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Effect of Root-Surface Modification of Human Teeth on Adherence of Fibronectin

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Summary.

Fibronectin [FN] is a promising agent in periodontal disease therapy for facilitating reattachment of connective tissue to human tooth-root surfaces. This study examined the influence of various surface treatments on promoting or hindering FN adherence to tooth roots. Rectangular sections of planed and non-planed tooth-root surfaces were acid-demineralized or incubated with collagenase. The tooth-root sections were treated with tritiated (FN) and its adherence was determined by liquid scintillation counting. The level of FN adherence was highest for non-planed acid-demineralized sections, and lowest for planed-only sections. This adherence varied with FN concentration and duration of contact for non-planed sections, but varied only with duration of contact for planed sections. Collagenase significantly reduced FN adherence to non-planed or planed acid-demineralized sections, but not to planed-only sections. These effects were attributed to tooth-root collagen, which can strongly bind FN.

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Introduction.

The adhesive glycoprotein, fibronectin (FN), consists of two 220 kilodalton polypeptide chains which possess specific regions responsible for a variety of histologic activities. FN mediates cell-to-cell and cell-to-extracellular matrix interactions, functions in cellular migration, morphology regulation, growth and alignment, and promotes wound healing (reviewed by Vartio, 1983; Yamada et al., 1985).

Fernyhough and Page (1983) demonstrated that FN greatly enhanced the attachment of human gingival fibroblasts to planed tooth-root surfaces. The purpose of the present study was to examine variables affecting FN adherence to non-planed as well as planed tooth-root surfaces in order to identify experimental treatments for optimizing FN adherence. Information of this nature could aid investigations designed to reattach fibroblasts to tooth roots.

Materials and Methods.

Root surface sections. Rectangular sections were cut from non-planed and from planed root surfaces of extracted human third molars that had not been involved in periodontal disease. The original tooth surface area for each section was calculated by caliper measurements, yielding a mean (± s.d.) sectional area of 12.62 ± 2.73 mm^2 (N = 208). For one portion of the study involving collagenase (Table 4), the conical end of a micropipette tip was attached to the original tooth surface of each section by dental sticky wax. Precise areas, with a mean (± s.d.) of 2.10 ± 0.04 mm^2 (N = 38), were thereby defined on the sections. The micropipette tips were in place only for the experimental steps involving collagenase or FN; they were removed prior to final rinsing procedures.

Acid demineralization and collagenase treatment of root surface sections. Sections were acid demineralized (AD) by exposure for 3 min to 25% (w/v) citric acid adjusted to pH 1.0 with HCl, after which they were washed 8X with phosphate-buffered saline (PBS), pH 7.4 (Voller, Bidwell and Bartlett, 1976). Certain sections were incubated with 700 units/ml of Type VII collagenase from *Clostridium histolyticum* (Sigma Chemical Co., St. Louis, MO, USA) for 30 min at 37°C, after which the sections were washed 7X with PBS.

Preparation of ^{3}H-FN. Tritiated fibronectin (^{3}H-FN) was prepared from tritium-labelled formaldehyde, as described previously (Pederson, Lamberts and Shklair, 1988). Five and ten µl samples of this solution were counted on a model LS 7500 liquid scintillation counter (Beckman, Irvine, CA, USA), yielding an average of 4,600 counts per minute (CPM) for each µg of labelled FN.

Root surface treatment. For treatment of tooth-root sections with 3H-FN, 5 to 20 sections at a time were placed one-per-well, with original root surfaces uppermost, in a 96-well microplate that had been pretreated with 1% bovine serum albumin and contained 100 µl PBS per well. All sections were incubated at 37°C for 1 h with 32, 40, 48, 80, 104, or 160 µg ^{3}H-FN per section, except for the use of 3 h for one series (Table 2). Excess ^{3}H-FN was removed after each incubation by nine washes with PBS containing 0.1% Tween 20. Bound ^{3}H-FN was removed only from the uppermost side of each section, representing the original tooth surface. The ^{3}H-FN was recovered from this surface as follows: Ten µl of freshly prepared 5% trypsin (Type III, Sigma Chemical Co., St. Louis, MO) in PBS was applied to the section, then removed after 4-6 min along with any released ^{3}H-FN. This step was repeated four times, after which the section was rinsed with five successive 10-µl volumes of PBS. All of the recovered fluids were pooled and combined with Ready-Solv HP counting fluid (Beckman) in a scintillation vial. Radioactivity was determined as disintegrations per minute (DPM), and the DPM per mm^2 calculated for each section. The mean DPM per mm^2 was then determined for each experimental and control group.

Results.

Data presented in the first three tables (not involving collagenase) were acquired from the entire upper surfaces of tooth-root sections. Table 1 demonstrates the effects of acid demineralization on FN adherence to planed and non-planed tooth-root sections. Although FN adherence was only about half as much for planed as for non-planed sections, acid demineralization increased FN adherence significantly in both cases.

TABLE 1.

INFLUENCE OF ACID DEMINERALIZATION [AD] ON FIBRONECTIN [FN] ADHERENCE TO NON-PLANED AND PLANED TOOTH-ROOT SECTIONS

SET	SURFACE TREATMENT	N	MEAN DPM*
1	Non-Planed	14	299 ± 144
2	Non-Planed + AD	15	725 ± 157
3	Planed	20	149 ± 21
4	Planed + AD	20	619 ± 141

* Mean ± s.d. per mm^2 section surface area.
104 µg FN applied per section, with N = number of sections

Analysis of variance (ANOVA) revealed statistically significant effects ($p<0.001$) both from acid-demineralization and from planing, but showed no significant interaction of these variables.

The effects of FN concentration on its adherence to the non-planed or experimentally modified tooth-root sections are presented in Table 2.

TABLE 2.

INFLUENCE OF FIBRONECTIN [FN] LEVEL ON FN ADHERENCE TO NON-PLANED, PLANED, AND PLANED ACID-DEMINERALIZED [PLANED AD] TOOTH-ROOT SECTIONS

SERIES	µg FN PER SECTION	MEAN DPM [± s.d.] PER mm² SECTION SURFACE		
		Non-Planed	Planed	Planed AD
1	32	115 ± 32	90 ± 14	256 ± 77
2	80	183 ± 103	123 ± 36	363 ± 55
3	160	215 ± 100	118 ± 19	571 ± 154

Seven sections were used per set within each series

ANOVA revealed a significant ($p<0.001$) main effect of condition within each test group. Post-hoc comparisons by the Least Significant Difference method showed that the non-planed and planed groups did not differ significantly from one another, but both groups differed significantly ($p<0.05$) from the planed acid-demineralized group. It is also evident that increased FN concentration had little effect on FN adherence to the planed sections but resulted in markedly increased FN adherence to the planed acid-demineralized sections.

Table 3 presents data on FN adherence after tooth-root:FN contact periods of 1 and 3 hours.

TABLE 3.

INFLUENCE OF TOOTH-ROOT/FIBRONECTIN [FN] CONTACT PERIOD ON FN ADHERENCE TO NON-PLANED, PLANED, AND PLANED ACID-DEMINERALIZED [PLANED AD] TOOTH-ROOT SECTIONS

S[illegible]S	FN CONTACT PERIOD [H]	MEAN DPM [± s.d.] PER mm² SECTION SURFACE		
		Non-Planed	Planed	Planed AD
1	1	130 ± 12	107 ± 9	225 ± 26
2	3	266 ± 54	245 ± 22	424 ± 77

Two sections were used per set within each series, and 48 µg FN was applied per section

ANOVA revealed statistically significant effects both from the type of surface treatment ($p<0.01$) and from the period of tooth root:FN contact ($p<0.001$); however, there was no significant interaction of the period of contact and surface treatment variables.

The application of collagenase (Table 4) demonstrated the effects of collagen destruction on FN adherence, there being a significant decrease in FN binding in all cases except for the planed tooth-root sections (t test).

TABLE 4.

INFLUENCE OF COLLAGENASE [C] ON FIBRONECTIN ADHERENCE TO NON-PLANED ACID-DEMINERALIZED [AD], PLANED, AND PLANED AD TOOTH-ROOT SECTIONS

SET	SURFACE TREATMENT	N	MEAN DPM*	SETS COMPARED	P
1	Non-Planed	4	11,049 ± 1,660	1 vs 2	<0.01
2	Non-Planed +C	5	5,260 ± 1,572		
3	Non-Planed +AD	4	20,203 ± 4,158	3 vs 4	<0.001
4	Non Planed +AD+C	5	4,784 ± 746		
5	Planed	5	638 ± 203	5 vs 6	NS
6	Planed +C	5	475 ± 118		
7	Planed +AD	5	1,128 ± 43	7 vs 8	<0.01
8	Planed +AD+C	5	652 ± 187		

* Mean ± s.d. per mm^2 section surface area
40 μg FN applied per section, with N= number of sections per set

The data obtained under these conditions also confirm the effects of planing and acid demineralization identified in Table 1 for whole section surfaces. As mentioned earlier, these tests were conducted on areas of sections that had been defined by micropipette tips. The application of FN to these areas produced an FN concentration at the tooth-root surfaces that averaged 2.8X higher than had occurred for the whole sections (Tables 1-3). The DPM per mm^2 data for Table 4 also averaged 5.5X higher for planed surfaces than the corresponding data of Tables 1-3. However, the data in Table 4 for non-planed surfaces averaged over 50X higher than the corresponding data of Tables 1-3. Although these findings could be explained in part as due to lack of surface saturation by FN in the whole section experiments (Tables 1-3), the reason for the unusually strong FN binding to the non-planed surfaces of Table 4 is not immediately apparent.

Discussion.

Although this study has been limited to the identification of tooth-surface treatments that will maximize FN binding, our findings could find application in clinical studies of methods to promote repair of periodontally diseased tissues. Caffesse et al. (1985) examined the effects of FN addition to citric acid-demineralized tooth roots of rhesus monkeys after mucoperiosteal flap surgery. An enhanced proliferation of connective tissue cells was observed, along with significant increases in connective tissue reattachment to the FN treated areas. Other investigators (Bowersox and Sorgente, 1982; Tsukamoto, Helsel and Wahl, 1981) have suggested that FN may promote wound repair by functioning as a chemoattractant for fibroblasts. Falcone et al. (1984) found that the breaking strength of wounds was 30% greater for wounds receiving FN application than for control wounds when examined 7 to 21 days after wound production.

We used root planing in this study even though the teeth employed had not been involved with periodontal disease. Fernyhough and Page (1983) determined FN adherence to root-planed dentinal surfaces, and found essentially no differences in data for surfaces of teeth that had or had not been involved in periodontal disease. Hence, this consideration would appear to be minor, particularly in view of the significantly higher FN adherence observed for planed acid-demineralized tooth-root sections compared to the planed-only or the non-planed control sections.

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