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ORIGINAL ARTICLE

A time-dependent effect of PDGF-BB on adhesion and growth of cultured fibroblasts to root surfaces

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OBJECTIVE: Platelet-derived growth factor (PDGF-BB) is suggested to be a potent stimulator and a strong mitogenic agent for human periodontal ligament cells (PDL). This study aimed at assessing the effectiveness of PDGF-BB application on periodontally diseased root surfaces through attachment and growth of fibroblast cells.

MATERIALS AND METHODS: Fifteen periodontally involved and five healthy teeth were selected, prepared from proximal surfaces and distributed into four groups (10 specimens per group): I: healthy; II: untreated diseased; III: scaling and root planning (SRP); and IV: SRP and PDGF-BB. Each group had three subdivisions (three specimens per group) which were incubated at three different time periods. The remaining specimen for each group was used to examine surface topography. Fibroblasts were pooled on root specimens and incubated. Results were evaluated by using scanning electron microscopy. Repeated cell counting was done within a representative standard area.

RESULTS: The best results regarding PDL cell shape and density were obtained at day 3 in all experimental groups, except the diseased group. Although SRP samples showed slightly higher results in numbers of attached fibroblasts than diseased samples, they demonstrated a similar negative effect denoting incompatible root surfaces for fibroblast attachment. SRP plus PDGF-BB and healthy samples showed a comparable positive effect, suggesting a good root surface biocompatibility. Intergroup differences showed no significant differences on day 1, but statistically significant differences were found on both day 3 and day 7 incubation periods favoring groups I and IV over groups II and III.

CONCLUSIONS: Platelet-derived growth factor showed a positive effect on adhesion and growth of cultured fibroblasts to periodontally diseased surfaces. Thus, PDGF-BB may have a promising role in clinical periodontics.
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Keywords: cell culture; human periodontal ligament fibroblast; PDGF-BB; scaling/root planning

Introduction

The removal of bacterial deposits, arrest of periodontal disease and regeneration of periodontal tissues that are lost due to the disease process constitute the ideal and main goal in periodontal therapy (O'Leary, 1986; American Academy of Periodontology, 2001). The biocompatibility of the root surfaces is of importance for achieving periodontal healing (Fernyhough and Page, 1983). Scaling and root planning (SRP) and platelet-derived growth factor (PDGF-BB) are among the different methods that are used in periodontal therapy to improve root surface biocompatibility, in a trial to favorably influence rate of healing. However, many reports showed that the conventional SRP alone cannot totally eliminate etiologic contaminants (Adriaens *et al*, 1988), and the formation of a smear layer after mechanical SRP may inhibit reattachment of cells to the root surface and thus may be detrimental to periodontal tissue healing (Blomlof *et al*, 1997).

Several studies have documented the role of growth factors in periodontal regeneration. PDGF-BB is a potent stimulator and acts as a strong mitogenic agent for human periodontal ligament cells (PDL) (Marcopoulou *et al*, 2003; Papadopoulos *et al*, 2003). PDGF-BB significantly stimulates human PDL fibroblasts to attach and adhere to periodontally involved root surfaces when applied in concentrations ≥50 ng ml^{-1}. Thus, PDGF-BB may regulate and modify cell activity (Gamal and Mailhot, 2000).

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Information about the mechanism of attachment of human PDL fibroblast cells and how to evaluate their shape and density across periodontally involved root surfaces is scarce. The aim of the present study was to evaluate the effectiveness of PDGF-BB application on periodontally diseased root surfaces by estimating both attachment and growth of human PDL fibroblast cells cultured *in vitro* at three different time periods.

Materials and methods

Sample collection

Fifteen freshly extracted human periodontally involved teeth from 11 patients (seven men and four women, aged 35–65 years) were obtained from the periodontal clinic. The teeth were extracted as part of the dental treatment, and selected using the following criteria: proximal attachment loss ≥7 mm; no history of scaling or root planing in the previous 6 months; no signs of caries, fractures or anatomic abnormalities on the root surfaces. Furthermore, patients were non-smokers and free from any significant systemic diseases that can affect the periodontium.

Five impacted teeth obtained from five patients (three women and two men, aged 19–26 years) from the Oral Surgery clinic served as a positive healthy control group.

In addition, six freshly extracted human healthy teeth from four patients (three women and one man, aged 18–28 years) were obtained from the Department of Oral Surgery, extracted either due to impaction (four teeth) or orthodontic reasons (two teeth) and used for PDL fibroblast cell culturing procedure. Informed consent was obtained from all patients prior to extraction, and approval for the experiment was obtained from the university ethical committee.

Cell culture procedure

Immediately following extraction, healthy teeth were placed in a sterile Hanks balanced salt solution. The teeth were then rinsed twice with a culture medium [Eagle's minimum essential medium (EMEM) supplemented with 10% heat inactivated fetal bovine serum (FBS), penicillin G potassium (100 U ml^{-1}), streptomycin sulfate (100 mg ml^{-1}) and 1.16% L-glutamine] (EMEM, 10% FBS, 1% P/S, and 1.16% LG).

Under sterile conditions, only PDL tissues that were attached to the middle third of the root were gently scrapped with a sterile sharp scalpel, peeled off and removed. PDL tissue explants were incubated in 35 mm^2 tissue culture plates with the same culture medium at 37°C in a humidified atmosphere of 95% air and 5% CO_2. The medium was replaced the following day and then subsequently every 3 days with the same culture medium (EMEM, 10% FBS, 1% P/S, and 1.16% LG).

When fibroblast-like cells grew from tissue biopsies, they were incubated and fed every 3 days until they became confluent, then transferred to 10 cm^2 tissue culture plates by the use of 0.08% trypsin/0.04% ethylenediaminetetraacetic acid, pH 7.2 and were designated at that time as first passage cells (P1). Subsequent passages are followed once cells become confluent again. This method of cell culture was based on the study of Somerman *et al* (1988) with some modifications. At P3, the cells were stored in a cell banker storage solution at −80°C, until further use. Usually, cells were used between the third and the ninth passage.

Specimen preparation and sectioning procedure

Before extraction, a pencil line was drawn at the level of the gingival margin of periodontally involved teeth for future identification of the portion of the tooth that was exposed to the area of the pocket below the gingival margin (Gamal *et al*, 1998). Extraction was performed without traumatizing or disturbing regions of interest on the root surfaces of both periodontally involved and healthy teeth. Immediately after extraction, all teeth were gently cleaned by saline irrigation.

Thorough SRP was carried out in 10 periodontally involved teeth on both mesial and distal root surfaces before separating specimens from the root surfaces. The SRP procedure was handled by the same experienced operator until the root surfaces were adequately debrided and planned. In accordance with previous studies (Gamal and Mailhot, 2000; Davenport *et al*, 2003), 15–20 strokes made with a sharp Gracey periodontal curette (7/8; HuFriedy, Chicago, IL, USA) were used to simulate periodontal treatment of the root surface.

Test areas were prepared by extending outlines from the pencil mark at the line angles toward connective tissue attachment at the base of the pocket. Under continuous water coolant, roots of all periodontally involved teeth were sectioned horizontally at the most apical part of the outlined extension, and vertically along sides of the outlined extension toward the crown. The roots were then sectioned longitudinally through the root canals to a point coronal to the test area, and another horizontal cut was made at this level to separate the test area from the tooth, thus forming the root surface specimens.

Test areas were prepared and cut from experimental mesial and distal root surfaces ($n = 15$ periodontally involved and 5 healthy teeth = 20 teeth × 2 = 40 specimens) with a size of 4 mm^2 and a thickness of 2 mm. The specimens were then sterilized by autoclaving (Feist *et al*, 2003), and stored in sterile saline at 0–5°C until starting the actual experiment.

Experimental groups

Thirty human periodontally-involved and 10 healthy root surface specimens were prepared and randomly distributed into four experimental groups (10 specimens per group) as follows:

Group I: Positive control group: healthy root surfaces, without receiving any treatment.
Group II: Negative control group: untreated periodontally involved root surfaces, i.e., without receiving any treatment.
Group III: root surfaces received thorough SRP using a sharp Gracey periodontal curette.

Group IV: root surfaces treated with SRP followed by PDGF-BB application with a concentration of 50 ng ml^{-1}.

Group subdivisions
Each group was further subdivided into three subdivisions (three specimens per group) to be incubated at three different time periods (days 1, 3, and 7), whereas the remaining specimen per each group was reserved for examining root surface topography by scanning electron microscopy (SEM).

Preparation and application of PDGF-BB
Recombinant human PDGF-BB was purchased commercially (Recombinant Human PDGF-BB Homodimer; Austral Biologicals, San Ramon, CA, USA), reconstituted by dissolving in distilled water at neutral pH and preserved at −20°C until use according to instructions of the manufacturer. The stock solution of PDGF-BB was diluted to the concentration of 50 ng ml^{-1}, immediately before cell culture procedure, with cell culture medium (EMEM, 1% P/S). PDGF-BB was applied to the selected root surface specimens for a period of 5 min, and then removed. PDL cells were then placed on the experimental root surfaces and incubated for the different selected time periods.

Primary PDL cell counting, pooling, and incubation
Cells were counted using a transmission light microscope and a counting grid. An area of exactly 4 mm^2 was scanned under the microscope, and the number of cells in the area was counted and divided by 4 to get cell density per mm^2.

Periodontal ligament cells were pooled onto each root surface specimen in different experimental groups with a concentration of 5×10^3 per 20 μl per sample. Cultures were then allowed to incubate for 1, 3, and 7 days at 37°C in an atmosphere of 95% humidified air and 5% CO_2.

SEM specimen preparation and examination
Following incubation for the selected time periods and gentle withdrawal of culture medium from tissue culture plates, the specimens were fixed for 2–5 h with 2.5% glutaraldehyde fixative solution and then washed overnight in 0.1 M phosphate-buffered saline. The next day, the specimens were post-fixed with 2% osmium tetraoxide for 2 h, and then dehydrated in increasing concentrations of ethyl alcohol (50%, 70%, 80%, 90%, 95%, and 100%). The specimens were then washed with 3-methyl butyl acetate (isoamyl acetate) for 20 min and dried to the critical point. The specimens were then attached to an aluminum mount using sliver paste and coated with 7 nm of titanium. Finally, the coated specimens were examined under a scanning electron microscope (S-4500 SEM; Hitachi Ltd, Hitachinaka, Japan) at different magnifications. All samples were investigated independently by two blinded examiners for both counting procedures and SEM examinations to evaluate number, adhesion, and growth of fibroblast cells on the root surfaces.

Secondary PDL cell counting procedure using SEM
Five SEM photomicrographs per each sample were selected (four images at four extremities of each specimen and the fifth image at its central area) and obtained at the same working distance (15 mm) using the same magnification (X350). Using these standardized conditions, repeated cell counting of PDL fibroblasts was made within a representative standard area (0.03 mm^2 surface area), three times blindly per each photomicrograph.

Statistical evaluation
Statistical analysis was performed using statistical software (SPSS version 9 for Windows; SPSS Inc., Chicago, IL, USA). Mean values and standard deviations of the cell counting procedure were then calculated for each group. One-way analysis of variance (ANOVA) with Scheffe test was used for multiple comparisons among different experimental groups, and paired *t*-test was used to compare between different incubation periods within each experimental group. $P \leq 0.05$ was considered as statistically significant.

PDL cell shape and density
The quality of PDL fibroblast cells was evaluated by recording their shapes in three forms: flat (the healthy form), oval (the intermediate form), and round (the diseased form). This method was similar to that used by Gamal and Mailhot (2000) but with some modifications, where they used four different scales: 1 – flat cells; 2 – combination of oval or round cells; 3 – oval cells; and 4 – round cells. These scores were averaged and groups were compared. The authors of the present study, preferred to use the above-mentioned three grades of PDL cell shapes (flat, oval and round) for easy discrimination and qualitative evaluation.

On the other hand, the quantity of PDL fibroblast cells is evaluated by recording their densities into the following grades:

- Negative (empty areas, i.e., no cells).
- Light (separately dispersed cells, with wide intercellular spaces).
- Intermediate (interlaced cells forming a network, with narrow spaces).
- Heavy (overlapped and multilayered cells, without spaces).

Cell density (negative, light, intermediate and heavy) was evaluated by obtaining nine SEM photomicrographs at low magnification (× 50) for each specimen in order to scan completely the whole specimen's surface. Thereafter, by using the above-mentioned cell density scale, the different cell density zones within each group were estimated.

Results

Root surface topography
The root surfaces of the four experimental groups are shown in Figure 1. The healthy specimen showed smooth homogenous structure with some small holes throughout the root surface. The untreated diseased specimen

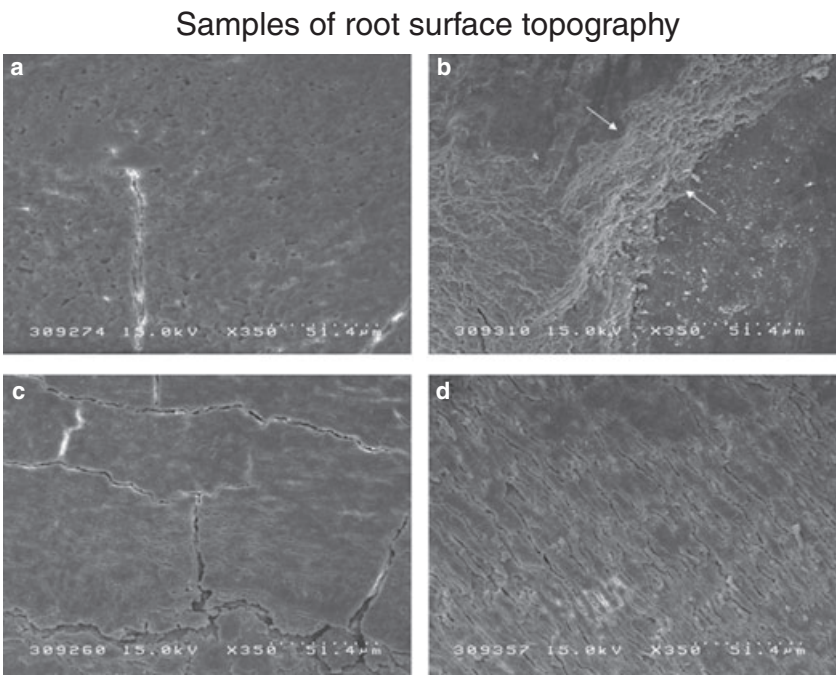

Figure 1 Scanning electron microscopy image of root surface topography. (a) Healthy specimen: homogenous smooth surface with tiny holes. (b) Diseased specimen: irregular and rough root surface due to surface blebs and calculus deposits (arrows). (c) Scaling and root planning specimen: some parallel striations and smear layer. (d) Scaling and root planning plus platelet-derived growth factor-BB specimen: parallel striations, removal of smear layer and opening of dentinal tubules. (×350)

showed irregular and rough root surface, with some calculus deposits distributed all over the root surface. The SRP specimen showed parallel striations and a smear layer covering the root surface. The SRP plus PDGF-BB specimen showed parallel striations along with removal of smear layer and opening of dentinal tubules.

Group I (healthy root surfaces; positive control)
Day 1
Specimens were characterized by the presence of separately dispersed flat PDL fibroblast cells with long cytoplasmic processes directed toward root surfaces. These cells are interspersed alternatively by oval and round cells with short to medium length cytoplasmic processes. The root surface was seen clearly within dispersed PDL cells. Cells had light density zones in some areas and intermediate density zones in other areas (Figure 2a). The mean number of attached cells to root surfaces was 11.7 ± 1.5 per unit area (Table 1).

Day 3
Specimens were characterized by the presence of overlapped flat PDL fibroblast cells in multilayers. Cells had smooth surfaces and formed interlacing and anastomosing networks that covered root surfaces almost completely. Generally, root surfaces could not be seen, except only in few limited areas, and neither oval nor round cells were found. Cells had heavy density zones dispersed all over the surface of the specimens (Figures 2b and 6a). The mean number of attached cells to root surfaces was 27.7 ± 3.5 per unit area (Table 1).

Day 7
Specimens were characterized by the presence of generally dispersed flat PDL fibroblast cells with smooth surfaces and long cytoplasmic processes directed toward root surfaces. Root surface was seen within observed cells. Cells had light to intermediate cell density zones all over the surface of the specimens (Figure 2c). The mean number of attached cells to root surfaces was 26.3 ± 2.5 per unit area (Table 1).

Group II (diseased root surfaces; negative control)
Day 1
Specimens were characterized by the presence of few separately dispersed flat PDL fibroblast cells with no cytoplasmic processes, except few and limited processes that were found occasionally. Sometimes, cells had bulled out surfaces directed away from root surfaces. Few isolated and single oval cells were also found in some areas. Root surfaces appeared mostly covered by rough surface blebs and some surface debris. Cells had light density zones, excepting few intermediate density zones (Figure 3a). The mean number of attached cells to root surfaces was 8.3 ± 1.5 per unit area (Table 1).

Day 3
Specimens were characterized by the presence of few isolated and single flat PDL fibroblast cells in some areas, and few oval to round isolated cells in other areas. Cells had only light density zones and in some areas they were directed away from root surfaces (Figures 3b and 6b). The mean number of attached cells to root surfaces was 7.7 ± 1.5 per unit area (Table 1).

Day 7
Specimens were characterized by the presence of flat PDL fibroblast cells in some areas interspersed with a mix of round to oval cells. Cells had intermediate density zones in some areas and light density zones in others (Figure 3c). The mean number of attached cells

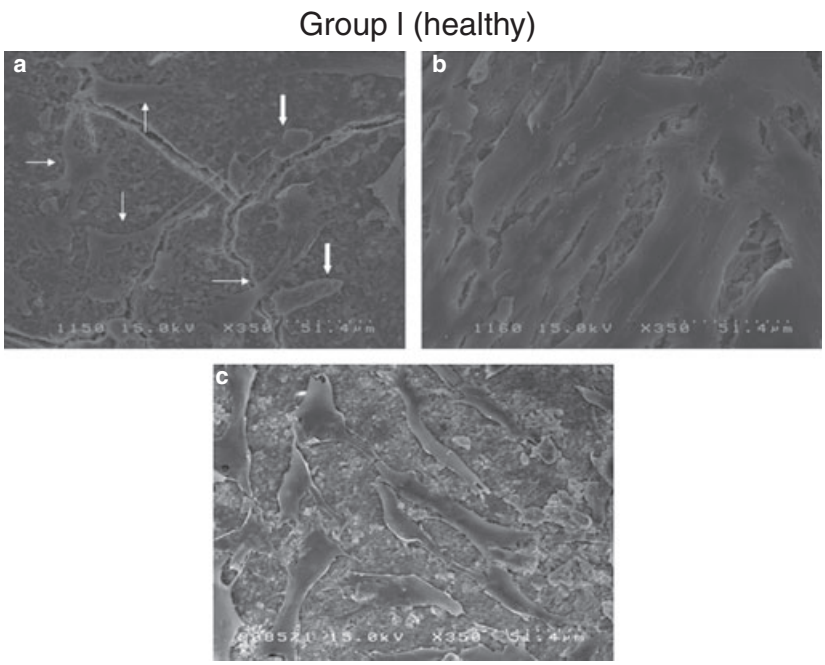

Figure 2 Scanning electron microscopy image of a healthy (positive control) root surface specimen showing attached periodontal ligament fibroblast cells after seeding at day 1. (a) [note flat cells (straight arrows) and oval or round cells (thick arrows)], day 3 (b) (note the overlapping flat cells in multilayers completely covering root surface), and at day 7 (c). (×350)

Table 1 Mean ± s.d. values of number of cell counting procedures among studied groups at each incubation period, along with significance level using paired *t*-test and ANOVA test

	Incubation periods (mean ± s.d.)			*Paired t-test*					
				Day 1 vs day 3		*Day 1 vs day 7*		*Day 3 vs day 7*	
Study groups	*Day 1*	*Day 3*	*Day 7*	*t*	*P*	*t*	*P*	*t*	*P*
G I	11.7 ± 1.5	27.7 ± 3.5	26.3 ± 2.5	−8.0	0.015*	−11.0	0.008*	2.00	0.184**
G II	8.3 ± 1.5	7.7 ± 1.5	12.7 ± 2.1	0.378	0.742**	−2.137	0.166**	−8.660	0.013*
G III	8.0 ± 1.0	9.3 ± 2.5	13.7 ± 1.5	−1.512	0.270**	−17.00	0.003*	−6.500	0.023*
G IV	10.7 ± 2.1	25.7 ± 4.5	24.3 ± 2.5	−9.820	0.010*	−41.00	0.001*	1.109	0.383**
ANOVA test									
F	3.822	32.266	31.144						
P-value	0.06**	0.000*	0.003*						
Scheffe test	–	G I *vs* II and III, G IV *vs* II and III / G I *vs* II and III, G IV *vs* II and III							

Results are expressed by mean number of cell counting/0.03 mm^2. G, group; Group I: Healthy root surface specimens. Group II: Periodontally diseased root surface specimens. Group III: Scaled and planed root surface specimens. Group IV: Scaled and planed plus PDGF-BB root surface specimens.
*Statistically significant at $P \leq 0.05$.
**Statistically not significant.

to root surfaces was 12.7 ± 2.1 per unit area (Table 1).

Group III (scaled and root planed surfaces)
Day 1
Specimens were characterized by the presence of round to oval PDL fibroblast cells dispersed all over root surfaces with light to intermediate cell density zones. Sometimes, single isolated flat PDL fibroblast cells that had rough surfaces and no cytoplasmic processes were also found. Root surfaces appeared mostly and continuously covered by an evident smear layer (Figure 4a). The mean number of attached cells to root surfaces was 8.0 ± 1.0 per unit area (Table 1).

Day 3
Specimens were characterized by the presence of flat PDL fibroblast cells dispersed all over root surfaces with occasionally few single isolated oval to round cells. Cells had mostly light cell density zones. Root surfaces also appeared mostly and continuously covered by a smear layer (Figures 4b and 6c). The mean number of attached cells to root surfaces was 9.3 ± 2.5 per unit area (Table 1).

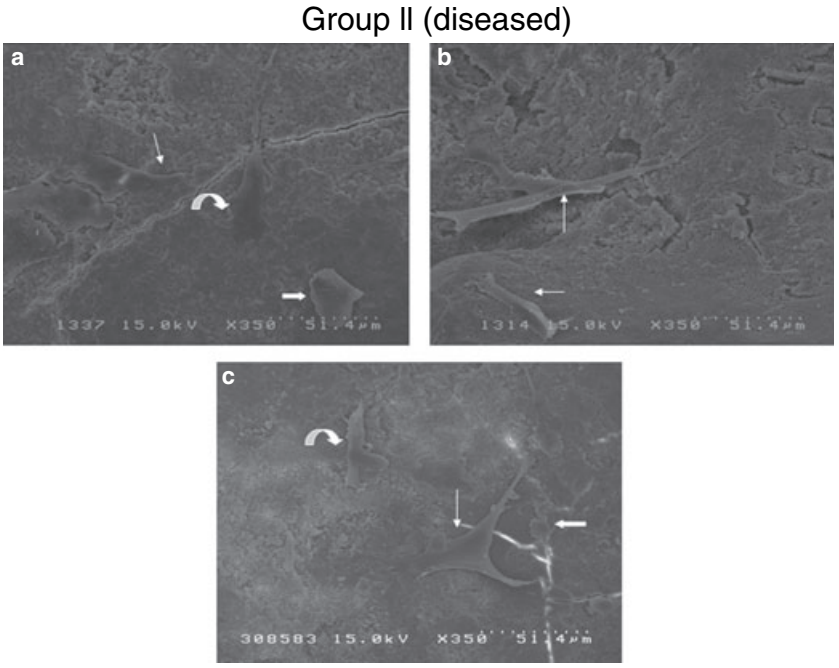

Figure 3 Scanning electron microscopy image of untreated periodontally diseased (negative control) root surface specimen showing attached periodontal ligament fibroblast cells after seeding at day 1 (a), day 3 (b) and day 7 (c). Note some cells with bulled out surface directed away from the root surface (curved arrows), flat cells (straight arrows) and oval or round cells (thick arrows). (×350)

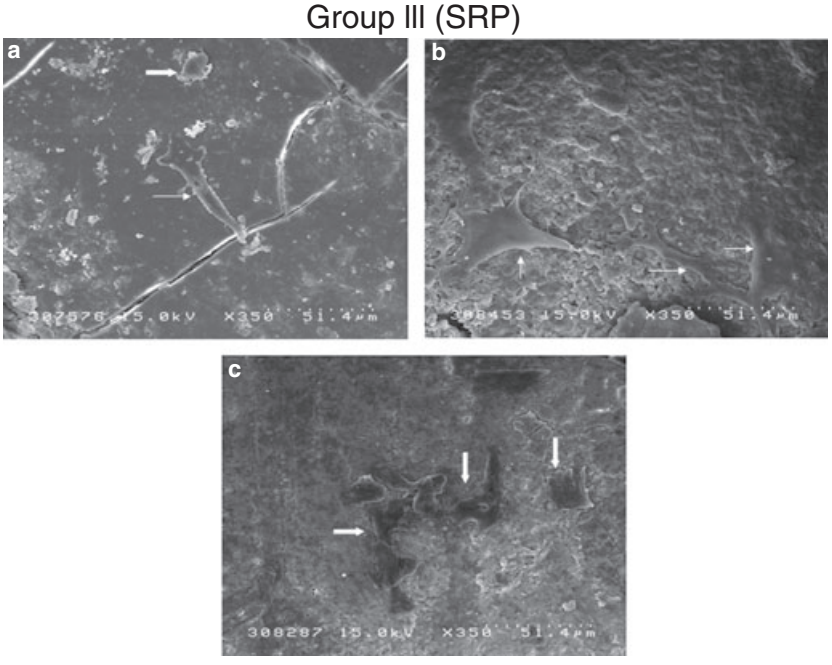

Figure 4 Scanning electron microscopy image of scaling and root planning root surface specimen showing attached periodontal ligament fibroblast cells after seeding at day 1 (a), day 3 (b), and day 7 (c). Observe the presence of a smear layer covering the root surface and cells with rough irregular surfaces (a, c). Note flat cells at straight arrows, and oval or round cells at thick arrows. (×350)

Day 7

Specimens were characterized by the presence of flat PDL fibroblast cells dispersed all over the root surface, with intermediate cell density zones in some areas and light cell density zones in other areas. These cells had irregular surfaces and did not show cytoplasmic processes, i.e., neither long nor short cytoplasmic extensions were found. Some oval and round cells with light density zones were also found. The root surface appeared to be covered by a smear layer, and some parallel striations were found occasionally (Figure 4c). The mean number of attached cells to root surfaces was 13.7 ± 1.5 per unit area (Table 1).

Group IV (scaled and root planed surfaces plus PDGF-BB)

Day 1

Specimens were characterized by the presence of several parallel striations due to manual instrumentation procedure that preceded PDGF-BB application. Meanwhile, complete removal of smear layers from the root surfaces along with opening of dentinal tubules were

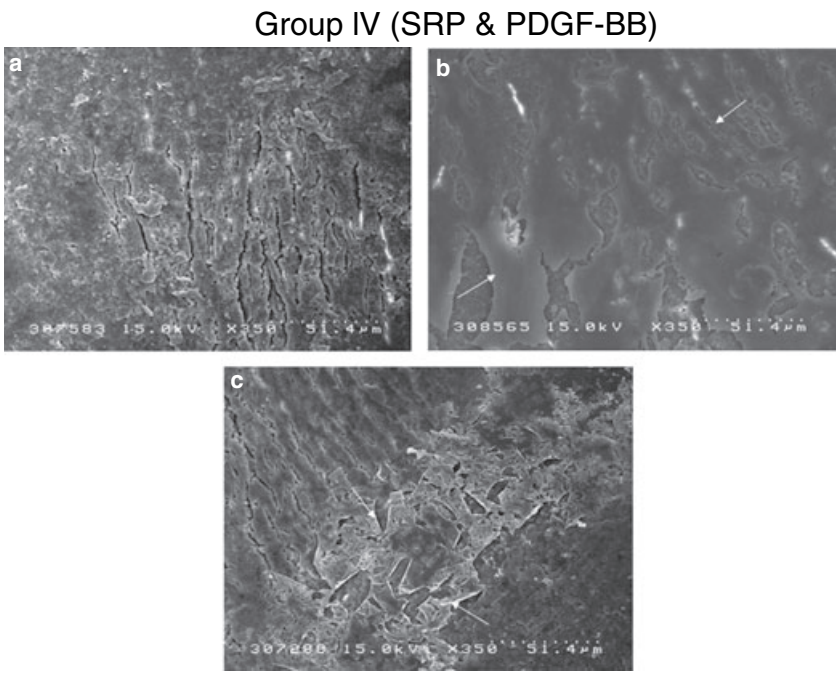

Figure 5 Scanning electron microscopy image of scaling and root planning plus platelet-derived growth factor-BB root surface specimen showing attached periodontal ligament fibroblast cells after seeding at day 1 (a), day 3 (b), and day 7 (c). Observe smear layer removal and opening of dentinal tubules in (a), and cells forming confluent melted monolayers in (b) and (c) at arrows. (×350)

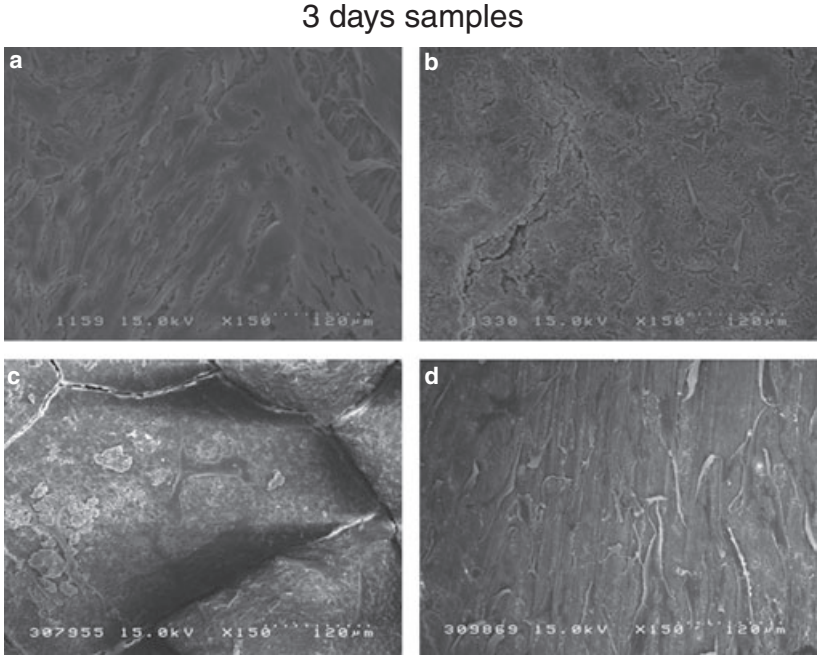

Figure 6 Scanning electron microscopy image of attached periodontal ligament fibroblast cells throughout root surfaces of all experimental groups at the day 3 incubation period (the most characteristic period). (a, d) Healthy and scaling and root planning (SRP) plus platelet-derived growth factor-BB respectively. Note the most abundant cell number covering almost all root surfaces. (b, c) Diseased and SRP groups respectively. (×150)

noticed. Few flat and oval PDL fibroblast cells with light cell density zones were found occasionally (Figure 5a). The mean number of attached cells to root surfaces was 10.7 ± 2.1 per unit area (Table 1).

Day 3

Specimens were characterized by the presence of clearly defined flat PDL fibroblast cells that had melted smooth surfaces, covered almost all of the root surfaces and formed overlapping multilayers to give a final characteristic shape of confluent monolayer. These cells mostly formed heavy cell density zones dispersed all over the surface of the specimen (Figures 5b and 6d). The mean number of attached cells to root surfaces was 25.7 ± 4.5 per unit area (Table 1).

Day 7

Specimens were characterized by the presence of several parallel striations from manual curette instrumentation. The smear layer was entirely eliminated from the root surface. Occasionally, flat PDL fibroblast cells with intermediate to heavy cell density zones were found on

the root surface. These cells formed an interlacing anastomosing network with some areas of the characteristic melted appearance and smooth cell surfaces, forming monolayers that completely cover the underlying root surface (Figure 5c). The mean number of attached cells to root surfaces was 24.3 ± 2.5 per unit area (Table 1).

Using paired *t*-test, groups I and IV showed statistically significant differences from day 1 to day 3, and also from day 1 to day 7, but no significant differences were observed from day 3 to day 7. Groups II and III did not show statistically significant differences from day 1 to day 3, but showed significant differences from day 3 to day 7. Meanwhile, when compared day 1 versus day 7, group III showed statistically significant differences, but group II did not. Using one-way ANOVA, there were no significant inter-group differences at day 1, but statistically significant differences were found at day 3 and day 7 for groups I and IV versus both groups II and III (Table 1).

Discussion

The least numbers of attached fibroblasts were obtained at day 1 period in all groups, except the diseased group where it was obtained at day 3. This may be explained by the small amount of culture medium that is used in the start of actual experiment, as PDL cells are pooled with 5×10^3 per 20 μl per specimen, allowed to incubate for 24 h, and then 3 ml of culture medium is added per each specimen at the end of both day 1 and day 3 incubation periods.

In general, cell morphology at the day 1 incubation period included the presence of few separately dispersed flat PDL fibroblast cells interspersed with few oval and round cells all over the sample surface. Therefore, cell density for the four groups showed mostly light density zones, although intermediate density zones were occasionally found in few areas.

The characteristic feature per each group at the day 1 incubation period was as follows: group I (healthy): the presence of long (for flat cells) and short to medium (for oval and round cells) cytoplasmic processes directed toward root surfaces suggesting good means of attachment; group II (diseased): no cytoplasmic processes with sometimes bulled out cell surfaces directed away from root surfaces suggesting no means of attachment; group III (SRP): root surfaces mostly covered by an evident smear layer and cells had rough surfaces denoting incompatible root surfaces; group IV (SRP and PDGF-BB): the smear layer is completely removed from root surfaces along with opening of dentinal tubules, suggesting good and valuable means of root surface biocompatibility.

In general, day 3 samples of groups II and III (diseased and SRP) showed cells that still had light density zones in the form of few flat PDL fibroblast cells alternatively distributed with few single isolated oval to round cells. The characteristic feature was the presence of a smear layer in SRP group III samples, and some cells appeared not well attached in the diseased group II samples where they were directed away from root surfaces. The presence of rough blebs and some debris on the root surfaces of diseased samples, and smear layer on surfaces of SRP samples may hinder PDL fibroblast cell attachment and growth, and thus may explain the presence of incompatible root surfaces in these groups. This is in agreement with the unfavorable findings of Gamal and Mailhot (2000) in their periodontally diseased control group that was pretreated by only SRP, where they found round PDL cells with short microvilli on the dentine specimens and noticed the presence of an organic smear layer that obscured the dentinal tubules. Meanwhile, Schwarz *et al* (2003) reported that scaled and root planed specimens exhibited considerably fewer attached and more round cells. In accordance with this, Rossa *et al* (2002) suggested that root surfaces subjected exclusively to conventional SRP were not conducive to cellular adhesion and proliferation.

Groups I and IV (healthy and SRP plus PDGF-BB) at the day 3 incubation period showed cells with continually heavy density zones in the form of overlapped flat PDL fibroblast cells in multilayers covering mostly the whole root surfaces. Neither oval nor round cells were observed. The characteristic feature was that cells in healthy group I samples formed an interlacing network in the form of sheets like a mat, while in group IV (SRP plus PDGF-BB), cells melted altogether on the root surfaces and formed fused confluent monolayers. This appeared only in these two groups and especially at the day 3 period, where the number of PDL cells was significantly and suddenly elevated in an enormous form, from day 1 to day 3. PDL cells increased tremendously at this time period (day 3) to the most abundant cell numbers reaching the highest levels amongst all experimental groups of the present study. This may be explained by achieving the best form of root surface biocompatibility, as the highest ratio of PDL cell attachment is obtained in these two groups (healthy and SRP plus PDGF-BB), that did not appear in any one of the other experimental groups.

Groups II and III (diseased and SRP) at the day 7 incubation period showed PDL fibroblast cells with a light to intermediate density zones in the form of flat cells interspersed with oval to round cells. The characteristic feature of SRP (group III) samples at day 7 is the presence of a smear layer covering the root surface, obscuring dentinal tubules. Occasionally, parallel striations are found due to manual instrumentation by the use of periodontal curette. PDL cells in the SRP group of the present study did not show cytoplasmic processes, had irregular shapes and appeared not well attached to root surface, as they had sometimes bulled out surfaces that directed away from the root surface, suggesting the presence of incompatible root surfaces. This is in accordance with the previous findings of Hatfield and Baumhammers (1971) and Polson *et al* (1984) who suggested that the production of a smear layer after mechanical root surface debridement with hand-held instruments may inhibit cell migration and attachment, and therefore may be detrimental to periodontal tissue

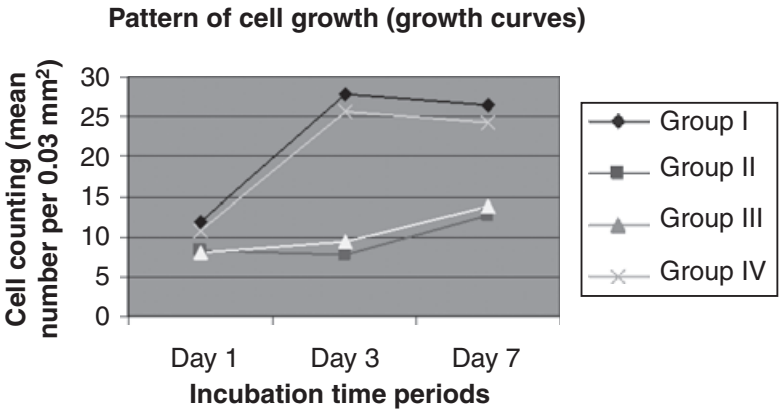

Figure 7 Pattern of cell growth (growth curves) among all experimental groups from day 1 to day 3 and day 7 incubation periods measured by mean number of cell counting procedure for each group.

healing. On the other hand, the characteristic feature of diseased samples at day 7 is the presence of some isolated PDL cells that are directed away from the root surface. This may suggest a strong inhibiting effect of root surface deposits for PDL fibroblast cell attachment in the diseased group.

The specimens of groups I and IV (healthy and SRP plus PDGF-BB) at the day 7 incubation period showed flat PDL fibroblast cells with long cytoplasmic processes directed toward the root surface, suggesting good cell attachment. Occasionally, cell density was intermediate in healthy samples and heavy in SRP plus PDGF-BB samples. The characteristic feature of the SRP plus PDGF-BB group when compared with the healthy group is the presence of an occasionally interlacing anastomosing network on the root surface with some limited areas showing the specific melted shape and smooth cell surfaces forming fused confluent monolayers. This is in agreement with the findings of Gamal and Mailhot (2000) who detected a highly significant increase in the number of adherent fibroblasts that were usually oriented in multilayer completely covering the dentine surface in PDGF-BB groups when compared to periodontally diseased treated control ($P \leq 0.05$), but there was no apparent difference between the morphology of the cells in these groups and the healthy control. However, PDGF-BB in the study of Gamal and Mailhot (2000) was reconstituted using a culture medium (EMEM/0.5% FBS and P/S) and their cultures were allowed to incubate for only 24 h, while PDGF-BB in the present study was reconstituted in sterile distilled water according to the manufacturer's instructions and cultures were incubated for 1, 3 and 7 day periods. The present study selected the use of PDGF-BB with a concentration of 50 ng ml^{-1}, where Gamal and Mailhot (2000) found similar effects when using higher concentrations (100, 200, and 300 ng ml^{-1}) as obtained by using 50 ng ml^{-1}, and they suggested the use of this concentration (50 ng ml^{-1}) for optimal stimulation of human PDL cells to adhere to periodontally affected root surfaces.

Analysis of individual results within each group using paired *t*-test (Table 1) revealed a significant increase in the number of attached PDL cells only from day 3 to day 7 in group II (diseased), while group III (SRP) showed a significant increase between both day 1 to day 7 and day 3 to day 7 incubation periods. This is considered in favor with a certain degree for results of SRP over diseased samples, although no statistically significant inter-group differences were found between the two groups. In contrast, groups I and IV showed a significant increase in the number of attached PDL cells between both day 1 to day 3 and day 1 to day 7, but not between day 3 and day 7 incubation periods. This means that the cell number is increased tremendously, reaching its maximum at day 3 and remaining almost stationary at day 7.

Although no significant inter-group differences were found among the four groups of the present study at day 1, statistically significant differences ($P \leq 0.05$) were found at both day 3 and day 7 for groups I and IV when compared with groups II and III. This refers to more favorable results of both healthy and SRP plus PDGF-BB groups over diseased and SRP groups (Table 1). This is in agreement with the findings of Gamal *et al* (1998) who evaluated human PDL fibroblast response to PDGF-BB and IGF-1 application on tetracycline HCL conditioned root surfaces, and found a significant increase ($P \leq 0.05$) in the number of attached fibroblast on dentine specimens pretreated with 50 ng ml^{-1} of PDGF-BB compared with non-tetracycline-conditioned (non-treated) and tetracycline-conditioned control groups.

As regards the SRP and SRP plus PDGF-BB groups, SRP samples had a much lower degree of root surface biocompatibility and PDL fibroblast cell attachment than both healthy and SRP plus PDGF-BB samples (statistically significant difference), but it did not show a pronounced improvement over diseased samples (no statistically significant inter-group differences). On the other hand, SRP plus PDGF-BB samples (group IV) showed a similar degree of root surface biocompatibility and PDL fibroblast cell attachment to healthy samples of group I (no statistically significant difference), although it had a characteristic form of PDL fibroblast cell fusion and melting, forming confluent monolayers all over the root surface. However, SRP plus PDGF-BB showed a greater degree of root surface biocompatibility and PDL fibroblast cell attachment than both diseased and scaled root surfaces (groups II and III).

In conclusion, the behavior of SRP samples (group III) was similar to the behavior of diseased root surface

samples (group II). However, SRP plus PDGF-BB root surface specimens (group IV) showed interesting significant positive features regarding cell adhesion and proliferation similar to the behavior of healthy (group I) root surface specimens (Figure 7). This suggests that SRP alone may not ensure an acceptable root surface biocompatibility, whereas PDGF-BB when applied following SRP may significantly modify the characteristics of diseased root surfaces in a positive way to mimic healthy root surfaces to attain good biocompatibility needed for fibroblast cell adhesion and proliferation.

Group IV mostly showed flat cells except very few isolated oval cells that appeared only at day 1 in some specimens. Most of the group III specimens (SRP) had an evident smear layer on the surface without significant increase in adhesion and/or proliferation of fibroblast cells, while group IV specimens (SRP and PDGF-BB) showed almost no smear layer and a significant increase in cells, denoting a strong stimulation of adhesion and/or proliferation of fibroblast cells either by direct effect on the biocompatibility of root surfaces, or indirectly by removing the smear layer and subsequently opening dentinal tubules, or both.

The present study was designed to determine the phenotype of cells along with primary cell counting procedures using microscopy by one blind examiner. However, further studies are required to prove the adhesive nature of cells for maintaining the phenotype in primary cells by using mRNA analysis, by RT-PCR and protein expression study, by Western blot/immunocytochemistry, etc. Despite the fact that the application of PDGF-BB to the root surface showed opening of dentinal tubules/removal of smear layer by SEM observation, it is necessary to clarify further how PDGF-BB acts on the root surface. The present study also did not notice a great difference between cells of the third and ninth passage. Therefore, any specific difference that may be found between cell passages should be evaluated in future studies.

In summary, PDGF-BB showed a good positive effect on adhesion and growth of cultured human PDL fibroblasts to periodontally diseased root surfaces. Thus, PDGF-BB might act as a good modulating agent for periodontally diseased root surfaces, enhancing regeneration during periodontal wound healing.

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