A comparative histologic analysis of tissue-engineered bone using BMSCs, alveolar bone cells, and periosteal cells

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감사의 글

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항상 힘이 되어주고 격려해주신 부모님과 그리고 추운 겨울이든 무더운 여름이든 가족을 위해 열심히 애쓰는 사랑스러운 아내 최향옥과 귀여운 딸 단영과 함께 이 보람의 기쁨을 나누고 싶습니다.

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저자 씀

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ABSTRACT

A comparative histologic analysis of tissue-engineered bone using BMSCs, alveolar bone cells, and periosteal cells

Objective: The aim of this study was to evaluate the osteogenic potential of bone marrow mesenchymal stem cells (BMSCs), alveolar bone cells and periosteal cells in tissue-engineered bone formation, and to compare the effects of platelet-rich plasma (PRP) and platelet-enriched fibrin glue on bone formation in tissue engineering.

Methods: BMSCs, alveolar bone cells and periosteal cells were isolated from dogs and expanded in vitro, and platelet-enriched fibrin glue and platelet rich plasma (PRP) were prepared using dog's blood. 12 nude mice were used for comparing donor cell-related differences in a tissue-engineered bone. The cultured BMSCs, alveolar bone cells and periosteal cells were mixed with BMP-2 and platelet-enriched fibrin glue, and injected into the subcutaneous space on the dorsum of nude mice. On the control group, BMP-2 and platelet-enriched fibrin glue without cells were injected; In addition, 6 nude mice were used for comparing the effects of PRP and platelet-enriched fibrin glue on bone formation in bone tissue engineering. PRP was mixed with periosteal cells and BMP-2, and then the composites were injected into the subcutaneous space on the dorsum of nude mice (PRP sides). On the contralateral side of the dorsum, platelet-enriched fibrin glue/periosteal cells/BMP-2 composites were injected. The bone formation was evaluated after 12 weeks.

Results: Histomorphometric analysis demonstrated that the subcutaneous nodules formed in nude mice contained 29.9% newly formed bone in the BMSCs,

42.9% newly formed bone in the alveolar bone cells, and 61.1% newly formed bone in the periosteal cells; in addition, it contained 26±3% newly formed bone at the PRP/periosteal cells/BMP-2 sides and 61.1±4.6% newly formed bone at the platelet-enriched fibrin glue/periosteal cells/BMP-2 sides.

Conclusion: The periosteal cells can form bone the most effectively; the osteogenic characteristics of platelet-enriched fibrin glue are superior to PRP; and the periosteal cells combining with platelet-enriched fibrin glue are indicated to enhance bone formation in bone tissue engineering.

Key words: Tissue engineering, periosteal cells, stem cell, alveolar bone cells, fibrin glue, PRP, injectable bone, bone regeneration

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I. INTRODUCTION

Tissue engineering approach is one of the most promising techniques of bone reconstruction, which is composed of cells, biomaterial scaffold and growth factors. In tissue-engineered bone formation, autogenous osteogenic cells are of paramount importance for successful bone formation. Several different types of cell have been used in tissue-engineered bone formation. Among those cells, bone marrow mesenchymal stem cells (BMSCs), alveolar bone cells and periosteal cells have been studied the most extensively in the oral maxillofacial region.

BMSCs, which were first identified in the pioneering studies of Friedenstein and Petrakova, [1] can be isolated from the marrow cavity as well as from the trabecular compartment. BMSCs are believed to be multipotent cells that can replicate as undifferentiated cells, and have the potential to produce mesenchymal tissues such as bone, cartilage, fat, tendon, muscle, and marrow stroma.[2] Some studies demonstrated BMSCs have capacity of forming new bone when transplanted,[3] or cultured in scaffolds and implanted into the subcutaneous space in mice and rats.[4,5,6] Yamada et

al[6] investigated the effect of mandibular defect repair using BMSCs in a canine model, which resulted in new bone formation.

Alveolar bone is one of the most active bones in human body. Osteoblast-like cells, which were isolated from alveolar bone chips via explant culture, display the osteoblast phenotype in culture.[7,8] Xiao et al[9,10] investigated cranial defect repair in an immunodeficient mouse model using alveolar bone cells in a three-dimensional collagen scaffold. The results indicated that cells derived from alveolar bone could induce new bone formation.

Bone tissue engineering using periosteal cells represents another approach. Peiosteum is a fibrous connective tissue on the outer surface of bone, it has been macroscopically divided into an inner proliferative or cambial layer and an outer fibrous layer.[11] The cambium layer contains a high number of osteogenic precursor cells, which under physiological conditions contribute to normal bone development and repair.[12] Fell[13] firstly reported on the culturing of periosteum and its cells, and showed *in vitro* that the bilayered membrane can form mineralized extracellular matrix under the appropriate conditions. Several studies have reported the osteogenic potential of periosteal cells in vivo.[14,15] For the repair of critical-size defects in the mandible, periosteal cells have been shown to induce bone formation in minipig models.[16] In addition, Vacanti's group[17] have firstly published a clinical case in which periosteal cells were applied in bone tissue engineering.

In order to capitalize on the ability of those cells to induce bone formation in vivo, it is necessary to investigate donor cell-related differences in terms of their efficiency and efficacy in bone formation. This approach would provide more comprehensive information for the success of cell-based strategies in tissue engineered bone formation.

In recent years, the use of an injectable scaffold has been believed to be ideal approach for tissue-engineered bone formation. The use of an injectable scaffold may offer several advantages over the preformed solid scaffold approach. A fluid material

can fill any defect shape, can incorporate various therapeutic agents (e.g., growth factors) by simple mixing, does not contain residual solvents that might be present in a preformed scaffold, and finally, does not require a complex surgical procedure for placement. It has been reported that successful bone formation in vivo in experimental animals using injectable biomaterials such as collagen gel,[18] polyethylene oxide,[19] fibrin glue,[20] and chitosan-alginate gel [4] etc. Yamada et al.[21,22] reported that a bone formation could be achieved using platelet-rich plasma (PRP) gel as a scaffold.[21,22,23] Specifically, PRP has been reported to enhance bone formation, [21,22,23] as it contains a large number of platelets that release significant quantities of growth factors known to promote wound healing.[24,25] However, the use of platelet-enriched fibrin glue as a scaffold for injectable tissue-engineered bone seems attractive because it contains high concentrations of fibrinogen, which can produce a dense fibrin clot with sufficient adhesive strength to maintain a required configuration. For these reasons, we used platelet-enriched fibrin glue in injectable tissue-engineered bone, and compared the osteogenic potential of PRP and platelet-enriched fibrin glue in tissue-engineered bone in this study.

II. MATERIALS AND METHODS

1. Animals

Six mongrel dogs (each weighing more than 15 kg) and eighteen athymic nude mice at 6 weeks of age were used in this experiment. All surgical procedures were performed under systemic (ketamine, 5 mg/kg and xylazine, 2 mg/kg i.m.) anesthesia.

2. The isolation and culture of BMSCs, alveolar bone cells and periosteal cells

1) BMSCs isolation and culture

Bone marrow samples were collected from the iliums of each dog by aspiration using a syringe with a No. 18 G spinal needle. The BMSCs were isolated and cultured using a slight modification of a previously reported method.[26] Briefly, approximately 3 ml of the bone marrow samples transferred to a sterile tube containing an equal volume of the growth medium, mixed well and centrifuged for 30 min at 1000×g. The supernatant fat was removed, and the remanent supernate and interface were collected and suspended in 10 ml of growth medium and plated in a 75cm^2 flask (NUNC, Roskilde, Denmark). (Fig. 1) These cells were incubated in a humidified atmosphere of 95% air and 5% CO_2 at 37ºC for 3 days, and the non-adherent cells were removed by replacing the medium. The growth medium consisted of Dulbecco's Modified Eagles Medium (Gibco, Grand island, U.S.A) supplemented with 20% fetal bovine serum, 1% nonessential amino acid (Gibco, Grand island, U.S.A), sodium pyruvate (100 ng/ml, Gibco), and antibiotics (100 U penicillin and 100 μg/ml streptomycin, Gibco). Once confluence reached 80% of substrate, the cells were dissociated with 0.05% trypsin-EDTA, divided in two, and transferred into two 75cm^2 flask to subculture. The BMSCs at passage 3 were used in our studies.

2) Alveolar bone cells isolation and culture

Alveolar bone cells used for this study were isolated from alveolar bone according to procedure described previously.[7,8] Briefly, under disinfected condition, alveolar

bone specimens were obtained from the lateral cortex of the mandible of each dog using Handpiece drill and pint-sized osteotome. The specimens were broken into small pieces and then used as explants for establishment of cell culture. The bone fragments were placed in 25cm^2 tissue culture flasks (FALCON, Franklin lakes, USA), (Fig.2) and just submerged with culture medium, and incubated at 37 ºC in a humidified atmosphere of 95% air and 5% CO_2. The culture medium was the same medium described above. Culture medium was first changed after 1 week of incubation, subsequently changed 2 times a week. Once cells confluence reached 80% of substrate, the cells were detached by trypsin treatment and transferred into a 75cm^2 flasks. The cultured alveolar bone cells at passage 3 were used in our studies.

3) Periosteal cells isolation and culture

Periosteal cells were isolated from the periosteum as described previously.[27] Briefly, under disinfected condition, Approximately 5 × 20 mm rectangular periosteal tissue was separated from the lateral cortex of the mandible of each dog. Care was taken to ensure the harvesting of the cambium layer. The periosteum was immediately placed in Ringer's buffer and under sterile conditions diced into 2-mm^2 pieces. Explants were transferred into a sterile tube and washed 3 times with serum-free medium. After the final washing, these explants were transferred into 25cm^2 tissue culture flasks with the osteogenic layer facing down and the growth medium described above was added moderately. (Fig.3) The explants were incubated at 37ºC in a humidified atmosphere of 95% air and 5% CO_2. Culture medium was first changed after 1 week of incubation, subsequently changed 2 times a week until cell outgrowth created a confluent monolayer. Once cells confluence reached 80% of substrate, the cells were detached by trypsin treatment and transferred into 75cm^2 tissue culture flasks. Third passage periosteal cells were used in this study.

3. Preparation of platelet-enriched fibrin glue, PRP and thrombin

Platelet-enriched fibrin glue, PRP and thrombin were prepared using a slight modification of a previously described methods.[28,29] (Fig. 4) Prior to surgery, 45 ml of blood of each dog was drawn using a syringe containing 5ml of 10% sodium citrate as an anticoagulant and immediately transferred into a sterile tube. The blood was

centrifuged at 327 ×g for 15 min at ambient temperature to remove blood cells. Approximately 22.5ml of plasma was obtained from the 45ml of whole blood. the 22.5ml of plasme was divided into 2.5ml to produce autologous thrombin and 20 ml to produce platelet-enriched fibrinogen solution) and PRP .

1) Preparation of thrombin

To 2.5 ml of the plasma, 22.5 ml of citric acid was added, and then the mixture was centrifuged for 5 min at 3000 ×g and at 4 ºC. After discarding the supernate, the precipitate was dissolved in 200 µl of 0.1M $CaCl_2$. The pH was adjusted to pH 7 by adding 80 to 100 µl of $NaHCO_3$. After clot formation, the thrombin solution was collected, and was diluted to 10% with a 0.05 M $CaCl_2$.

2) Preparation of platelet-enriched fibrinogen

The fibrinogen solution was prepared using by cold ethanol precipitation. To 10 ml of the plasma, 300 µl of tranexamic acid and 1000 µl of ethanol were added, and the mixture was then placed in an ice-water bath for 20-30 min to a temperature of approximately 0 ºC. The precipitated fibrinogen was separated by centrifugation at 3000 ×g for 8 min at 0-4 ºC. After discarding the supernate, the fibrinogen precipitate was redissolved by incubation at 37 ºC, and was diluted to 50% with 0.9% NaCl.

3) Preparation of PRP

The remained 10 ml of plasma was centrifuged at 3000 × g for 8 min to separate the PRP from the supernatant platelet-poor plasma (PPP) portions. Platelet counts were then conducted for each dog, yielding a mean PRP platelet count of 1,490,000 (with a range of 1,020,000 to 2,140,000).

4) Gels formation

Just before application, platelet-enriched fibrinogen and PRP were activated with thrombin solution in ratio 3:1(Vol./Vol.) to form a gel.(Fig. 5)

4. Surgical procedure

In order to investigate donor cell-related differences in a tissue-engineered bone, 12 nude mice were assigned to 4 different groups and used for a surgical protocol as follows.

Group 1 (n = 3): Approximately 1×10^7 of periosteal cells were mixed with 300 µl

of the platelet-enriched fibrinogen solution and 2 μg of rhBMP-2 (R&D System, Minneapolis, USA). The platelet-enriched fibrinogen/BMSCs/rhBMP-2 mixture was loaded in a 1ml syringe, and then mixed with 100 μl of thrombin solution by aspiration to activate fibrinogen to coagulate. An approximately 400 μl of composite, viz. the platelet-enriched fibrin gel containing periosteal cells and rhBMP-2 was formed in the syringe. This composite was injected through an 18-gauge needle subcutaneously into the backs of the nude mice. A total of 6 injections were performed (i.e., two injections per mouse). (Fig. 6)

Group 2 (n = 3): This group of nude mice was injected with 400 μl of the platelet-enriched fibrin gel containing 1×10^7 of the alveolar bone cells and 2 μg of rhBMP-2.

Group 3 (n = 3): This group of nude mice was injected with 400 μl of the platelet-enriched fibrin gel containing 1×10^7 of the periosteal cells and 2 μg of rhBMP-2.

Group 4 (n = 3): As a controls, this group of nude mice was injected with 400 μl of the platelet-enriched fibrin gel containing 2 μg of rhBMP-2 alone.

In addition, to compare the effects of PRP and platelet-enriched fibrin glue on bone formation in bone tissue engineering, 6 nude mice were used for a surgical protocol as follows.

Approximately 1×10^7 of periosteal cells were mixed with 300 μl of PRP and 2 μg of rhBMP-2. The PRP/BMSCs/rhBMP-2 admixture was loaded in a 1ml syringe, and mixed with 100 μl of thrombin solution by aspiration to activate fibrinogen to coagulate. An approximately 400 μl of composite, viz. the PRP gel containing periosteal cells and rhBMP-2 formed in the syringe. The composites were injected through an 18-gauge needle subcutaneously into the backs of the nude mice (PRP side). On the contralateral side of the dorsum, 400 μl of the platelet-enriched fibrin gel containing 1×10^7 of periosteal cells and 2 μg of rhBMP-2 were injected (fibrin side). A total of 12 injections (n = 6) were performed (two injections per mouse).

5. Sample preparation

All of the animals were sacrificed at 12 weeks after the injections to harvest the

specimens. The specimens were fixed in 10% buffered formalin, decalcified, and then cut through their middle plane. Histological sections were stained with haematoxylin and eosin, and the sections were examined under a light microscope.

6. Histomorphometry

Computer-assisted histomorphometric measurements of the newly formed bone were obtained using an image analysis system (IBAS, Contron, Erching, Germany). The regenerated bone was distinguished by the morphology of the trabecular bone. The perimeter around the newly formed bone was traced, and the enclosed area was determined in mm^2 using image analysis software. The percentages of the newly formed bone within the specimen outline were then calculated.

7. Statistical analysis

For the histomorphometric analysis, significant differences in the amount of new bone formed in response to the different cells were identified by ANOVA (analysis of variance), and Wilcoxon's signed rank test for paired samples was used to calculate statistical differences between PRP/periosteal cells/BMP-2 sides and platelet-enriched fibrin glue/periosteal cells/BMP-2 sides. Data were considered significant with a p value < 0.05, and statistical analysis was performed using a statistical software package (SPSS for Windows).

III. RESULTS

After injecting platelet-enriched fibrin glue/BMSCs/BMP-2 composites into first group of nude mice, platelet-enriched fibrin glue/alveolar bone cells/BMP-2 composites into the second group of nude mice, platelet-enriched fibrin glue/periosteal cells/BMP-2 composites into the third group of nude mice, subcutaneous nodules were noted to have formed by 12 weeks. Those formed nodules were hard and resisted compression. Upon dissection from the subcutaneous tissue, the composite nodules also had well-defined margins. Their volume was 150±25 μl at the platelet-enriched fibrin glue/MSCs/BMP-2 groups, 70±18 μl at the platelet-enriched fibrin glue/alveolar bone cells/BMP-2 groups, and 185±15 μl at the platelet-enriched fibrin glue/periosteal cells/BMP-2 groups, (Fig. 7) demonstrating that there were significant differences in the volume of the nodules formed in response to the different cells. However, after injecting platelet-enriched fibrin glue/BMP-2 composites into the fourth group of nude mice, the composites failed to form nodules, all of the composites were gradually absorbed and completely vanished within 2 month.

A histological examination of the nodules from different donor cell groups revealed that they were encapsulated with a fibrous capsule and there was trabecular bone as well as an amorphous calcified matrix in the nodules of all samples. The trabeculae contained many osteocytes and were regularly lined with many osteoblasts, indicating bone-forming activity. (Fig. 9) In the periphery of new-formed bone, a laminar pattern can be observed to be similar to normal bone. (Fig. 10) There was no evidence of inflammation or foreignbody reaction in the host tissue adjacent to the new bone, and there was no evidence of cartilage generation. The percentages of newly formed bone in response to different cells, were 29.9±5.2% in the platelet-enriched fibrin glue/MSCs/BMP-2 implants, 42.9±4.3% in the platelet-enriched fibrin glue/alveolar bone cells/BMP-2 implants, 61.1±4.6% in the platelet-enriched fibrin glue/periosteal cells/BMP-2 implants, (Fig. 8) demonstrating that there were significant differences in the amount of new bone formed in response to different cells ($p<0.05$). (Fig. 11)

After injecting the PRP/periosteal cells/BMP-2 composites into one side of each mouse's dorsum and injecting platelet-enriched fibrin glue /periosteal cells/BMP-2 into the contralateral side, subcutaneous nodules were noted to have formed by 12 weeks, and these nodules were hard and resisted compression. Upon dissection from the subcutaneous tissue, the composite nodules also had well-defined margins. The nodules' volumes were 55±18 μl at the PRP/periosteal cells/BMP-2 sides and 175±17μl at the platelet-enriched fibrin glue/periosteal cells/BMP-2 sides respectively. This finding demonstrated that there were significant differences in the volume of the nodules formed in response to the different gels.

A histological examination of the nodules from both PRP and fibrin sides revealed that they were encapsulated with a fibrous capsule, and there was trabecular bone in the nodules of all the samples. The trabeculae contained many osteocytes and were regularly lined with many osteoblasts, indicating bone-forming activity. At the periphery of the nodules, the bone had a laminar pattern similar to normal bone. There was no evidence of inflammation or foreign-body reaction in the host tissue adjacent to the new bone, and there was no evidence of cartilage generation. The percentages of trabecular bone were: 36±3% at the PRP/periosteal cells/BMP-2 sides and 60±5.3% at the platelet-enriched fibrin glue/periosteal cells/BMP-2 sides. (Fig.12) This difference was statistically significant ($p<0.05$).

IV. DISCUSSION

Different types of cell have been used in tissue-engineered bone formation, including bone marrow stem cells,[30,31] periosteal cells,[32,33] skeletal muscle cells[34] and cells derived directly from bone.[9,35,36] Kasperk et al[37] reported that there are differences between bone cells from different skeletal sites with respect to phenotype and cellular proliferation. One might expect that there would be donor cell-related differences in terms of their efficiency and efficacy in bone formation. To our knowledge, no study has yet directly addressed this issue. This study evaluated bone marrow mesenchymal stem cells, alveolar bone cells and periosteal cells for their in vivo potential to form bone. There were significant differences in the amount of new bone formed in response to different cells, suggesting that there are donor cell-related differences in a tissue-engineered bone formation. An interesting finding in this study was that periosteal cells formed bone the most effectively. An explanation of this observation might be that periosteal cells possess the highest osteogenic potential.

Vacanti et al[17] were the first to publish a clinical case in which periosteal cells were applied in bone tissue engineering for replacement of an avulsed phalanx. Schmelzeisen et al[38] reported clinical cases in which these cells were applied for augmentation in the posterior maxilla prior to implant insertions, and reported that the harvesting procedure of the periosteum at the mandibular angle via an intraoral mucosal incision under local anesthesia was tolerated well by all patients. When we consider that periosteal cells possess high osteogenic potential and are easy to obtain, one might expect that bone engineering using periosteal cells will offer great potential for craniomaxillofacial surgery and bone reconstruction procedures in other parts of the skeleton

The use of BMSCs for bone regeneration is in vogue.[39,40] Their multilineage differentiation potential, their relative availability in terms of cell harvesting, and their capacity to undergo extensive replication without a loss of that multipotential capacity make them an attractive cell source for cell-based therapeutic approaches.[2,41] Several

experiments have demonstrated that BMSCs can be induced into osteoblasts.[2,39] However, directing these cells into osteogenic differentiation is still a major obstacle.[9] When specifically comparing the bone formation of BMSCs with periosteal cells, this study found that BMSCs formed significant less bone quantitatively and qualitatively as compared to periosteal cells.

Bone cells derived from intraoral osseous tissue proved to be an important source of the osteoprogenitor cells required for alveolar bone healing.[7] Alveolar bone is an easily accessible site for harvesting bone cells in the oral cavity. In particular, sites affected by periodontitis, alveolar cysts, atrophic maxilla and sites around osseointegrated implants are believed to be prime areas where alveolar bone cells with the same origin and physiology as the damaged alveolar bone could be used.[7,8,9] However, the volume of newly formed tissue was much smaller than the original size of the implants (only 18%). This is believed to have been caused by the faster degradation of the fibrin glue as compared to the capacity of the alveolar bone cells to form new bone.

In recent years, the use of an injectable scaffold has been believed to be ideal approach for tissue-engineered bone formation. There have been reports of successful in vivo bone formation in experimental animals using several injectable biomaterials as scaffold.[4,18,19,20,21,22,23,42] Yamada et al.[21,22] reported that a bone formation could be achieved using platelet-rich plasma (PRP) gel as a scaffold,[21,22,23] and PRP has been reported to enhance bone formation. However, the use of platelet-enriched fibrin glue as a scaffold for injectable tissue-engineered bone seems attractive because it contains high concentrations of fibrinogen, which can produce a dense fibrin clot with sufficient adhesive strength to maintain a required configuration. This study determined whether the combination of platelet-enriched fibrin glue and periosteal cells results in better bone formation than does the combination of PRP and periosteal cells. The present study showed that when a combination of platelet-enriched fibrin glue and periosteal cells was used in bone tissue engineering, the volume of the new bone formation was significantly higher than in the group treated with a combination of PRP and periosteal cells. In addition, it showed significantly higher percentages of

the newly formed bone within the specimen outline of the platelet-enriched fibrin glue and periosteal cells composites as compared to those of PRP and periosteal cells composites. This suggests that periosteal cells exhibit a more positive effect when combined with platelet-enriched fibrin glue, and this is probably due to the properties of the platelet-enriched fibrin glue. Thorn et al.[28] reported that the concentration of fibrinogen in platelet-enriched fibrin glue was approximately 12 times that found in PRP. The fibrin matrices might encourage periosteal cells adhesion, proliferation, and differentiation, thus eliciting bone formation. In addition, when we consider that fibrinogen concentrations have an effect on the degradation of the glue,[43] platelet-enriched fibrin glue had a slower degradability rate than PRP. This property will allow the retention of growth factors and cells for a longer period of time and thus will produce more bone in the platelet-enriched fibrin glue than in the PRP. Today, bone morphogenetic proteins are recognized as being key factors in the field of bone tissue engineering.[44] However, the practical application of these proteins will depend on the carrier system used for delivery to the site of the hard tissue repair or restoration.[44,45] This proteins need to be released continuously over a sufficiently long period of time to induce bone formation.[46] Based on the present animal experiment, the platelet-enriched fibrin glue appeared to act as an effective delivery system for BMP-2.

Fibrin glues were first used to establish hemostasis at the beginning of the last century. In 1940, Young and Medawar[47] mixed bovine thrombin with plasma fibrinogen to produce the first biologic adhesive. Several commercial fibrin glues are presently available. If these products are compared to the fibrin glue prepared using the technique described in this study, the major incentive for using the fibrin glue is that it contains high concentrations of platelets, whereas the platelets are not present in the commercial products. Therefore, platelet-enriched fibrin glue offers a significant additional benefit in accelerating wound healing, which is aided by the presence of high concentrations of growth factors in the platelets.[48,49,50] Growth factors released from the platelets have been shown to include platelet-derived growth factor, transforming growth factor ß, platelet-derived epidermal growth factor, platelet-derived angiogenesis factor, insulin-like growth factor 1, and platelet factor 4.[24,25]

These growth factors are known to promote cell proliferation, cell differentiation, motility, and matrix synthesis, either alone or together, by binding to the specific cell surface receptors.[51] It was assumed that growth factors, including BMP-2 and others released from the platelets, might interact with the neighboring periosteal cells to induce them to proliferate and differentiate into osteoblasts, and to subsequently form new bone. More basic research investigating the mechanism by which this occurs is clearly necessary in order to capitalize on the ability of growth factors to enhance bone formation in vivo.

V. CONCLUSION

Comparing the osteogenic potential of BMSCs versus alveolar bone cells versus periosteal cells in the field of bone engineering, our results indicated that periosteal cells can form bone the most effectively. Comparing the osteogenic potentials of PRP and platelet-enriched fibrin glue in bone tissue engineering, the osteogenic characteristics of platelet-enriched fibrin glue show being superior to PRP. Finally, periosteal cells combining with platelet-enriched fibrin glue are indicated to enhance bone formation in bone tissue engineering.

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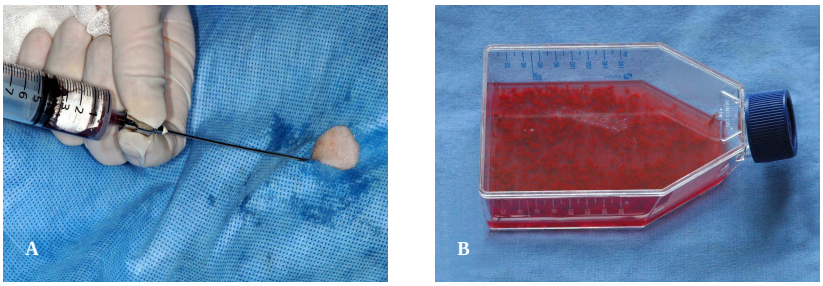

Figure 1. The harvest of bone marrow (A) and the primaryculture of BMSCs (B).

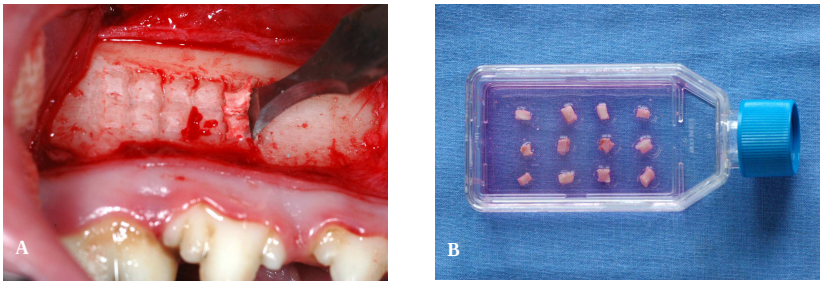

Figure 2. The preparation of alveolar bone chip (A) and the primary culture of alveolar bone cells (B).

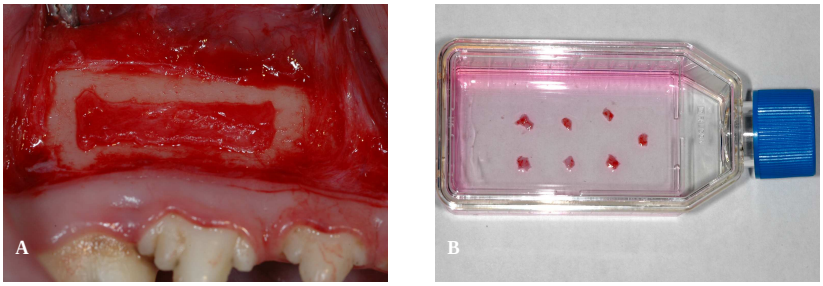

Figure 3. Excised periosteum (A) and primary culture of periosteal cell (B).

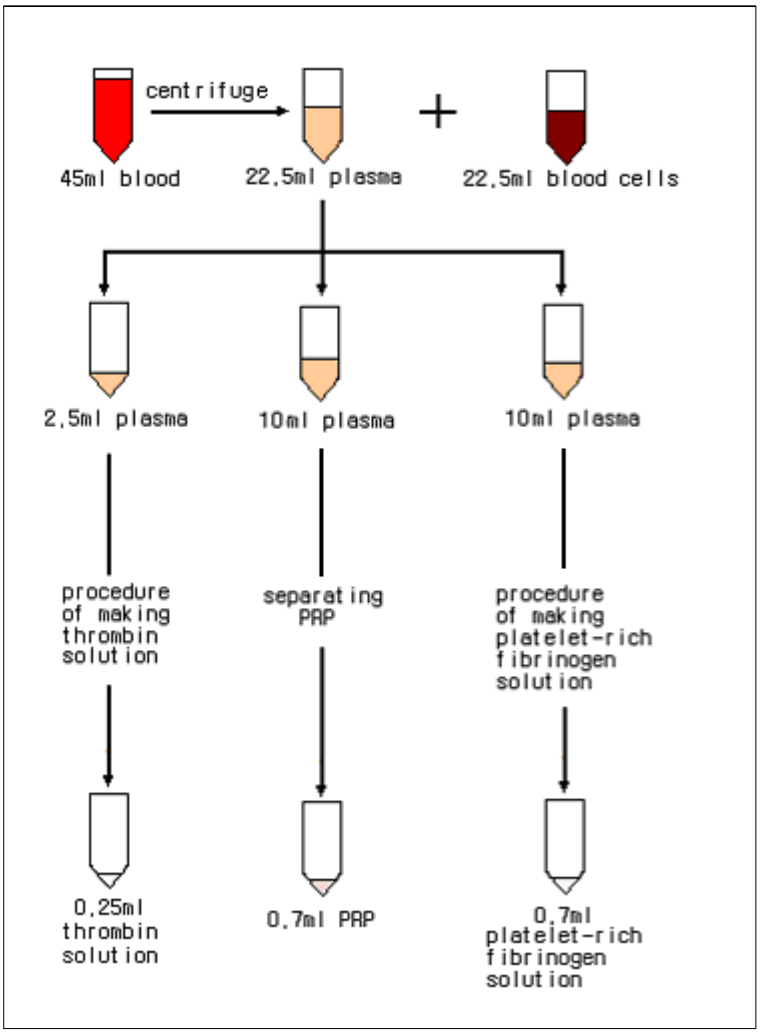

Figure 4. Illustration of preparation of thrombin, platelet-enriched fibrinogen and PRP.

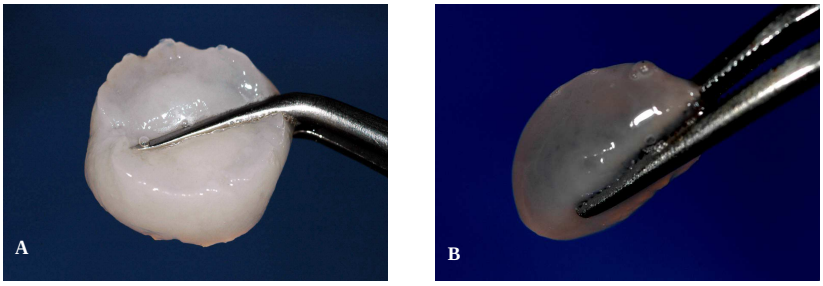

Figure 5. Comparison of the gels formed from the platelet-enriched fibrin glue (A) or the PRP (B).

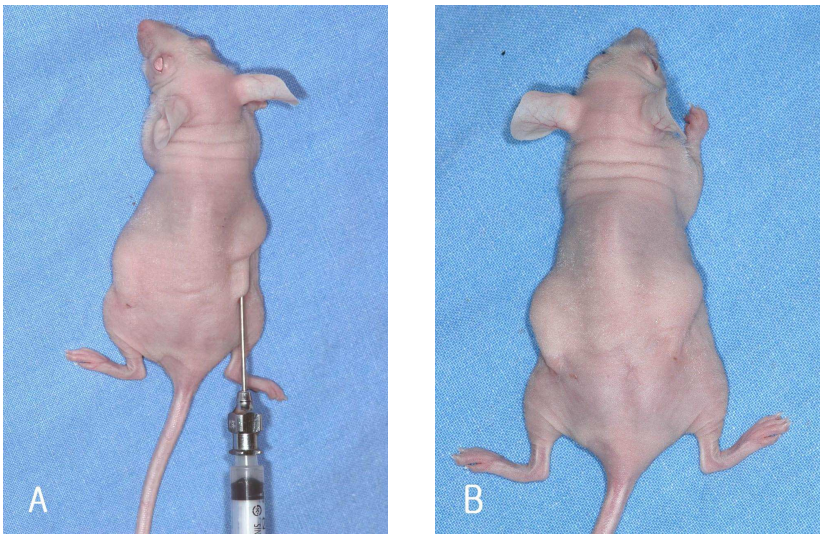

Figure 6. The subcutaneous injection of implants.

A: Subcutaneous injection. B: Immediate status after injection.

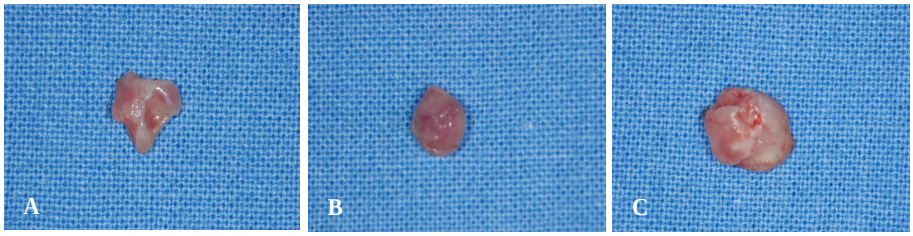

Figure 7. Macroscopic comparison of specimens.

The specimen was developed from the platelet-enriched fibrin glue/MSCs/BMP-2 admixture (A), platelet-enriched fibrin glue/alveolar bone cells/BMP-2 admixture (B) and platelet-enriched fibrin glue/periosteal cells/BMP-2 admixture (C).

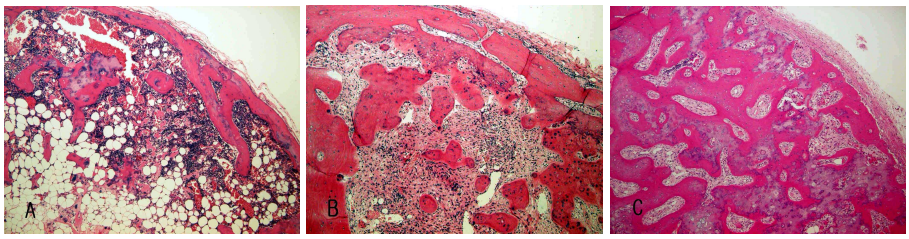

Figure 8. Histological comparison of specimens. (HE, ×40)

The specimen was developed from the platelet-enriched fibrin glue/MSCs/BMP-2 admixture (A), platelet-enriched fibrin glue/alveolar bone cells/BMP-2 admixture (B) and platelet-enriched fibrin glue/periosteal cells/BMP-2 admixture (C).

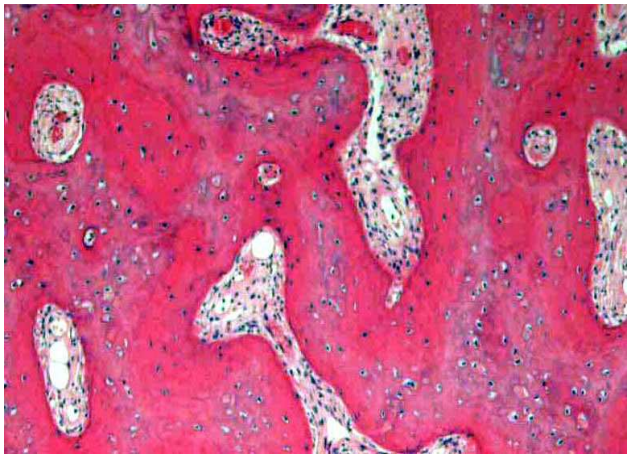

Figure 9. The specimen showing the trabeculae containing many osteocytes.

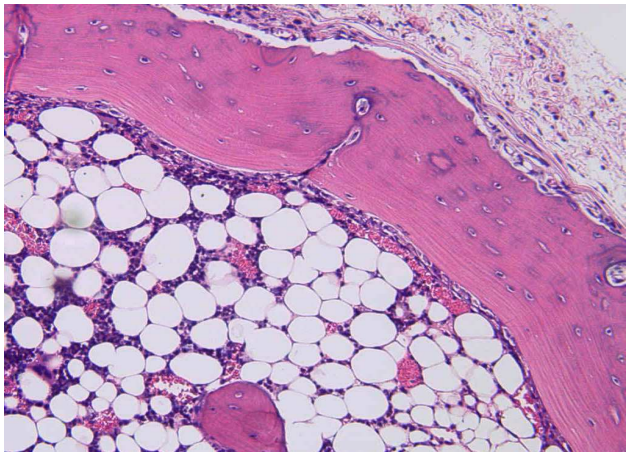

Figure 10. The specimen showing a laminar pattern.

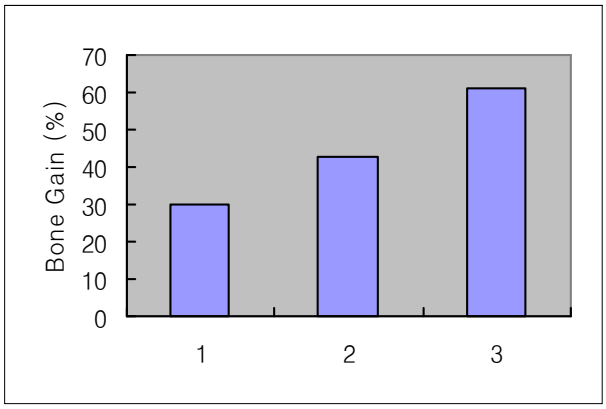

Figure 11. Histomorphometric data for the percents of the newly formed bone within the specimens.
(1) Fibrin glue/MSCs/BMP-2, (2) Fibrin glue/alveolar bone cells/BMP-2, (3) Fibrin glue/periosteal cells/BMP-2. Data are presented as means±SD, N= 6; * : P < 0.05.

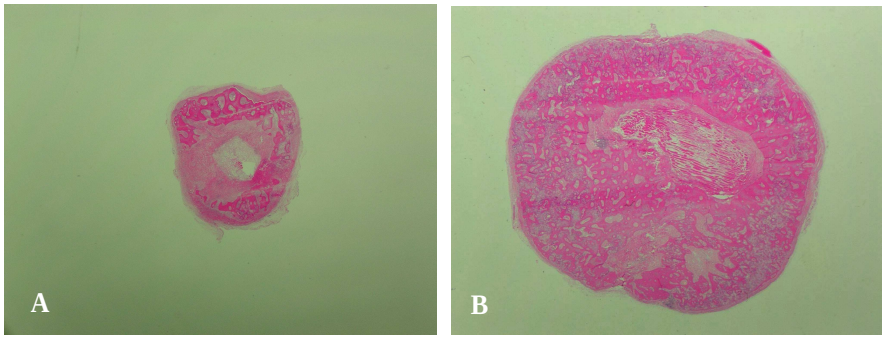

Figure 12. Histological comparison of specimens. (HE, ×4)
The specimen was developed from the PRP/periosteal cells/BMP-2 mixture (A) and the platelet-enriched fibrin glue/periosteal cells/BMP-2 mixture (B).

국문요약

연구목적: 조직공학을 이용한 골 형성 방법에서 골세포의 종류에 따라 골형성에 차이가 있는지를 평가하기 위하여 골수줄기세포, 치조골세포, 골막세포의 골형성 능력을 비교하고자 하였고 또한 세포운반체의 종류에 따른 차이가 있는지 평가하기 위하여 platelet-rich plasma (PRP)와 platelet-enriched fibrin glue의 골 형성효과를 비교하고자 하였다.

방법: 성견에서 골수줄기세포, 치조골세포, 골막세포를 분리 배양하고 동일 성견의 혈액을 채취하여 platelet-enriched fibrin glue 와 PRP를 제조하였다. 배양한 골수줄기세포, 치조골세포, 골막세포를 각각 BMP-2 와 platelet-enriched fibrin glue과 혼합하여 9마리의 누드마우스를 3그룹으로 나누어 누드마우스의 등에 피하 주사하였고 대조군(3마리)에서는 세포 없이 BMP-2와 platelet-enriched fibrin glue를 혼합하여 주사하였다. 그리고 PRP와 platelet-enriched fibrin glue의 골 형성 효과를 비교하기 위하여 PRP에 골막세포와 BMP-2를 혼합하여 6마리의 누드마우스 등의 한쪽에 피하 주사하였고 등의 다른 쪽에는 platelet-enriched fibrin glue에 골막세포와 BMP-2를 혼합하여 피하 주사하였다. 주입 12주후 골 형성을 평가하였다.

결과: 누드마우스 피하에 형성된 결절을 조직형태 계측학적 분석을 시행한 결과 골수줄기세포에서 29.9±5.2%, 치조골세포에서 42.9±4.3%, 골막세포에서 61.1±4.6%의 신생골이 형성되였고 PRP/골막세포/BMP-2 복합체를 주입한 군에서는 36±3%, platelet-enriched fibrin glue/골막세포/BMP-2 복합체를 주입한 군에서는 60±5.3%의 신생골이 형성되였다.

결론: 골수줄기세포, 치조골세포 및 골막세포를 이용하여 조직공학 방법으로 골형성 능력을 비교해 볼 때 골막세포가 가장 효과적이였고, platelet-enriched fibrin glue 가 PRP 보다 더 우수하였다. 그러므로 본 연구에서는 조직공학을 이용하여 골형성을 높이기 위하여 골막세포의 이용과 platelet-enriched fibrin glue의 이용을 제안한다.