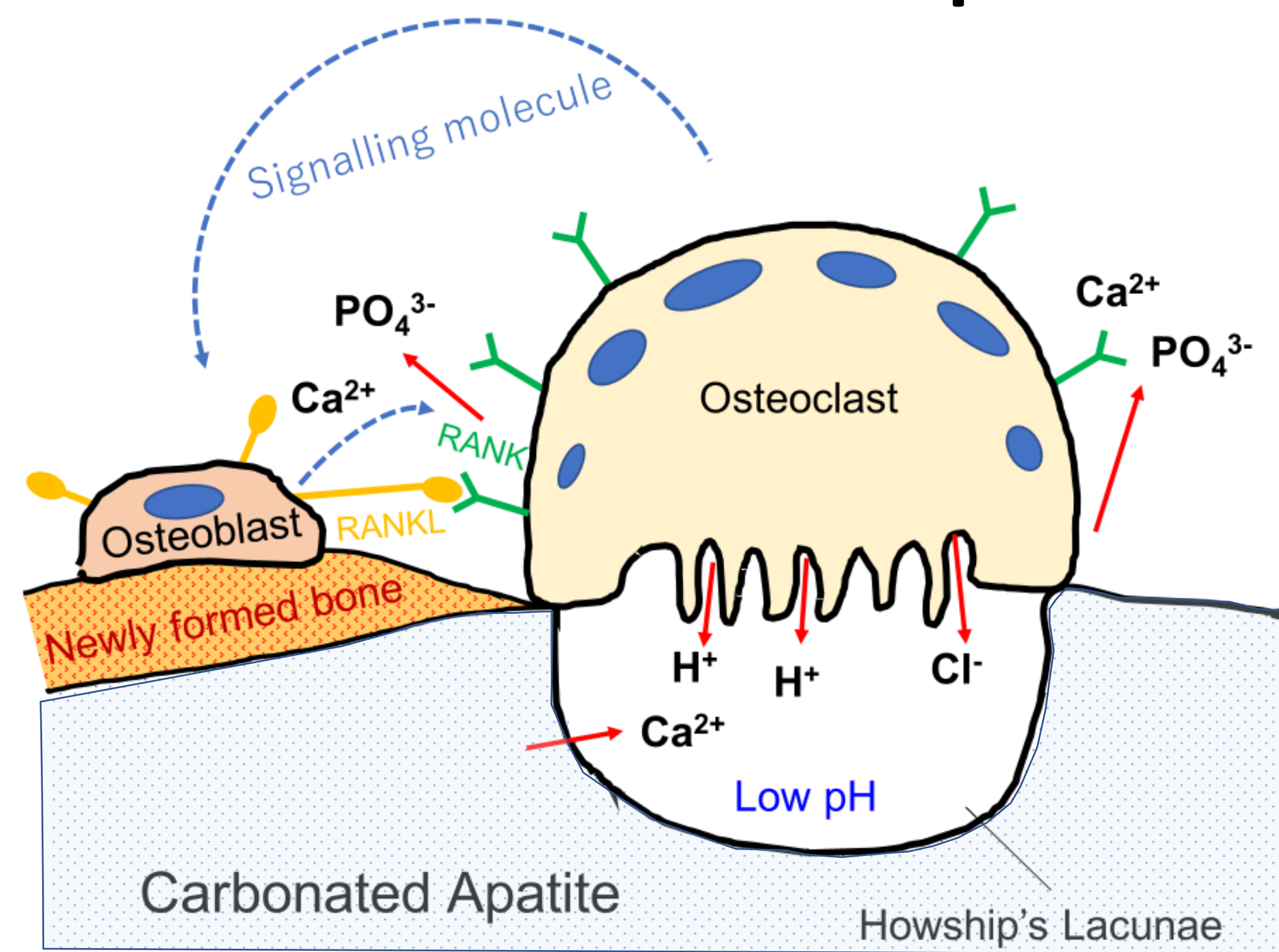


Figure 6. Mechanism of bone remodeling

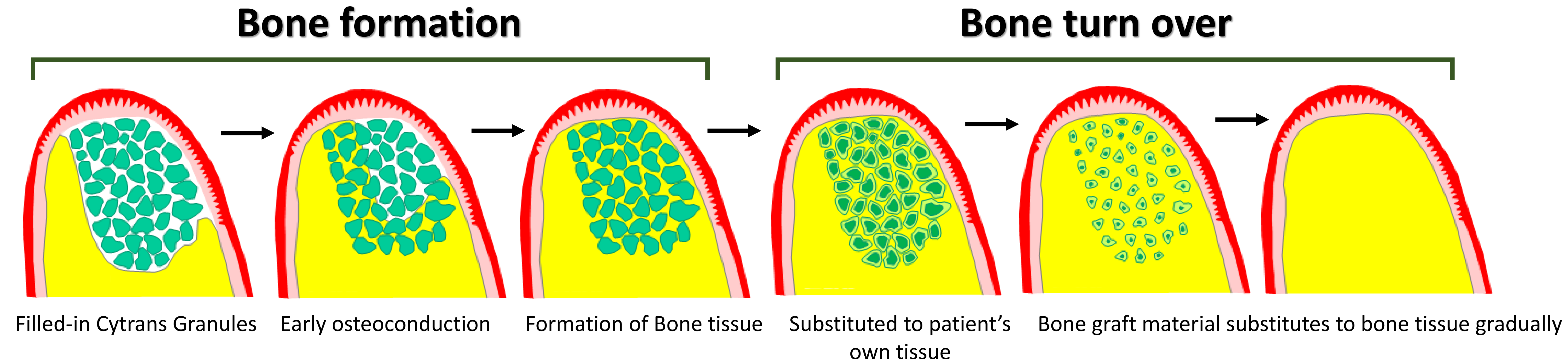


Figure 7. Mechanism of bone formation

CONCLUSION

[2] Fujioka-Kobayashi, M et al, *Journal of Biomedical Material Research A*, 2022 110 1524

[3] Nakamura, M et al, *Journal of Biomedical Research A*, 2013 101 3141 51

[4] Nagai, H et al, *Journal of Material Science Materials in Medicine*, 2015 26 99

This study suggests the efficacy of using CO₃Ap granules in buccal dehiscence type bone defects.