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Injectable Porous Bioresorbable Composite Containing Fluvastatin for Bone Augmentation

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ABSTRACT

The purpose of this study was to evaluate the effects of an injectable composite made up of calcium sulfate (CAS), fluvastatin (FS) and atelocollagen on bone augmentation in rats. Porous structures and the compressive strength of composites were evaluated. The cumulative release kinetics of FS were determined *in vitro* by a spectrophotometer. To observe bone regeneration *in vivo*, five different materials (normal saline; atelocollagen gel only; composite of CAS and atelocollagen; composite containing 0.5% FS; and composite containing 1.0% FS) were injected in extraction sockets and on the crania of rats. Micro-computed tomography (micro-CT) and histological evaluation were performed after 2, 4, and 8 weeks of healing time. The composites had high porosity (greater than 55%). FS kept a slow and stable release for >30 days. *In vivo* results demonstrated that, more new bone was formed in the FS groups compared with other groups, both bone mass and bone density had prominent increased in maxillae and crania. Resorption of the composite was also observed for cranial tissues. In conclusion, this composite can be applied percutaneously, without any incision. It has excellent properties with replaceability into bone and anabolic effects for bone formation, as well as a drug delivery system for bone formation.

KEYWORDS: *calcium sulfate, fluvastatin, bone regeneration, injectable bone substitute, drug release, porous material*

1. INTRODUCTION

Currently, dental implants have become an increasingly acceptable therapy for partial and fully edentulous patients. However, tooth extraction leads to alveolar bone resorption.[1] Serious resorption can result in an inadequate amount of bone at the implant site. In such cases, bone augmentation is becoming a focus for dental implant treatment.

For bone augmentation, autograft is sometimes selected, but any additional complex surgeries are operated, which can produce huge injury and pain. Meanwhile, harvesting of bone can lead to donor site morbidity.[2] Allograft has risk of immunological rejection, virus and bacteria transmission and additional bone processing procedure are used to reduce risk which will also affect its biological activity.[3,4] Instead, artificial bone is often used for bone augmentation. Artificial bone usually needs to be pre-formed as particle or block which is inconvenient to fill them into intraoral recipient site. And the pre-formed shape may not adapt to the shape of the defect site, especially for some cases where the bone defect is small. Besides, surgery causes trauma, and dehiscence and infection may occur. Here, we generated an injectable composite, which is more convenient to be operated in the mouth. Transmucosal injection or injection via minimally invasive fenestration will reduce risk, as well as both physical and mental burden of patient.

Various materials such as hydroxyapatite (HAp), tricalcium phosphate (TCP), and calcium sulfate (CAS) are used for bone augmentation. However, HAp has a low resorption rate,[5] which leads to insufficient bone-carrier integration and replacement of tissue. TCP is dissolved faster than HAp, but with less regenerated bone ingrowth.[6] CAS is a cheap and easily available bone substitute that has been used for more than 100 years and is rapidly resorbed.[7-9] Furthermore, it

is osteoconductive, allows adherence of osteogenic cells,[10] promotes formation of blood vessels.[11] Disadvantages of CAS include its rapid resorption rate that prevents its maintenance, thereby limiting bone regeneration.[12,13] The rapid dissolution of CAS elicited a mild inflammatory response.[14] Thus, CAS has less obvious advantages for bone augmentation.[14,15] To improve its effect on bone regeneration to match its resorption rate, the combination of other material seems to be possible.

Statins play roles in stimulating bone regeneration via the mevalonate pathway, where they inhibit 3-hydroxy-3-methylglutaryl-coenzymeA (HMG-CoA) reductase,[16] thereby, affects growth factors in osteoblastic cells such as the upregulated expression of BMP-2 and VEGF.[16,17] *In vivo*, statins promoted bone healing and significant new bone growth, enhanced bone strength,[18] and increased bone volume.[19] In addition, statins promoted bone formation around implants when injected systemically or locally.[20,21] However, the effect of statins was only maintained for 5 days when used without an effective drug delivery system (DDS).[22] So, we made a composite made up of αTCP and atelocollagen as a drug carrier of fluvastatin and induced new bone formation.[23] However, this composite was unfavorable because the dense carrier hindered drug release and bone ingrowth. Thus, a better drug releasable material with the long-term stable release of bioactive substances such as statins for osteoinductivity are expected.

Here, we generated an injectable composite of fluvastatin, CAS and atelocollagen. CAS solidifies too fast when it is mixed with water. To make sure its injectability, atelocollagen gel was used to instead of water.[23] Atelocollagen has high moisture retention capacity and excellent biocompatibility. It is often used as a tissue engineering scaffold to culture cells.[24] Meanwhile,

atelocollagen have active effect on guiding tissue regeneration,[25,26] it promotes osteoblast differentiation and type I collagen production, thus caused bone regeneration.[27]

In the present study, structures and *in vitro* release kinetics were investigated. *In vivo* studies investigated the effects of this composite on bone augmentation via immediate injection. We expect this composite induces new bone formation, under the influence of statins with excellent bone regeneration properties.

2. MATERIALS AND METHODS

2.1. Materials

Calcined gypsum, the main component of CAS, was purchased from Nacalai Tesque (Kyoto, Japan). Fluvastatin sodium salt (FS) was obtained from Toronto Research Chemicals (Toronto, Canada). Atelocollagen gel was purchased from Koken (Tokyo, Japan).

2.2. Injectable Composite Preparation

CAS and atelocollagen gel were mixed respectively on mixing paper pad for 30 seconds using five powder-to-liquid ratios (P/L ratio)— 1:2, 1:1, 5:4, 3:2, and 2:1. Each composite was injected into a wax mold with a diameter of 5 mm and a height of 10 mm. Time measurement was started when the molds were placed in a vacuum oven (AVO-250NB; AS ONE, Osaka, Japan) at 37°C. Six replicates of each ratio were prepared. In addition, about 0.3 ml of each composite was injected into 50 ml of phosphate buffered saline solution (PBS, pH 7.4) using a 2.5 ml syringe (Terumo, Tokyo, Japan) with a 21G needle (Terumo, Tokyo, Japan) to observe the diffusion state of these pastes in PBS solution.

2.3. Physical Characteristics

For the composite containing FS, different amounts of FS were added and thoroughly mixed with CAS before adding atelocollagen gel. Composites with three different doses of FS were produced: no FS (CS), 0.5% FS (FS-0.5), and 1% FS (FS-1) (powder weight ratio).

2.3.1. Internal Structure and Porosity

Three composite pastes were molded respectively in a wax mold with a diameter of 5 mm and height of 10 mm. The filled molds were placed in a vacuum oven for 7 days at 37°C. The cylindrical specimens were coated with gold (MSP-1S; Vacuum Device, Mito, Japan) and examined with a scanning electron microscope (SEM, S-4800, Hitachi, Tokyo, Japan) at 15 kV. Each group had six specimens.

Computer-based image analysis was used to measure porosity.[28-30] A porosity calculation model was built based on Image-pro Plus 6.0 software (Media Cybernetics, Rockville, MD, USA) as previously described.[28] The cross-sections of each specimen were scanned and six 400× magnification images were obtained. First, binarization images were obtained through threshold segmentation by the software. In the binarization images, pores (black) phases were distinguished from the wall (white) phase. The porosity was calculated from the measurement of the total area of pores (pixel value) divide the whole area of the image (pixel value). And the porosity of one specimen is the mean value of the six images. To reduce visual errors, a multiple-rating method was adopted, in which three individuals (authors) were selected to evaluate the threshold selection of each image. Then, the mean threshold of image segmentation was evaluated. Pore size was measured also based on 400× magnification images. Diameters of three hundred pores were measured for one specimen by image J (NIH, Bethesda, MD, USA).

2.3.2. Mechanical Testing

Cylindrical specimens were fabricated as above. Compressive strength testing was performed with a universal testing machine (Autograph AG-IS 10 kN, Shimadzu, Kyoto, Japan), using a speed of 5 mm/min. The maximum compressive strength values were tested for a total of six replicates of each type of composite.

2.4. *In vitro* FS Release

The cumulative release of FS was determined with a spectrophotometer (Biospec-mini 100 V; Shimadzu) at 238 nm. Two types of composite with 0.5% FS (1.5 mg) and 1% FS (3.0 mg) were injected into the same molds as above, respectively. Cylindrical samples were removed from molds after 7 days at 37°C. Six replicates of each type of composite were prepared. The samples were immersed in 50 ml PBS (pH 7.4) in a 37°C water bath. Then, 70 μl of PBS supernatant was obtained daily for 30 days, and absorbance values were measured. After each sampling, 70 μl of PBS was replenished. A standard curve was prepared using known concentrations of FS (0.2, 0.4, 0.8, 1.2, and 1.6 μg/ml) and the corresponding absorbance values were measured. Then, the concentration of FS was determined by using the equation: $A = 0.5627C + 0.5974$ ($R=0.9998$); where A is the absorbance value at 238 nm, and C is the concentration of FS (μg/ml).

2.5. *In vivo* Animal Experiments

2.5.1. Animals

Animals were handled according to the guidelines for animal care established by Kyushu University. The study protocol was approved by the committee for Animal Research of Kyushu University (approval number: A29-155-0). Ninety male Wistar rats (4-weeks-old) weighing 60-90 g were used in the study.

2.5.2. Surgical Procedures

The right maxillary first and second molars were extracted under general anesthesia. At 4 weeks after extraction, all animals were divided into 5 groups: normal saline (CON); atelocollagen gel only (AC); composite of CAS and atelocollagen (CS); composite containing 0.5% FS (FS-0.5); and composite containing 1.0% FS (FS-1). Each rat received injections using a 21G needle in the extraction region of the maxilla (about 0.1 ml) and percutaneous injection over periosteum of crania (about 0.3 ml) with one of the materials as described previously.[23, 31]

2.5.3. Micro-CT Evaluation of Maxillae

After 2, 4 and 8 weeks, rats were deeply anesthetized and perfused intracardially with normal saline, followed by 4% paraformaldehyde (pH 7.4). The maxillae were harvested with mucosa. Meanwhile, crania were harvested together with the periosteum at 2, 4, 8 weeks. All tissues were immersed in 4% paraformaldehyde for 24 hours. Maxillae were examined by micro-CT (SkyScan1076KHS; SkyScan, Antwerp, Belgium), and imaged with 40 kV voltage and 249 μA current. Images were reconstructed by software (NRecon, SkyScan). The volume of the extraction region of maxillae (from the proximal end of maxillary first molar to the distal end of maxillary second molar) was measured by 3D numerical analysis software (CT-Analyzer, SkyScan).

2.5.4. Histological Preparation and Histomorphometrical Evaluation

After micro-CT evaluation, all tissues were decalcified in 20% EDTA solution (pH 7.4) for 3 weeks. Specimens were dehydrated completely in ethyl alcohol solution and embedded in paraffin wax. Sections of 7 μm were prepared using a microtome (Leica RM2235; Leica Biosystems, Wetzlar, Germany) and stained with Ladewig's stain.[19]

Six sections from each specimen were observed and photographed under light microscope. (BIOREVO BZ-9000; Keyence, Osaka, Japan). Images were analyzed by image software (ImageJ). The new bone thickness (NBT) of crania and bone density (BD) of crania and maxillae were measured as the previous studies described.[19,23] The NBT was defined as the ratio of the thickness of newly formed bone to the thickness of the whole bone. The BD of cranium was defined as the ratio of the new bone area to the total induced tissue and the BD of maxilla was the ratio of bone area to whole tissue area of extraction socket. **(Figure 1)** BD of each section was calculated based on the hole image. The mean NBT of each section was calculated by measuring NBT at five different positions of one image.

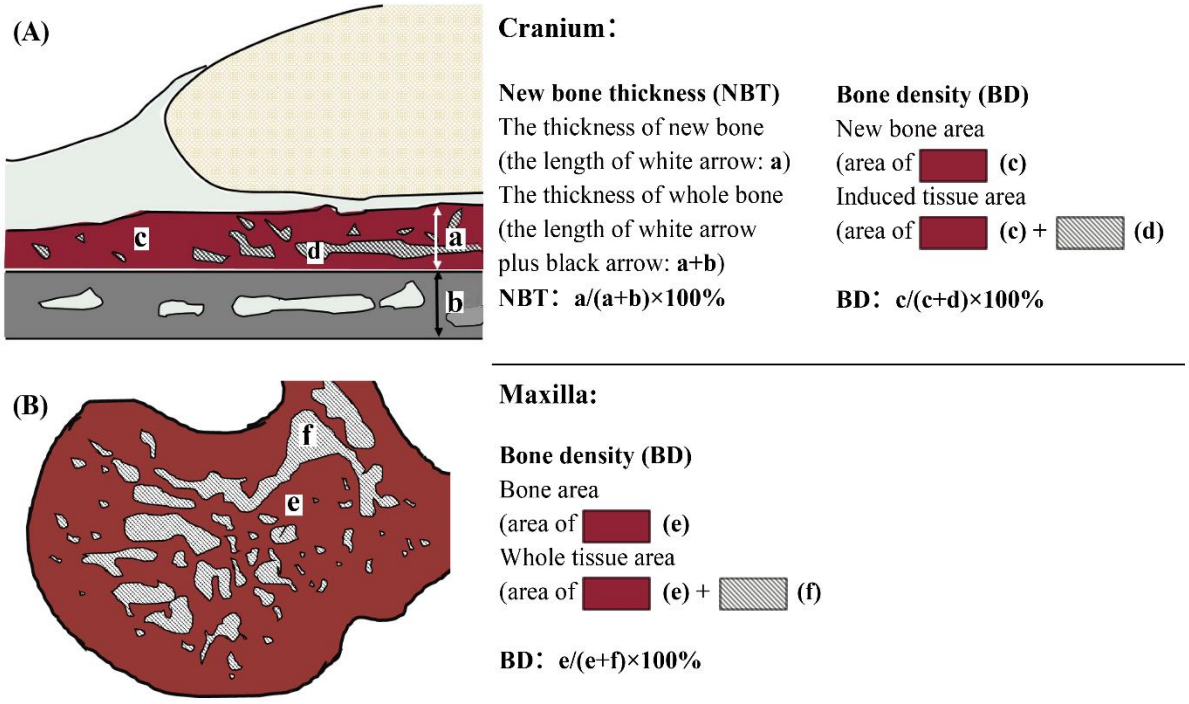

Figure 1. The calculation method sketch of new bone thickness (NBT) and bone density (BD) of cranium (A) and maxilla (B) of rat. NBT (%) was defined as the ratio of newly formed bone thickness to the whole bone thickness. BD (%) of cranium was defined as the ratio of the new bone area to the total induced tissue. BD (%) of maxilla was the ratio of bone area to the whole tissue area of extraction socket.

2.6. Statistical Analysis

Data collected were presented as the mean values ± standard deviations (mean ± SD). All-data

were analyzed using one-way ANOVA with Scheffe's post-hoc test. The results were considered statistically significant when $p < 0.05$. All statistical analysis was performed using IBM SPSS Statistics 19.

3. RESULTS

3.1. Fluidity and Setting Time

The fluidity and setting time of composites with different P/L ratios are shown in **Tables 1** and **2**. No temperature increase was observed during the setting. At a ratio of 1:2, the composite could be smoothly injected, but it dispersed in the PBS solution. At this ratio, it took more than 7 hours to harden. The setting time for a ratio of 1:1 required more than 2 hours to harden. At a ratio of 3:2, the composite was set within 35 minutes, and its shape was maintained as strip slurry. The ratio of 5:4 was not dispersed in the PBS solution, and the ratio of 2:1 was difficult to push out from the syringe. Therefore, a ratio of 3:2 was selected as the best ratio of powder/liquid in the composite.

Table 1. Fluidity of composites with different P/L ratios. + (smooth and no spread in PBS), ++ (thin), +++ (too thin to maintain its shape in PBS), × (difficult to inject).

P/L ratio	1:2	1:1	5:4	3:2	2:1
Fluidity	+ + +	+ +	+	+	×

Table 2. Setting time of composites with different P/L ratios.

P/L ratio	1:2	1:1	5:4	3:2	2:1
Setting time	7:15′	2:29′	42′	34′	23′

3.2. Physical Characteristics

The big pores of all materials were observed at 100× magnification **(Figure 2 A)**. CS had a flat surface, by contrast, many pores of varying sizes were observed in FS groups (FS-0.5 and FS-1). At 800× magnification, CS was a uniform prismatic crystal structure while the FS groups were composed of many irregular particles (**Figure 2 B**). In FS groups, many small pores were observed to constitute of big irregular pores. The small pores of all materials were observed at 2000× magnification (**Figure 2 C**). In the CS, regular compact prismatic crystals made up of many small pores (diameters: 2–10 μm). For comparison, FS groups had pores with various sizes, and most pores were bigger than that of CS. The pore size of CS was between 2–120 μm, FS groups were all between 3–270 μm. However, in FS-0.5, only about 1/3 of pores ≥ 50 μm, while in the FS-1, half of pores ≥ 50 μm.

The porosities of the three indurated composites CS, FS-0.5 and FS-1 were 52.54 ± 1.38%, 55.70 ± 2.34% and 59.96 ± 1.81%, respectively (**Figure 2 D**). Porosities of all groups were significantly different from each other. FS-1 had the highest porosity compare with the other groups.

The compressive strength decreased with increasing dose of FS: 28.32 ± 1.93 MPa for CS, 23.18 ± 1.60 MPa for FS-0.5, and 14.63 ± 2.05 MPa for FS-1 (**Figure 2 E**). The compressive strength was markedly different between groups.

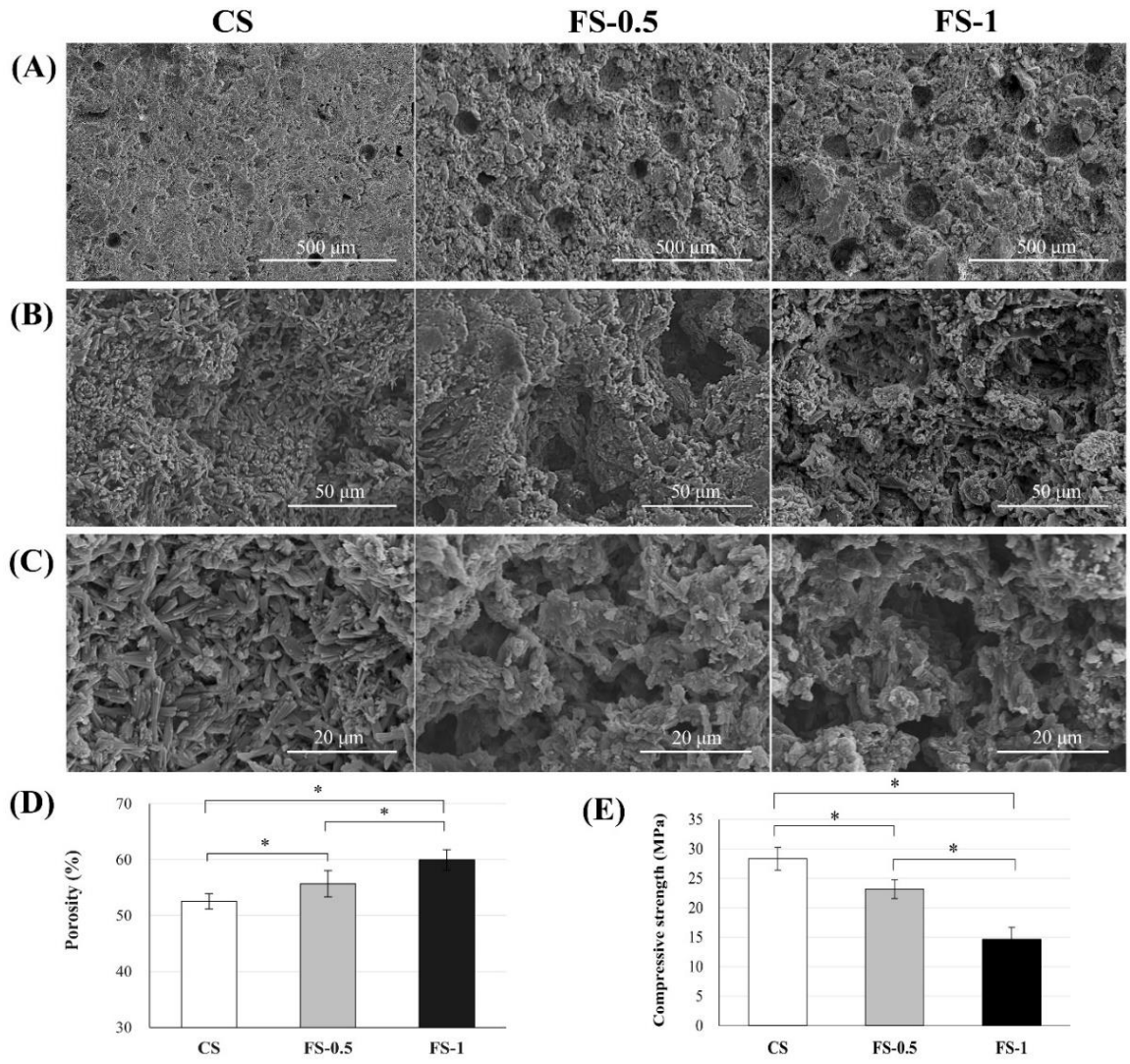

Figure 2. SEM micrographs (100×) of CS, FS-0.5 and FS-1 (A). SEM micrographs (800×) of three groups (B). SEM micrographs (2000×) of three groups (C). In CS group, regular compact prismatic crystals made up of many small pores. FS groups had a variety of pores and big pores were composed of small pores. Porosity of the three groups (n=6) (D). Compressive strength of the three groups (n=6) (E). One-way ANOVA with Scheffe's post-hoc test; * $p < 0.05$ compared with all groups.

3.3. FS Release

The cumulative release amounts of FS from the composites are shown in **Figure 3**. The FS maintained a slow and sustained release. The release rate of FS in FS-0.5 was decreased, while

FS in FS-1 continued to be released faster in larger amounts.

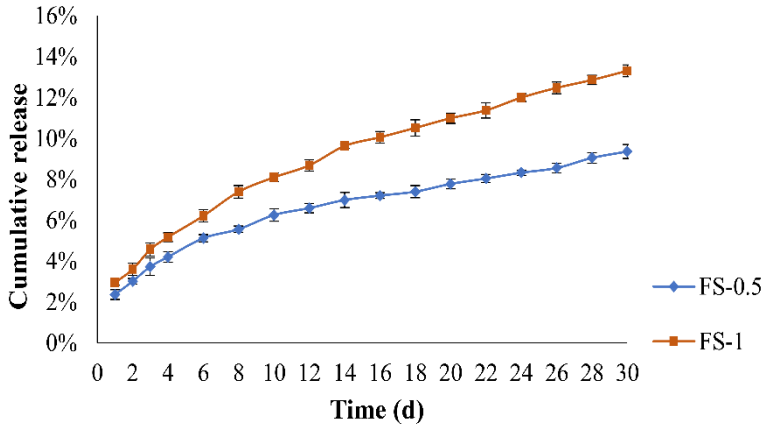

Figure 3. Cumulative release percentage of fluvastatin from FS-0.5 and FS-1. (n=6)

3.4. Maxillary Bone Volume

After micro-CT reconstruction, the bone volume of the maxillae was calculated (**Figure 4**). Different levels of bone mass increase were observed for all the groups over time. A prominent increase of bone volume was found in the FS groups compared with the other three groups at 2 and 8 weeks. The difference in bone volume between FS-0.5 and FS-1 was greater at 2 weeks.

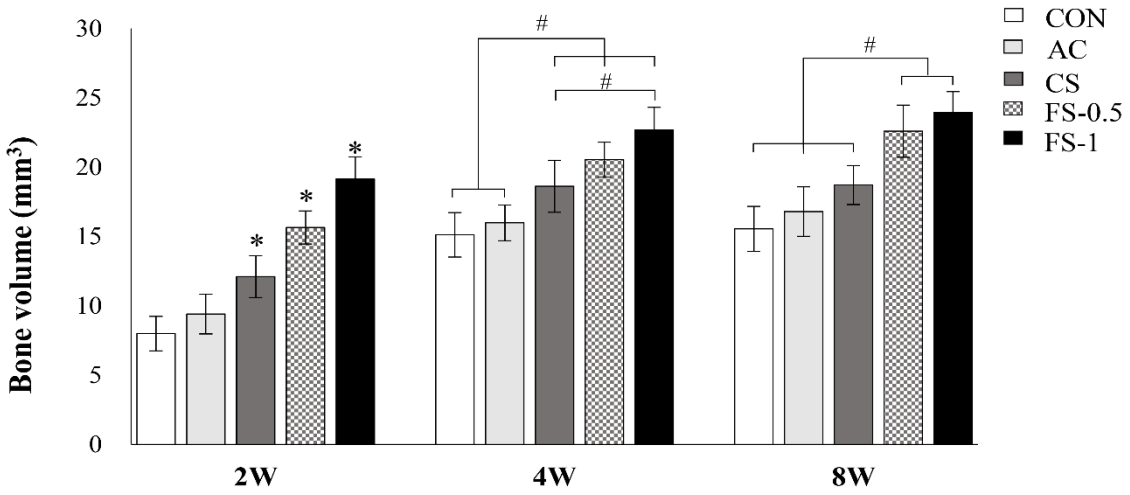

Figure 4. Bone volume of maxillae at 2, 4 and 8 weeks. One-way ANOVA with Scheffe's post-hoc test (n=6); * $p < 0.05$ compared with all groups. # $p < 0.05$ between the indicated groups.

3.5. Histological and Histomorphometrical Evaluation

The histological appearances of maxillae are shown in **Figure 5 A**. After 2 weeks, CON, AC and CS had big marrow cavities and loose trabecular structure. In contrast, the extraction sockets were filled with bone trabeculae in FS groups. At 4 weeks, the CON also had bone marrow cavity. Abundant bone trabeculae in the AC and CS were more compacted while higher density structures of bone were observed in the FS groups. More abundant vessel and dense structure of new bone could be found in the FS-0.5 than the CS (**Figure 5 B**). The degree of calcification was enhanced at 8 weeks, particularly in the FS-0.5. The BD of FS groups were significantly higher compared with other three groups at 2 and 4 weeks (**Figure 5 C**). BD of the FS-0.5 was higher compared with the CON, AC and CS at 8 weeks.

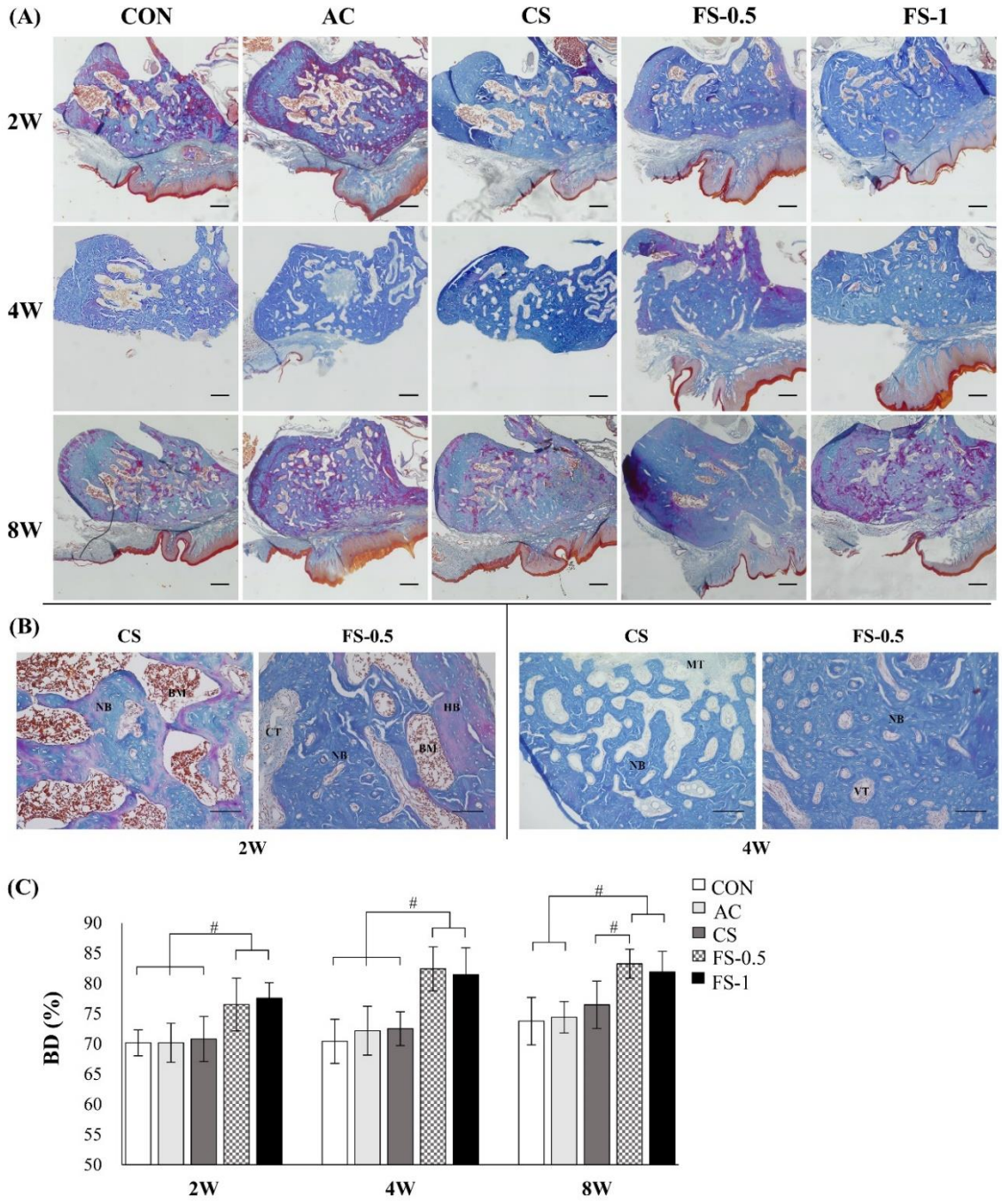

Figure 5. Histological micrographs of maxillae at 2, 4 and 8 weeks at low magnification (A). Scale bar = 300 μm. After 2 weeks, the extraction sockets were filled with bone trabeculae in FS groups, different from big marrow cavities and loose trabecular structure in the other three groups. At 8 weeks, the degree of calcification was enhanced, particularly in the FS-0.5. Histological micrographs of maxillae at 2 and 4 weeks at high magnification of CS and FS-0.5 group) (B). Scale bar = 100 μm. New bone (NB), bone marrow (BM), fibrous connective tissue (CT), host bone (HB), material (MT), vascular tissue (VT). More vessel and dense structure of new bone could be found in the FS-0.5 than the CS. Bone density (BD) of newly formed bone in maxillae at 2 and 4 weeks (C). One-way ANOVA with Scheffe's post-hoc test (n=6); # $p < 0.05$ between the indicated groups.

Residual composites in all groups were observed on crania, and the volume was reduced with time after 2, 4 and 8 weeks (**Figure 6 A**). As shown in **Figure 6 B,** continuous newly formed bone under the periosteum contact to host bone directly in FS-0.5 at 4 weeks. Material was degraded and surrounded by fibrous connective tissue. Primitive mesenchyme also could be observed.

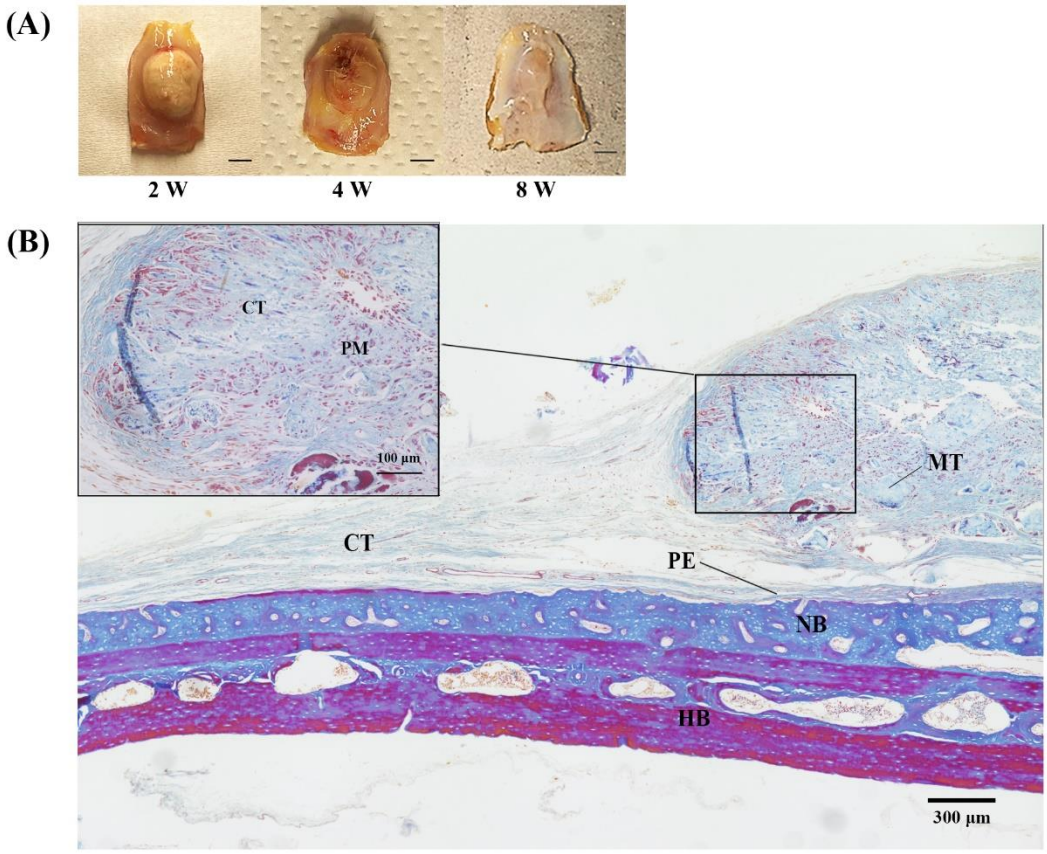

Figure 6. Residual composites on crania at 2, 4 and 8 weeks (A). Scale bar = 4 mm. The volume of residual composite was reduced with time after 2, 4 and 8 weeks. Histological micrographs of cranium at 4 weeks in the FS-0.5 (B). New bone (NB), host bone (HB), periosteum (PE), fibrous connective tissue (CT), material (MT), primitive mesenchyme (PM). Continuous newly formed bone under the periosteum contact to host bone directly in FS-0.5 at 4 weeks.

The histological micrographs at high magnification of crania are shown in **Figure 7**. In the CON, newly formed bone was not present over the calvaria in all specimens. At 2 weeks, new bone formation was not observed in the AC and only a small amount of bone formation was

seen in the CS. In comparison, new bone was significantly formed in the FS groups. At 4 weeks, there was more newly formed bone in the FS groups than in the other groups, and the new bone in the FS-1 was thicker than that in the FS-0.5. After 8 weeks, all specimens in the FS-0.5 showed a large amount of new bone formation, which was similar to that in the FS-1.

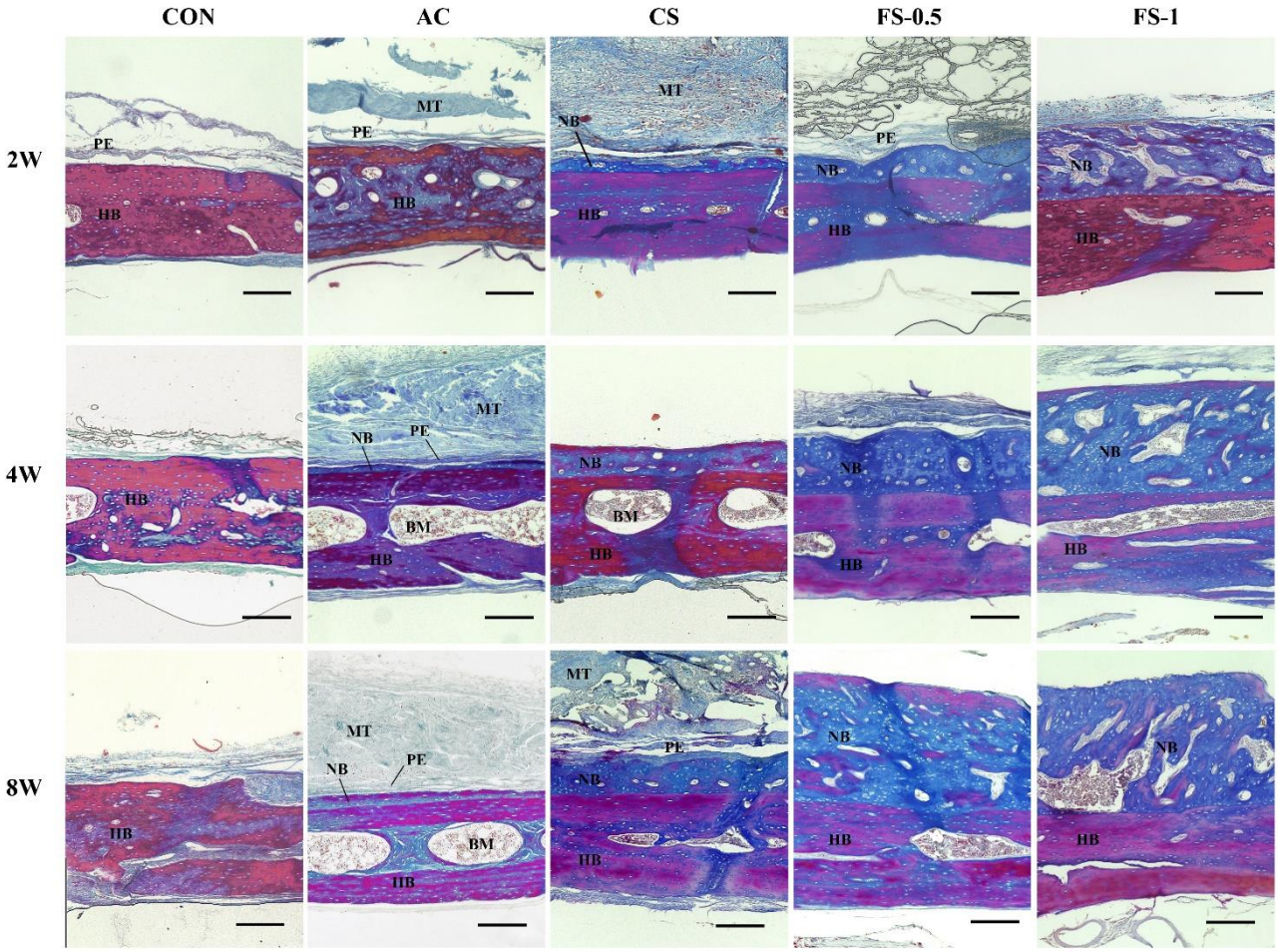

Figure 7. Histological appearance in crania at 2, 4 and 8 weeks. New bone (NB), host bone (HB), bone marrow (BM), periosteum (PE), material (MT). Scale bar = 250 μm. Newly formed bone in the FS groups (FS-0.5 and FS-1) is significantly thicker than in the other groups (CON, AC and CS).

New bone formation capacity is shown in **Figure 8 A**. At 2, 4 and 8 weeks, the FS groups had markedly greater bone formation compared with the other groups. BD values of FS-0.5 were markedly higher than that of FS-1 at 2 weeks (**Figure 8 B**). At 4 and 8 weeks, BD value

of AC and CS groups were higher than that of FS groups.

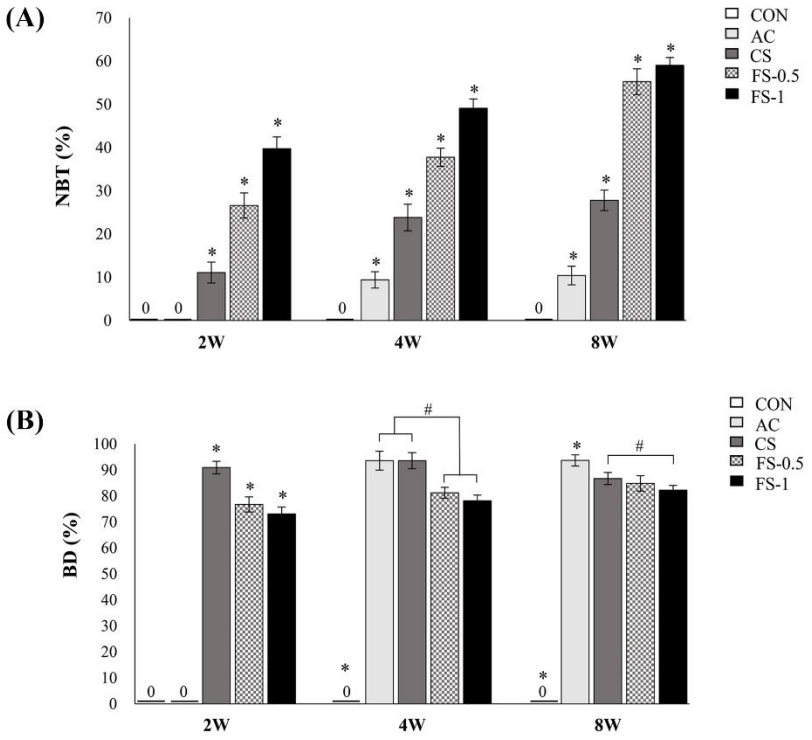

Figure 8. New bone thickness (NBT) on crania at 2, 4 and 8 weeks in all treatment groups (A). Bone density (BD) of newly formed bone on crania at 2, 4 and 8 weeks (B). NBT and BD were analyzed using one-way ANOVA with Scheffe's post-hoc test (n=6); * $p < 0.05$ compared with all groups. # $p < 0.05$ between the indicated groups.

4. DISCUSSION

In the present study, we demonstrated that a single-shot injection of FS with an atelocollagen-CAS composite successfully induced and increased the bone formation. In this study, the setting time mainly depended on the powder-to-liquid ratio. Too fast setting process may lead to composite harden during injection, and the needle is clogged. Initial setting time (T_I) is the time when material loose liquidity while final setting time (T_F) is the time when material has hardened sufficiently. The appropriate setting time for clinical application of calcium phosphate cement (CPC) is $3 \leq T_I < 8$ min, and $T_F \leq 15$ min.[32] In this case, the setting time in **Table 2**

refer to T_F. The T_I should be moderated while T_F is allowed a properly extend.[33] Earlier study also showed that, the T_F of different commercial CPC is higher than 1h generally.[33] In addition, our material is applied using injection. It means that the hardening reaction of this material proceeds underneath the mucosa. Thus, patient need not await setting, even setting time is longer than they expected. The setting time of composite (P/L ratio of 3:2) can meet the need of clinical application.

Greater porosity allows cell and vessel growth into the material.[34] When porosity reached 36%, active osteoblasts lined the pore areas of a porous material.[35] Porous structures are important for bone graft substitutes to promote osteoconductivity.[36] Tissues around porous ceramics grew faster and exhibited thinner fibrous encapsulation than non-porous structures.[37] The pore size is also important for osteoconductivity. It was reported that 150 μm is suitable as a pore size that does not prevent the entry of cells and blood vessels.[38] Some researchers believed that the pore size should be ≥ 100 μm, that minimum osteon could grow in it.[39] Another study proved that, HAp with 50 μm pores also had osteocondutivity.[36] In addition, interconnected micropores (2–10 μm) promoted osteoinduction in the scaffolds, when porous material was implanted under skin.[40] Our study revealed high porosity (> 55 %), and pore size of FS groups is believed to be adequate for tissue ingrowth.

In the present study, an increase in porosity would lead to an unnecessary decrease in mechanical strength. When the porosity was increased to 62%, the diametral tensile strength value of calcium phosphate cement was zero.[41] Consistent with previous studies, incorporating drugs can impair the compressive strength of bone substitutes.[42] Gbureck et al.[43] fabricated TCP with porosity between 30% and 42% and compressive strength of 1–10 MPa. Lopez-Heredia et

al.[44] used PMMA added to calcium phosphate and sodium carboxymethylcellulose with a strength of 220 MPa. Despite the low compressive strength of the FS-0.5 group, the value (23.18 ± 1.60 MPa) was analogous to that of cancellous bone (5–10 MPa). [45] The composite can meet the demands of maintaining a space that the osseous tissue can grow into.

Drug carriers consist of a substance that is incorporated to prolong the delivery and effectiveness of drugs. The release rate of FS-1 was faster than that of FS-0.5. It is speculated that the drug release behavior of this composite related to the dose of FS. Generally, diffusion mechanism played an important role in drug release when calcium sulfate was used as drug carrier.[46,47] It means that, any difference in diffusion controlling factors such as high porosity can lead to fast drug release and high total released amount.[46,48] Higher dose of FS lead to higher porosity to promote the drug diffusion from the composite. Meanwhile, small number of interconnected pores resulted in a low release rate of both groups.[49] *In vitro* experiments showed a stable and long-term continuous release profile of both doses. Therefore, the composite is considered an effective DDS.

Previous studies showed no significant osteogenesis when materials such as HAp and octacalcium phosphate were implanted above the calvarial periosteum.[50,51] In contrast, our study showed new bone formation on crania in AC and CS. The reason for this discrepancy is unknown, but it can be speculated that atelocollagen or CAS injected in this study stimulated periosteum promoting bone formation. Above all, NBT in CS group was larger than that in AC group. This can be speculated that the resorption of CAS creates a source of calcium ions, which may enhance the differentiation of mesenchymal stem cells into osteoblasts.[52,53] Our study showed that more new bone contacted to host bone directly in FS groups compared to the

previous studies using αTCP as a drug carrier for FS.[23] It is supposed that the new bone was made by osteoblasts which were differentiated from mesenchymal cells in periosteum by a series of factors such as BMP-2. Statin may control the expression of them.

The BD values of crania for non-FS groups (AC and CS) were higher than that for FS groups. New bone in the FS groups was much thicker and to develop large amount of bone, a lot of nutrient and oxygen may be required. Consequently, it can be speculated that the vasculature was developed among the newly formed bone and BD was decreased.

In the present study, we showed a mean release of about 8.26 μg FS/day (FS-0.5) and about 23.47 μg FS/day (FS-1) *in vitro*, the doses of FS were similar to that were injected on crania. The optimal dose of statin used for bone formation has not been confirmed;[19-23] however, the doses of statin used in our study were close to the normal range for bone formation because no typical findings of inflammation or other adverse symptoms was observed.

Some modifications of the composite can be further explored by adjusting the dose of FS and increasing its strength without reducing porosity. Future studies focus on its efficiency to repair big bone defects and improving bone formation around implants.

5. CONCLUSIONS

An injectable CAS loaded with FS was developed for bone augmentation. Histological study using maxilla indicated that this porous material was rapidly replaced by newly formed bone and promoted bone regeneration. As a DDS, it enlarged the vertical height of rat calvarial bone without any surgical intervention other than injection.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Injectable Porous Bioresorbable Composite Containing Fluvastatin for Bone Augmentation

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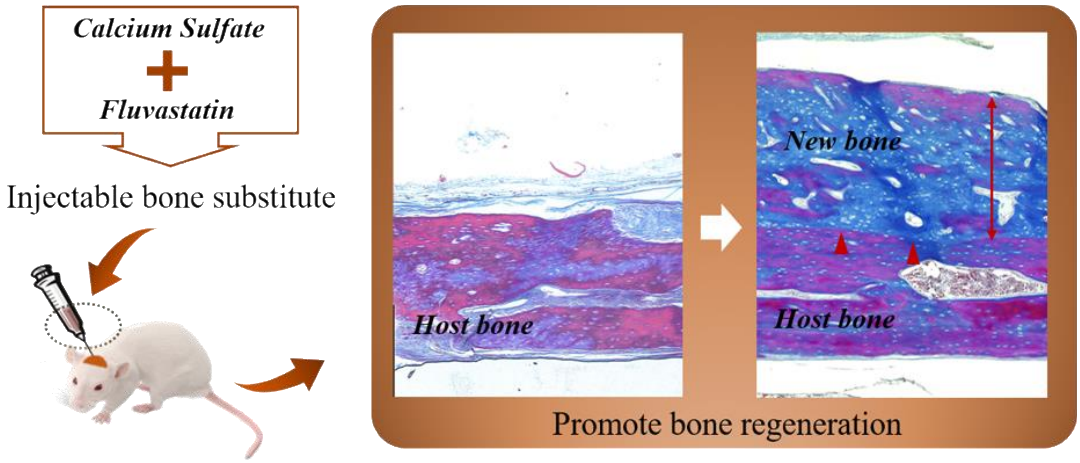

A percutaneous injection over periosteum of calcium sulfate containing fluvastatin was promoted bone regeneration and enlarged the vertical height of rat calvarial bone.