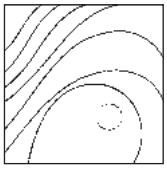
Bone Regeneration with Self-Assembling Peptide Nanofiber Scaffolds in Tissue Engineering for Osseointegration of Dental Implants



Tomoyuki Kohgo, DDS[1]/Yoichi Yamada, DDS, PhD[2]/Kenji Ito, DDS, PhD[3]
Akihiro Yajima, DDS, PhD[4]/Ryoko Yoshimi, DDS[5]/Kazuto Okabe, DDS[5]
Shunsuke Baba, DDS, PhD[6]/Minoru Ueda, DDS, PhD[7]

The aim of this study was to evaluate the correlation between the osseointegration of dental implants and tissue-engineered bone using a nanofiber scaffold, PuraMatrix (PM). The first molar and all premolars in the mandibular regions of dogs were extracted, and three bone defects were prepared with a trephine bur on both sides of the mandible after 4 weeks. The experimental groups were as follows: (1) *PM,* (2) *PM and dog mesenchymal stem cells (dMSCs),* (3) *PM, dMSCs, and platelet-rich plasma, and* (4) *control (defect only). Implants were placed in the prepared areas 8 weeks later and were assessed by histologic and histomorphometric analyses (bone-to-implant contact [BIC]). The BICs for groups 1, 2, 3, and 4 were 40.77%, 50.35%, 55.64%, and 30.57%, respectively. The findings indicate that PM may be useful as a scaffold for bone regeneration around dental implants.* (Int J Periodontics Restorative Dent 2011;31:e9–e16.)

[1]Department of Oral and Maxillofacial Surgery, Nagoya City University School of Medical Sciences, Nagoya, Japan; Formerly, Student, Department of Oral and Maxillofacial Surgery, Nagoya University Graduate School of Medicine, Nagoya, Japan.
[2]Assistant Professor, Center for Genetic and Regenerative Medicine, Nagoya University Graduate School of Medicine, Nagoya, Japan.
[3]Assistant Professor, Department of Clinical Cell Therapy and Tissue Engineering, Nagoya University School of Medicine, Nagoya, Japan.
[4]Chief, Department of Oral and Maxillofacial Surgery, Hoshigaoka Maternity Hospital, Nagoya, Japan.
[5]Student, Department of Oral and Maxillofacial Surgery, Nagoya University Graduate School of Medicine, Nagoya, Japan.
[6]Chief, Department of Regenerative Medicine, Institute of Biomedical Research and Innovation, Kobe, Japan.
[7]Professor and Chair, Department of Oral and Maxillofacial Surgery, Nagoya University Graduate School of Medicine, Nagoya, Japan.

Correspondence to: Dr Yoichi Yamada, 65 Tsuruma-cho, Showa-ku, Nagoya 466-8550, Japan; fax: +81-52-744-2352; email: yyamada@med.nagoya-u.ac.jp.

Bone regeneration and augmentation for dental implant placement are frequently carried out using autografts, allografts, xenografts, or synthetic materials.[1–4] Among these materials, autogenous bone grafts are considered an ideal graft material and the gold standard. However, the harvesting of bone grafts injures otherwise healthy sites, causing morbidity.[5,6] Presently, bone substitutes such as hydroxyapatite, tricalcium phosphate, or inorganic porous animal-derived bone minerals[7–9] are used to provide alternatives to autogenous bone for improvement of the bone volume; these materials need to be researched more regarding their safety and effectiveness in clinical practice.[10,11] Moreover, these materials and internal structures may not provide a particularly favorable environment for cell survival and bone regeneration, and the microscale environment of these materials needs to serve as an extracellular matrix (ECM) in nature.

The present study considered the use of the novel matrix material PuraMatrix (PM; 3-D Matrix),

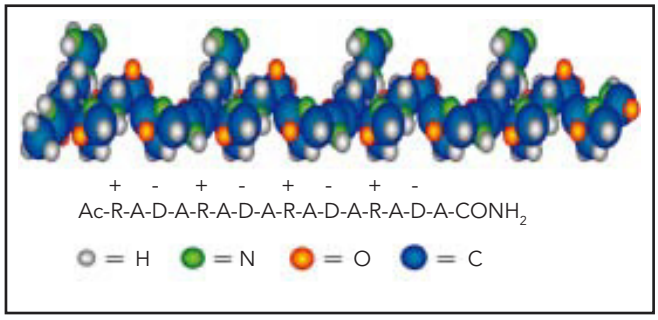


Fig 1 *Molecular structure of PM. Molecular models of the amphiphilic self-complementary peptide have 16 amino acids with an alternating polar and nonpolar pattern. A = alanine; R = arginine; D = aspartic acid; + and – refer to the positively and negatively charged residues, respectively.*

which is synthesized by chemical peptide methods, has similarities to the fibers and pore sizes found in ECM, is not animal-derived, and shows fluid characteristics.[12] Therefore, PM exhibits good plasticity, absorption, biocompatibility properties, and is devoid of animal-derived pathogens and antigens.[5]

Recently, a concept of tissue engineering whereby isolated cells, signal molecules, and scaffolds are necessary for tissue regeneration has been proposed.[13] Successful tissue engineering involves the implantation of living cells along with synthetic materials and results in new tissue regeneration.[14] Tissue engineering procedures for bone augmentation offer significant advantages compared with conventional grafts since there is minimal or little donor site morbidity.[15] Within this concept, the authors suggest the use of mesenchymal stem cells (MSCs) as the cells, platelet-rich plasma (PRP) as the source of signal molecules,[16] and PM as the scaffold. Generally, in tissue engineering, the cells are typically seeded in a three-dimensional (3D) scaffold, which not only provides the anchorage for cell adhesion, but also creates a suitable cellular microenvironment through adhesions that regulate cell differentiation and cell-cell signaling.[13]

Method and materials

All animal experiments undertaken were performed in strict accordance with protocols approved by the Institutional Animal Care Committee. After a period of acclimation, hybrid dogs with a mean age of 2 years were operated on under general anesthesia. The first molar and all premolars in the mandible were extracted, and extraction sites were allowed to heal for 4 weeks. Bone defects on both sides of the mandible were prepared with a 10-mm (diameter and depth) trephine bur.[17–19] The defects received implants using graft materials as follows: group 1, PuraMatrix (PM); group 2, PM and dog MSCs (dMSCs; PM/dMSCs); group 3, PM, dMSCs, and PRP (PM/dMSCs/PRP); and group 4, control (defect only). Without any differences between the various sites in terms of bone healing, three defects per side of the mandible were prepared, and the four groups of materials were implanted in a random and non–site-specific manner. After 8 weeks, bone biopsies were taken using a 2-mm-diameter trephine bur, and Brånemark implants (8.5 mm in length, 3.75 mm in diameter; Nobel Biocare) were placed into the bone regeneration areas.

Cell cultivation

Bone marrow cell isolation and expansion were performed according to previous methods.[14] The cells were incubated at 37°C in a humidified atmosphere containing 95% air and 5% carbon dioxide. In culture, dMSCs were trypsinized and used for implanting.

PM is a synthetic peptide consisting of a sequence of 16 amino acids (Ac-RADARADARADARADA-$CONH_2$) (Fig 1). PM peptide hydrogel was sonicated for 30 minutes using a sonic machine (Branson). Phosphate-buffered saline (100 μL) was layered over the top of the hydrogel, allowed to sit at room temperature for 1 minute, and then mixed gently. PRP was prepared according to previous methods.[17] Then, the PRP and dMSCs (1.0×10^7 cells/mL) or dMSCs alone (1.0

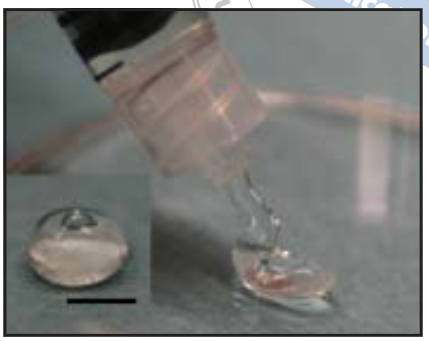


Fig 2 *PM is an injectable scaffold and shows good plasticity. Bar = 5 mm.*

$\times$ 10^7 cells/mL) were mixed with PM and thrombin/calcium chloride. The contents assumed a gel-like form to produce an insoluble gel.

Histologic and histomorphometric analyses

Each implant site was excised with a 2-mm-diameter trephine bur after 8 weeks of implantation. Specimens were fixed in 10% formalin, decalcified, and stained with hematoxylin-eosin. At 2 and 4 weeks after implant placement, 20 mg of oxytetracycline hydrochloride/kg and 5 mg calcein/kg were administered intravenously (Wako Pure Chemical Industries). The dogs were sacrificed 8 weeks later. Nondecalcified (ground) sections were processed according to the method of Donath and Breuner.[20] Sections with a thickness of approximately 10 μm were stained with toluidine blue. Histologic and histomorphometric analyses were conducted using a BIOZERO fluorescent microscope (BZ-8000, Keyence).

The bone-to-implant contact (BIC) was calculated as the total length of bone contact divided by the total length of the implant surface and multiplied by 100 to obtain a percentage.

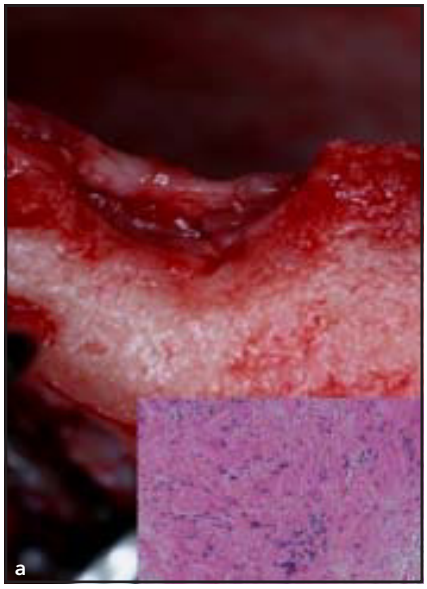
Statistical analysis

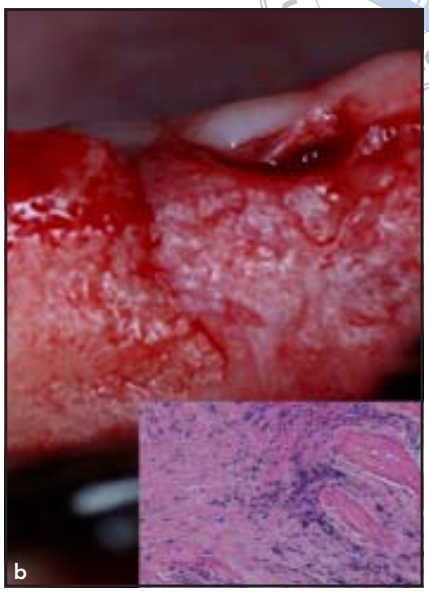
Group means and standard deviations were calculated for each experimental area. The data were compared using the Tukey-Kramer test following one-way analysis of variance between the control, PM, PM/dMSCs, and PM/dMSCs/PRP groups. A P value of $< .05$ indicated significance.

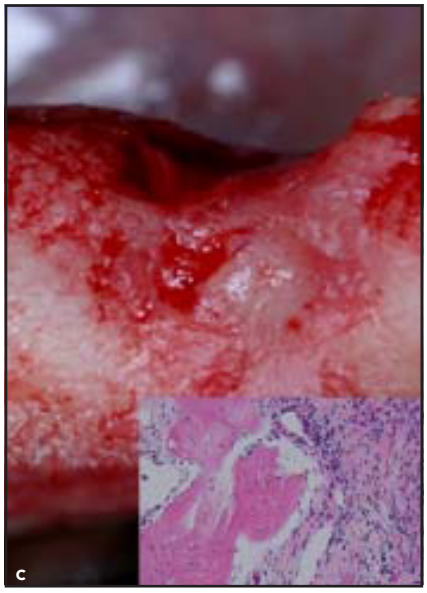
Results

PM is a gel with improved tissue retention (Fig 2), and it can be injected into bone defects. Macroscopic findings showed that bone regeneration formed in the control, PM, and PM/dMSCs groups was incomplete. In the histologic observations, the control (Fig 3a) and PM (Fig 3b) groups showed cavities invaded by fibrous tissue, PM/dMSCs sites showed new partial bone formation (Fig 3c), and PM/dMSCs/PRP sites showed mature bone (Fig 3d). Therefore, when dental implants were inserted into these areas, the exposure of the dental implant thread in the control (Fig 4a), PM (Fig 4b), and PM/dMSCs (Fig 4c) groups was observed, but exposure of the dental implant thread was only minimally observed in the PM/dMSCs/PRP group (Fig 4d). It was labeled green by tetracycline at 2 weeks and blue by calcein at 4 weeks after dental implant insertion, as fluorescent markers of new bone formation (Fig 5). The regenerated bone volume was greater in PM/dMSCs and PM/dMSCs/PRP sites than in control and PM sites. In addition, regenerated bone showed the highest level of maturity around dental implants in PM/dMSCs/PRP sites.

BIC was 30.57% ± 2.50% (control), 40.77% ± 12.85% (PM), 50.35% ± 8.02% (PM/dMSCs), and 55.64% ± 4.97% (PM/dMSCs/PRP) 8 weeks after implant placement (Table 1). The BIC of the PM/dMSCs/PRP group showed a significant increase in the implant surface compared with the control ($P < .01$) and PM ($P < .05$) groups. The BIC of the PM/dMSCs group showed a significant increase in the implant surface compared with the control ($P < .05$, Table 1).







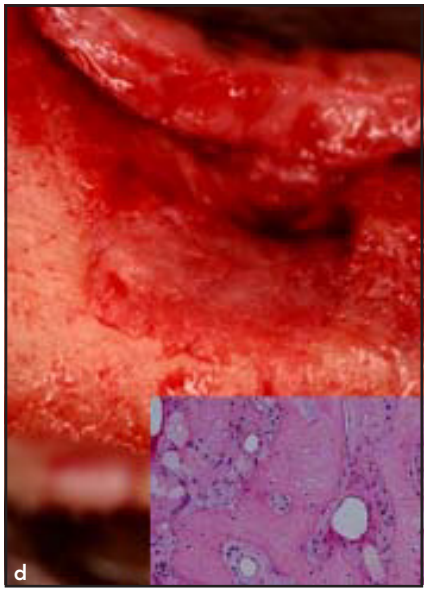
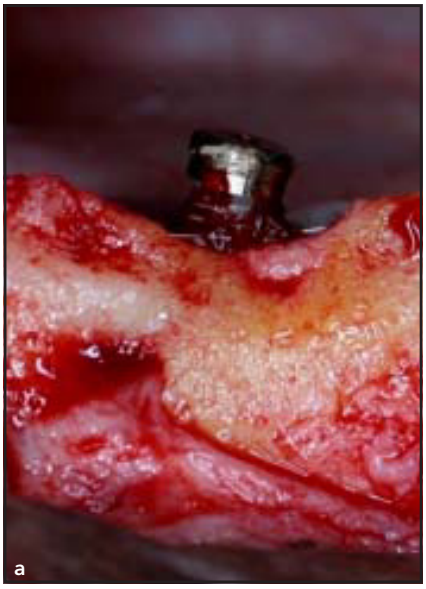
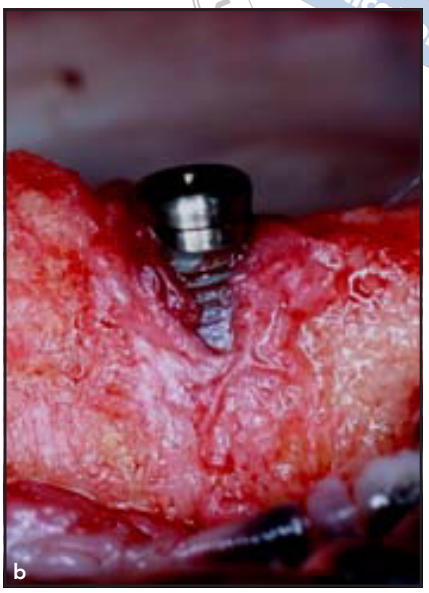
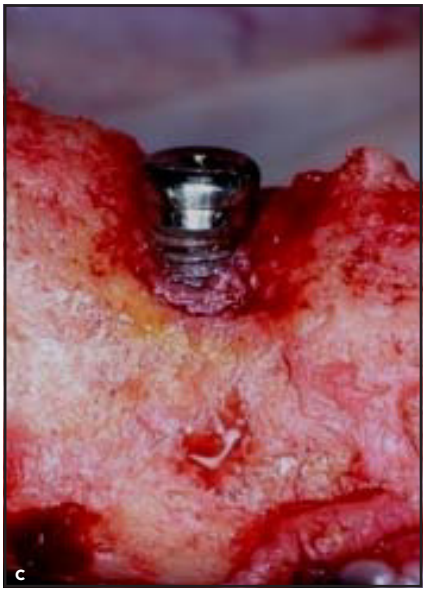


Fig 3 *Macroscopic observations of the* (a) *control,* (b) *PM,* (c) *PM/dMSCs, and* (d) *PM/dMSCs/PRP groups after 8 weeks of material implantation. The inner box shows the histologic evaluation (hematoxylin-eosin, original magnification ×100).*







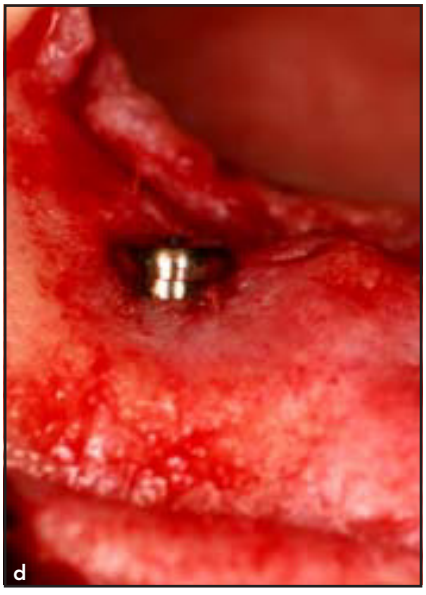


Fig 4 *Macroscopic observations 8 weeks after those in Fig 3, after implant insertion.* (a) *Control,* (b) *PM,* (c) *PM/dMSCs, and* (d) *PM/dMSCs/PRP.*

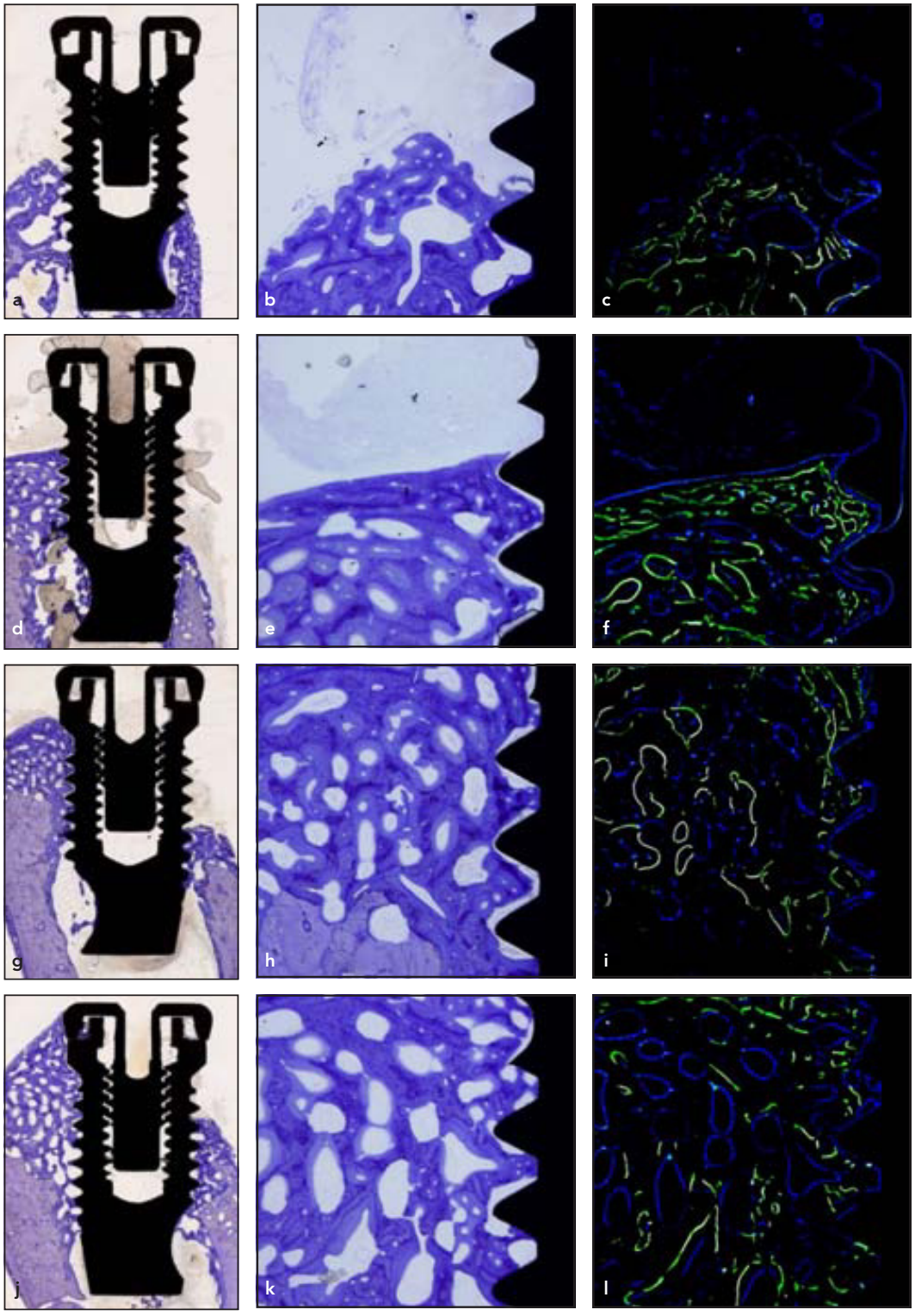


Fig 5 *Photographs of histologic sections, as seen on light microscopy, 8 weeks after implant placement.* (a to c) *In the control group, the buccal and lingual walls were not sufficiently regenerated for dental implants.* (d to f) *In the PM group, slight bone regeneration in the lingual wall was observed.* (g to i) *However, slightly more could be seen in the PM/dMSCs group.* (j to l) *On the other hand, the amount of regenerated bone was greatest in the PM/dMSCs/PRP group* (a, d, g, *and* j, *magnification ×12.5;* b, e, h, *and* k, *magnification ×200;* c, f, i, *and* l, *magnification ×200).*

Table 1 **Results of BIC (mean ± SD)†**

Graft materials	BIC
Control	30.57% ± 2.50%
PM	40.77% ± 12.85%
PM/dMSCs	50.35% ± 8.02%
PM/dMSCs/PRP	55.64% ± 4.97%

BIC = bone-to-implant contact; SD = standard deviation; PM = PuraMatrix; dMSCs = dog mesenchymal stem cells; PRP = platelet-rich plasma
*$P < .01$; **$P < .05$.
†Data were compared using the Tukey-Kramer test following one-way analysis of variance between control, PM, PM/dMSCs, and PM/dMSCs/PRP groups.

Discussion

Tissue engineering for bone regeneration has been shown to be an attractive alternative to autogenous or synthetic bone substitutes. Recently, tissue engineering approaches have been attempted, and scaffolding has been discussed extensively.[21,22] Scaffolds are requested to have the ability to induce bone formation at nonbony sites, provide a scaffold for new bone formation, be safe for the host, and harmonize with the timing of tissue repair. But the ability of bone regeneration in various scaffolds would not have been sufficient.[17,23,24] In this study, a new bone biomaterial composite, PuraMatrix, which forms a unique gel that is absorbable and nanofibered for tissue engineering, was investigated.[25]

This study confirmed the compatibility of dMSCs and PM. PM is a synthetic peptide consisting of a 16–amino acid sequence (Ac-RADARADARADARADA-$CONH_2$). The RAD (arginine-alanine-asparate) repeats in the peptide are similar to the ubiquitous integrin receptor binding site RGD (arginine-glycine-asparate) sequence, which was identified as the main structural motif of ECM.[26] It has been reported that PM may be able to mimic the structure and biologic function of ECM, both in terms of the chemical composition and physical structure.[27] In these reports, it might be inferred that cells survived and maintained favorable conditions in PM.[5,6] In fact, the PM/dMSCs group showed new areas of bone formation compared with the PM and control groups on histologic observation (see Figs 3 and 4). Hamada et al[28] reported that MSCs survive in PM hydrogel and that 3D osteogenic differentiation can occur. PM alone might not be able to stimulate sufficient bone formation for dental implants, but a PM scaffold with dMSCs might encourage MSC adhesion, proliferation, and differentiation, and thus induce effective bone formation.

On histomorphometric evaluation, the PM/dMSCs and PM/dMSCs/PRP groups were significantly different from the control (defect only). In this study, the BICs of the PM/dMSCs and PM/dMSCs/PRP groups were 50.35% ± 8.02% and 55.64% ± 4.97%, respectively (Table 1). In a previous study, the BIC of autogenous particulate cancellous bone and marrow (PCBM) was 49.9% ± 8.2%.[23] These results indicate that bone regenerated with PM/dMSCs was as satisfactory as that with PCBM. The new technology developed, injectable tissue-engineered bone,[18,19] was based on tissue engineering concepts.[29] Therefore, using MSCs and PM, bone formation could be induced with artificial 3D scaffolds. To achieve greater bone formation around dental implants, it might be advisable to use PRP containing some growth factors as signal molecules.

Conclusion

The results suggest that tissue-engineered bone can integrate well around dental implants. PM is a 3D structure that may have the potential to be a scaffold applicable in bone tissue engineering. In the future, the authors would like to clinically apply bone regeneration for dental implants using tissue engineering technology.

Acknowledgments

The authors would like to thank Nobel Biocare, ArBlast, and 3-D Matrix Japan for their assistance.

References

1. Piattelli A, Degidi M, Di Stefano DA, Rubini C, Fioroni M, Strocchi R. Microvessel density in alveolar ridge regeneration with autologous and alloplastic bone. Implant Dent 2002;11:370–375.
2. Norton MR, Odell EW, Thompson ID, Cook RJ. Efficacy of bovine bone mineral for alveolar augmentation: A human histologic study. Clin Oral Implants Res 2003;14:775–783.
3. Artzi Z, Dayan D, Alpern Y, Nemcovsky CE. Vertical ridge augmentation using xenogenic material supported by a configured titanium mesh: Clinicohistopathologic and histochemical study. Int J Oral Maxillofac Implants 2003;18:440–446.
4. Ganz SD, Valen M. Predictable synthetic bone grafting procedures for implant reconstruction: Part two. J Oral Implantol 2002;28:178–183.
5. Kisiday J, Jin M, Kurz B, et al. Self-assembling peptide hydrogel fosters chondrocyte extracellular matrix production and cell division: Implications for cartilage tissue repair. Proc Natl Acad Sci U S A 2002;99: 9996–10001.
6. Holmes TC. Novel peptide-based biomaterial scaffolds for tissue engineering. Trends Biotechnol 2002;20:16–21.
7. Nunens CR, Simske SJ, Sachdeva R, Wolford LM. Long-term ingrowth and apposition of porous hydroxylapatite implants. J Biomed Mater Res 1997;36:560–563.
8. Richardson CR, Mellonig JT, Brunsvold MA, McDonnell HT, Cochran DL. Clinical evaluation of Bio-Oss: A bovine-derived xenograft for the treatment of periodontal osseous defects in humans. J Clin Periodontol 1999;26:421–428.
9. Bucholz RW. Nonallograft osteoconductive bone graft substitutes. Clin Orthop Relat Res 2002;(395):44–52.
10. Nishikawa T, Masano K, Tominaga K, et al. Bone repair analysis in a novel biodegradable hydroxyapatite/collagen composite implanted in bone. Implant Dent 2005;14:252–260.
11. Tamimi FM, Torres J, Tresguerres I, Clemente C, López-Cabarcos E, Blanco LJ. Bone augmentation in rabbit calvariae: Comparative study between Bio-Oss and a novel beta-TCP/DCPD granulate. J Clin Periodontol 2006;33:922–928.
12. Yokoi H, Kinoshita T, Zhang S. Dynamic reassembly of peptide RADA16 nanofiber scaffold. Proc Natl Acad Sci U S A 2005;102:8414–8419.
13. Khademhosseini A, Langer R, Borenstein J, Vacanti P. Microscale technologies for tissue engineering and biology. Proc Natl Acad Sci U S A 2006;103:2480–2487.
14. Kadiyala S, Young RG, Thiede MA, Bruder SP. Culture expanded canine mesenchymal stem cells possess osteochondrogenic potential in vivo and in vitro. Cell Transplant 1997;6:125–134.
15. Bruder SP, Kurth AA, Shea M, Hayes WC, Jaiswal N, Kadiyala S. Bone regeneration by implantation of purified, culture-expanded human mesenchymal stem cells. J Orthop Res 1998;16:155–162.
16. Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR. Platelet-rich plasma: Growth factor enhancement for bone grafts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998; 85:638–646.
17. Yamada Y, Ueda M, Naiki T, Takahashi M, Hata K, Nagasaka T. Autogenous injectable bone for regeneration with mesenchymal stem cells and platelet-rich plasma: Tissue-engineered bone regeneration. Tissue Eng 2004;10:955–964.
18. Yamada Y, Ueda M, Naiki T, Nagasaka T. Tissue-engineered injectable bone regeneration for osseointegrated dental implants. Clin Oral Implants Res 2004;15:589–597.
19. Ito K, Yamada Y, Nagasaka T, Baba H, Ueda M. Osteogenic potential of injectabe tissue-engineered bone: A comparison among autogenous bone, bone substitute (Bio-Oss), platelet-rich plasma, and tissue-engineered bone with respect to their mechanical properties and histological findings. J Biomed Mater Res A 2005;73:63–72.
20. Donath K, Breuner G. A method for the study of undecalcified bones and teeth with attached soft tissues. The Säge-Schliff (sawing and grinding) technique. J Oral Pathol 1982;11:318–326.
21. Ohgushi H, Caplan AI. Stem cell technology and bioceramics: From cell to gene engineering. J Biomed Mater Res 1999;48:913–927.
22. Mistry AS, Mikos AG. Tissue engineering strategies for bone regeneration. Adv Biochem Eng Biotechnol 2005;94:1–22.
23. Yoshikawa T, Ohgushi H, Tamai S. Immediate bone forming capability of prefabricated osteogenic hydroxyapatite. J Biomed Mater Res 1996;32:481–492.
24. Fariña NM, Guzón FM, Peña ML, Cantalapiedra AG. In vivo behaviour of two different biphasic ceramic implanted in mandibular bone of dogs. J Mater Sci Mater Med 2008;19:1565–1573.
25. Zhang S, Holmes TC, DiPersio CM, Hynes RO, Su X, Rich A. Self-complementary oligopeptide matrices support mammalian cell attachment. Biomaterials 1995; 16:1385–1393.
26. Ruoslahti E, Pierschbacher MD. New perspectives in cell adhesion: RGD and integrins. Science 1987;238:491–497.
27. Hosseinkhani H, Hosseinkhani M, Tian F, Kobayashi H, Tabata Y. Osteogenic differentiation of mesenchymal stem cells in self-assembled peptide-amphiphile nanofibers. Biomaterials 2006;27:4079–4086.
28. Hamada K, Hirose M, Yamashita T, Ohgushi H. Spatial distribution of mineralized bone matrix produced by marrow mesenchymal stem cells in self-assembling peptide hydrogel scaffold. J Biomed Mater Res A 2008;84:128–136.
29. Langer R, Vacanti JP. Tissue engineering. Science 1993;260:920–926.