# The Effect of Platelet-Derived Growth Factor on the Cellular Response of the Periodontium: An Autoradiographic Study on Dogs

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PLATELET-DERIVED GROWTH FACTOR (PDGF) is a polypeptide growth factor considered to have a role in the proliferation and migration of fibroblasts at a wound healing site. The aim of this investigation was to determine if PDGF, when applied to root surfaces, would stimulate the proliferation of fibroblasts and further enhance regeneration. Six mongrel dogs with healthy periodontia were selected for this study. Using a closed wound surgical model, standardized 4 × 4 mm fenestration defects were created into dentin on the mid-facial of the mesial and distal roots of 4 mandibular posterior teeth in each quadrant. Each defect received either: 1) saline solution (C); 2) expanded polytetrafluoroethylene (ePTFE) membrane; 3) PDGF; or 4) ePTFE + PDGF. $^{3}$H-thymidine was administered 1 hour prior to animal sacrifice at 1, 3, and 7 days postsurgery. Each time period included 2 dogs with each dog undergoing the four different treatments. Slides were prepared for autoradiography. $^{3}$H-thymidine-labeled cells were counted and results were statistically analyzed using the Bonferroni (Dunn) *t* test on the SAS program. Results indicated PDGF enhanced fibroblast proliferation when compared to the groups without PDGF. Significant differences ($P < 0.05$) were noted at day 1 and 7 when PDGF and PDGF + GT were compared to C and GT groups. No significant differences were observed in labeled fibroblasts between the C and GT groups at any time period. These findings suggest that PDGF enhances fibroblast proliferation in early periodontal wound healing, whether used alone or in combination with the ePTFE membrane. *J Periodontol 1994;65:429–436.*

**Key Words:** Fibroblasts; membranes, barrier; growth factors, platelet-derived; polytetrafluoroethylene/therapeutic use.

The treatment of periodontal disease by traditional methods results in wound healing by the formation of a long junctional epithelium.[1-3] The ultimate goal of periodontal therapy is to regenerate the periodontal supporting tissues lost to the inflammatory disease process. There has been a major effort to achieve this goal by using either resorbable and nonresorbable physical barriers to impede epithelial migration or to treat diseased root surfaces with various agents (root conditioning solutions or growth factors).[4-6] These mechanical barriers work by creating a space beneath the surgical flap which would allow the periodontal ligament and/or bone cells to proliferate, while at the same time blocking cells from the gingival tissues and epithelium from gaining access to the exposed root. Recent investigations focusing on regeneration of the periodontium have attempted to define factors involved in the formation of a new connective tissue attachment to periodontally-diseased or denuded root surfaces.[7-8] After cell adhesion to substrate, one of the biological events involved in tissue regeneration is specific cell-directed migration, or chemotaxis, which is an essential feature of many biological processes in both health and disease.[7]

Several growth factors have been shown to possess chemoattractant activity including platelet-derived growth factor (PDGF), nerve growth factor, epidermal growth factor, and transforming growth factors-α and β.[9-10] PDGF, a

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principal source of mitogens present in whole blood serum, was discovered in 1974 when it was shown to be released from platelets and was responsible for the growth of many cells in culture that are serum-dependent.[11-12] PDGF is a highly cationic, dimeric protein (pI = 9.8), with molecular masses ranging from 28,000 to 35,000 Da.[13-14] There are two different PDGF polypeptides: PDGF-A and PDGF-B that are 56% homologous and encoded by different genes.[15-17] The PDGF-A and PDGF-B genes are located on chromosomes 7 and 22, respectively, and are independently regulated.[17] PDGF isolated from human platelets consists of homodimers (AA or BB) or heterodimers (AB) of the two PDGF gene products, with the heterodimer predominating.[18] Recent evidence indicates that the PDGF-AB heterodimer and the PDGF-BB homodimer have equivalent activity and are equally potent in stimulating DNA synthesis in human fibroblasts.[19-20]

Studies reporting the use of growth factors in vivo for periodontal regeneration have recently begun to enter the scientific literature. Lynch and coworkers have published several articles on the use of a combination of PDGF and IGF-1 (insulin-like growth factor-1) in beagle dogs.[4,6] A pilot study was performed in 1989[4] which examined the application of an aqueous gel of PDGF and IGF-1 on the periodontitis-affected root surfaces of 3 dogs following open flap debridement. Sections of the experimental sites and controls were examined histologically 2 weeks after treatment. The control specimens revealed a long junctional epithelial attachment with no new bone formation. In contrast, the growth factor-treated sites exhibited significant amounts of new bone and cementum formation. A nearly continuous layer of osteoblasts lined the newly formed bone, and there was a dense cellular "front" at the coronal extent of the new bone.

Lynch's group later reported the short-term effects of application of a combination of PDGF and IGF-1 on periodontal wound healing.[5] This study compared the growth factor-containing gel to controls in 13 beagle dogs. The results showed a statistically significant 5- to 10-fold increase in new bone and cementum in PDGF-B/IGF-1-treated sites at 2 and 5 weeks following surgery compared to controls. The authors cautioned, however, that additional studies were necessary to evaluate the proper combinations, the optimum dose, and number of doses to be used. The exact mechanism(s) by which PDGF/IGF-1 acts to enhance periodontal regeneration remains to be fully elucidated. Recent studies by Matsuda et al.[21] and Oates et al.[22] indicated that PDGFs are potent mitogenic agents for human PDL cells in vitro. The American Academy of Periodontology's position paper[23] on growth factors summarizes that these polypeptide growth factors possess greater ability to control cell growth and differentiation than any other class of molecules currently used in dentistry. Their clinical applicability to periodontics awaits their large-scale production, purification, and formulation into delivery systems which control and maximize their actions. If these obstacles can be overcome, growth factors hold great promise for providing the periodontist with a mechanism for stimulating true periodontal regeneration. Therefore, the purpose of this investigation was to study the effect of platelet-derived growth factor on the pattern of proliferation and migration of fibroblasts with and without barrier membranes.

## MATERIALS AND METHODS

### Animal Selection

Six adult mongrel dogs (mean weight 13 kg) with clinically healthy periodontia and complete dentitions were selected for this study. The research protocol was approved by The University of Michigan Use and Care of Animal Committee. Two weeks prior to surgery each dog was sedated with 2.5% thiamylal sodium 15 to 20 mg/kg of body weight. Initial prophylaxis consisted of hand scaling, root planing, and tooth polishing. Subsequently, oral hygiene was maintained by daily brushing with 0.12% chlorhexidine digluconate solution 5 times per week. Photographs and x-rays were taken to document the oral health and condition of the dental supporting tissues before and after surgery. Four birooted teeth in both quadrants of the mandibular arch were used; they included the second (P2), third (P3), and fourth premolars (P4), and first molars (M1). The buccal surface of each root was used as a treatment site. This provided 16 treatment sites per dog. Two dogs were used for each time period (1, 3, and 7 days). The sites were randomly assigned to one of four treatment modalities: 1) sterile water (C); 2) expanded polytetrafluoroethylene (ePTFE) membrane alone; 3) PDGF (PDGF); or 4) PDGF plus ePTFE (ePTFE + PDGF). Eight roots (n = 8) were evaluated for each different treatment at different time periods. The PDGF-BB homodimer used in this study was obtained from human platelets and procured from a commercial firm¶ (10 μg/vial). The PDGF-BB homodimer solution was prepared at the time of surgery by adding 1 ml of sterile saline to obtain a solution with a concentration of 10 μg/ml. The ePTFE membrane# was selected as the nonresorbable barrier material.

### Surgical Procedure

Each dog was anesthetized before surgery with pentobarbital sodium** (30 mg/kg of body weight) and an endotracheal tube was placed in order to maintain a patent airway. To control excessive gingival bleeding, 1.8 ml of 2% lidocaine hydrochloride†† with epinephrine 1:50,000 was injected locally in each quadrant. A full-thickness mucoperiosteal flap was reflected on the buccal aspect of each experimental tooth using a reverse bevel, scalloped incision. Vertical releasing incisions were placed mesial to each cuspid and distal to each first molar, extending from the

¶Collaborative Research Inc., Bedford, MA.
#Gore-Tex, W.L. Gore & Assoc., Inc., Flagstaff, AZ.
**Nembutal, Abbott Laboratories, Abbott Park, IL.
†† Xylocaine, Astra Pharmaceutical Products Inc., Westboro, ME.

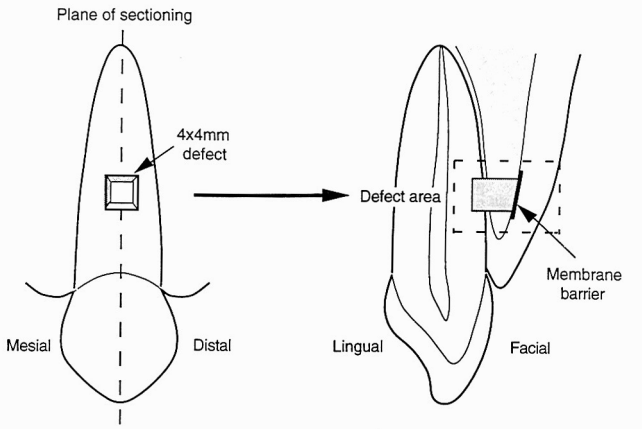


*Figure 1. A. (left) Facial perspective of surgical site. B. (right) Profile view of surgical defect.*

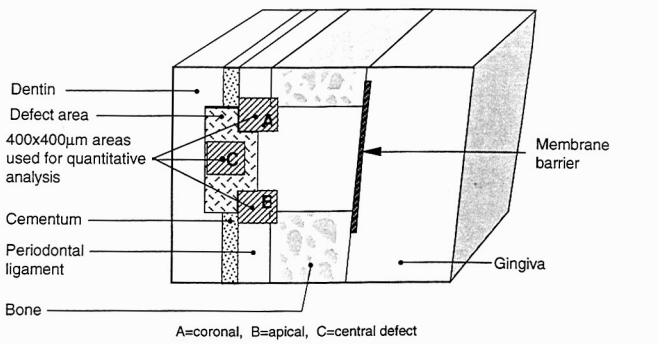


*Figure 1C. Enlargement of surgical defect area.*

free gingival margin to the mucogingival junction. Following the reflection of the gingival flap, 4 × 4 mm square areas were outlined on the surface of bone using a lead pencil to indicate the location of the fenestrations. With a slow speed end cutting bur and continuous saline drip, fenestrations were cut through the bone to expose the middle half of the roots. The cementum was completely removed and the dentin exposed (Figs. 1A and B). The defects were rinsed and blotted dry with sterile cotton pellets. After these procedures were completed, the defects were treated with one of the following modalities: C, ePTFE, PDGF, or ePTFE + PDGF. In teeth treated with PDGF, the solution (10 μg/ml) was applied to the root surface with a cotton pellet for 1 minute. For teeth which received ePTFE, the membranes were trimmed, adapted, and extended 2 to 3 mm beyond the borders of the fenestrations to ensure complete coverage of the defect regions. The flaps were repositioned and the implant alignment was verified by lifting the flap and reexamining. Firm pressure was applied over the flaps for 3 minutes prior to suturing to ensure good flap adaptation.

### Laboratory Procedures

Under general anesthesia with pentobarbital sodium, the dogs were sacrificed at 1, 3, and 7 days following surgery by exsanguination via the femoral arteries. One hour prior to sacrifice, the animals were injected with tritiated thymidine (0.5 μCi/gm body weight) through the cutaneous veins of the legs. The use of tritiated thymidine allowed for the labeling of the cells in the "S" phase of mitosis in order to permit the autoradiographic analysis of the specimens.[24] Following sacrifice, the head and neck of each dog was perfused with a 10% buffered formalin solution through the common carotid arteries and refrigerated for 24 hours. The mandibles were then dissected free and processed for histologic specimens.

### Histologic Techniques

The blocks were rinsed in water and placed in 5% formic acid for decalcification. The formic acid solution was changed once every 3 days to allow decalcification to progress at a fast rate. When the blocks were completely decalcified, they were cut into halves, the cutting plane passing through the axis of the experimental root in a buccolingual plane of orientation. The sections (5 μm) were prepared for histological examination. Three sections representing the breadth of each defect were processed for autoradiographic evaluation. For autoradiography, 5-μm sections were coated with film,‡‡ exposed for 14 days, developed, and stained lightly with Harris' hematoxylin and eosin.

### Identification of Cell Types

Criteria used for identification of cell types were based on location in relationship to blood vessels, cementum, and bone, and on their cellular outlines. The following were features used to define each cell type. Fibroblasts: stellate cells located within the extracellular matrix of the PDL and not associated with mineralized tissues or vascular structures. Cementoblasts: round, flattened or short columnar cells intimately associated with the mineralized or premineralized matrix within the iatrogenically induced defects. Osteoblasts: round, flattened, or short columnar cells intimately associated with osteoid of the alveolar bone proper. Perivascular cells: cells in close proximity, but not in intimate contact with, vascular structures within the defect or PDL. Endothelial cells: cells intimately associated with the basement membrane of vascular, externally, or intima, internally.

### Quantification of the Cellular Response

The source of the cellular response refers to the area(s) of the periodontium from which the proliferating cells needed for the healing of the surgical defect originated. Distinct quadrangular areas of approximately 400 × 400 μm were selected for determining the source and counting of active labeled cells (Fig. 1C). Two areas were identified in that part of the periodontal ligament and alveolar bone that were aligned either with the apical or coronal border of the dental defect. A third area was located in the central part of the

‡‡Kodak NTB-2, Eastman Kodak Company, Rochester, NY.

defect adjacent to the dentinal defect. These areas were used primarily for determining the source and quantification of active cells in the specimens. Cells having undergone active proliferation were marked in the "S" phase of the mitosis by tritiated thymidine. A cell was considered to be in active proliferation and therefore marked if it contained a minimum of three reduced silver halide grains. The calculation of the number of cells in each area was obtained by utilizing a 400 × 400 μm square grid × 400. Labeled cells were identified and placed into one of the following categories: Fibroblasts, osteoblasts, cementoblasts, perivascular, endothelial, or inflammatory cells.

### Statistical Analysis

For analysis of data, analysis of variance (ANOVA) was utilized. For testing differences between the four treatments, a Bonferroni *t*-test was performed in each time point. A Bonferroni *t*-test was also employed to test the differences between means for all time effects in each treatment area. These statistical computations were performed on the SAS program.[§§]

## RESULTS

### Total Labeled Non-Inflammatory Cells (Mean ± Standard Error)

There was an increase in mean labeled cells when the four treatments and all cell types (excluding inflammatory cells) were totaled (Table 1) at day 7 (57.9 ± 6.44 cells) compared to days 1 (21.1 ± 1.8) or 3 (15.5 ± 2.0). When all time periods and noninflammatory cell types were totaled both PDGF (46.8 ± 7.9) and ePTFE + PDGF (44.8 ± 4.6) showed increased cellular proliferation when compared with either the C (15.3 ± 1.8) or ePTFE (18.9 ± 2.3) treatments (Table 2).

### Total Labeled Cells (Including Inflammatory Cells)

The inclusion of the inflammatory cells obscured the trends previously noted for the early time periods (days 1 and 3) due to the predominance of inflammatory cells in most slide sections (Table 2). This was particularly evident for the C groups (mean at day 1 = 115.31, day 3 = 48.3, day 7 = 15.4). As the mean number of inflammatory cells decreased from day 1 to day 7 (Table 3), the trend of increased labeled cells for the PDGF containing groups became more evident. At day 7 both PDGF and ePTFE + PDGF treatments had significantly ($P < 0.05$) more active cells than either the C or ePTFE groups.

Table 4 portrays the pattern of cellular labeling when the 5 types of noninflammatory cells (fibroblasts, cementoblasts, osteoblasts, perivascular, and endothelial cells) are viewed by time, treatment, and area. At days 1 and 3 there was a trend for more labeling for both the PDGF and the ePTFE + PDGF groups although this was statistically significant ($P < 0.05$) only for ePTFE + PDGF compared to the C and ePTFE groups. By day 7 both PDGF and ePTFE + PDGF treatments demonstrated significantly ($P < 0.05$)

[§§]SAS 6.03, SAS Institute, Cary, NC.

**Table 1. Total Labeled Cells (excluding inflammatory cells) in All Areas for All Times and Treatments (mean ± standard error)**

| Day | Control | ePTFE | PDGF | ePTFE + PDGF |
|---|---|---|---|---|
| 1 | 16.83 ± 2.85 | 14.17 ± 2.67 | 27.30 ± 5.58 | 31.84 ± 3.93*† |
| 3 | 14.73 ± 3.67 | 12.60 ± 3.25 | 14.17 ± 3.65 | 21.44 ± 5.61 |
| 7 | 13.64 ± 2.74 | 37.60 ± 6.42 | 87.7 ± 17.46‡§ | 73.96 ± 9.54* |

**Table 2. Total Labeled Cells (including inflammatory cells) in All Areas for All Times and Treatments**

| Day | Control | ePTFE | PDGF | ePTFE + PDGF |
|---|---|---|---|---|
| 1 | 115.3 ± 22.09 | 67.78 ± 14.22 | 61.37 ± 14.84 | 98.07 ± 12.37 |
| 3 | 48.29 ± 12.58 | 82.36 ± 21.20‡ | 30.67 ± 8.66 | 58.69 ± 15.10 |
| 7 | 15.44 ± 3.24 | 48.80 ± 10.46 | 107.1 ± 20.8‡§ | 104.9 ± 13.42*§ |

**Table 3. Total Labeled Inflammatory Cells in All Areas for All Times and Treatments**

| Day | Control | ePTFE | PDGF | ePTFE + PDGF |
|---|---|---|---|---|
| 1 | 98.56 ± 20.14 | 53.62 ± 12.57 | 34.07 ± 9.96 | 66.22 ± 10.80 |
| 3 | 33.56 ± 10.40 | 69.76 ± 18.77 | 16.50 ± 5.38† | 37.26 ± 10.02† |
| 7 | 1.80 ± 0.74 | 11.20 ± 4.59 | 19.33 ± 4.12 | 30.98 ± 4.68*† |

Significant at $P < 0.05$.
*Between C and GT + PDGF.
†Between GT and GT + PDGF.
‡Between GT and PDGF.
§Between C and PDGF.

**Table 4. Labeled Cells by Time and Area for All Treatment Groups (Mean ± Standard Error)**

| | Day 1 | Day 3 | Day 7 |
|---|---|---|---|
| Control | | | |
| Apical | 21.82 ± 4.66 | 12.73 ± 2.95 | 5.46 ± 1.07† |
| Middle | 68.19 ± 13.46 | 22.69 ± 6.38 | 5.54 ± 1.53† |
| Coronal | 25.30 ± 6.01 | 12.87 ± 4.12 | 4.44 ± 1.20† |
| ePTFE Treatment | | | |
| Apical | 13.55 ± 2.76 | 20.89 ± 6.03 | 11.30 ± 1.86 |
| Middle | 45.10 ± 11.59 | 37.96 ± 9.57 | 23.50 ± 6.84 |
| Coronal | 9.13 ± 1.91 | 23.51 ± 6.24‡ | 14.00 ± 2.44 |
| PDGF | | | |
| Apical | 13.37 ± 3.03 | 7.88 ± 2.00 | 21.12 ± 3.06‡ |
| Middle | 31.63 ± 10.11 | 13.98 ± 4.70 | 70.73 ± 16.09‡ |
| Coronal | 16.37 ± 3.44 | 8.82 ± 2.48 | 15.26 ± 2.66 |
| ePTFE + PDGF Treatment | | | |
| Apical | 19.29 ± 3.02 | 15.08 ± 3.81 | 18.67 ± 1.94 |
| Middle | 53.18 ± 9.15 | 29.62 ± 8.06 | 70.14 ± 10.74‡ |
| Coronal | 25.60 ± 2.88 | 14.00 ± 4.10 | 16.14 ± 1.73† |

Significant at $P < 0.05$.
*Between day 1 and day 3.
†Between day 1 and day 7.
‡Between day 3 and day 7.

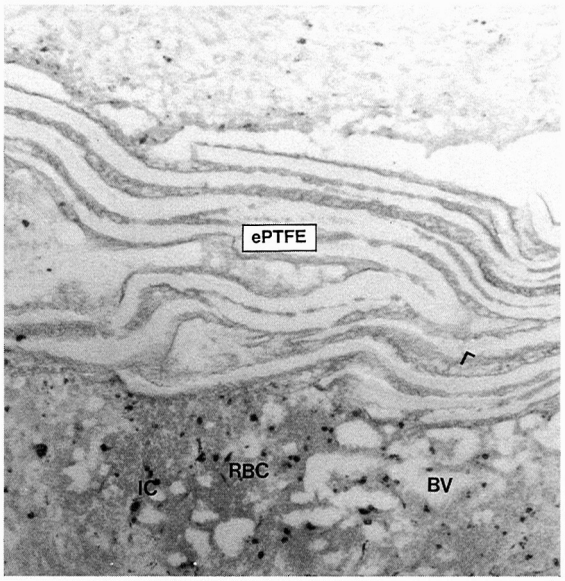


*Figure 2. Magnified view of the ePTFE-treated site at day 7. Expanded polytetrafluoroethylene membrane separating tissues from inside to outside of the defect. The newly-formed granulation tissues were evident on the inside of the defect. ePTFE = expanded polytetrafluoroethylene membrane; RBC = red blood cells; BV = blood vessels; IC = inflammatory cells (original magnification × 100; H & E stain).*

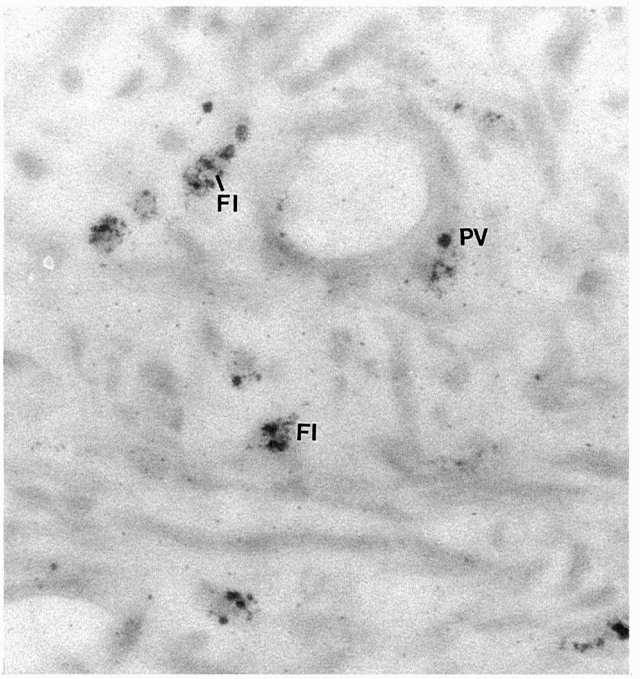


*Figure 3. Higher magnification view of the ePTFE-treated site at day 7. Labeled fibroblasts (FI) and perivascular cells (PV) were demonstrated (original magnification × 400; H & E stain).*

increased labeling as compared with C while the PDGF group had significantly ($P < 0.05$) more cellular labeling than ePTFE.

**Total Labeled Cells by Time, Area, and Treatment (Table 4)**

***Treatment C.*** Labeled cells significantly decreased from day 1 to day 7 for all three observed areas ($P < 0.05$). Labeled cells were found predominantly in the middle region.

***Treatment ePTFE (Figs. 2 and 3).*** Labeled cells were highest in mean numbers in the middle region for all times although not significantly. The apical area showed slightly more activity at day 1 and less at days 3 and 7 in comparison to the coronal area.

***Treatment PDGF (Fig. 4).*** Cellular labeling was again seen to be more pronounced for the middle area but in contrast to the previous two treatments the most activity was noted at day 7 compared to day 1 and day 3 ($P < 0.05$). Figure 4 demonstrate sections from the PDGF treatment group which show herniation and then proliferation of the PDL fibroblasts into the defect with cellular labeling.

***Treatment ePTFE + PDGF (Fig. 5).*** This treatment group had a similar pattern of cell activity compared to the PDGF group. There was more labeling in the middle portion of the defect and moderate labeling apically and coronally. Statistical differences existed between the coronal areas at days 1 to 3 and days 1 to 7 and also between the middle regions at days 3 and 7. Figure 5 shows a typical example of the defect area with the membrane in place. It appears as though the PDL fibroblasts are bridging across the defect with moderate amounts of cellular labeling present.

**Labeled Cells for All Times and Treatments by Cell Type (Table 5)**

***Fibroblasts.*** The general trend was of moderate cellular activity at day 1, decreasing at day 3, and a dramatic increase at day 7. At day 1 however, significant differences ($P < 0.05$) existed between the PDGF and ePTFE groups and between the ePTFE + PDGF and both ePTFE and C groups. No statistically significant differences were noted at day 3. By day 7, labeling in the PDGF group was much greater ($P < 0.05$) than either C or ePTFE. The ePTFE + PDGF treatment also was significantly ($P < 0.05$) more effective at enhancing cellular activity at day 7 than the C treatment.

***Cementoblasts.*** Labeling was least at day 3 and greatest at day 7 for all but the C group. Significant differences ($P < 0.05$) existed only between PDGF and C at day 7.

***Osteoblasts.*** Labeling was greatest for the PDGF group at day 7 (similar to the cementoblasts) which was significantly different ($P < 0.05$) compared to the C group.

***Perivascular cells.*** Day 7 exhibited the highest degree of cell activity for all but the C group. Both PDGF treatment groups were significantly greater than the C group at day 7.

***Endothelial cells.*** The trend of highest activity for the PDGF and ePTFE + PDGF groups at day 7 held for this

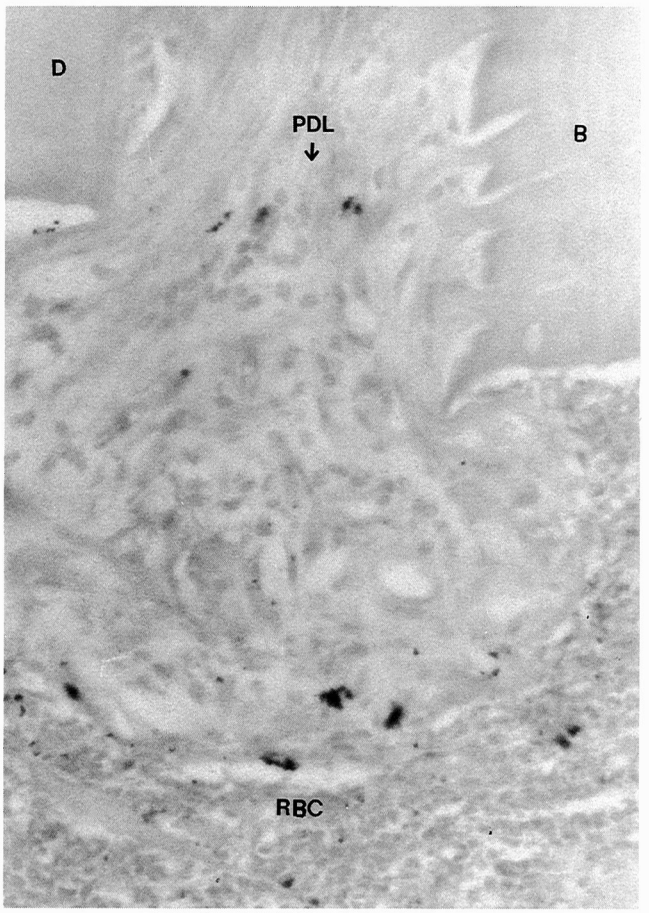


*Figure 4. Magnified view of the PDGF-treated site at day 1. Fibroblast proliferation with evidence of newly-formed granulation tissues (original magnification × 250; H & E stain).*

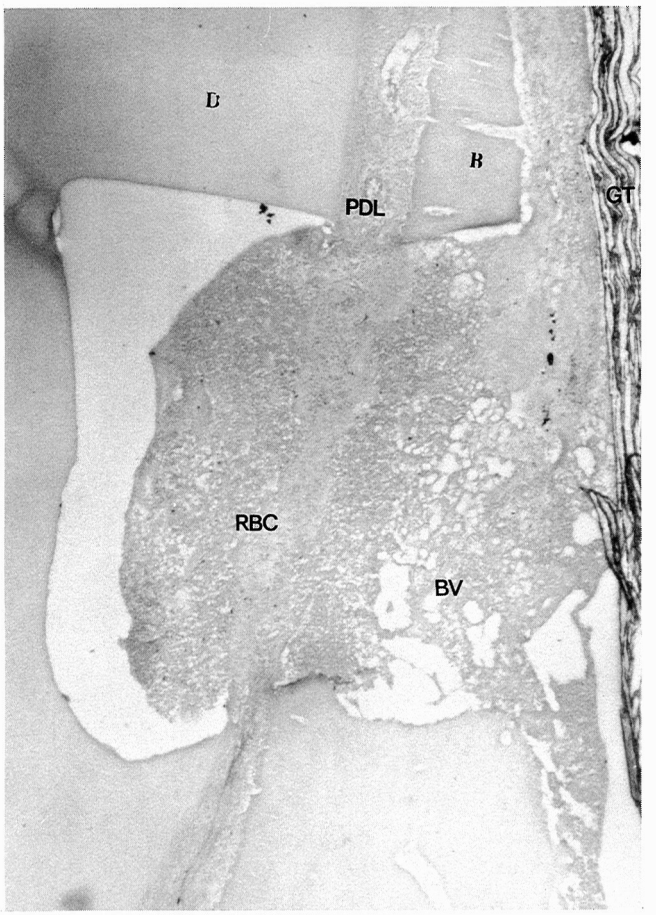


*Figure 5. Lower magnified view of the PDGF + ePTFE-treated site at day 1. Defect filled with RBCs and inflammatory cells (IC) which do not contact the tooth surface. There is apparent "bridging" of PDL cells (--) from the coronal to apical regions. D = dentin; B = bone, PDL = periodontal ligament; BV = blood vessels; ePTFE = expanded polytetrafluoroethylene membranes (original magnification ×40; H & E stain).*

cell type. Statistical differences existed only between the ePTFE + PDGF and C groups at day 7, however.

## DISCUSSION

Periodontal wound repair is the consequence of an organized series of cellular and molecular events in the structurally and functionally impaired tissue. The repair of injury begins as soon as tissue damage occurs, and the release of polypeptide growth factors from injured cells and inflammatory cells is a critical part of this process.[25] This study was initiated to provide an understanding of the effect of PDGF, with and without barrier membranes, on cellular proliferation during early wound healing in the dog model.

The data obtained in this research demonstrated that the short-term exposure of periodontal tissues to PDGF can stimulate the proliferation of fibroblasts when compared to control or ePTFE membrane specimens. Similar findings were reported by Matusda et al., who showed that PDGFs have potent and comparable mitogenic effects on PDL fibroblastic cells.[21] Our results were also in agreement with Oates et al., who reported that PDGF-AA and PDGF-BB are potent mitogenic agents for human PDL cells and may play critical roles in modulating oral fibroblast cell proliferation.[22] The addition of an ePTFE membrane did not enhance the results obtained by the use of PDGF alone. No significant differences were observed in labeled fibroblasts between the C and ePTFE groups at any time period. This is in agreement with the findings of Aukhil and Iglhaut[26] and Iglhaut et al.,[27] which indicated that there was no difference in the healing process using membrane barriers. They found that the premitotic labeling with or without barriers was the same.

In this study, gingival and PDL fibroblasts were exposed to PDGF in the PDGF group whereas only the PDL fibroblasts were exposed to the growth factor in the ePTFE + PDGF group. It has been suggested that gingival fibroblasts may not have the regenerative potential of periodontal ligament fibroblasts,[1,3] which play a most critical role in periodontium regeneration.[21] No significant differences were noted between labeled fibroblasts for either the PDGF or the ePTFE + PDGF groups. It is, however, difficult to

**Table 5. Total Count of Specific Labeled Cells for All Times and Treatments (Mean ± Standard Error)**

| | C | ePTFE | PDGF | ePTFE + PDGF |
|---|---|---|---|---|
| Fibroblasts | | | | |
| Day 1 | 10.3 ± 1.73 | 8.9 ± 1.63 | 18.9 ± 3.81* | 22.9 ± 2.91†§ |
| Day 3 | 7.2 ± 1.83 | 8.2 ± 2.18 | 10.1 ± 2.62 | 13.5 ± 3.59 |
| Day 7 | 9.3 ± 1.90 | 25.6 ± 5.61 | 70.8 ± 14.58*‡ | 55.1 ± 7.12† |
| Cementoblasts | | | | |
| Day 1 | 1.41 ± 0.36 | 0.6 ± 0.18 | 1.26 ± 0.4 | 1.47 ± 0.29 |
| Day 3 | 0.91 ± 0.33 | 0.67 ± 0.2 | 0.46 ± 0.16 | 0.85 ± 0.35 |
| Day 7 | 0.38 ± 0.17 | 1.97 ± 0.71 | 2.51 ± 0.52‡ | 1.94 ± 0.31 |
| Osteoblasts | | | | |
| Day 1 | 2.41 ± 0.6 | 1.13 ± 0.24 | 1.89 ± 0.54 | 1.80 ± 0.32 |
| Day 3 | 1.82 ± 0.59 | 1.24 ± 0.33 | 1.29 ± 0.37 | 1.64 ± 0.45 |
| Day 7 | 0.54 ± 0.19 | 1.93 ± 0.36 | 3.59 ± 0.74‡ | 2.22 ± 0.40 |
| Perivascular Cells | | | | |
| Day 1 | 1.52 ± 0.48 | 2.63 ± 0.69 | 3.89 ± 0.87 | 4.22 ± 0.80† |
| Day 3 | 2.93 ± 0.70 | 1.98 ± 0.53 | 1.98 ± 0.54 | 3.59 ± 0.93 |
| Day 7 | 2.69 ± 0.63 | 6.63 ± 0.93 | 7.24 ± 1.33 | 10.06 ± 1.17† |
| Endothelial Cells | | | | |
| Day 1 | 1.17 ± 0.29 | 0.90 ± 0.22 | 1.33 ± 0.38 | 1.44 ± 0.34 |
| Day 3 | 1.87 ± 0.65 | 0.47 ± 0.18 | 0.35 ± 0.18 | 1.82 ± 0.58 |
| Day 7 | 0.72 ± 0.21 | 1.47 ± 0.29 | 3.69 ± 0.98 | 4.65 ± 1.17† |

Significant at $P < 0.05$
*Between ePTFE and PDGF
†Between C and ePTFE + PDGF
‡Between C and PDGF
§Between ePTFE and ePTFE + PDGF

differentiate the origin of the fibroblasts in the PDGF group since these cells may have come from either the gingival or PDL compartments. Perhaps either type of fibroblast is capable of providing progenitor cells for repopulating the wound area.

Fibroblast proliferation increased greatly from day 1 to day 7 in both groups treated with PDGF. According to general wound healing principles, the number of fibroblasts begins to increase in the wound at 2 to 4 days and, following a "lag phase" in which there are no new proteins produced in the wound, the fibroblasts begin to synthesize collagenous and noncollagenous connective tissue proteins actively at about 5 days.[28,29] This is in agreement with the trend seen in this study where there was a decrease in cell activity at day 3 and increase at day 7. The 7-day PDGF specimens exhibited areas of cell rich connective tissue attachment without inflammatory cells as well as areas showing the fibrin clot in various stages of resorption. Lynch et al. determined that the half-life of PDGF was 4.2 hours with less than 4% of the growth factor remaining present by 96 hours.[6] They felt that, although the growth factor was absent in the later time periods (beyond 2 weeks), it stimulated a cascade of wound healing events that continued on even in its absence. In our research the greatest increase in fibroblasts labeling occurred at day 7 when most of the PDGF had become extinct.

The proliferation of inflammatory cells decreased from day 1 to day 7. This is in agreement with typical wound healing where the early hours following wounding consists of clot formation and a nonreparative inflammatory phase.[28] The experimental groups (ePTFE, PDGF and ePTFE + PDGF) exhibited fewer inflammatory cells than the control at day 1 with a sharp decrease in inflammatory cells for all groups by day 7. As the inflammatory cells decreased they were replaced by noninflammatory cells. Perhaps the PDGF was actually toxic at the early stages of healing thus inhibiting the early inflammatory response and clot formation. While some investigators have found PDGF to have a limiting acting time at wound sites,[30] the optimal in vivo dose is still unknown[5] and excessive doses may be initially toxic. PDGF is a cytokine which works in concert with other inflammatory type cytokines, such as interleukin-1β. Inflammatory cells have been shown to express growth factors and may be essential for true regeneration to occur.[31] A sustained inflammatory response may sponsor sustained cell recruitment via the production of one or more growth factors.

Both labeled cementoblasts and osteoblasts were found in relatively low numbers throughout all three time periods, with significant increases found only between the PDGF and C groups at day 7. This is somewhat in agreement with the studies, which have shown that PDGF enhances proliferation and migration of osteoblastic cells.[32-33] Wound healing following full-thickness mucoperiosteal flaps typically results in osteoclastic activity between the fourth and tenth days of healing[34] and is thus in agreement with these observations. The control group, however, demonstrated decreased cementoblastic labeling from day 1 to day 7, which is the opposite of what would be expected. Lynch and coworkers found significantly more bone and cementum (5 to 10 times more) in sites treated with PDGF and IGF-I over controls both at 2 and 5 weeks.[6] Osteoblasts are generally not active until 7 to 14 days into the healing process while cementogenesis does not begin for 10 to 18 days.[29] Therefore, it is not unexpected to see little activity for these mineralized tissue forming cells in this 7-day experiment.

Labeled perivascular and endothelial cells showed a general decrease from day 1 to day 3 and an increase at day 7 in ePTFE, PDGF, and ePTFE + PDGF groups. Typically, revascularization of wounds begins at about 2 days as angioblasts proliferate from cut surfaces of regional capillaries and form nonpatent buds. With time, the buds fuse with each other to form patent capillary loops, which themselves show evidence of additional budding.[28]

Future studies should include a larger sample size and a longer observation period to ensure that the entire healing period is evaluated. The optimal dosage of PDGF to enhance the early wound healing is yet to be determined. Within the limits of this study, the following conclusions were drawn: 1) the application of PDGF in periodontal closed wounds enhanced the proliferation of fibroblasts; 2) the implantation of ePTFE membranes had no effect on the total fibroblast proliferation at the wound sites, but the exclusion of the gingival connective tissue cells may have resulted in stronger cellular activity at the periodontal ligament; 3) the prevalence of inflammatory cells was greater

in the groups treated without PDGF than the groups treated with PDGF; and 4) discrete cellular activity was observed among cementoblasts and osteoblasts in all experimental groups at 7 days. Perivascular and endothelial cells were slightly more active in specimens treated with PDGF.

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