The Putative Collagen Binding Peptide Hastens Periodontal Ligament Cell Attachment to Bone Replacement Graft Materials

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Background: Bone replacement graft (BRG) materials are often used to treat periodontal defects, to promote cellular invasion, and to encourage bone regrowth. Periodontal ligament fibroblasts (PDLF) incorporate these materials and form the basis of the renewed connection between the existing and newly formed alveolar bone and the tooth surface. A peptide (P-15) that mimics the putative cell-binding domain of collagen has been reported to promote dermal fibroblast attachment and proliferation.

Methods: PDLF were quantitatively examined for their ability to adhere to a variety of BRG materials fluorometrically. In addition, scanning electron microscopy was used to examine the changes in morphology exhibited by these cells as they attached and spread on several BRG materials. Finally, BRG materials containing the P-15 peptide were quantitatively examined for their ability to promote PDLF attachment and proliferation.

Results: Freeze-dried allograft bone supports greater PDLF attachment than does several xenograft and alloplastic anorganic bone replacement materials. An anorganic BRG material containing the P-15 peptide promoted more rapid cell attachment and spreading than a similar anorganic BRG material lacking this peptide. Finally, none of the BRG materials examined promoted PDLF proliferation.

Conclusions: Our data indicate that the addition of the P-15 peptide increases the rapidity of PDLF attachment to xenogeneic bone replacement materials. This increase in the rate of attachment may have clinical significance in the context of the dynamic regulation of cell attachment during periodontal regeneration. However, this peptide does not promote an increase in stable cell attachment or proliferation in vitro. *J Periodontol 2001;72:990-997.*

KEY WORDS

Grafts, bone; periodontal pockets/therapy; bone regeneration; collagen; fibroblasts; cell adhesion; periodontal regeneration; peptides/therapeutic use.

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Contemporary periodontal therapy seeks to reverse the plaque-induced resorption of bone and periodontal ligament (PDL) collagen by promoting the regeneration of the periodontium.[1,2] This process requires the appropriate positioning of cells capable of synthesizing collagen, cementum, and bone. Although the source of competent cells in the adult for such repair remains uncertain, PDLF have been proposed as likely candidates.[3-5] Recognized therapeutic strategies such as guided tissue regeneration[6] and coronally positioned flaps designed to exclude non-PDL cells from regenerating areas[7] provide indirect evidence that the PDL is the source of such cells. Moreover, PDLF express alkaline phosphatase,[8,9] a marker for developing bone cells that is either not expressed by gingival fibroblasts[10] or is expressed at significantly lower levels.[11] In addition, PDLF are a major cellular component of the PDL.[12-14] Past studies have presented evidence that PDLF are capable of converting from fibroblasts to osteoblasts,[15-18] are chemotactically responsive to appropriate growth factors,[19] and participate in defect repairs at surgical sites.[4,20]

Implantation of bone replacement graft (BRG) materials to stimulate bone deposition has been used orthopedically since the 1950s and in periodontal therapy since the 1970s.[21-25] BRG materials that maximize functional bone deposition are considered more clinically useful. Currently a wide variety of materials including demineralized and non-demineralized freeze-dried bone allo-

graft, various types of natural and synthetic hydroxyapatites, ceramics, calcium carbonate (natural coral), silicon-containing glasses, and synthetic polymers are utilized as BRG materials. This variety of BRG materials reflects attempts to identify BRG materials with high osteogenic potential and low clinical risk.[26,27] Such an awareness has led to the incorporation of hydroxyapatite coatings on dental implants[28] and the use of growth factors and attachment proteins to stimulate osteoprogenitor cells, including PDLF.[29] An additional goal of the use of these BRG materials is to promote the regeneration of the more complex periodontal attachment apparatus, including the original and repaired bone, the periodontal ligament, and cementum.

Recently, a synthetic 15-amino acid sequence (P-15) with steric similarities to the cell binding site of Type I collagen was demonstrated to promote concentration-dependent binding of cultured human dermal fibroblasts to anorganic bovine bone material (ABM) to which the P-15 sequence had been adsorbed (ABM.P-15). ABM.P-15 also enhanced expression of alkaline phosphatase, as well as increased nucleic acid and protein synthesis in dermal fibroblasts grown on ABM.P-15 versus cells grown on ABM alone.[30-32]

Since the attachment of PDLF to BRG materials is of importance in periodontal therapy involving placement of BRG materials, we investigated the interactions of cultured human PDLF with a variety of BRG materials including demineralized (DFBA) and non-demineralized (NDFDBA) freeze-dried bone allograft, ABM with and without adsorbed P-15, and several alloplastic materials. This was done to determine: 1) if the synthetic peptide enhanced cell attachment and proliferation for PDLF as it had for dermal fibroblasts and 2) how the time course of PDLF spreading, as assessed by scanning electron microscopy (SEM), compared among the graft materials.

MATERIALS AND METHODS

Periodontal Ligament Fibroblasts

Stock cultures of human polyclonal PDLF (a gift from Dr. D. Robinson, University of California, San Francisco) were maintained in 25 cm^3 tissue culture flasks‡ at 37.5°C in a humidified incubator gassed with 5.0% CO_2 for 15 to 24 passages. Two polyclonal PDLF populations were tested and found to behave identically in all subsequent tests. PDLF cultures were grown in minimum essential medium-alpha (MEM-α)§ complete with minerals and vitamins, and supplemented with 10% fetal calf serum and antibiotics. Cells were harvested using trypsin-EDTA.

Bone Replacement Graft Materials

A variety of BRG materials were examined in this study (Table 1). BRG materials were rinsed extensively in PBS prior to use for adhesion or cell proliferation experiments.

Table 1.

Bone Replacement Graft Materials

Number	Name	Source	Particle Size (μm diameter)
1	Non-demineralized freeze-dried bone (NDFDBA)		200-500
2	Demineralized freeze-dried bone (DFDBA)		200-500
3	Osteograf N-300 (ABM)	Bovine bone	250-420
4	PepGen P-15 (ABM.P-15)	Bovine bone	250-420
5	Osteograf LD-300		250-420
6	Bio-OSS	Bovine bone	200-600
7	Bio-Coral	Coral	300-450
8	HTR-24		>500
9	HTR-40		>500
10	Perioglas 58s		200-500
11	Calcitite		200-400
12	Perioss		200-600
13	Osteograf-AR		>500
14	Osteogen		300-400

Manufacturer information is provided in the footnotes on pages 991-994.

The putative collagen binding peptide, P-15 (GTPGPQGIAGQRGVV) was available commercially bound to ABM.‖

Scanning Electron Microscopy (SEM)

For culture on bone replacement graft materials (BRG materials), PDLF were plated onto BRG particles that had been stabilized in gelled 3.0% agarose¶ in the bottoms of wells of 24-well culture dishes.‡ BRG material-containing wells were constructed by adding 0.7 ml of liquid 3.0% agarose to each well and allowing the agarose to solidify. Subsequently, 0.1 ml of the various BRG materials were added to each well and were pressed into the agarose gel so that portions of the particles protruded above the agarose surface and were available for cell binding. PDLF were plated at a density of 6.5×10^3 cells/ml (1 ml/well) and cultured from 5 minutes to 2 days. For baseline observations cells were grown on polystyrene. All cultures were fixed in 4.0% glutaraldehyde in 0.1M sodium cacodylate buffer for 1 hour (room temperature), post-fixed in

‡ Corning, Corning, NY.
§ Gibco, Grand Island, NY.
‖ Osteograf CS-300 or Pepgen P-15, CeraMed Dental LLC, Lakewood, CO.
¶ Invitrogen, Carlsbad, CA.

1.0% osmium tetroxide in the same buffer for 1 hour (room temperature), and dehydrated in an ascending series of alcohols (50 to 100%).[33] The agarose disk containing the BRG particles was then removed and critical point dried in a Tsoumis and critically-point drying apparatus. Samples were then sputter-coated with gold-palladium in a sputter-coating device[#] and examined in a scanning electron microscope[**] at an accelerating voltage of 10 kV. Cells grown on polystyrene were treated similarly except that the bottom of the dish was cut out with an electric "hot wire." Images were recorded on film.[††]

Quantitation of Cell Attachment

Cell attachment was measured to BRG materials fluorometrically. Cells were grown in MEMα containing 10% fetal bovine serum (FBS). BRG materials were added to Eppendorff tubes to a volume of between 25 to 40 µl. BRG particle size varied from 200 to greater than 500 µm in diameter (Table 1). BRG materials were rinsed 3 times with 1 ml of PBS prior to the addition of cells. 2×10^4 PDLF were added to BRG materials proteins in a volume of 500 µl of MEMα in the presence or absence of 10% FBS. Cells were allowed to adhere to the substratum for 15 minutes to 48 hours, as noted for each experiment. In all cases, 8 samples were prepared for each BRG material for each experiment. Non-adherent cells were removed by 3 washes of 500 µl of PBS, and the adherent cells lysed by freezing at –80°C for 1 hour. Adherent cells were quantified fluorometrically, using fluorescent dye[‡‡] and measured in a fluorescent microplate reader.[§§] This dye fluoresces at 530 nm when bound to DNA, and was prepared per the manufacturer's instructions. This dye fluoresces only when in the presence of nucleic acids and did not fluoresce after exposure to BRG materials in the absence of cells (data not shown). Since the nucleic acid content of cells is fairly constant, this measurement was used to represent the relative number of cells attached to each BRG material examined. For each sample, 300 µl of the dye solution was added to each sample, vortexed vigorously, and incubated for 1 hour at 25°C in the dark; 200 µl of the resulting supernatant was transferred to a 24-well microplate. The data presented represent the mean and standard deviation of 3 experiments, each containing 8 samples. Student *t*-test was used to determine the significance of the differences in adhesion seen between samples.

Cell Proliferation Assay

To determine the effect of various BRG materials on PDLF proliferation, 5,000 PDLF cells were incubated with 25 µl of BRG materials in 500 µl of MEMα with 10% FBS for 1, 2, 3, or 4 days at 37°C. This represents 10% of the cells used in the quantitative cell adhesion experiment to ensure that available BRG particle surface would not be limiting. After incubation, the excess media were removed, and the relative cellular content of each sample determined using the cell attachment protocol described above, with the exception that no effort was made to remove non-adherent cells. The data presented represent the mean and standard deviation of 8 samples.

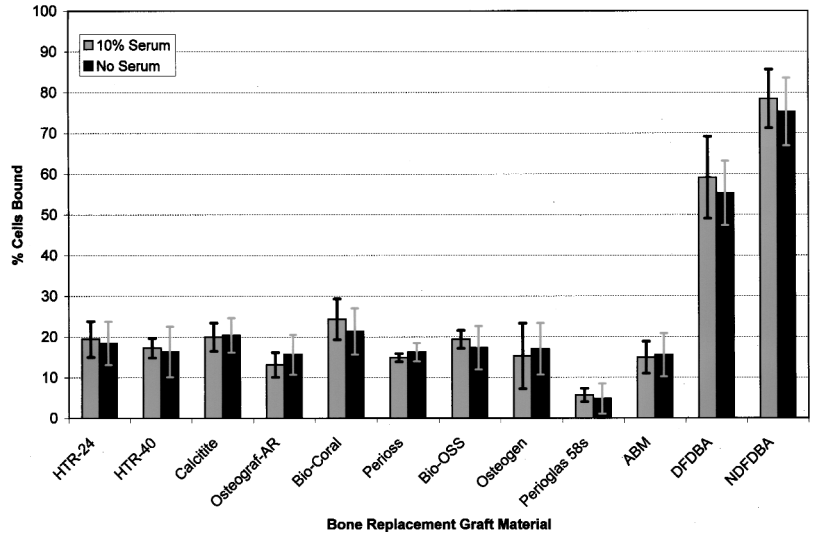

Figure 1.
Quantitative analysis of PDL fibroblast attachment to numerous BRG materials. PDL fibroblast attachment to a variety of commercially available BRG materials was quantified in the presence or absence of 10% fetal bovine serum.

RESULTS

Quantitation of Periodontal Ligament Fibroblast Attachment to BRG materials

A variety of BRG materials are currently used to replace bone and promote fibroblast invasion in the treatment of periodontal disease. In order to quantify their potential clinical usefulness, we compared a variety of commercially-available BRG materials for the ability to promote PDLF attachment (Fig. 1). In general, the use of NDFDBA and DFDBA supported the greatest amount of PDLF attachment (about 70% and 60%, respectively). This was significantly greater than any other of the plain BRG materials examined within this study ($P < 0.01$). In contrast, the alternative plain BRG materials (HTR-24,[||||] HTR-40,[||||] Bio-Coral,[¶¶] Cal-

Anatech, San Diego, CA.
** Model T-300, JEOL, Peabody, MA.
†† 4127 Graphic Arts film, Kodak, Rochester, NY.
‡‡ Cyquant, Molecular Probes, Eugene, OR.
§§ FL500, Biotek, Winooski, VT.
|||| Bioplant Inc., Norwalk, CT.
¶¶ INOTEB, Saint-Gonnery, France.

citite,[##] Bio-OSS,[***] Perioss,[†††] Osteogen,[‡‡‡] Osteograf N-300,[§§§] Osteograf-AR,[§§§] and Perioglas-58s)[‖‖‖] supported much less attachment (<25%). Cell attachments among the alternative plain BRG materials tested were not significantly different (P <0.05). The presence or absence of serum (10%) did not significantly affect cell adhesion to any of the BRG materials examined (P <0.05).

Periodontal Ligament Fibroblast Attachment and Morphology on Bone Replacement Graft Materials

All of the BRG materials utilized in this study were capable of supporting attachment of PDLF. The most dramatic morphological differences were observed after short culture intervals. PDLF attached to all of the BRG materials and polystyrene within 5 minutes in culture (Fig. 2A, D, G, and J), and were still rounded and had numerous surface irregularities. Cells cultured on polystyrene, NDFDBA, and ABM.P-15 (Fig. 2A, D, and G) had already begun to send out lamellaepodia and filopodia, while those cultured on ABM without P-15 did not possess lamellaepodia, and filopodia were either short or absent (Fig. 2J). Cells plated on polystyrene showed the most rapid cell spreading, while those grown on ABM without P-15 were slowest. The spreading response was characterized by: 1) attachment of rounded cells to the substratum; 2) extension of filopodia and abbreviated lamellaepodia; 3) expansion of lamellaepodia; and 4) continued flattening of the cell. After 30 minutes in culture (Fig. 2) PDLF cultured on ABM without P-15 were less well spread than those on polystyrene, NDFDBA, or ABM.P-15. Cells cultured on polystyrene, NDFDBA, and ABM.P-15 had undergone extensive spreading and were almost completely flattened except for a prominent nuclear bulge (Fig. 2B, E, H). Cells cultured for 30 minutes on ABM without P-15 were still rounded, had smaller lamellaepodia, and more irregular surfaces than those cultured on the other materials (Fig. 2K). Two hours after plating, PDLF cultured on ABM without P-15 were still not as spread as PDLF cultured on polystyrene, NDFDBA, or ABM.P-15 (Fig. 2L versus Figs. 2C, F, and I). After 1 day in culture, PDLF had completely flattened out on all of the substrata (not illustrated).

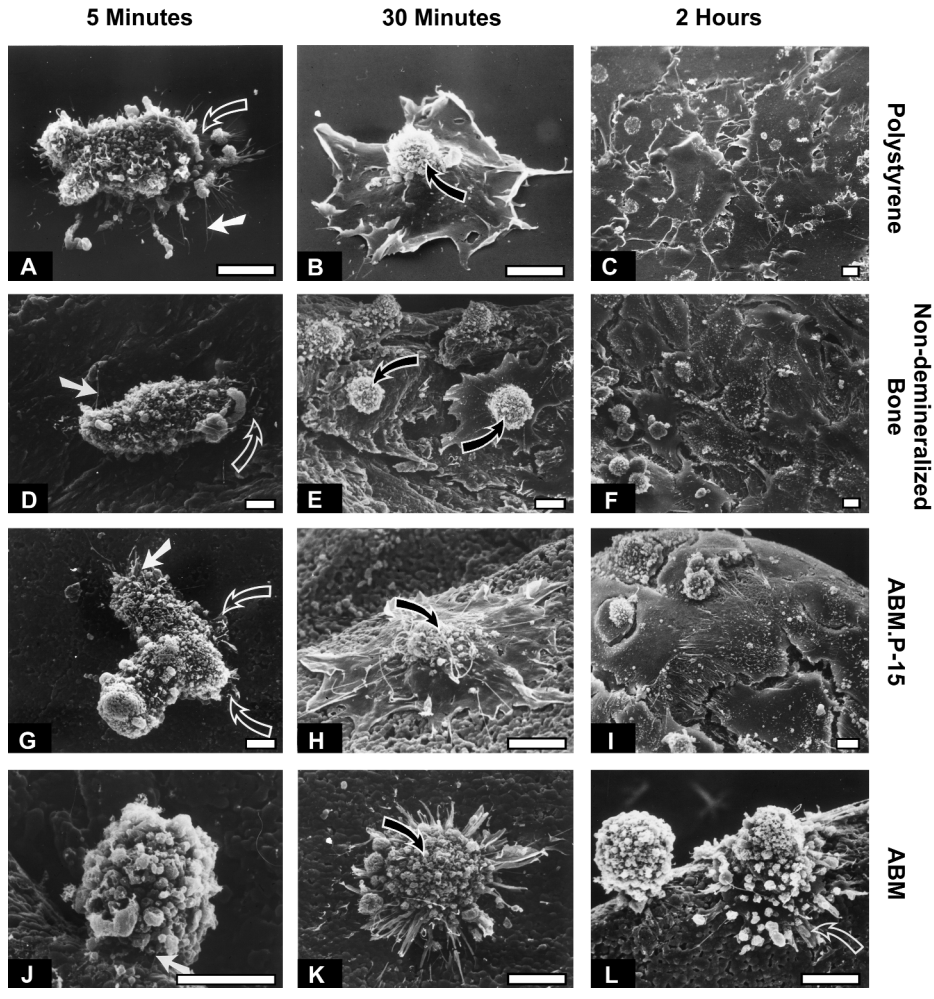

Figure 2.
Effect of P-15 peptide on the morphology of PDL fibroblast to BRG materials. PDL fibroblasts grown on polystyrene ***(A-C),*** *NDFDBA* ***(D-F),*** *ABM.P-15* ***(G-I),*** *and ABM* ***(J-L)*** *for 5 minutes* ***(A, D, G,*** *and* ***J),*** *30 minutes* ***(B, E, H,*** *and* ***K),*** *and 2 hours* ***(C, F, I,*** *and* ***L).*** *Note the lamellaepodia (open arrows) and filopodia (solid arrows) associated with the basal membranes of PDL fibroblasts grown on polystyrene, NDFDBA, ABM.P-15, and ABM (A, D, G, and J). Cells are rounded regardless of substrate after 5 minutes (A, D, G, and J). After 30 minutes, PDL fibroblasts grown on polystyrene, NDFDBA, and ABM.P-15 (B, E, and H) are substantially more spread than cells grown on ABM (K). Note the prominent nuclear bulges (dark arrows) on the cultured cells (B, E, H, and K). After 2 hours, PDL fibroblasts are completely spread out on polystyrene, NDFDBA, and ABM.P-15 (C, F, and I) whereas lamellaepodia (open arrow) are still being put forth from PDL fibroblasts grown on ABM (L).*

Quantitation of Periodontal Ligament Fibroblast Attachment to P-15 on BRG materials

In order to substantiate the functional relevance of the apparent differences in cellular morphology in indi-

Sulzer Calcitek, Carlsbad, CA.
*** Osteohealth, Inc., Shirley, NY.
††† Miter Inc., Indianapolis, IN.
‡‡‡ Impladent, Jackson Heights, NY.
§§§ CeraMed Dental LLC, Lakewood, CO.
‖‖‖ Dbek Drug Co., Inc., Jersey City, NJ.

vidual cells seen with SEM, we quantitatively measured the attachment of PDLF to a battery of these materials (Fig. 3). In order to promote cell attachment to xenogeneic natural hydroxyapatite BRG materials (such as Osteograf N-300), the putative collagen binding peptide P-15 has been adhered to the surface of hydroxyapatite ABM (PepGen P-15). After 2 hours of incubation, 60% to 70% of PDLF adhere to demineralized and non-demineralized bone (Fig, 3). In contrast, less than 15% of PDLF attach to ABM-without P-15 (Osteograf N-300). The addition of P-15 to ABM (PepGen P-15) did not significantly increase PDLF adhesion to these particles. Of potential clinical interest, the low-density hydroxyapatite BRG material¶¶¶ (Osteograf LD-300) promoted significantly more adhesion of PDLF (about 30% of cells (P <0.01)). After 24 hours of incubation, adhesion of PDLF to all the various ABM was increased slightly (about 5%). Under these conditions, PDLF adhesion to demineralized and non-demineralized bone increased to a greater extent (10% and 30%, respectively). No significant changes in adhesion were observed in cells allowed to adhere for 48 hours (P >0.05). The only noticeable difference observed with PDLF attachment to ABM.P15 versus ABM was in the initial phase of cell attachment at 15 minutes (P <0.01). These studies suggest that the P-15 peptide does not enhance stable PDLF attachment to BRG materials, but may promote more rapid, dynamic adhesion associated with cell migration and tissue repair. They also demonstrate that fragmented bone (whether demineralized or non-demineralized), supports PDLF attachment to a greater extent than xenogeneic or alloplastic hydroxyapatite based BRG.

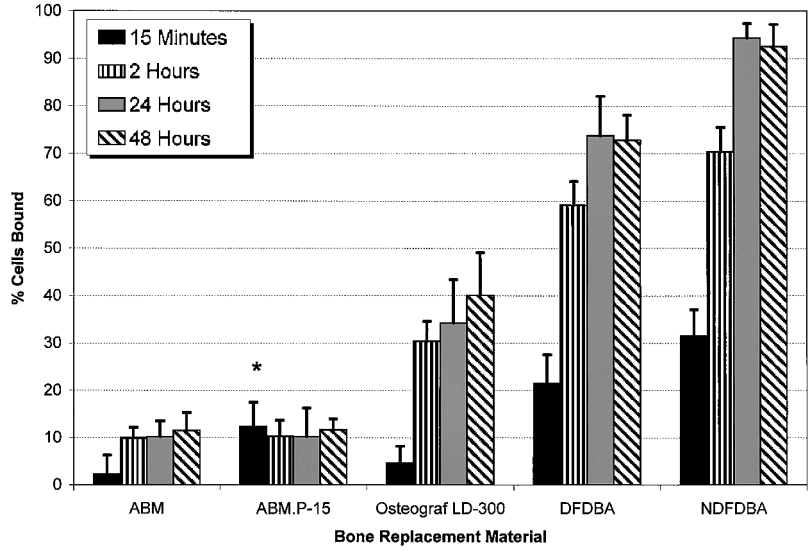

Figure 3.
*Effect of P-15 peptide on PDL fibroblast attachment to BRG materials. PDL fibroblast attachment to commercially available BRG materials (Osteograf N-300 [ABM], PepGen P-15 [ABM.P-15], Osteograf LD-300, demineralized bone allograft [DFDBA]and non-demineralized bone allograft [NDFDBA]) was quantified. PepGen P-15 (ABM.P-15) contains the putative collagen binding peptide, P-15. No significant difference was observed in the attachment of either PDL fibroblast cell lines to any of the BRG materials tested. *A significant difference in adhesion between non-P-15 and P-15 ABMs. Error bars represent the standard deviation of the mean.*

Effects of BRG Materials on Periodontal Ligament Fibroblast Proliferation

Another potential result of cell interaction with collagen is the stimulation of cell division. Thus, the putative collagen cell-binding peptide (P-15) could potentially stimulate PDLF to proliferate after invading a recently filled defect. We therefore quantitatively compared PDLF proliferation on cells grown on several BRG with polystyrene (Fig. 4). On polystyrene, PDLF divided rapidly, doubling first with 24 hours, and then again within another 24 hours. Thereafter, cell proliferation slowed, but remained substantial through the fourth day in culture. In contrast, PDLF grown on non-demineralized bone proliferated, but failed to double their number by the fourth day in culture. Cell proliferation was even slower on both ABM (Osteograf N-300) and ABM.P-15 (PepGen P-15). A direct comparison of these 2 growth conditions demonstrated no significant difference in the response of PDLF to ABM or ABM.P-15. Finally, no cell proliferation was detected in PDLF grown on low-density hydroxyapatite (Osteograf LD-300). Therefore, P-15 did not significantly affect PDLF proliferation. In addition, all of the BRG materials promoted only minimal cell proliferation when compared to tissue culture plastic, thus indicating that BRG materials may potentially inhibit cell proliferation. Since non-demineralized bone supported nearly 95% PDLF attachment at 24 hours (Fig. 3), but failed to support significant cell proliferation (Fig. 4), we conclude that the inhibition observed in proliferation in not due simply to a failure in cell anchorage.

DISCUSSION

Our results indicate that xenogeneic and synthetic BRG materials are inferior to human-derived bone (whether demineralized or non-demineralized) in promoting PDLF cell attachment. We also demonstrate that cultured human PDLF spread out more rapidly on ABM coupled to a synthetic attachment peptide (P-15) than on ABM without the adsorbed peptide, and that the time course of cell spreading on ABM.P-15 is similar to that observed in PDLF cultured on either polystyrene or NDFDBA. This enhanced initial cell interaction indicates that such agents may be clinically useful, since

¶¶¶ Osteograf LD-300, CeraMed Dental LLC.

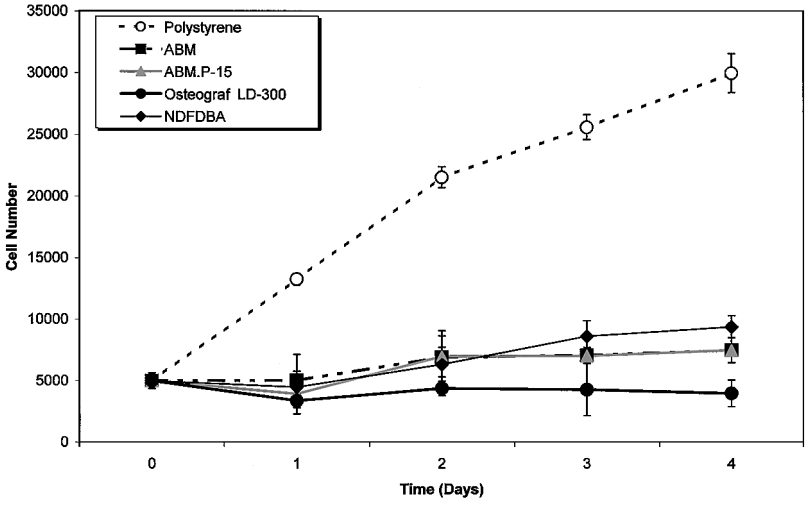

Figure 4.
Effect of P-15 peptide on PDL fibroblast proliferation on BRG materials. PDL fibroblast proliferation on commercially available BRG materials was quantified. No significant difference was observed in the proliferation of either PDL fibroblast cell lines to any of the BRG materials tested. Error bars represent the standard deviation of the mean.

rapid functional interaction of cells capable of promoting periodontal regeneration with implant materials is important in initiating repair. ABM.P-15 did quantitatively promote more rapid attachment of PDLF than ABM alone. However, subsequent PDLF attachment to ABM.P-15 was quantitatively identical to that seen on ABM alone. This may reflect an increase in the rapid and dynamic cell attachments on ABM.P-15 associated with motile cells that occur during tissue repair, compared to a more stable cell attachment characteristic of stationary cells on ABM alone. It is also possible that any clinical benefit observed with ABM.P-15 could result from another function of the cell-collagen interaction, for instance the promotion of either cell proliferation or differentiation. In testing the potential cellular functions, we found that ABM.P15 promoted PDLF proliferation no better that ABM alone.

In our experiments, only the initial attachment of cultured human PDLF to ABM was enhanced by the P-15 peptide. Subsequent PDLF attachment was unaffected by the presence of the P-15 peptide. Previously, the P-15 peptide was demonstrated to enhance attachment of dermal fibroblasts in a sustained manner, with observable differences at 4 days in culture.[30] The differences in these observations may be due to different sources of fibroblasts (dermal versus PDL), the number of cells plated onto a larger surface area in the above study, or different sampling times (24 hours versus 1 week). The accelerated binding of PDLF over dermal fibroblasts may reflect greater efficiency on the part of PDLF to bind to bone minerals such as hydroxyapatite. Interestingly, the cellular morphology of PDLF spreading on polystyrene and cells spreading on NDFDBA, ABM, and ABM.P-15 is very similar. The initial binding and spreading of PDLF to polystyrene is probably mediated by serum vitronectin and possibly fibronectin.[34-39] Nevertheless, the slower spreading of PDLF grown on ABM compared to those grown on ABM.P-15, polystyrene, and NDFDB indicates that the nature of the substratum is also important in determining the nature and dynamics of the interactions between PDLF and BRG materials.

Our results also differ quantitatively from those previously reported for ABM.P-15.[30] Specifically, dermal fibroblasts displayed a small (15%) but significant increase in attachment to ABM.P-15. In our hands, PDLF attached no better to ABM.P-15 than to ABM. In addition, dermal fibroblasts were previously reported to increase their DNA synthesis and protein synthesis when grown on ABM.P-15. This was reported to indicate an increase in cell adhesion, but may also reflect an increase in cell proliferation. In contrast, PDLF displayed no increase in cell proliferation when exposed to ABM.P-15. In fact, all of the BRG tested appear to inhibit cell proliferation, when compared to tissue culture plastic. Together, these data may indicate a stimulation of PDLF differentiation by BRG, resulting in a coincident decrease in cell proliferation. ABM.P-15 has been demonstrated to induce alkaline phosphatase activity in dermal fibroblasts.[30] In addition, very favorable clinical results with ABM.P-15 have been demonstrated in periodontal osseous defects.[40] Further studies will be required to investigate the relationship between clinical efficacy and PDLF differentiation and proliferation.

PDLF proliferation and differentiation and the colonization of postsurgical sites by PDLF are of critical importance in determining clinical success of procedures using BRG materials in periodontal therapy.[41,42] Early stages of osseous repair depend on interaction of PDLF and HA[43] and conversion of PDLF to osteoblasts to effect bone formation. Cultured PDLF can differentiate into either osteoblasts and express osteoblastic characteristics such as alkaline phosphatase or retain gingival fibroblast phenotypes.[11,13,44] Our data indicate that the clinical benefits observed while using ABM.P-15 in osseous repair are not due to an increase in cell adhesion or proliferation. Nevertheless, ABM.P-15 may play a beneficial role in promoting fibroblast invasion of the graft site and their subsequent differentiation. Future studies should allow an assessment of which, if any, of the BRG materials promote expression of proteins such as alkaline phosphatase and osteonectin in cultured PDLF.

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REFERENCES

1. Cochran DL, Wozney JM. Biological mediators for periodontal regeneration. *Periodontol 2000* 1999;19:40-58.
2. Hempton TJ, Wilkins E, Lancaster D. Periodontal regeneration. A review of periodontal attachment and guided tissue regeneration. *RDH* 1998;18:30-32, 34, 36; quiz 37.
3. Gaunt WA, Osborn JW, TenCate AR. The periodontal ligament. In: *Advanced Dental Histology.* Baltimore: Williams and Wilkins Co; 1971.
4. Beertsen W, McCulloch CA, Sodek J. The periodontal ligament: a unique, multifunctional connective tissue. *Periodontol* 2000 1997;13:20-40.
5. Tenenbaum HC. Cellular origins and theories of differentiation of bone-forming cells. In: Hall BK, ed. *Bone: The Osteoblast and Osteocyte,* vol 1. Caldwell, NJ: Telford Press; 1990:41-69.
6. Caton J, Greenstein G, Zappa U. Guided tissue regeneration using Vicryl periodontal mesh. *Compendium* 1992;13:202, 204, 206-208.
7. Martin M, Gantes B, Garrett S, Egelberg S. Treatment of periodontal furcation defects. (I). Review of the literature and description of a regenerative surgical technique. *J Clin Periodontol* 1988;15:227-231.
8. Groeneveld MC, Everts V, Beertsen W. Alkaline phosphatase activity in the periodontal ligament and gingiva of the rat molar: its relation to cementum formation. *J Dent Res* 1995;74:1374-1381.
9. Yamaguchi M, Shimizu N, Shibata Y, Abiko Y. Effects of different magnitudes of tension-force on alkaline phosphatase activity in periodontal ligament cells. *J Dent Res* 1996;75:889-894.
10. Giannopoulou C, Cimasoni G. Functional characteristics of gingival and periodontal ligament fibroblasts. *J Dent Res* 1996;75:895-902.
11. Ohshima M, Kuwata F, Otsuka K, et al. Alkaline phosphatase activities of cultured human periodontal ligament cells. *J Nihon Univ Sch Dent* 1988;30:208-217.
12. Phipps RP, Borrello MA, Blieden TM. Fibroblast heterogeneity in the periodontium and other tissues. *J Periodont Res* 1997;32:159-165.
13. Lekic P, McCulloch CA. Periodontal ligament cell population: the central role of fibroblasts in creating a unique tissue. *Anat Rec* 1996;245:327-341.
14. Schroeder HE, Munzel-Pedrazzoli S, Page R. Correlated morphometric and biochemical analysis of gingival tissue in early chronic gingivitis in man. *Arch Oral Biol* 1973;18:899-923.
15. Urist MR, Sato K, Brownell AG, et al. Human bone morphogenetic protein (hBMP). *Proc Soc Exp Biol Med* 1983; 173:194-199.
16. Reddi AH. Cell biology and biochemistry of endochondral bone development. *Coll Relat Res* 1981;1:209-226.
17. Ripamonti U, Reddi AH. Tissue engineering, morphogenesis, and regeneration of the periodontal tissues by bone morphogenetic proteins. *Crit Rev Oral Biol Med* 1997;8:154-163.
18. Bernick S, Paule W, Ertl D, Nishimoto SK, Nimni ME. Cellular events associated with the induction of bone by demineralized bone. *J Orthop Res* 1989;7:1-11.
19. Nishimura F, Terranova VP. Comparative study of the chemotactic responses of periodontal ligament cells and gingival fibroblasts to polypeptide growth factors. *J Dent Res* 1996;75:986-992.
20. Narayanan AS, Page RC. Connective tissues of the periodontium: a summary of current work. *Coll Relat Res* 1983;3:33-64.
21. Mellonig JT, Seamon BC, Gray JL, Towle HJ. Clinical evaluation of guided tissue regeneration in the treatment of grade II molar furcation invasions. *Int J Periodontics Restorative Dent* 1994;14:254-271.
22. Rabie AB, Deng YM, Samman N, Hagg H. The effect of demineralized bone matrix on the healing of intramembranous bone grafts in rabbit skull defects. *J Dent Res* 1996;75:1045-1051.
23. Hosokawa R, Kubo T, Wadamoto M, Sato Y, Kimoto T. Direct bone induction in the subperiosteal space of rat calvaria with demineralized bone allografts. *J Oral Implantol* 1999;25:30-34.
24. Edwards JT, Diegmann MH, Scarborough NL. Osteoinduction of human demineralized bone: characterization in a rat model. *Clin Orthop* 1998;357:219-228.
25. Carnes DL Jr, De La Fontaine J, Cochran DL, et al. Evaluation of 2 novel approaches for assessing the ability of demineralized freeze-dried bone allograft to induce new bone formation. *J Periodontol* 1999;70:353-363.
26. Damien CJ, Parsons JR. Bone graft and bone graft substitutes: a review of current technology and applications. *J Appl Biomater* 1991;2:187-208.
27. Bauer TW, Muschler GF. Bone graft materials. An overview of the basic science. *Clin Orthop* 2000;371:10-27.
28. Lacefield WR. Hydroxyapatite coatings. *Ann NY Acad Sci* 1988;523:72-80.
29. Terranova VP, Wikesjö UM. Extracellular matrices and polypeptide growth factors as mediators of functions of cells of the periodontium. A review. *J Periodontol* 1987; 58:371-380.
30. Qian JJ, Bhatnagar RS. Enhanced cell attachment to anorganic bone mineral in the presence of a synthetic peptide related to collagen. *J Biomed Mater Res* 1996;31: 545-554.
31. Bhatnagar RS, Qian JJ, Wedrychowska A, Sadeghi M, Wu YM, Smith N. Design of biomimetic habitats for tissue engineering with P-15, a synthetic peptide analogue of collagen. *Tissue Eng* 1999;5:53-65.
32. Bhatnagar RS, Qian JJ, Gough CA. The role in cell binding of a beta-bend within the triple helical region in collagen alpha 1 (I) chain: structural and biological evidence for conformational tautomerism on fiber surface. *J Biomol Struct Dyn* 1997;14:547-560.
33. Moses RL, Claycomb WC. Differentiated membrane specializations and myofibrillar breakdown and recovery in cultured adult cardiac myocytes treated with TPA and diacylglycerol. *J Cell Sci* 1989;93:95-105.
34. Ruoslahti E. Fibronectin and its integrin receptors in cancer. *Adv Cancer Res* 1999;76:1-20.

35. Schvartz I, Seger D, Shaltiel S. Vitronectin. *Int J Biochem Cell Biol* 1999;31:539-544.
36. Hayman EG, Pierschbacher MD, Ruoslahti E. Detachment of cells from culture substrate by soluble fibronectin peptides. *J Cell Biol* 1985;100:1948-1954.
37. Hayman EG, Pierschbacher MD, Suzuki S, Ruoslahti E. Vitronectin–a major cell attachment-promoting protein in fetal bovine serum. *Exp Cell Res* 1985;160:245-258.
38. Felding-Habermann B, Cheresh DA. Vitronectin and its receptors. *Curr Opin Cell Biol* 1993;5:864-868.
39. Miyamoto S, Katz BZ, Lafrenie RM, Yamada M. Fibronectin and integrins in cell adhesion, signaling, and morphogenesis. *Ann NY Acad Sci* 1998;857:119-129.
40. Yukna RA, Callan DP, Krauser JT, 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.
41. Schroeder HE. Biological problems of regenerative cementogenesis: synthesis and attachment of collagenous matrices on growing and established root surfaces. *Int Rev Cytol* 1992;142:1-59.
42. Herr Y, Matsuura M, Lin ML, Genco RJ, Cho MI. The origin of fibroblasts and their role in the early stages of horizontal furcation defect healing in the beagle dog. *J Periodontol* 1995;66:716-730 (erratum: 1995;66:915-923).
43. Alliot-Licht B, De Lange GJ, Gregoire M. Effects of hydroxyapatite particles on periodontal ligament fibroblast-like cell behavior. *J Periodontol* 1997;68:158-165.
44. Piche JE, Carnes DL Jr, Graves DT. Initial characterization of cells derived from human periodontia. *J Dent Res* 1989;68:761-767.

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