Healing of Periodontal Flaps Following Use of MBR 4197 (Flucrylate) in Rhesus Monkeys

A Biometric and Histometric Evaluation*

by

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SUTURING IS USED for fixation of periodontal mucoperiosteal flaps, but it is a time consuming and tedious procedure. Tears associated with less than perfect sutures also may occur. Sutures may act as a wick absorbing microorganisms beneath the surgical flap. Hemorrhage is not always controlled with sutures.

Tissue adhesives are being developed which may eliminate the need for sutures while accomplishing the goal of flap fixation and adaptation as well as hemorrhage control. Cyanoacrylate tissue adhesives may reduce surgical time, are easy to apply, and are effective for hemostasis.

A fluoroalkyl cyanoacrylate was reported in 1968. Tested in animals, it showed to have desirable physical properties with low systemic and local toxicity.[1] This product (MBR 4197-Flucrylate) has shown promising results in periodontal surgery. No delay in healing has been reported and in many instances, it has been claimed to be faster when this material was used as a periodontal dressing.[2] Recently we reported the clinical and histologic results obtained with MBR 4197 when compared to conventional sutures in promoting tissue readaptation and healing after modified Widman flap surgery in monkeys.[3]

The purpose of this study is to report the biometric and histometric results obtained when using this cyanoacrylate compound (trifluorisopropyl cyanoacrylate) and conventional suturing following modified Widman flap surgery in monkeys. As a consequence this report supplements the previous publication from our laboratory.[3]

MATERIALS AND METHODS

Six adult Rhesus monkeys, five males and one female, of average weight 7 kg (ranging from 5½ to 8 kg) were used in this study. All animals had a moderately severe chronic gingivitis with supragingival and subgingival calculus. A few areas of localized recession and attachment loss were found. Full mouth roentgenograms were taken prior to the experimental procedures.

Scaling and rubber cup polishing were performed at weekly intervals both before and after the surgery, up to the time of sacrifice. There was marked reduction in the severity of gingivitis after institution of the weekly prophylaxes. There was, however, a tendency for persistence of mild marginal inflammation.

Two days prior to surgery, clinical photographs and full mouth charting were obtained for each animal. A thin periodontal probe‖ was used to take measurements on all experimental teeth as described for the selected teeth in the PDI index.[4] Measurements were taken from the cemento-enamel junction to the free gingival margin and from the free gingival margin to the base of the clinical sulcus. Each tooth was measured at six points: the mesio-facial and mesio-lingual, facial and lingual midpoints, and disto-facial and disto-lingual. The interproximal measurements were made as close as possible to the contact area. Buccal and lingual measurements for multirooted teeth were obtained from the most prominent aspect of the mesial root.

Difficulty was encountered in locating the cemento-enamel junction interproximally on maxillary posterior teeth. Notches were placed interproximally on these teeth with a No. 1 round bur. The notches, placed 1 to 2 mm coronal to the gingival margin, served as reference points on the maxillary posterior teeth.

The monkeys were randomly divided into three groups of two monkeys each, to be sacrificed at 14 days, 35 and 180 days postsurgery.

The jaws of each animal were divided into three maxillary and mandibular segments. The cuspid teeth marked the division between the segments. The cuspids and third molars were not included in the study.

Sodium pentobarbital IV (Nembutal¶) was the general anesthetic used for initial scaling and all surgical procedures. Local anesthesia (lidocaine hydrochloride, 2% with 1:50,000 epinephrine**) was also used during surgical procedures to aid in control of hemorrhage. For the weekly prophylaxes, ketamine hydrochloride, IM (Vetalar††) adequately sedated the monkeys for instrumentation.

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‖ M-1 probe, Marquis Manufacturing Company, Denver, Colorado.

¶ Abbott Laboratories, North Chicago, Ill. Dose 30 mg/kg body wt.

** Astra Pharmaceutical Products Inc., Worcester, Mass.

†† Parke-Davis, Detroit, Mich. Dose 200 mg per animal.

The surgical treatment consisted of modified Widman flaps[5] in all segments.

Since the monkeys did not have appreciable periodontal pockets, it was necessary to remove some crestal bone in order to achieve flap adaptation comparable to humans with periodontal disease. Furcations were not opened.

At completion of all surgical procedures, the flaps were thoroughly irrigated with sterile saline and closely adapted to the necks of the teeth. All surgery was performed by one operator.

Prior to securing the flaps, measurements were taken from the cemento-enamel junction to the alveolar crest at the facial and lingual midpoints.

Following surgery, experimental and control segments were determined by the "flip of a coin". The posterior segments were sutured on one side and cyanoacrylate was used on the other side. Anterior segments were treated with cyanoacrylate in the maxilla and sutures in the mandible. The reverse order was used for the other monkey in each experimental time period. All segments were operated except for one monkey in the 6 month group. Due to the absence of lower incisors, the anterior segments of this monkey were not included in the study.

Suture material was 0000 braided black silk* with a curved, atraumatic needle. No conventional periodontal dressing were used.

MBR 4197 (Flucrylate†) was supplied in liquid and aerosol forms by the manufacturer.† It was determined from trial operations that thin, even application of monomer was difficult to obtain with the liquid form compared to the aerosol. Aerosol spray of flucrylate was used for all applications in this study. Monomer was applied rapidly while another investigator held the flaps securely in place. A thin even coating of adhesive resulted from this technique in a matter of a few seconds. Occasionally, a local area was resprayed to control hemorrhage.

Face masks were used for protection of operator personnel during surgery and application of the adhesive spray. The monkeys were protected from the spray with gauze placed over the eyes and throat packs.

After the flaps were secured, clinical measurements were taken of the flap margins. Two measurements were taken on each tooth: one oral midpoint from the cusp tip to the flap margin and a mesial lingual measurement from the marginal ridge to the flap on all teeth except the maxillary posteriors. For these teeth, the mesial measurement was taken from the notch to the flap margin.

A plastic collar was placed on all monkeys for 1 week following surgery to prevent disruption of the operated areas. Each animal was kept on systemic antibiotics‡ and a soft diet for 1 week. Sutures were removed and a rubber cup polish accomplished at 1 week postoperatively. A rubber cup polish was done at weekly intervals until sacrifice.

One monkey of the 14 day group died approximately 12 hours after surgery, of postanesthetic complications unrelated to the material being tested. All other animals were sacrificed by exsanguination according to the experimental schedule of 14, 35, and 180 days.

Periodontal charting of all experimental teeth according to the PDI technique were obtained 1 week prior to sacrifice. No follow-up scoring was done on the 14 day group. The 180 day group were charted at the same time as the 35 day group in addition to 1 week prior to sacrifice.

Following sacrifice, the jaws were dissected and processed to secure bucco-lingual sections of posterior teeth for histometric evaluation. The anterior teeth were not used for this purpose.

Histometric measurements were made facially and lingually in posterior teeth for the distances: (a) From the cemento-enamel junction to the apical base of the junctional epithelium. (b) From the cemento-enamel junction to the alveolar crest.

The histometric measurements were made with a screw micrometer eyepiece,§ held parallel to the specimen surface in a standard microscope.‖ Three measurements were taken from each point and were averaged. Histometric values were converted to millimeters by calibrating the eyepiece with the grid on a hemacytometer.¶

Statistics

Statistical analysis was performed using the Michigan Interactive Data Analysis System (MIDAS).** Measurements were subjected to analysis of covariance of postoperative scores at the indicated time intervals with initial scores as covariates for each variable.

Pairwise *t* tests were used to evaluate the change in clinical scores from initial to 35 days postoperative. Pairwise *t* tests were also used to compare the histometric measurements at 35 and 180 days.

Results

Biometrics

Analysis of covariance of postoperative clinical scores with initial scores as covariates revealed that the monkeys were not similar enough to group all of the scores together for a given time interval. Significant differences among the initial scores of the group indicated statistical evaluation was required on an individual basis.

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* Ethicon Inc, Somerville, NJ.

† Minnesota Mining and Manufacturing Company, St. Paul, Minn.

‡ Cosa Terramycin R., Department of Veterinary Medicine, Pfizer Inc., New York, NY. Daily dose 100 mg dissolved in water.

§ Bausch and Lomb, Filar Micrometer Eyepiece, Rochester, New York 14602 Cat. #31-16-50.

‖ American Optical Instrument Company, Buffalo, New York 14215

¶ Spencer Bright Line Hemacytometer, American Optical Instrument Company, Buffalo, New York 14215. Cat. #4011.

** Amdahl 470V/6 computer.

Measurements from each animal were subjected to analysis of covariance comparing adjusted means of suture with cyanoacrylate treated sides at 35 and 180 days, except for the following variables:

1. Mesio-lingual, free gingival margin to notch in monkey No. 82 at 35 days.
2. Disto-facial, free gingival margin to cemento-enamel junction in monkey No. 84 at 35 and 180 days.
3. Lingual pocket depth in monkey No. 85 at 35 and 180 days.

For these measurements, comparison of suture and cyanoacrylate was performed by analysis of variance. Comparison of the clinical distance from the free gingival margin to notch on disto-lingual surfaces of monkey No. 82 (35 days) was obtained by analysis of variance of the change from preoperative to 35 days. No significant differences were found from these analyses of variance tests.

There were no significant differences in the analysis of covariance for each monkey except for the following pocket depth adjusted means at 35 days (Table 1):

1. mesio-facial: monkey No. 83.
2. facial midpoint: monkey No. 82.
3. disto-facial: monkey No. 84.
4. lingual midpoint: monkey No. 82, 83.

For those adjusted means which showed statistical differences, the pocket depths were shallower for cyanoacrylate compared to suture for monkey No. 82 (facial and lingual) and No. 84 (disto-facial), while adjusted means were less for suture compared to cyanoacrylate in monkey No. 8 (mesio-facial and lingual).

All other clinical variables at 35 days showed no significant difference between adjusted means for suture and cyanoacrylate treated sides for all monkeys. There were no significant differences between adjusted means for all variables in both animals at 180 days.

Change in clinical pocket depths and location of the free gingival margin from preoperative to 35 days was subjected to a pairwise *t* test comparing suture to cyanoacrylate treated sides. Results from the pairwise *t* test of change in clinical scores from four monkeys at 35 days revealed no significant differences between suture and cyanoacrylate treatment for all the variables tested. There was a general trend toward recession and slightly deeper pocket depths postoperative for both modalities of treatment.

Histometrics

Mean histometric values were subjected to a pairwise *t* test for suture and cyanoacrylate treated sides at 35 and 180 days (Tables 2 and 3). Two monkeys were included at each sacrifice period of 35 and 180 days. No significant differences were found between treatment sides at either 35 or 180 days.

Table 4 presents the results of a pairwise *t* test of the clinical distance from the cemento-enamel junction to alveolar crest at the time of surgery. This analysis was performed only on those teeth evaluated histometrically. The results indicated that there were no significant differences in level of the alveolar crest between treatment sides at surgery following bone removal.

Discussion

Biometrics

The biometric evaluations assessed pocket depths and attachment levels related to fixed reference points on the teeth. All data were analyzed to assess for differences between operated areas treated with sutures and cyanoacrylate. Analysis of covariance for all of the animals revealed that enough variables were significantly different to prevent grouping scores of all monkeys in statistical analysis. This finding was not surprising because

Table 1. *Adjusted Means Which Demonstrate Statistically Significant Differences Between Suture and Cyanoacrylate 35 Days*

	Monkey #82		Monkey #83		Monkey #84		Monkey #85	
Var.	S	C	S	C	S	C	S	C
DF FGM-BP	2.479	2.438	2.566	2.982	2.701	2.174*	2.726	2.918
F FGM-BP	1.826	1.258**	2.481	2.142	1.250	1.500	1.339	1.356
MF FGM-BP	2.333	2.417	2.282**	3.242	2.250	2.125	2.970	2.611
L FGM-BP	2.011	1.734*	1.897*	2.261	1.869	2.256	1.727[1]	1.583[1]

** p < .01
* p < .05
1 = means computed from analysis of variance

Table 2. *Pairwise t Test of Mean Histometric Measurements From the Cemento-Enamel Junction to the Apical Base of Junctional Epithelium In Millimeters*

	FACIAL				LINGUAL			
Time	S	C	T-Stat	Sig.	S	C	T-Stat	Sig.
35 days	1.206	1.049	.737	NS (.596)	1.298	1.070	4.436	NS (.141)
180 days	1.147	1.810	4.154	NS (.151)	1.039	1.070	.081	NS (.948)

TABLE 3. *Pairwise t Test of Mean Histometric Measurements From the Cemento-Enamel Junction to the Alveolar Crest In Millimeters*

	FACIAL				LINGUAL			
Time	S	C	T-Stat	Sig.	S	C	T-Stat	Sig.
35 days	1.822	1.683	.639	NS (.638)	2.057	1.811	4.260	NS (.147)
180 days	1.942	2.415	5.942	NS (.106)	1.654	1.675	.395	NS (.760)

TABLE 4. *Pairwise t Test of Mean Clinical Distances From Cemento-Enamel Junction to Alveolar Crest at Facial and Lingual Midpoints at Time of Surgery In Millimeters*

	FACIAL				LINGUAL			
	S	C	T-Stat	Sig.	S	C	T-Stat	Sig.
Monkey #82 & 83 (35 day group)	2.125	2.083	.200	NS (.874)	2.292	2.033	.633	NS (.640)
Monkey #84 & 85 (180 day group)	1.833	1.833	0.	NS (1.0)	1.900	2.000	-1.000	NS (.500)

initial clinical examination revealed general differences in periodontal status.

Analysis of variance was performed on an individual animal basis comparing suture to cyanoacrylate. With initial scores as covariates, evaluation was based on comparison of adjusted means. At 35 days (four animals) the following pocket depth adjusted means demonstrated significant differences (Table 1):

1. Monkey no. 83: mesio-facial.
2. Monkey No. 82: facial midpoint.
3. Monkey No. 84: disto-facial.
4. Monkey No. 82, 83: lingual midpoint.

These variables which demonstrated statistical significance represent only five out of 64 variables scored at 35 days. No statistical trend was observed: three scored better results with cyanoacrylate and two scored better with sutures. The shallow pocket depth for suture was influenced in monkey No. 83 by the extraction of tooth No. 8 which probably contributed to shallower pocket depths on the mesial of No. 7 and No. 9 (which were treated by sutures). However, statistical differences remained even when anterior teeth were eliminated from new calculations of mesio-facial pocket depth adjusted means in monkey No. 83.

The differences may be a result of local operative technique which may not have allowed similar flap adaptation between sides. Flap adaptation has been shown to be a highly significant means of optimizing potential for reattachment and prevention of recurrent pocket depth.[6] No differences were observed in adjusted means of all other pocket depths at 35 days.

There were no significant differences between treatment modalities for adjusted means of recession for all measurement points at 35 days.

When analysis of covariance was performed on the remaining two monkeys at 180 days, no statistically significant differences between treatment modalities were observed for pocket depth and recession.

Five variables were subjected to analysis of variance rather than analysis of covariance.

1. Mesio-lingual, free gingival margin to notch in monkey No. 82 at 35 days.
2. Disto-facial, free gingival margin to cemento-enamel junction in monkey No. 84 at 35 and 180 days.
3. Lingual pocket depth in monkey No. 85 at 35 and 180 days.

This was necessary because all of the initial measurements were the same and equal for suture and cyanoacrylate. With the initial measurements equal, it became mathematically impossible to compute covariance.

It was mathematically impossible to compute analysis of variance as well as covariance for the clinical distance from the free gingival margin to the notch on disto-lingual surfaces of monkey No. 82. The problem was that all initial values were zero except one. To effectively compare for differences between clinical measurements of suture and cyanoacrylate, analysis of variance was performed on the change in values from preoperative to 35 days. No significant differences between treatment modalities were found in these special statistical situations.

To further clarify the variables which demonstrated statistically significant differences (Table 1), a pairwise *t* test was used to compare treatment modalities with regard to change from initial to 35 day measurements for: (a) pocket depth, (b) level of free gingival margin. Of the 16 variables subjected to the pairwise *t* test, none showed statistical differences between treatment modalities.

It was not possible to perform a pairwise *t* test on the 180 days group changes because only two monkeys were available. This allowed only one degree of freedom, which was insufficient for this kind of evaluation.

The surgically produced defects created optimal potential for reattachment because the root surfaces had not been contaminated by exposure to the septic environment of the periodontal pocket. However, the monkeys had no significant attachment loss or pockets at the time of surgery so that recession produced open inter-

proximals. This interproximal recession led to increased accumulation of debris. The interproximal debris produced greater inflammation than existed preoperatively in spite of the weekly prophylaxes.

Histometrics

Results from the pairwise *t* test of mean values of histometric measurements demonstrated no significant differences in level of attachment and alveolar crest between treatment modalities at 35 and 180 days (Tables 2 and 3). No statistical trends were observed to favor one treatment over the other. The pairwise *t* test of mean clinical distances from the cemento-enamel junction to the alveolar crest at the time of surgery demonstrated that insignificant differences in bone level existed at the time of surgery (Table 4). Due to the fact that one monkey from the 14 day group died the first night, only one animal was left in the 14 day group. This left insufficient degrees of freedom to perform a pairwise *t* test. The remaining 14 day animal was not included in the statistical data.

The histometric results may be of limited significance due to the following difficulties encountered:

1. The histologic material was shared with another investigation[3] which made it difficult to obtain the desired plane of orientation in all cases, since mesiodistal sections were also processed for histologic evaluation. Some sections were unacceptable for measurement because of an oblique plane of orientation during preparation.
2. Several teeth were found without an observable cemento-enamel junction because of instrumentation at the time of surgery.
3. Insufficient time was allowed for decalcification of the 180 day specimens which caused processing artifacts and difficulty in obtaining histometric measurements.

As a result, an average of only five teeth each for suture and cyanoacrylate were suitable from each monkey for histometric measurement.

Clinical Significance

The use of MBR 4197 aerosol dramatically reduced the time of flap fixation to a matter of seconds compared to 10 to 15 minutes for suturing a quadrant of periodontal flap surgery. The rapid adhesion produced effective hemostasis immediately in almost all cases. Occasionally, when a small amount of hemorrhage persisted, respraying with MBR 4197 quickly resolved the bleeding. The aerosol form of MBR 4197 was far superior to the dropper bottle for application on a thin, even film of monomer on the tissues.

MBR 4197 provided secure fixation for conservative mucoperiosteal flaps not reflected beyond the mucogingival junction. Flaps fixed with MBR 4197 did not have as much resistance to tension as flaps fixed with sutures. The material may not provide the strength to resist muscle pulls on flaps reflected far beyond the mucogingival junction. In these cases, a combination of sutures and cyanoacrylate may be advisable.

Conservative well adapted flap margins minimize the chance of cyanoacrylate slipping beneath the flaps and acting as a barrier to healing. There was no histologic evidence of cyanoacrylate beneath the tissues of the monkeys used in this study.[3] Less clinical inflammation was found in the early stages of healing with MBR 4197 as compared to sutures. Reduced inflammation of the cyanoacrylate treated sides in the early stages of healing may be due to mechanical protection at the flap margins plus the bacteriostatic effect of MBR 4197. In addition, sutures act as a source of irritation by trapping bacteria.

MBR 4197 is certainly much less bulky than conventional periodontal dressings. Patient preference has been reported with the use of normal butyl cyanoacrylate[7] and isobutyl cyanoacrylate[8] intraorally. However, care must be exercised when handling the material because cyanoacrylate monomer is highly irritating to the respiratory tract and ocular tissues.[2]

Summary

Modified Widman flap surgery was performed on six Rhesus monkeys for the purpose of comparison of flap fixation by conventional sutures and trifluoroisopropyl cyanoacrylate (MBR 4197). Experimental time intervals were 14, 35 and 180 days. One monkey died approximately 12 hours after surgery, one was sacrificed at 14 days, and two each were sacrificed at 35 and 180 days.

Clinical measurements were obtained for pocket depths and for the distance from the free gingival margin to the cemento-enamel junction or to an experimentally produced bur notch. Measurements were taken preoperatively, and at 35 and 180 days.

Split mouth comparison of treatment modalities was used for all posterior teeth. For the incisors, one method was used for the maxilla and the other for the mandible. The pattern was reversed for the other monkey from each experimental time interval.

Following sacrifice, histologic sections of the posterior teeth were prepared and the following distances measured histometrically:

1. Cemento-enamel junction to the apical base of junctional epithelium, facial and lingual midpoints.
2. Cemento-enamel junction to the alveolar crest, facial and lingual midpoints.

Analysis of covariance over all the monkeys revealed that each monkey should be analyzed separately for differences between suture and cyanoacrylate. Analysis of covariance within each monkey produced five variables (out of 64) at 35 days which showed statistically significant differences. However, no statistical trends were observed and the differences were not attributed to the use of cyanoacrylate or sutures. Analysis of covariance within each monkey at 180 days showed no differences. When the clinical data was analyzed by a pairwise *t* test of the change in scores from preoperative to 35 days, no differences were observed.

Histometric data was subjected to a pairwise *t* test for the values obtained at 35 and 180 days with no statistical differences found.

Conclusions

Within the limits of this study, it may be concluded that:

1. Following modified Widman flap surgery, the use of MBR 4197 does not alter pocket depth and attachment level biometrically and histometrically as compared to conventional suturing.
2. Aerosol spray of MBR 4197 is easily applied and provides rapid, effective flap fixation and hemostasis when used for modified Widman flap surgery.

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Abstracts

Note on Treating a Case of Acute Lymphocytic Leukemia Resembling Necrotizing Ulcerative Gingivitis: A Case History

Aker, F., Magera, Jr., and Vernino, A.
Quintessence Int **9:** 51, March, 1978.

A 17-year-old male patient complained of blood oozing from the gingiva and sore mouth, and a diagnosis of acute necrotizing ulcerative gingivitis (ANUG) was made on the clinical findings of edematous gingiva, blunted papillae, heavy plaque, and spontaneous bleeding. A blood analysis showed a 220,000 white count with immature lymphocytes. After use of Vincristine and Prednisone for 1 week, despite his continued neglect of oral hygiene, the gingival condition improved and his white count had fallen to 5,300. In the treatment phase, an antibiotic is not recommended, since it would suppress any bacterial agent sufficiently to mask any systemic disorder. It was emphasized that, in cases of apparent ANUG, a blood count should be included as well as a careful physical examination. *U.S. Navy Dental Corps., N MCB-40 FPO, San Francisco, Calif 96601.* Dr. Yau-Fwu Huang

The Sensory Mechanism in Human Dentin as Revealed by Evaporation and Mechanical Removal of Dentin

Brännström, M. and Johnson, G.
J Dent Res **57:** 49, January, 1978.

The pain sensation in human dentin was tested clinically on 39 contralateral pairs of premolars in young individuals. In each of 25 pairs, one exposed dentinal surface was selected to be chiseled under water flow, while the opposite tooth was chiseled under dry conditions. In ten pairs, anesthesia was used, while in the 15 others an intrapair sensitivity procedure using a dry and wet routine was employed. In a group of 14, sensitivity was compared after chiseling by keeping the area dry or moist with saline. The teeth were extracted for orthodontic purposes, decalcified and stained for histologic study. The dry chiseling was found to cause greater pain than wet chiseling, but pain ceased when the exposed dentin was remoistened. Mechanical stimulus may be due to removal of fluid from the dental tubules and may produce pain in the same way as evaporation. *Department of Oral Pathology, and Department of Cariology, School of Dentistry, Karolinska Institute, Stockholm, Sweden.* Dr. Yau-Fwu Huang

The Development of a Maximal Clenching Force Between Two Antagonistic Teeth

van Steenberghe, D., and De Vries, J. H.
J Periodont Res **13:** 91, January, 1978.

To study the reproducibility of maximal clenching forces between antagonistic canine teeth, nine volunteer patients were subjected to measurement by means of a dynamometer mounted with acrylic blocks. The patients had sound dentitions and supporting structures with the Gingival Index at the start averaging 0.89 and pockets averaging 1.40 mm in depth. Clenching began at a moderate pace and increased by degrees until a plateau was reached. Each clench lasted 4 seconds at 15-second intervals with 2-minute rest periods. These were consistently reproducible. When the process had continued 10 successive minutes, the plateau level could not again be attained and maintained. No conclusions were made as to what caused the inability to reach the original peak level of clenching again. *Department of Periodontology, Tandheel Kundig Instituut, Louwesweg 1, Amsterdam (Slotervaart), The Netherlands.* Dr. Larry Johnson