Morphologic Studies on the Biologic Seal of Titanium Dental Implants. Report I. In Vitro Study on the Epithelialization Mechanism Around the Dental Implant

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To propose a mechanism for apical epithelialization at the implant-tissue interface, cell contact to titanium surfaces and adhesive strength of epithelial-like (HGE) and fibroblastic (HGF) cells derived from human gingiva were investigated under three different media conditions containing plaque extracts: nonfiltered, 5-µm pore filtered, and 0.22-µm pore filtered. The plaque extracts had a greater effect in decreasing the growth rate of the HGF than of the HGE. Similarly, the HGE exhibited greater adhesive strength than the HGF. These differences in the cells' resistance to plaque extracts were also observed using light and electron microscopy. Evidence from this study suggests that the difference in growth, contact, and adhesive strength of the HGE and HGF cells to titanium surfaces may promote apical epithelialization under the pathologic condition.
(INT J ORAL MAXILLOFAC IMPLANTS 1998;13:457–464)

Key words: biologic seal, cell culture, epithelialization, plaque extracts

Dental implant rejection occurs as a result of apical epithelialization at the implant-tissue interface. Thus, it is critical to prevent apical epithelialization for the body to retain the dental implant.[1,2] The two factors affecting apical epithelialization are: *(1)* the macro-(mm)/micro-(µ) geometric structure of the implant surface, which governs the adhesion and orientation of epithelial cell growth,[3,4] and *(2)* the biochemical and biophysical compatibility of the implant surface, which dictate the adhesion of epithelial and connective tissues. As evidenced from scanning electron microscopy (SEM) investigations, these two factors are easily disturbed under the pathologic condition of peri-implant soft tissue as a result of bacterial invasion, food debris ingress, and physicochemical irritation of restorative materials and setting cement.[5] However, the mechanism that causes apical epithelialization is not fully understood. To elucidate the phenomenon that controls apical epithelialization, this study investigated the effect of plaque extracts on the in vitro response of epithelial-like cells and fibroblastic cells to titanium (Ti) surfaces.

Materials and Methods

Cells. Epithelial-like cells (HGE) and fibroblastic cells (HGF) were derived from the gingival soft tissue of a 23-year-old Japanese male. The Institutional Review Board's approval and the informed consent of the participating subject were obtained prior to the start of the experiment. The explant tissue from the

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human gingiva was cleaned and disinfected with 1,000 mL phosphate-buffered saline solution (PBS) containing 20,000 U penicillin and 50 mg streptomycin. The explant tissue was then diced into 2-mm^3 fragments and treated with 0.25% trypsin solution at 4°C for 5 hours. The trypsin solution was recanted and the fragments were then magnetically stirred in 0.02% ethylenediaminetetraacetic acid PBS at room temperature for 7 minutes. The stirred tissue fragments were inoculated in a 3.5-cm polystyrene tissue culture dish (76-058-05, Dainihon Seiyaku, Suita, Osaka, Japan) containing minimal essential media (MEM) and 10% fetal calf serum. The tissue culture medium was renewed weekly. Three weeks after inoculation, the cells reached the log phase in their proliferation. The cells were then isolated using 0.05% trypsin solution, and the suspended cells (containing both the HGE and HGF cells) were seeded in a 17-mm-diameter multiwell tissue culture dish (76-063-05, Dainihon Seiyaku). After three or four subcultivations, some wells contained either HGE or HGF cells. These unestablished lines of the HGE and HGF cells were then subcultured separately.

Human Plaque Extracts. Three plaque extracts were used in this study. A 1,000-mg human plaque containing soft tartar was diluted with 60 mL PBS and triturated with glass pestle and mortar. After trituration, the plaque-tartar-PBS mixture was centrifuged at 1,500 rpm for 10 minutes. The supernatant of homogeneously translucent solution was used as original plaque extract (OPE) without filtration. Filtered plaque extracts were produced by filtering the OPE using Millipore filters with pore sizes of 5.0 μm and 0.22 μm (Millipore Japan Yonezawa Manufactory, Yamagata, Japan). The filtered plaque extracts were labeled 5 FE and 0.22 FE.

Growth Rate Assay. In a single-blind evaluation, the effect of plaque extracts on multiplication of the HGE and HGF cells was measured using cell growth rate as a marker. The substrates used for this in vitro study were polyacrylic plates (10 mm × 10 mm) coated with Ti film. The HGE and HGF cells were then seeded on the Ti surfaces at a density of 50 cells/mm^2. Two days after seeding, the media were replaced with 18 mL MEM (with 10% fetal calf serum) and 2 mL of either OPE, 5 FE, or 0.22 FE. The cells were exposed to the plaque extracts for 5 days. For the control wells, the cell culture medium (MEM and 10% fetal calf serum) was used. At 0 days, 2 days, 4 days (2 days of plaque extract exposure), and 7 days (5 days of plaque extract exposure) after seeding, the growth rates of the HGE and HGF cells were measured. Using a sample size of six for each treatment, the growth rate was determined by counting the number of cells/mm^2 with CELCOM (Aoi System, Osaka, Japan).[6] The results were statistically compared using the analysis of variance (ANOVA). The alpha level for data analysis was set at $\alpha = .05$, and differences were considered significant if $P < .05$.

Cell Morphology Assay. Cell morphology on Ti surfaces was examined using a single-blind evaluation. The HGE and HGF cells were seeded on the Ti surfaces at a density of 50 cells/mm^2. Two days after seeding, the media were replaced with 18 mL MEM (with 10% fetal calf serum) and 2 mL of either OPE, 5 FE, or 0.22 FE. The cells were exposed to the plaque extracts for 2 days. For the control wells, the cell culture medium (MEM and 10% fetal calf serum) was used. The morphology of the HGF and HGE cells was examined using light microscopy after 2 days of plaque extract exposure.

Cell Adhesive Strength to Ti Substratum. In addition to the growth rate and cell morphology assays, the effect of cell adhesive strength to Ti substratum was measured using a single-blind evaluation. The HGE and HGF cells were seeded on the Ti surfaces at a density of 50 cells/mm^2. Two days after seeding, the media were replaced with 18 mL MEM (with 10% fetal calf serum) and 2 mL of either OPE, 5 FE, or 0.22 FE. The cells were exposed to the plaque extracts for 2 days. For the control wells, the cell culture medium (MEM and 10% fetal calf serum) was used. Two days after plaque extract exposure, the adhesive strength of the HGE and HGF cells to Ti substrates was measured by means of a viscometric method. A shear stress of 3.7 dyne/cm^2 was applied for 20 minutes, and the final cell number was measured automatically with CELCOM. Using a sample size of five for each treatment, the relative adhered cell number (RACN) value was then calculated by means of the following equation:[7,8]

$$\text{RACN} = \frac{\text{No. of adhered cells after shear stress}}{\text{No. of adhered cells before shear stress}}$$

The RACN values were statistically compared using the ANOVA. The alpha level for data analysis was set at $\alpha = .05$, and differences were considered significant if $P < .05$.

Effect of Plaque Extracts on the HGE and HGF Cocultivation. Similar to the above assays, the effect of plaque extracts on the HGE and HGF cocultivations was measured using a single-blind evaluation. Cocultivation was performed by seeding an equal density (1×10^4 cells/mL) of the HGE and HGF cell suspension. Two days after seeding, the cocultivated cells were exposed to 5 FE for 24 hours. The morphologic changes of both cells after plaque extract exposure were investigated using light and electron microscopy.

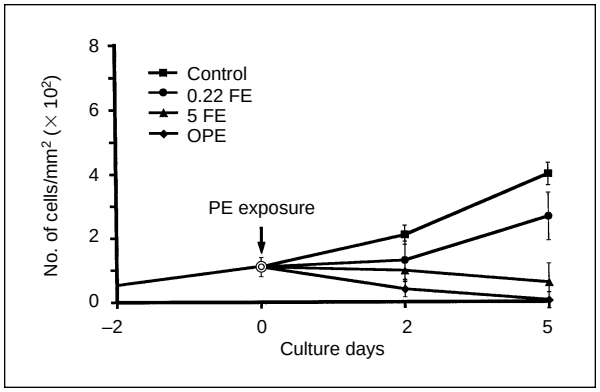

Fig 1 Effect of plaque extracts on the multiplication of HGF cells. The error bar represents one standard deviation (n = 6). (PE = plaque extract; OPE = unfiltered plaque extract.)

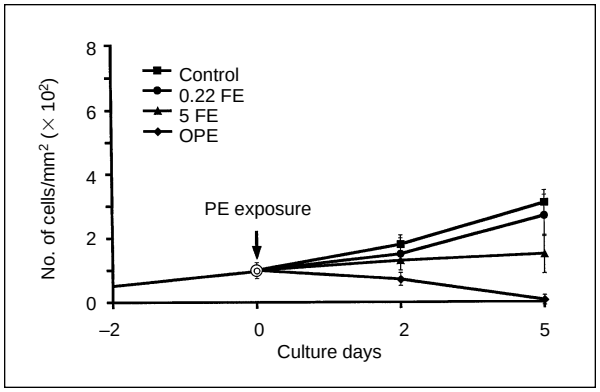

Fig 2 Effect of plaque extracts on the multiplication of HGE cells. The error bar represents one standard deviation (n = 6). (PE = plaque extract; OPE = unfiltered plaque extract.)

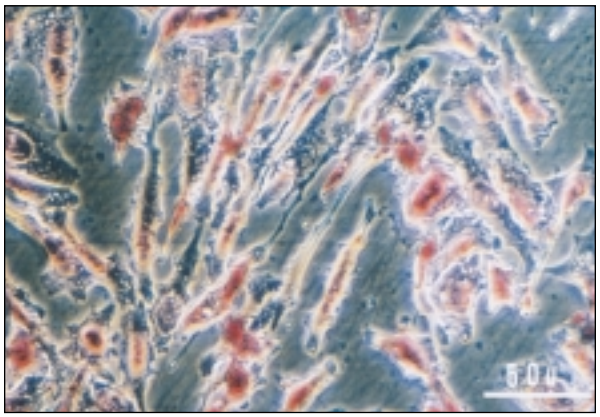

Fig 3 Fibroblastic cells (HGF) derived from the human gingiva after 4 days' cultivation in the control medium.

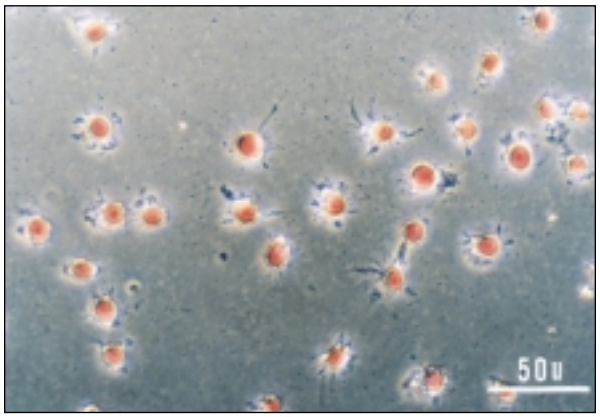

Fig 4 HGF cells, shrunken and detached from the titanium substratum after 2 days of exposure to 5 FE.

Results

Growth Rate Assay. The presence of plaque extracts was observed to have had a significant effect on the growth rate of the HGE and HGF cells. As shown in Figs 1 and 2, the OPE had the most significant cytotoxic effect, followed by the 5 FE. The 0.22 FE had the least cytotoxic effect on the growth rate of the HGE and HGF cells. Compared to that of the HGE, the growth rate of the HGF was significantly reduced. After 5 days of exposure to the plaque extracts, the mean cell densities of the HGF in the presence of the control media, OPE, 5 FE, and 0.22 FE were 400 cells/mm^2, 5 cells/mm^2, 60 cells/mm^2, and 267 cells/mm^2, respectively (Table 1). Similarly, after 5 days of exposure to the plaque extracts, the mean cell densities of the HGE in the presence of the control media, OPE, 5 FE, and 0.22 FE were 310 cells/mm^2, 7 cells/mm^2, 150 cells/mm^2, and 270 cells/mm^2, respectively (Table 1 and Fig 2).

Table 1 Mean Growth Rates (± SD) of HGE and HGF Cells After 5 Days of Exposure to Plaque Extracts*

Cells	HGE (cell no./mm^2)	HGF (cell no./mm^2)
Control**	310 ± 21	400 ± 34
0.22 FE	270 ± 17	267 ± 49
5 FE	150 ± 50	60 ± 46
OPE	7 ± 7	5 ± 7

*Plaque extracts = unfiltered plaque extract (OPE), 5.0-µm-pore filtered extract (5 FE), and 0.22-µm-pore filtered extract (0.22 FE).
**Control = the cell culture medium without plaque extracts.

Cell Morphology Assay. *HGF.* The spindle shape or fibrous morphology of normal HGF cells was shrunken and rounded after 2 days of exposure to the 5 FE (Figs 3 and 4). The HGF cells had a similar shrunken morphology after 2 days of exposure to the OPE and 0.22 FE. The mean density of the HGF cells after 2 days of exposure to the 5 FE was 98 cells/mm^2 (Fig 1). For the same cultivation

period, the HGF in the control media exhibited a mean density of 210 cells/mm^2. This indicates a 53.3% reduction in the growth rate of the HGF when exposed to the 5 FE.

HGE. After 2 days of exposure to the 5 FE, the cell-cell attachment of the pavement-like monolayer cell sheet found in normal HGE cells was partially detached and separated. Cytoplasmic shrinkage also was observed. In addition, the rounded or ellipsoid nuclei found in normal HGE cells became deformed and irregular. However, the close cell-cell contact in the HGE colony sheet was partially retained, and no cytoplasmic vacuolization was observed (Figs 5 and 6). The mean cell density of the HGE after 2 days of exposure to the 5 FE was 132 cells/mm^2 (Fig 2). For the same cultivation period, the HGE in the control media exhibited a mean cell of 189 cells/mm^2. Thus, after 2 days of exposure to the 5 FE, a 30.1% reduction in the growth rate of the HGE cells was observed. Based on these findings, it is evident that the HGE cells have a higher resistance to plaque extracts than the HGF cells.

Table 2 Mean Adhesive Strength of the HGE and HGF Cells After 2 Days of Exposure to Plaque Extracts

Cells	HGE (RACN value)	HGF (RACN value)
Control	90 ± 10.6	87 ± 8.9
0.22 FE	86 ± 12.3	71 ± 14.2
5 FE	36 ± 11.8	20 ± 13.7
OPE	11 ± 9.3	4 ± 4.5

RACN = relative adhered cell number.

Adhesive Strength of Cells. The mean adhesive strength of the HGE cells to Ti substrates was statistically greater than that of the HGF cells after 2 days of exposure to the plaque extract (Table 2 and Fig 7). After 2 days of exposure to the 0.22 FE, 5 FE, and OPE, the RACN values for the HGF were 71, 20, and 4, respectively. For the same cultivation period, the RACN value for the HGF in the control media was 87. Thus, the RACN values for the HGF exposed

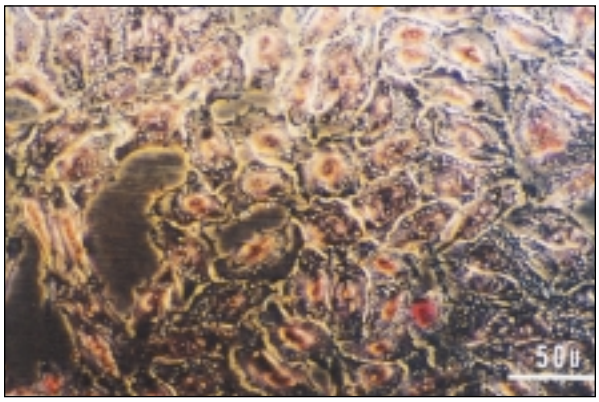

Fig 5 HGE cells exhibit monolayer colony sheet with close cell-cell contact after 4 days' cultivation in the control medium.

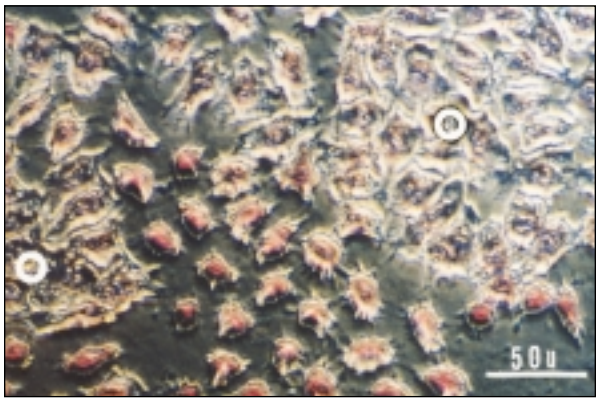

Fig 6 Close cell-cell contact of HGE colony sheet, detached and separated (O) because of cytoplasmic shrinkage after 2 days of exposure to 5 FE.

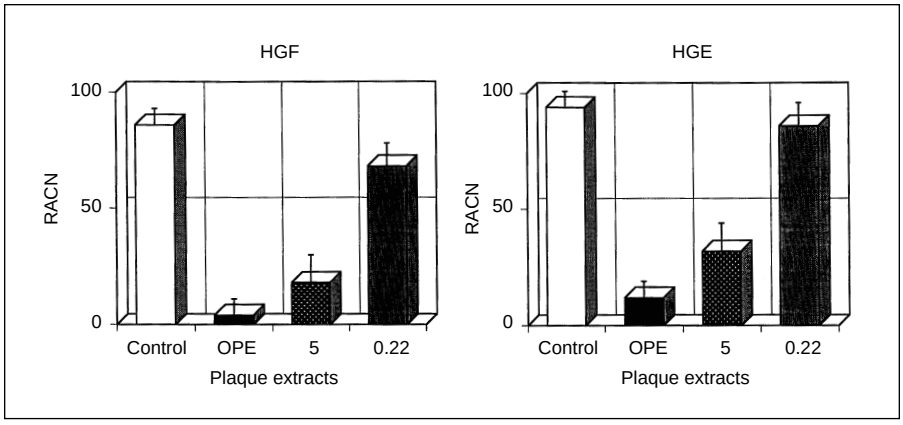

Fig 7 Effect of plaque extracts on the adhesion of fibroblast (HGF) and epithelial (HGE) cells derived from human gingival tissue. RACN refers to the apparent adhesive strength (n = 5) after 2 days of exposure to plaque extracts. (OPE = unfiltered plaque extract.)

to 0.22 FE, 5 FE, and OPE decreased by 18%, 77%, and 95%, respectively.

A similar trend was observed for the HGE exposed to plaque extracts. After 2 days of exposure to the 0.22 FE, 5 FE, and OPE, the RACN values for the HGE were 86, 36, and 11, respectively. For the same cultivation period, the RACN value for the HGE exposed to the control media was 90. The 0.22 FE exposure exhibited the least effect (4% decrease) on adhesive strength, followed by the 5 FE exposure (60% decrease) and the OPE exposure (87% decrease). The RACN values for HGE were constantly higher than the RACN values for HGF in each condition.

Effect of 5 FE on the HGE and HGF Cocultivation. In the cocultured wells of the HGE and HGF, a dense colony of HGE cells, with close cell-cell contact, was observed after 2 days (Fig 8). In contrast, the HGF cells exhibited a dispersed colony, with loose cell-cell contact. When the cocultured colonies were exposed to the 5 FE for 1 day, the HGE cells showed close adhesion and stretching on the Ti substrate. Moreover, the HGE intruded into the detached space of the HGF and adhered closely to the Ti substrates. The intrusion caused the HGF to loosen and "pile up" on the HGE (Figs 9a and 9b). This "pile up" phenomenon may be a result of the HGE cells' greater resistance, cytoplasmic locomotion, and stretching in the presence of plaque extracts as compared to the HGF cells. As shown in Fig 10, extracellular glue, focal contact, and cell-metal fusion were observed, with the HGE adhering to Ti substrates.

Using transmission electron microscopy (TEM), differences in cellular behavior in the presence of plaque extracts were observed for the HGE and HGF cells. Confirming the light microscopy analyses, the TEM micrographs revealed intrusion of the HGE and the "pile up" phenomenon of the HGF after exposure to 5 FE for 1 day. The cytoplasmic extension of the HGE slipped into the detached space of the HGF, thereby allowing the HGE to closely adhere to the Ti substrates. The production of extracellular glue, cell-metal fusion, and focal contact were observed at the HGE-Ti film interface, and production of a hemidesmosome-like structure of HGE was observed at the HGE-HGF interface (Figs 11a and 11b).

Discussion

Although dental implants made from metallic, ceramic, or polymeric materials are biocompatible, they are nonetheless different from natural tissues, which may react to them as to a foreign body. As a result, epithelialization around the dental implant occurs naturally, causing the living tissues to reject

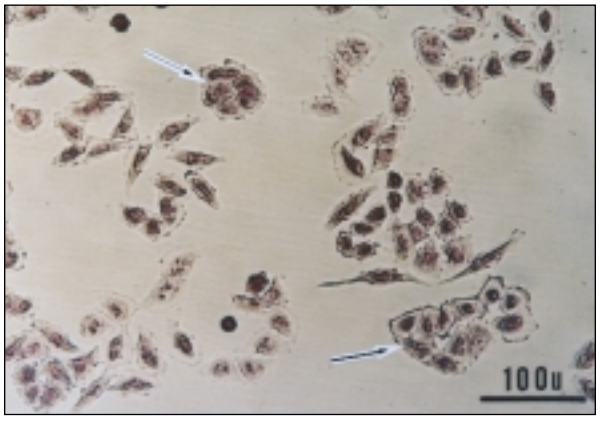

Fig 8 Close cell-cell contact of HGE *(arrows)* and loose contact of HGF after 3 days' cocultivation in the control medium.

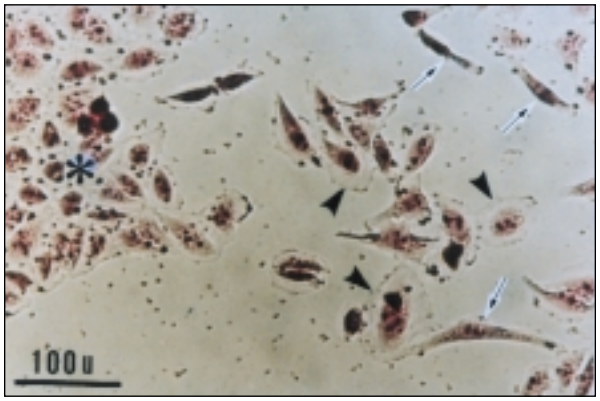

Fig 9a HGF *(arrows)*, HGE (*), and dispersed HGE *(arrowheads)* after 1 day of exposure to 5 FE.

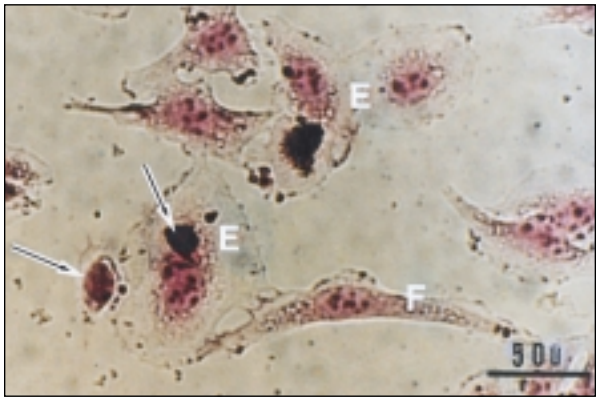

Fig 9b Enlarged magnification of Fig 9a showing "pile up" phenomenon with the degenerated HGF *(arrows)* and HGE (E) cells (F = HGF).

the implant. However, the factors causing apical epithelialization are apparently understood. Histopathologic investigations have indicated that the development and promotion of apical epithelialization are caused by: *(1)* the increasing multiplication potential, and *(2)* the increasing cell adhesion of the epithelial cells on/to the implant surface. In this study, the mechanism of the apical epithelialization at the implant-tissue interface was evaluated in vitro using both epithelial-like and fibroblastic cells derived from human gingiva around Ti surfaces.

Cell Multiplication. At the implant crevice, degenerative reactions of the epithelial cells have been observed at the outermost layer of the epithelium. Such reactions occur as a result of physicochemical irritation of exogenous factors, such as food debris, bacteria, setting cements, and restorative materials. At the inner layers of the epithelium, the epithelial cells were slightly agitated, thereby activating and increasing their multiplication.[9] Such an increase in epithelial cell multiplication has been suggested to promote apical epithelialization.

In this in vitro study, the presence of plaque extracts was observed to have an inhibiting effect on multiplication of the HGE and HGF cells. This result was in direct contrast to the accelerating effect observed in vivo on the multiplication of epithelial cells, suggesting that the in vivo observations may have been caused by a cascade of biologic events, including immunologic reactions. As observed in this study, the inhibiting effect of plaque extracts on the multiplication of HGF cells was greater than that on HGE cells. The HGE cells exhibited a higher resistance to exogenous toxic factors than the HGF cells. This higher resistance was likely the result of the intrinsic behavior of the epithelial cells to protect the inside of propria mucosa from outside factors.

The cells exposed to 5 FE were observed to have a greater decline in growth rate than the cells exposed to 0.22 FE. This finding suggests that bacteria may play a role in the growth rate of these cells. In the 0.22 FE, which was filtered with Millipore filters of 0.22-μm pore size, bacteria were screened. However, bacteria were not screened in the 5 FE, which was filtered with Millipore filters of 5-μm pore size. As a result, the media containing 5 FE and OPE were thoroughly contaminated with bacteria that originated from the plaque extracts after 5 days of exposure. The microparticles of the filtered tartar and plaque, which may contain cytotoxic elements such as proteases, col-

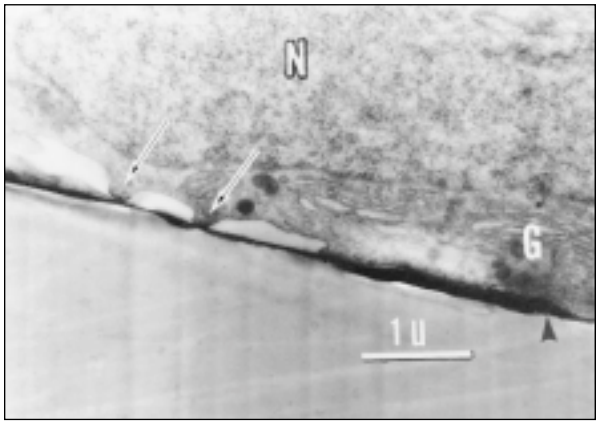

Fig 10 Focal contact *(arrows)* and cell-metal fusion *(arrowhead)* to titanium substratum. HGE after 3 days' cultivation in the control medium (N = nucleus; G = golgi apparatus).

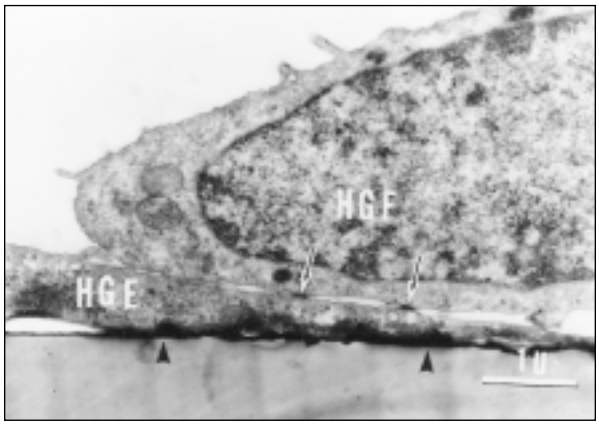

Fig 11a HGE and HGF cells after 2 days' cocultivation and 1 day of exposure to 5 FE. Note cytoplasmic intrusion of HGE into the detached space of HGF. The HGE was in contact with the titanium substratum, indicating cell-metal fusion *(arrowheads)*. The HGE was also in contact with HGF and hemidesmosome-like apparatus of HGE *(arrows)*.

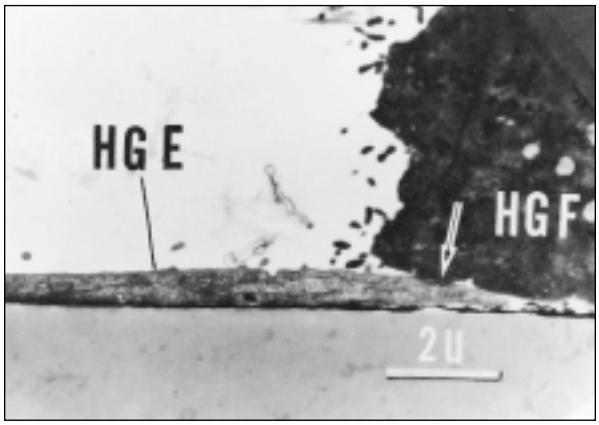

Fig 11b HGE and HGF cells after 2 days' cocultivation and 2 days of exposure to 5 FE. Note cytoplasmic extension of HGE into the space between degenerated HGF cells and the titanium substratum. *Arrow* indicates hemidesmosome-like apparatus.

lagenase, and lipopolysaccharides, should also be considered,[10] and these cytotoxic elements may have an inhibiting effect on the multiplication and adhesion of HGF and HGE cells. The inhibiting effect caused by the cytotoxic elements in filtered tartar and plaque will be addressed in future studies.

Cell Adhesion. In the presence of cytotoxic elements, cytoplasmic shrinkage of cells usually occurs. In addition, cell-cell contact and cell-substratum adhesion are loosened, resulting in detachment and separation of the cell from the substratum. Separation and detachment may be caused by the deterioration of extracellular glue at the cell-substratum interface, indicating the acceleration of autolysis and/or sublethal autolysis by lysosomal and nuclear enzymes under the lethal and/or sublethal condition.

Cell-Cell Contact. A dense HGE colony with close cell-cell contact was observed in this study. In contrast, a sparse HGF colony with loose cell-cell contact was also observed. The dense colonization of the HGE cells was suggested to be the result of the intrinsic behavior of epithelial cells, thereby promoting apical epithelialization at the implant-tissue interface.

The formation of dense colonization in vivo has been suggested to be the result of cellular selectivity. Cellular selectivity is a process in which cells of a given kind associate and combine with other cells during tissue repair after the gingival aggression. This process of cellular selectivity, whereby HGE and HGF cells formed independent colonies in the cocultivation, was observed in this in vitro study. This finding of a cellular selectivity process was in agreement with the findings of other studies indicating the resettlement of destroyed bone marrow from the circulated intact stem cells and the selective fusion of tissue in wound healing with self-sorting of cell types after random scrambling in vitro.

Cell Adhesion to Substratum. It has been reported that epithelial cells generally have greater adhesive strength to the substrate than fibroblasts.[8,11] The poor adhesive strength of the HGF cells observed in this study suggests that connective tissue may be more easily detached from the implant surface than epithelial tissue. The observed adhesive strength also implies that the epithelial tissues strongly adhered to the implant surface during the development of apical epithelialization. Absence of connective tissue adhesion to the implant surface was suggested during the development of apical epithelialization. Differences between the adhesive strength of the HGE and HGF were statistically increased under the sublethal condition. These increased differences suggest that bacterial contamination in plaque may be one of the possible mechanisms for the active promotion of apical epithelialization.

As observed under TEM, adhesion of the cocultivated HGE and HGF cells in the presence of 5 FE indicated the occurrence of apical epithelialization under the sublethal condition. The HGF cells were shrunken and detached from the substratum. The HGE cells were still intact, closely adhered to the Ti substrates, and exhibited focal contact and cell-metal fusion. Furthermore, cytoplasmic elongation and locomotion of the HGE were observed, with cells slipping into the detached space of the HGF.

At the material-cell interface, the existence of hemidesmosomes in vitro has been reported in epithelial cells adhered to cellulose film. The presence of hemidesmosomes in vivo, especially with metallic implants, has also been suggested by many investigators.[9,12] In ultrastructural analyses, the metallic implant–epithelial cell interface indicated the presence of hemidesmosomes and other types of attachment complexes.[13,14] However, with the exception of the study on ceramic implants by McKinney et al,[14] in these ultrastructural analyses, the hemidesmosomes did not exhibit typical structure. It has also been acknowledged by many investigators that the typical structure of hemidesmosomes at the cell–metal implant interface will not be observed in in vitro and in vivo testings.[5,15,16] Focal contact and cell-metal fusion at the interface have been reported mainly at the epithelial cell–metallic implant interface. Thus, this study suggested that the adhesive strength of epithelial cells to metallic implants may be dependent on extracellular glue, focal contact, and cell-metal fusion.

Conclusion

In this study, plaque extracts were observed to have a greater effect in decreasing the growth rate of HGF cells as compared to HGE cells. Similarly, the HGE cells exhibited greater adhesive strength compared to the HGF cells. Using light and electron microscopy, differences in the cells' resistance to plaque extracts were also observed. The HGF cells were observed to exhibit a shrunken and rounded morphology, with loose cell-cell contact after plaque exposure. Cytoplasmic shrinkage, with irregular and deformed nuclei, was observed in the HGE cells. In addition, the HGE partially retained its cell-cell contact after plaque exposure. In the HGE and HGF cocultivation, the HGE cells were observed to move into the detached space of the HGF cells. Evidence from this study suggested that the differences in the growth, contact, and adhesive strength of the HGE and HGF cells to Ti surfaces may promote apical epithelialization under the pathologic condition.

Acknowledgments

Financial support for this study was provided by a grant from the Training Institute of the Japanese Society of Oral Implantology (JSOI). The authors would like to thank Y. Mimura, DDS, and Ms Y. Ohta for their helpful suggestions and assistance in the study.

References

1. Steflick DE, McKinney RV, Parr GR, Sisk AL, Koth DL. What we know about the interface between implants and soft tissues. Oral Maxillofac Surg Clin North Am 1991;3:775–793.
2. Kawahara H. Biological seal. In: Kawahara H (ed). Oral Implantology, Vol 1. Tokyo: Ishiyaku, 1991:187–190.
3. Kawahara H, Moriwaki S, Takashima Y, Iwao T. Studies on the reconstruction and function of tissue under the heterogenous environment, Report 1: Effect of heterogenous environment upon the morphological and functional behavior of cell in vitro. J Oromaxillofac Biomech 1995;1:11–16.
4. Chehroudi B, Gould TRL, Brunette DM. Effects of a grooved epoxy substratum on epithelial cell behavior in vitro and in vivo. J Biomed Mater Res 1988;22:459–473.
5. Kawahara H. Biomaterials for dental implants. In: Wise DL, Trantolo DJ, Altobelli DE, Yaszemski MJ, Gresser JD, Schwartz ER (eds). Encyclopedic Handbook of Biomaterials and Bioengineering, Vol 2. New York: Marcel Dekker, 1995:1469–1524.
6. Kawahara H, Imai K, Kawahara D. Photo-pattern analysis and computation in the evaluation of the cytotoxicity of dental materials in vitro. Int Endod J 1988;21:100–105.
7. Kawahara H, Mimura Y, Oki M, Soeda Y, Nomura Y. In vitro study on cell adhesive strength to titanium with anodic oxidation and nitridation. In: Kawahara H (ed). Oral Implantology and Biomaterials. Amsterdam: Elsevier, 1989:169–175.
8. Kawahara H, Maeda T, Iseki T, Sanchez AJ. Studies on cell adhesion to biomaterials by viscometric method, in vitro, Report 2: On the adhesive strength of cells to glass surface. J Jpn Soc Biomater 1984;2:53–58.
9. Albrektsson T, Dahl E, Enbom L, Engevall S, Engquist B, Eriksson AR, et al. Osseointegrated oral implants. A Swedish multicenter study of 8,139 consecutively inserted Nobelpharma implants. J Periodontol 1988;59:287–295.
10. Nelson SK, Knoernschild KL, Robinson FG, Schuster GF. Lipopolysaccharide affinity for titanium implant biomaterials. J Prosthet Dent 1997;77:76–82.
11. Kawahara H. Cell adhesion to biomaterials, in vitro. In: Lin OCC, Chao ERS (ed). Perspectives on Biomaterials. Amsterdam: Elsevier, 1986:30–38.
12. Hanson HA, Albrektsson T, Brånemark P-I. Structural aspects of the interface between tissue and titanium implants. J Prosthet Dent 1983;50:108–113.
13. Gould TRL, Westbury L, Brunette DM. Ultrastructural study of the attachment of human gingiva to titanium in vivo. J Prosthet Dent 1984;52:418–420.
14. McKinney RV, Steflik DE, Koth DL. Evidence for a junctional epithelial attachment to ceramic dental implants: A transmission electron microscopic study. J Periodontol 1985;56:579–591.
15. Jansen JA. Epithelial Cell Adhesion to Dental Implant Materials. The Netherlands: Krips Repro Meppel, 1984.
16. Jansen JA, de Wijn JR, Wolters-Lutgerhorst JML. Ultrastructural study of epithelial cell attachment to implant materials. J Dent Res 1985;64:891–896.