HUMAN HISTOLOGIC REPAIR AND REGENERATION AFTER BIOLOGIC PREPARATION OF DISEASED ROOT SURFACES

M. R. WIRTHLIN

NAVAL DENTAL RESEARCH INSTITUTE

Naval Medical Research and Development Command
Bethesda, Maryland

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M. R. WIRTHLIN

Research Progress Report NDRI-PR 83-19
Work Unit 63706N M0095PN003.3017
Naval Medical Research and Development Command
Naval Medical Command, National Capital Region
Bethesda, Maryland 20814

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G. E. CLARK
Captain, Dental Corps,
United States Navy
Commanding Officer
The treatment of the diseased root surface in humans is probably as important as methods of handling the gingiva and bone for attempts to achieve repair or regeneration of periodontal attachment loss, due to inflammatory periodontal disease (1). Most procedures for new attachment achieve a new dentogingival junction comprised almost entirely of junctional epithelium (2). Reviews of the long term results of therapy indicate that such results are maintainable with adequate levels of personal hygiene by the patient and frequent professional care (2-4). There has been a concern that a dentogingival junction of epithelium may be subject to failure (5); however a recent report by Ramfjord (6) indicated that the attachment does not open and close, but is stable.

A connective tissue new attachment would be teleologically desirable, and may give support to the tooth, but there is no information on the relative strength of epithelial versus connective tissue attachments, nor how long the "ideal" junctional epithelium attachment should be, postoperatively, in order to form a "seal".

Attempts to achieve connective tissue new attachment have been directed at removing the epithelium lining the pocket, implanting of material to regenerate lost bone, and root planing to prepare the root surface (2). Experimental methods of therapy recently reported have used chemicals as adjuncts to root preparation. Human clinical and histological responses following the use of citric acid (7-11), trichloracetic acid (12), formalin (13) and strongly alkaline sodium hypochlorite (14) on the diseased root surface, have interested clinicians.

Wirthlin and Hancock have reported on the use of biological products for detoxification of the diseased root surface (15-18). Their method used sodium deoxycholate to dissociate endotoxin, and human plasma fraction Cohn IV, to bind it. This approach might avoid invasive, deep, root planing or harsh chemicals. This report is an evaluation of that approach histologically in humans.

MATERIALS AND METHODS

Patients selected for this study were under treatment at the Naval Training Center, Great Lakes, Illinois, and had been independently diagnosed as having generalized, severe periodontitis, and requiring immediate complete dentures. Written informed consent was obtained from each patient.

Presurgical preparation in all cases consisted of oral hygiene instruction, periodontal scaling and polishing. Two of the patients permitted surgical removal of six treated teeth for histologic study. Local anesthesia was used in all surgical procedures.

Patient "A" was a 62-year-old black male who claimed to be a diet-controlled, adult-onset diabetic. This was confirmed by his physician, and his current blood sugar was 140 mg/100 ml. The mean pocket depth of all his remaining maxillary teeth was 5.0 mm. The maxillary incisors were extruded, had a clinical mobility of three, and, because of a collapsed bite, were driven labially upon closure in habitual centric. He complained that he could not bite on these teeth because of soreness of the teeth and gingiva. The gingivae were fibrotic, stippled, dull red at the margins, and pus was expressed upon palpation between the maxillary central incisors (Fig. 1).
Patient "B" was a 60-year-old white male who denied any significant medical problems. He had recently lost the lower left first premolar and lower right lateral incisor because of the bone loss beyond the apex from periodontal disease. The remaining mandibular anterior teeth were mobile and not suitable denture abutments. Periodontal pockets on the facial of the lower canine teeth extended 4 to 5 mm, and were apical to the mucogingival junction. The gingivae were firm and pink. Calculus deposits were heavy, despite frequent brushing and use of a pulsing irrigator twice a day. The patient was asked to discontinue the use of the irrigator and was shown how to remove bacterial plaque deposits by rubbing with toothpicks and floss (Fig. 2).

Surgical Procedures

Two weeks after periodontal scaling and polishing, a scalloped, inverse bevel incision was made through the gingival margin to the alveolar crest and mucoperiosteal flaps were elevated for 2 mm past the alveolar crest. The pocket lining and connective tissue coronal to the base of the pocket were removed. The relationship of the cementoenamel junction to the depth of probing and the alveolar crest are presented in Table 1 for the sites from which surgical specimens were later recovered. For patient "B", the pocket base was at the alveolar crest. The roots were lightly nicked with the scalpel blade to mark the pocket base. Any residual calculus deposits were removed with curette scalers, but no root planing was done. The experimental root surfaces were treated by rubbing with sterile cotton pellets moistened with a 2% solution of sodium deoxycholate* in sodium chloride injection, USP,** for one minute, rinsed with physiological saline, then rubbed with 5% solution of human alpha globulins plasma fraction Cohn IV,*** for one minute, and rinsed again. The plasma fraction was tested previously and found free of hepatitis B surface antigen. The control surfaces were rubbed with cotton pellets moistened with phosphate buffered physiological saline (PBS) for one minute. The gingival flaps were returned as closely as possible to their original location, held for two minutes with finger pressure over saline-moistened gauze, and stabilized with interrupted 0000 silk sutures mounted in atraumatic 3/8 circle reverse cutting needles, and a periodontal cement dressing.**** No antibiotics were prescribed. The patients were seen weekly for postoperative care. The cement and sutures were removed at one week, and the teeth polished at each weekly visit. At 28 postoperative days, the teeth were removed with the gingiva attached; these sections were each approximately 3 mm wide and included about 2 mm of the alveolar crest. All extraction sites healed uneventfully. The immediate dentures, delivered at the time of teeth removal, were adjusted as required. Specimens were fixed in formalin, decalcified in 4% nitric acid, and embedded in paraffin. Serial sections were cut at 8 μ and stained with hematoxylin and eosin.

Clinical Observations

Patient "A" was unable to retain the cement dressing on the palatal surface, tearing the suture out between teeth 8 and 9. This patient presented at each weekly postoperative visit with food impactions and plaque interdentally. 

* Sodium deoxycholate, Pfaltz and Bauer, Stanford, CT 06902.
** Sodium chloride injection, USP, Abbott Labs, North Chicago, IL 60064.
*** Human Alpha Globulins Factor IV-1, Miles Research Products, Elkhart, IN 46514.
**** Coe-Pak (no eugenol, Coe Laboratories, Inc., Chicago, IL 60658.
and on the palatal aspect of the incisors. The teeth remained loose and the gingival margin on the palatal surface was inflamed. During removal of the specimens, the tissue attachment to the mesial surface of tooth 6 was inadvertently separated.

Patient "B" also lost some cement dressing, but the gingival tissue had a good appearance. Except for some shrinkage and recession, the postoperative course was uneventful, and the gingivae were firm, pink and well adapted to the teeth 28 days postoperatively.

Histologic Observations

Patient "A". Control surfaces treated with PBS. On the mesial surface of tooth 6 the gingiva was separated from the root. There were some shreds of connective tissue on the root apical to the nick marking the pocket base, but no cells were found coronal to the nick. On the distal surface of tooth 8 the pocket epithelium was hyperplastic to the level of new plaque deposits. Junctional epithelium extended apically, and about 0.6 mm past the nick along the root surface. The connective tissue was infiltrated with lymphocytes subjacent to the pocket and junctional epithelium (Fig. 3).

Patient "A". Experimental surfaces. On the distal surface of tooth 9 there was hyperplastic pocket epithelium found to the level of new plaque deposits. Junctional epithelium extended apically on the root surface, stopping 0.1 mm coronal to the nick. A separate small clump of epithelium was found in the nick, probably residual epithelium left from the surgery. The connective tissue was infiltrated with lymphocytes subjacent to the pocket and junctional epithelium (Fig. 4). There was hyperplastic pocket epithelium on the mesial surface of tooth 11 to the level of the apical extent of plaque deposits. Apically, junctional epithelium six to eight cells thick, but with some rete extensions into the connective tissue, was attached to the root surface to a point about 0.7 mm coronal to the nick. Lymphocytes infiltrated the connective tissue subjacent to the pocket and junctional epithelium. Between the apical junctional epithelial cell and the nick the connective tissue fibers and flattened fibroblasts were oriented parallel to the root surface (Fig. 5).

Patient "B". Control surface treated with PBS. On the facial surface of tooth 22 there was a shallow gingival sulcus, and long junctional epithelium attached to the root surface, extending about 1.0 mm from the base of the sulcus to just past the nick in the root. A light infiltration of lymphocytes was seen in the connective tissue subjacent to the junctional epithelium (Fig. 6).

Patient "B". Experimental surface. On the facial surface of tooth 27 there was a shallow gingival crevice, and a junctional epithelium attached to the root surface for a distance of about 0.6 mm. There was a scant infiltration of lymphocytes subjacent to the epithelium. From the apical junctional epithelial cell to the nick, a distance of about 0.5 mm, there was connective tissue (Fig. 7). The connective tissue was separated from the root surface up to 0.04 mm, and it was interspersed with rounded and flattened nuclei of connective tissue cells. The surface facing the separation had a thin layer of pink refractile material thought to be cementoid (Fig. 8).
DISCUSSION

The results presented constitute case reports, which, due to difficulties associated with obtaining good histologic material from humans, provide only an indication of the treatments possible with the biologic preparation of diseased root surfaces. The treatment approach tested has yet to withstand a clinical trial and rigorous statistical analysis of clinical results. Also, there have not been independent investigations for corroboration of the efficacy of the approach or the preliminary studies in its development.

The therapeutic goal is to maintain the dentition in health and good appearance for a lifetime. Oral hygiene is the most important factor in the prevention and treatment of inflammatory periodontal diseases. Personal hygiene by the patient, daily and thoroughly, is a prerequisite for successful treatment. Once the patient has understood his role, the clinicians assist by scaling off calculus deposits, and polishing away plaque, stain, and pellicle. This alone can result in shrinkage and healing of pockets, both of these reducing the pocket depth (19). Normally, at least four to six weeks are allowed for the healing in our practice. Less time was allowed in this report because of the requirement to deliver the final restoration to the patient. Upon reevaluation the clinician has the option to repeat the scaling in areas that fail to resolve, or to expose the site surgically for access, to complete the removal of plaque, calculus, and pellicle from the root surfaces. Surgical procedures may also be done to repair a residual deformity. With gingival flap curettage procedures, sometimes combined with bone implants, the clinician often attempts to obtain a new attachment of the dentogingival junction. The result, histologically, is most often a dentogingival junction with a long junctional epithelial attachment (2). Since the periodontal probe penetrates the pocket to the point where healthy connective tissue backs up the junctional epithelium (20) the clinician does not know the exact nature of the new attachment — connective tissue, epithelium, or some combination thereof. To date, the long junctional epithelial attachment has formed whether the root surface was scaled, planed, or even denuded of cementum. Recently, more attention has been paid to the nature of the changes which occur in a diseased root surface, and to the search for root surface treatment methods that would promote connective tissue new attachment. The diseased root surface is hypermineralized (21), and it adsorbs toxins (22,23) and antigens (24). Extensive root planing to remove the cementum often results in a hypersensitivity most uncomfortable for the patient (25). The use of acids or other strong chemicals seems to be harsh treatments to use in a surgical procedure.

The hypothesis which is the basis for the biological preparation of diseased root surfaces is that endotoxin is an adsorbed noxious agent which destroys the viability of the diseased root surface and inhibits connective tissue cell adherence (26). Endotoxin is a complicated lipopolysaccharide which can be dissociated by sodium deoxycholate (bile salt) or detergent compounds. The dissociation reduces its pyrogenicity, which is regained when the bile salt is diluted, for the endotoxin reaggregates (27).

Besides dissociating endotoxin, bile salt could have other cleaning effects by its detergent like action. It can remove residual junctional epithelium (28) and has antibacterial properties (29), probably acting on the phospholipids in cell membranes.
Certain plasma factors can bind the dissociated endotoxin and prevent its reaggregation. Human plasma fraction Cohn IV has been reported to have this activity (30). After numerous in vitro tests on diseased root surfaces of extracted human teeth, the combination of bile salt followed by plasma fraction was found to produce a statistically significant result in adherence of gingival fibroblasts (5,16). Since trials with gamma globulins and albumin-rich biological materials did not give significant results, the activity was thought to lie in the alpha and beta globulins. In particular, the alpha-2-surface binding glycoproteins can opsonize endotoxin (31). Fibronectin may play this role (32). Recent experiments with fibronectin and Cohn IV, have demonstrated amorphous ring-like attachments to reaggregated endotoxin particles adsorbed to surfaces, and also within phagocytic vacuoles of fibroblasts. Thus fibronectin could promote the clearance of adsorbed endotoxin from a contaminated surface. Fibronectin also promotes cell adherence, and enhances fibroblast cell attachment to endotoxin-adsorbed surfaces better than any biological agent yet tested (33). Fibronectin could possibly promote the coronal migration of cells from the periodontal ligament and result in connective tissue new attachment by cementogenesis. One unresolved question has been the depth of endotoxin contamination in diseased root surfaces. Recent experiments suggest it is only a superficial surface adsorption (34).

The control to the in vitro tests was phosphate-buffered physiological saline. Rubbing diseased root surfaces with this may also be an effective treatment, if one considers the root surface similar to a hydroxyapatite column used in the laboratory, and from which certain material can be eluted by use of buffers. There is some indication buffers promote connective tissue cell attachment (35).

The findings presented above support the hypothetical treatment approach, but since endotoxin has many biological activities, in vitro tests were necessary. Using chronic periodontal pockets created in monkeys, the combination bile salt and plasma fraction treatment not only produced a viable surface for a new dentogingival junction but appeared to promote connective tissue new attachment and cementogenesis. The first qualitative result on maxillary incisors (17) was supported by a qualitative and statistically significant quantitative result on the proximal surfaces of posterior teeth (18). Therefore, permission was obtained for a limited clinical trial in human volunteers.

Patient "A" was not the best of patients for such a trial because of the unmanageable situation of the dentition. Our interpretation of the connective tissue attachment result on the mesial of tooth 11 was that of a "collagen adhesion". Patient "B" appeared to be a better subject, and there seemed to be a clear qualitative difference in the results on his experimental site, tooth 27. The evidence is quite limited, however. More experiments are anticipated to develop the hypothesis for the biological treatment of diseased root surfaces.

SUMMARY AND CONCLUSION

Two human volunteers contributed teeth and a small portion of the periodontium for histological evaluation of a new approach to the biological treatment of diseased root surfaces. Two of the three specimens treated by rubbing with cotton pellets moistened in physiological saline healed with
long junctional epithelial attachments, and the third specimen was inadvertently lost. Three specimens treated with bile salt and plasma fraction to detoxify the diseased root surface healed with 0.1, 0.5 and 0.7 mm of connective tissue attachment, and one of these appeared to present new cementum formation at 28 days after the surgical treatment.

The hypothetical basis for the biological treatment was briefly reviewed, and it appears to offer a rational alternative to invasive, deep root planing or the use of harsh and caustic chemicals. The treatment consists of surgical access by periodontal flap curettage, scaling to remove residual plaque and calculus, and detoxification of the diseased root surface with biological products.
REFERENCES


Table 1
Clinical Measurements for Control and Experimental Defects at Surgery, in Millimeters

<table>
<thead>
<tr>
<th>Patient</th>
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<th>Control Surfaces*</th>
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<th>Experimental Surfaces</th>
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<td>CEJ to Gingival Margin</td>
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*M, mesial; F, facial; D, distal; L, lingual.
Figure 1. Patient A. Radiographs present 30 to 70% bone loss. Radiographs are viewed from the lingual, with tooth 11 to the reader's left. Charting of conditions at the time of surgical treatment. Tooth 6 mesial and tooth 8 distal served as control sites treated with PBS on the root surface. Tooth 9 distal and tooth 11 mesial were experimental sites. The diagonal lines denote missing teeth, the vertical line shading denotes the pocket form, and the heavy black line denotes the mucogingival junction. The Roman numerals indicate mobility.
Figure 2. Patient B. Radiographs present 30 to 60% bone loss, with intrabony defects to the apices of teeth 21 and 26. The charting of conditions at the time of surgical treatment presents pockets apical to the mucogingival junction on the facial of tooth 22, control site, and tooth 27, experimental site.
Figure 3. Human specimen from Patient A control site removed 28 days after surgery. Tooth 8 distal surface presents the apical junctional epithelial cell (black line) about 0.6 mm apical to the nick (white line). (H&E. Original magnification X16).
Figure 4. Human specimen from patient A experimental site removed 28 days after surgery. Tooth 9 distal surface presents the apical junctional epithelium (black line) about 0.1 mm coronal to the nick (white line). A separation of epithelium is found in the nick. (H&E. Original magnification × 10).
Figure 5. Human specimen from patient A experimental site removed 28 days after surgery. Tooth 11 mesial surface presents the apical junctional epithelial cell (black line) about 0.7 mm coronal to the nick (white line). (H&E. Original magnification X32).
Figure 6. Human specimen from patient B control site removed 28 days after surgery. On the facial surface of tooth 22 the apical junctional epithelial cell (black line) is about 0.1 mm apical to the nick (white line). (H&E. Original magnification X16).
Figure 7. Human specimen from patient B experimental site removed 28 days after surgery. On the facial surface of tooth 27 the apical junctional epithelial cell (black line) is about 0.5 mm coronal to the nick (white line). (H&E. Original magnification X16).
Figure 8. Human specimen from patient B tooth 27 facial surface experimental site. The connective tissue coronal to the nick (white line) has separated from the acellular cementum surface. On the separated connective tissue surface there was a pink-stained, amorphous, refractile thin layer of material thought to be cementoid. The separation is thought to be an artifact. (H&E. Original magnification X40).
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Naval Dental Research Institute
Naval Base, Building 1-H
Great Lakes, Illinois 60088

Naval Medical Research and Development Command
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Bethesda, Maryland 20814

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Case reports of two human volunteers are presented with histologic evaluation of the results 28 days after periodontal flap surgery. Three specimens were treated with phosphate-buffered physiological saline applied to the diseased root surface for one minute (control), and three specimens were treated with an experimental combination of 2% bile salt followed by 5% human plasma traction for one minute each. There are indications that a connective tissue new attachment may be possible following the biologic preparation of diseased root surfaces.