Effect of synthetic cell–binding peptide on the healing of cortical segmental bone defects

Gokhan Cakmak, MD, Selcuk Bolukbasi, MD, Aykin Simsek, MD, Ozlem Erdem, MD, Guldal Yilmaz, MD, Alpaslan Senkoylu, MD.

ABSTRACT

Objectives: To determine the effect of inorganic bone matric/Pepgen P-15 (ABM/P-15) on the healing of a critical sized segmental defect in a rat radius using a radiological and histological grading system.

Methods: We carried out this study at the Research Laboratories, Gazi University School of Medicine in 2004. Critical sized segmental defects were created in the radius of 36 Wistar rats. Thirteen defects were filled with ABM/P-15 Flow (gel form), 12 defects were filled with ABM/P-15, and 11 defects were used as a control group. The rats were sacrified at the tenth week, and healing of the defects was evaluated radiographically and histologically.

Results: The usage of ABM/P-15 and ABM/P-15 Flow were demonstrated to improve healing of segmental bone defects compared with the control group. Statistical evaluation showed that there were significant differences between control sites, and the sites treated with P-15 and P-15 Flow (p=0.011). The highest radiological and histological grades were achieved by P-15.

Conclusion: Segmental cortical bone defects may be treated with ABM/P-15 instead of bone allografts, and autografts. According to the radiological and histological parameters measured in this study, the implantation of ABM/P-15 resulted in optimum healing of the segmental cortical bone defects. Pepgen P-15 has a positive effect on bone healing, without any immunogenic features and disease transmission risk. Therefore, ABM/P-15 can also be used for orthopedic surgery.

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Type 1 collagen acts as a template for cell migration and penetration in tissues. The bond between cells and collagen is formed by the help of highly specific receptors. The skeletal structure of the cell is produced by this bond and has a role on diversion of mechanical energy to the biochemical procedures. It also has a role in cell differentiation and morphogenesis.[1] The cell binding region of type 1 collagen was defined at a1 (I) chain. This sequence is specific for collagen. A synthetic peptide (P-15) was formed according to this sequence and it was seen that this sequence was a ligand with high affinity to collagen binding receptors. Attachment, migration and differentation of bone producing cells was aimed by placing the cell binding activity of type 1 collagen on the bioceramic skeletons and the polymer carrier matrices in a 3-dimensional way.[1,2] Inorganic bone matrix (ABM) is the source of calcium phosphate and natural anatomic matrix needed for cellular invasion. The P-15 regulates the cellular binding, migration, proliferation and differentiation. The ABM/P-15 combination binds to the cells, especially to fibroblasts and osteoblasts.[3] Bone formation was

From the Department of Orthopedics and Traumatology (Cakmak, Bolukbasi, Simsek, Senkoylu) and the Department of Pathology (Erdem, Yilmaz), Gazi University School of Medicine, Ankara, *Turkey*.

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Address correspondence and reprint request to: Dr. Gokhan Cakmak, Gokkusagi Sitesi 10/28 Yesil Blok Cigdem Mah, 06530 Ankara, *Turkey*. Tel/Fax. +90 (242) 5112350. E-mail: gcakmak@ttnet.net.tr/gokhancakmak75@hotmail.com

shown in experimental studies.[4] This osteoconductive combination had been used on periodontal defects clinically.[5,6] The P-15 has no antigenic effect and therefore there are no immunological reactions.[7,8] The aim of this study is to determine the effect of ABM/P-15 on the healing of a critical sized segmental defect in a rat radius by histological and radiological evaluation.

Methods. The study was carried out at the Research Laboratories, Gazi University School of Medicine in 2004, and used 36 female Wistar. The rats weighed approximately 300 ± 20 grams, and were kept in different cages in the same institution. The same surgeon under ketamine and xylazine anesthesia carried out the surgical procedures. The right forelimbs of the rats were shaved, prepared and draped in a sterile fashion. A longitudinal incision was made on the radius, and the overlying tissues were dissected. The middle third of the radius diaphysis was osteotomized, and a segmental defect was formed. The defect was 2 times greater than the radius of the bone diaphysis. After this procedure, the defect was irrigated by saline solution. The rats were divided into 3 experimental groups as follows: Group 1 - segmental bone defects were filled with ABM/P-15 Flow (gel form). Group 2 - segmental bone defects were filled with ABM/P-15. Group 3 - segmental bone defects were used as a control group and left empty. After the procedure, the skin was sutured primarily. After 10 weeks healing period, the rats were sacrificed by high dose ketamine and xylazine. The right forelimbs were dissected without causing any harm on the callus tissues and classified according to the designated groups. The specimens were subjected to radiographic examination and they were put in 10% formaldehyde solution. Anteroposterior (A-P) radiographs were taken for each specimen. The radiographic osseous union was graded according to the radiographic evaluation scale, which was prepared by Cook et al.[9] No device was used for radiodensity measurement because the previous studies showed that there is no need for any device for radiographic evaluation.[9-11] The specimens were decalcified in 10% formic acid for 10 days and 5mm slides were prepared. The specimens were stained by hemotoxylene and eosin. Histopathologic evaluation was made under light microscope. Bone healing was determined at the defect sites. Histological evaluation was carried out according to the histopathologic evaluation scale used by Salkeld et al.[10,12]

Statistical analyses were performed using SPSS software Version 11.0 (SPSS Inc. Chicago, Ill, USA) A probability value of less than 0.05 was considered statistically significant. Kruskal-Wallis and Mann-Whitney U tests were used for the statistical evaluation of the data obtained for the different groups statistically.

Results. At the end of 10 weeks bone healing period, the A-P radiographs of the rats' forelimbs were taken. Osseous union was graded according to the radiographic evaluation scale prepared by Cook et al.[9] Kruskal-Wallis test revealed that there was a significant difference between the 3 groups ($p<0.001$). There was also a significant difference between these groups radiologically according to Mann Whitney U test: group 1-3 ($p<0.001$), group 2-3 ($p<0.001$) and group 1-2 ($p=0.011$). When group 1 and group 2 were compared with the control group, bone union was observed (**Figure 1**). Also, when group 1 and group 2 were compared with each other, group 2 had better bone union than group 1 radiographically All the specimens were investigated under light microscope and histologically graded. The results were defined statistically. When the groups were evaluated according to the quality of union, the following results were obtained. There was no difference between group 1 and group 2 ($p=0.247$). There was difference between group 1 and 3 $p<0.001$). There was difference between group 2 and 3 ($p<0.001$). When group 1 and 2 were compared with control group, the bone union was recognized histologically (**Figures 2 & 3**). When the groups were evaluated according to the bone-graft incorporation and new bone formation, the following findings were found: There was no difference between group 1 and 2 ($p=0.852$). There was a difference between group 1 and 3 ($p=0.003$), and there was a difference between group 2 and 3 ($p<0.001$). The histological comparison of group 1 and 2 with the control group revealed bone-graft incorporation and new bone formation. The callus formation and mineralization were observed at the bone defects site of group 1 and group 2. Significant osteoblastic activity was seen in group 2 (**Figure 3**).

Discussion. Bone grafts are often used during orthopedic surgery. Today, bone grafts substitutes are frequently used for suitable cases. The most important point is to use the graft materials for appropriate cases and indications. The ABM/P-15 was produced for filling periodontal defects, and there were many experimental and clinical studies with this substitute. Yukna et al,[8,12] carried out 2 prospective clinical studies on periodontal defects and filled these defects with ABM/P-15. They found that the clinical results were better with ABM/P-15 than ABM alone. Bovine derived hydroxyapatites carry the risk of transmitting diseases (for example, Jakob Creutzfeld disease) with proteins and prions when they are

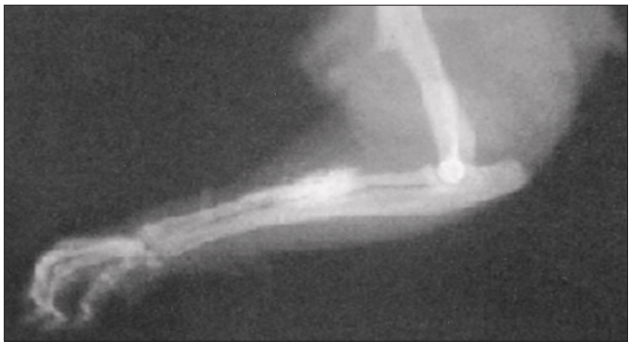

Figure 1 - Radiographic view of an inorganic bone matrix P-15 treated defect after 10 weeks.

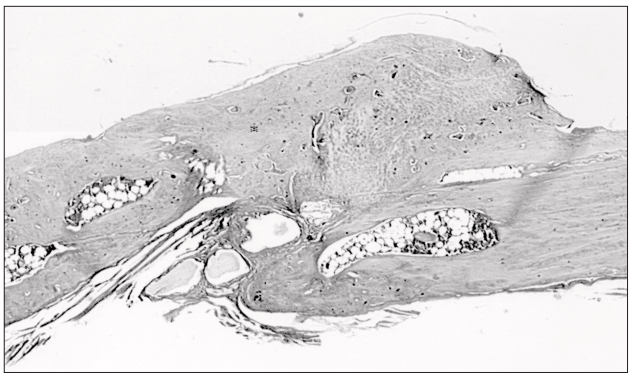

Figure 2 - The increase of osteoblastic activity and callus formation could be seen at the osteotomy site after 10 weeks (Group 1), hematoxylin and eosin X 12.5.

prepared at low temperatures. The bovine derived hydroxyapatites prepared at high temperature do not pose this risk. Therefore, ABM has this advantage. To increase the rate of cell binding to the graft material, the ABM surface was covered with cell binding peptide.[14] Cell binding peptide regulate apoptosis and improves cell binding, cell numbers and tissue structure.[15] Qian et al showed that ABM/P-15 had great effect on bone healing by alkalen phosphatase staining tissues in vitro.[16] The ABM/P-15 has shown good results on alveolar bone defects in long term clinical studies.[16,17] After filling the alveolar defects with ABM/P-15, an increase in osteogenic cells and revascularization was histologically observed.[18,19] Krauser et al[3] filled a maxillary sinus defect with inorganic bovine bone mineral and demineralized bone matrix. However, it was not successful and they then applied a ABM/P-15 combination to the defect. The defect filled with bone in a short time period radiologically and histologically. The ABM/P-15 has biocompatible hydrogel and chips forms. This hydrogel is composed of carboxymetylcellulose and glycerol. Hydrogel fills the gap between particles

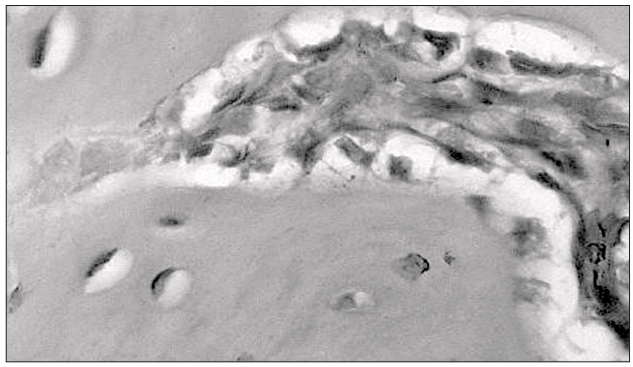

Figure 3 - Significant osteoblastic activity was seen around inorganic bone matrix P-15 at group 2 than group 1 after 10 weeks (hematoxylin and eosin, X 400).

and allows the cells to penetrate these P-15 cell binding areas. The previous studies showed that the hydrogel form of ABM/P-15 produced a more rapid bone formation than the other forms.[8,14] However, our study revealed that the ABM/P-15 chip form resulted in more osteoblastic activity. Kübler et al[14] compared the effects of 5 different graft materials on human bone tissue culture. These materials are phythogen hydroxyapatite, a-tricalcium phosphate, bovine derived hydroxyapatite produced at low temperature, bovine derived hydroxyapatite produced at high temperature and bovine derived hydroxyapatite produced at high temperature-P-15 combination (ABM/P-15). The effects of these different materials on osteoblasts were evaluated. Cell proliferation and presence were defined by alkalen phosphatase levels. The tissue cultures were investigated under light and electron microscopes. The ABM/P-15 had the most cell proliferation and differentiation than the other substitutes. Bovine derived hydroxyapatite produced at high temperature (Osteograft) was the other most effective graft material after ABM/P-15.[20] Most of the clinical and experimental studies were carried out on periodontal surgery and alveolar bones. Only one experimental animal study was affected by M/P-15 on cortical bones.[21] In that study, the tibial critical sized cortical defects were filled with ABM/P-15 Flow and ABM/P-15. These 2 groups had better bone healing than the control group after histological investigation. However, the group to which ABM/P-15 Flow was applied had less bone formation. The shortcomings of this study were that there was no histological and radiological grading system.[21] Our study provided histological and pathological support to it. Alizarin Red S histological staining was performed after applying the P-15 on human tissue culture studies. These cultures were positively stained and showed that mineralization occurred. Also alkaline phosphatase, TGF-B-1, type 1 collagen, osteocalcine and osteonectine levels were measured

to be higher than other tissue cultures. This shows that ABM/P-15 accelerates bone healing.[1,2,22] In short and long-term clinical studies, in which ABM/P-15 was applied for periodontal treatments, radioopacity was observed around the implants. This showed that new bone formation had occurred. Our experimental study, with a higher number of test subjects, showed the affectivity of ABM/P-15 radiographically and histologically by grading systems.

In conclusion, ABM/P-15 and ABM/P-15 Flow produced new bone formation at segmental cortical bone defects, especially at the peripheral sites. This could be observed radiologically and histologically. The ABM/P-15 had more osteogenic proliferation than ABM/P-15 Flow. Previous clinical and experimental studies on periodontal alveolar bone defects showed that ABM/P-15 Flow had better bone healing effect. By this experimental study, we evaluated the difference in the effectivity of these 2 products and obtained a different result. Also, by this study we combined histological and radiological evaluation of this product and had a different point of view. The only limitation of this study is the absence of the control group in which ABM alone will be used, but this is our preliminary results, and also by the previous in vitro and in vivo studies the affectivity of ABM/P-15 combination was approved. Synthetic cell binding peptide (P-15), which is used in periodontal procedures, clinically has a positive effect on bone healing, with no immunogenic feature and disease transmission risk. This product can be found and produced easily. Based on these findings, ABM/P-15 will most probably be used during orthopedic surgery in the future.

References

1. Bhatnagar RS, Qian JJ, Wedrychowska A, Smith N. Construction of biomimetic environments with a synthetic peptide analogue of collagen. ***Materials Reseach Society Symposium Proceedings*** 1998; 530: 43-54.
2. Bhatnagar RS, Qian JJ, Wedrychowska A, Dixon E, Smith N. Biomimetic habitats for cells: Ordered matrix deposition and differentiation in fibroblasts cultured on hydroxyapatite coated with a collagen analogue. ***Cells and Materials*** 1999; 9: 93-104.
3. Krauser JT, Rohrer MD, Wallace SS. Human histologic and histomorphometric analysis comparing OsteoGraf/N with PepGen P-15 in the maxillary sinus elevation procedure: a case report. ***Implant Dentistry*** 2000; 9: 298-302.
4. Lallier TE, Yukna R, St Marie S, Moses R. The putative collagen binding peptide hastens periodontal ligament cell attachment to bone replacement graft materials. ***J Periodontol*** 2001; 72: 990-997.
5. Açil Y, Springer IN, Broek V, Terheyden H, Jepsen S. Effects of bone morphogenetic protein-7 stimulation on osteoblasts cultured on different biomaterials. ***J Cell Biochem*** 2002; 86: 90-98.
6. Caffesse RG, de la Rosa M, Mota LF. Regeneration of soft and hard tissue periodontal defects. ***Am J Dent*** 2002; 15: 339-345.
7. Walters SP, Greenwell H, Hill M, Drisko C, Pickman K, Scheetz JP. Comparison of porous and non-porous teflon membranes plus a xenograft in the treatment of vertical osseous defects: a clinical reentry study. ***J Periodontol*** 2003; 74: 1161-1168.
8. Yukna RA, Callan DP, Krauser JT, Evans GH, Aichelmann-Reidy ME, Moore K, et al. Multi-center clinical evaluation of combination anorganic bovine-derived hydroxyapatite matrix (ABM)/cell binding peptide (P-15) as a bone replacement graft material in human periodontal osseous defects. 6-month results. ***J Periodontol*** 1998; 69: 655-663.
9. Cook S, Baffes G, Wolfe M, Sampath T, Rueger D. Recombinant human bone morphogenetic protein-7 induces healing in a canine long-bone segmental defect model. ***Clin Orthop*** 1994; 301: 302-312.
10. Lane JM, Sandhu H. Current approaches to experimental bone grafting. ***Orthop Clin North Am*** 1987; 18: 213-225.
11. Tiedeman J, Lippiello L, Connolly J, Strates B. Quantitative roentgenographic densitometry for assessing fracture healing. ***Clin Orthop*** 1990; 253: 279-286.
12. Salkeld SL, Patron LP, Barrack RL,Cook SD. The effect of osteogenic protein-1 on the healing of segmental bone defects treated with autograft or allograft bone. ***J Bone Joint Surg*** 2001; 83-A: 803-816.
13. Yukna RA, Krauser JT, Callan DP, Evans GH, Cruz R, Martin M. Multi-center clinical comparison of combination anorganic bovine-derived hydroxyapatite matrix (ABM)/cell binding peptide (P-15) and ABM in human periodontal osseous defects. 6-month results. ***J Periodontol*** 2000; 71: 1671-1679.
14. Kübler A, Neugebauer J, Oh J, Scheer M, Zöller J. Growth and Proliferation of Human Osteoblasts on Different Bone Graft Substitutes An In Vitro Study. ***Implant Dent*** 2004; 13: 171-177.
15. Hanks T, Atkinson B. Comparison of cell viability on anorganic bone matrix with or without P-15 cell binding peptide. ***Biomaterials*** 2004; 25: 4831-4836.
16. Qian JJ, Bhatnagar RS. Enhanced cell attachement to anorganic bone mineral in the presence of a synthetic peptide related to collagen. ***J Biomed Mater Res*** 1996; 31: 545-554.
17. Yukna RA, Krauser JT, Callan DP, Evans GH, Cruz R, Martin M. Thirty-six month follow-up of 25 patients treated with combination anorganic bovine-derived hydroxyapatite matrix (ABM)/cell-binding peptide (P-15) bone replacement grafts in human infrabony defects. I. Clinical findings. ***J Periodontol*** 2002; 73: 123-128.
18. Scarano A, Iezzi G, Petrone G, Orsini G, Degidi M, Strocchi R, et al. Cortical bone regeneration with a synthetic cell-binding peptide: a histologic and histomorphometric pilot study. ***Implant Dent*** 2003; 12: 318-324.
19. Valentin A, Weber, J. Receptor technology – cell binding to P-15: a new method of regenerating bone quickly and safely preliminary histomorphometrical and mechanical results in sinus floor augmentations. ***Keio J Med*** 2004; 53: 166–171.
20. Yukna R, Salinas TJ, Carr RF. Periodontal regeneration following use of ABM/P-1 5: a case report. ***Int J Periodontics Restorative Dent*** 2002; 22: 146-155.
21. Taniguchi T, Matsumoto T, Shindo H. Changes of serum levels of osteocalcin, alkaline phosphatase, IGF-I and IGF-binding protein-3 during fracture healing. ***Injury*** 2003; 34: 477-479.
22. Scarano A, Iezzi G, Petrone G, Orsini G, Degidi M, Strocchi R, et al. Cortical bone regeneration with a synthetic cell-binding peptide: a histologic and histomorphometric pilot study. ***Implant Dent*** 2003; 12: 318-324.
23. Turhani D, Item C, Thundler D, Kapral D, Cvikl B, Weissenbock M, et al. Nachweis der osteokalzin expression osteoblastarer zellen mandibularen ursprungs, wachsend auf biomaterialien, mittels RT-PCR und SDS-PAGE/ western blotting. ***Mund Kiefer Gesichtschir*** 2003; 7: 294-300.