

# Histological evaluation of gingival damage by toothbrushing

An experimental study in dog]

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Anneroth, G. & Poppelman, Å. Histological evaluation of gingival damage by toothbrushing. An experimental study in dog. *Acta Odont. Scand.* 33: 119–127, 1975.

The purpose of the investigation was to present an experimental model for a comparative histological evaluation of the effect on gingiva using two different types of bristle material at tooth brushing. Fifteen dogs constituted the material. Two different teeth or groups of teeth were selected on each dog. One tooth was brushed with a nylon brush. The diameter of the bristles was approximately 0.25 mm. The other tooth was brushed with a polytene brush. The diameter of the bristles vary between 0.7 mm at the base of the bristle to 0.25 mm in the near end of the bristle. Manual brushing during 10–60 seconds was carried out in 7 dogs and the other eight dogs were brushed with a mechanical device for 10–30 seconds. A histological evaluation of the relative degree of tissue damages based upon a grading of these damages in terms of a 0–3 point scale was carried out by two independent observers. Wilcoxon's matched-pair signed rank test was applied for a statistical analysis of the result of the histological examination. The conclusion was that the two different types of brushes most likely cause different damages on the gingival tissue and that the polytene brush appeared to cause less tissue damage than the nylon brush.

*Keywords:* Gingiva; dogs; toothbrushing; histology.

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## 1. INTRODUCTION

The plaque-removing capacity of different tooth brushes has been presented in several clinical investigations. The results point to soft tooth brushes as being more effective (Bergenholtz, Hugoson & Sohlberg, 1967; Bergenholtz *et al.*, 1969; Bay, Kardel & Skougaard, 1967; von Barnarius, Mieler & Singert, 1967; Gülzow & Busse, 1970; Kardel & Olesen, 1969). In spite of this, hard brushes are often preferred because the hardness is apprehended as a

guarantee for quality and durability (Fanning, 1967).

Since a long time authors have warned against tooth brushing with hard and stiff bristles which can give traumatic effects on the gingival margin. This traumatic effect can be aggravated by using an inadequate brushing technique. (Hirschfeld, 1931, 1934; Spies, 1934; Bass, 1948; McCauley, 1946; Miller, 1950; Hine, 1956; Glickman, 1967; Kimmelman & Tassman, 1957; Kimmelman *et al.*, 1958; Morris,

1958; Moskow & Bressman, 1965; Riethe, 1965; Henning & Fanning, 1967; Focke, 1951, 1952; Gorman, 1967; Storsberg, 1955; Löbbe, 1956; Pückeroff, 1956).

No general criterion or standards is indicated in the literature regarding the hardness and the rigidity of the bristle material (Frandsen *et al.*, 1970). There are also very few experimental studies presented where different experimental factors such as the brushing technique and the working pressure used are standardized. It is therefore difficult to make a comparative evaluation of the results, achieved from different investigations, as regards the effect of tooth brushing on the teeth and their surrounding tissues.

The purpose of this investigation is to present an experimental model for a comparative histological evaluation of the effect on the gingiva at toothbrushing.

#### MATERIAL AND METHODS

15 dogs aged from 8 to 84 months constituted the material. The body weight was between 5 and 16 kg. Four dogs had a moderate general gingivitis, while the others had a normal clinically healthy gingiva.

Two different teeth or groups of teeth were selected on each dog. In nine cases contralateral teeth were used (Table I). One tooth was brushed with a conventional soft toothbrush\* (N—S) and the other tooth was brushed with a Mölnlycke »disposable toothbrush»\*\*, (Po—N).

Manual brushing during 10—60 seconds was carried out in 7 dogs and eight dogs were brushed with a mechanical device for 10—30 seconds (Table I). The brushing

movements were horizontal and linear. The working pressure applied to the tooth and the surrounding gingiva was kept between 260—300 p by the aid of a pressure meter. In average about 350 single brushing movements per minute were made which appears to be in agreement with the number of brushing movements at normal tooth brushing of man. A special technique was applied at the mechanical brushing. The dogs were suspended on a dog stand with slings according to Saxe *et al.*, 1967. The stroke length of the machine was 26 mm.

The shape and size of the bristles were examined using scanning electron microscopy. After covering the bristles *in vacuo* with a gold foil of about 200 Å thickness they were studied in a Cambridge scanning microscope with an accelerating voltage of 20 kV.

The conventionally made toothbrush was fabricated in nylon by using a stamped method with extruded nylon bristles. The bristles had an even thickness and a cross-section in the form of a circle, the diameter of which was approximately 0.25 mm. The length varied between 9 and 13 mm. The bristles had been cut to suitable lengths in tufts of 50 bristles in each tuft (Fig. 1), and the bristles are stamped into the brush head with a metal bleach. The ends of the bristles are sharp as they are cut obliquely owing to the technique used (Fig. 2). The total number of bristles in one Jordan toothbrush is approximately 800.

The Mölnlycke »disposable toothbrush» is fabricated in a different way. The bristles, the brush head and the handle are produced in one unit of a thermoplastic material (polythene). The length of the bristles varies between 9 and 13 mm and they are grouped to fit the anatomy of the row of the teeth. The bristles are tapering towards the apices and have rounded ends

\* Nylon toothbrush, leading brand.

\*\* Disposable toothbrush, Mölnlycke AB.



Fig. 1. Bristles from a new nylon toothbrush. There are 50 bristles in each tuft. The length of the bristles varies between 9 and 13 mm. The diameter of each bristle is approximately 0.25 mm. Scanning EM.  $\times 22$ .



Fig. 2. The ends of the bristles of a new nylon toothbrush. The bristles are sharp due to the oblique cutting. The bristles have an even thickness and a cross-section in the form of a circle. Scanning EM.  $\times 220$ .

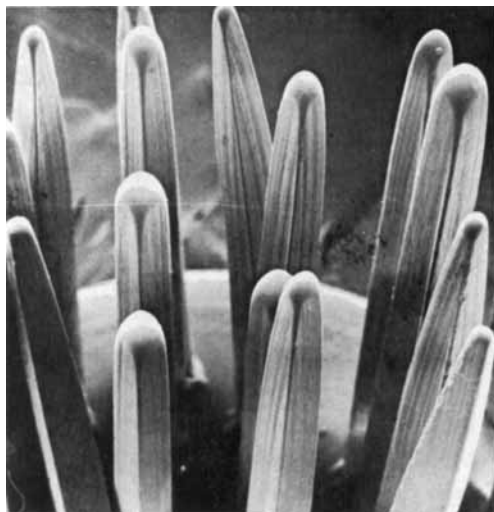


Fig. 3. The bristles, brush head and handle of the Mölnlycke »disposable toothbrush». The bristles are tapering towards the apices. The ends are rounded. Scanning EM  $\times 21$ .

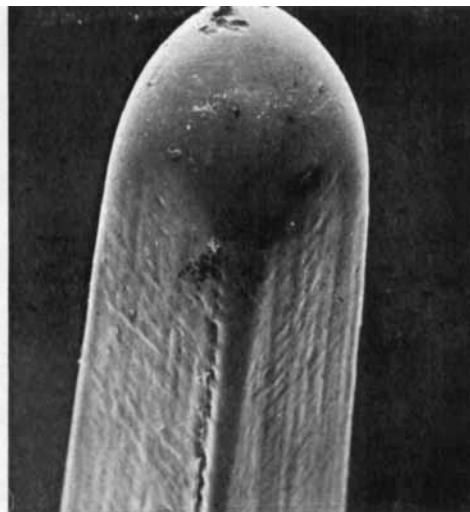


Fig. 4. The ends of the bristles of the Mölnlycke »disposable toothbrush» are rounded and the bristle has a cross-section of a square. Scanning EM.  $\times 230$ .

(Figs. 3 and 4). The diameter of the bristles vary from 0.70 mm at the base of the bristle to 0.25 mm in the near of the end of the bristle. The bristles have a cross-section of a square (Fig. 3). The total number of bristles is approximately 200.

When the brushing experiment was completed the dogs were sacrificed by injection of 20 cc Nembutal. In order to preserve the tissue relations between hard and soft tissue the jaws were immediately refrigerated in floating nitrogen. After dissection the experimental teeth with surrounding soft and hard tissues were fixed in 10 % neutral formalin according to Lillie (1954). After decalcification in 5% HNO<sub>3</sub> the material was embedded in paraffin and sectioned in 5  $\mu$ m thick sections. The stainings used were Mayer's haematoxylin-eosin and van Gieson.

For the histological evaluation of the reaction of the gingiva to the brushing carried out during the experiment, two regions — each including the epithelium and the subepithelial connective tissues — were selected for examination as follows: — the marginal sulcular epithelium (Region I), and the marginal oral epithelium (Region II). These regions correspond to the area normally denominated as »crestal» or »marginal» epithelium.

In order to compare the influence on the tissue of the two different toothbrushes, double blind estimation of the reaction of the gingival tissue within each region was carried out by two independent observers. It was agreed upon that if some of the sections showed intercellular rupture and intracellular damage or desquamation, it should be considered as tissue damage. The evaluation of the relative degree of tissue damage was based upon a grading of the tissue changes in terms of a 0—3 point scale, with the

following histological characteristics (Figs. 5—8):

- 0 = No damage
- 1 = Only the superficial epithelial cell layers were damaged
- 2 = The epithelium damage included more than half the thickness of the epithelium
- 3 = Tissue damages included the epithelium and the subepithelial connective tissue. In Figs. 5—8 examples are given to illustrate the application of the grading system.

#### *Statistical analysis*

It was assumed at the statistical analysis that the different values showing the frequency of the damages were not normally distributed and therefore a non-parametric test was found suitable. In this connection a method was used for the matched pairs in accordance with which the subject of the experiment is used as its own check of the intermediate variables which are of no interest for the investigation but can be expected to exercise some influence on the result.

For the statistical test Wilcoxon's matched-pairs signed rank test was applied which makes use not only of the difference in direction between the pair values but also of the power of this difference. In this case the experiment variable was the type of toothbrush.

$d_i$  is equal to the difference between the estimated values of the tissue damages (0—3) for dog No.  $i$ , obtained by two different methods during the experiment (= type of brush). All the  $d_i$  values are ranked disregarding the signs. Then each rank is given the sign which characterizes the difference, namely, + och —. If the brush types are equivalent, i.e. if the 0-hypothesis ( $H_0$ ) is true, it appears that

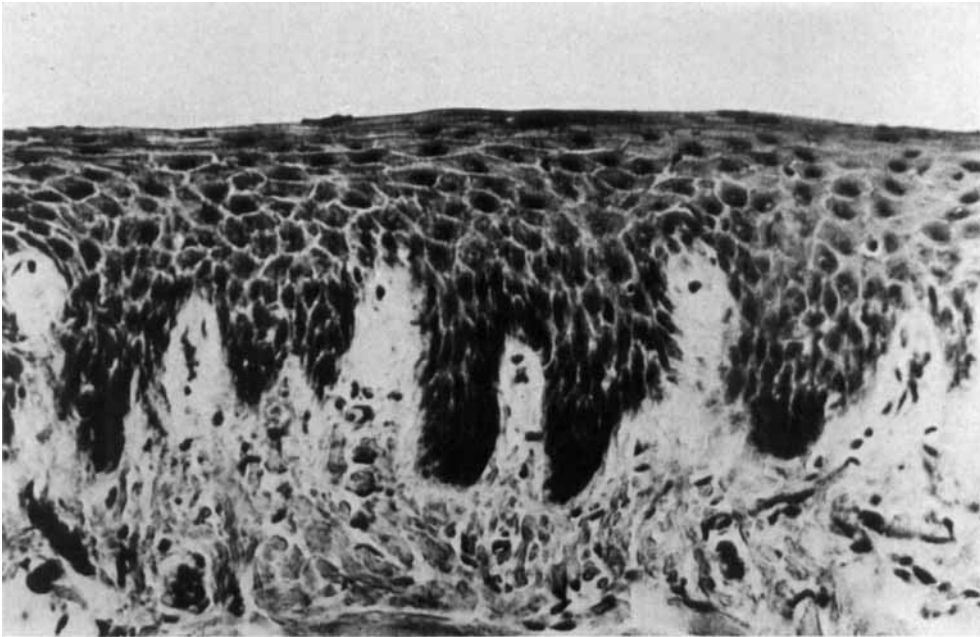


Fig. 5. Gingival tissue with no damage after tooth brushing.

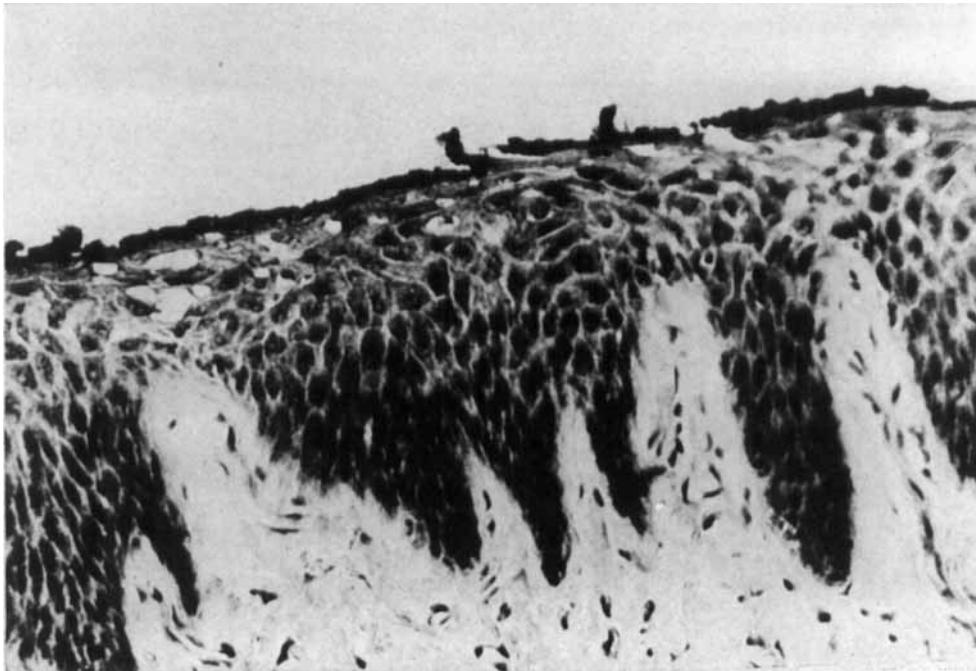


Fig. 6. Gingival tissue exposed to tooth brushing. Only the superficial epithelial cell layers are damaged due to tooth brushing.

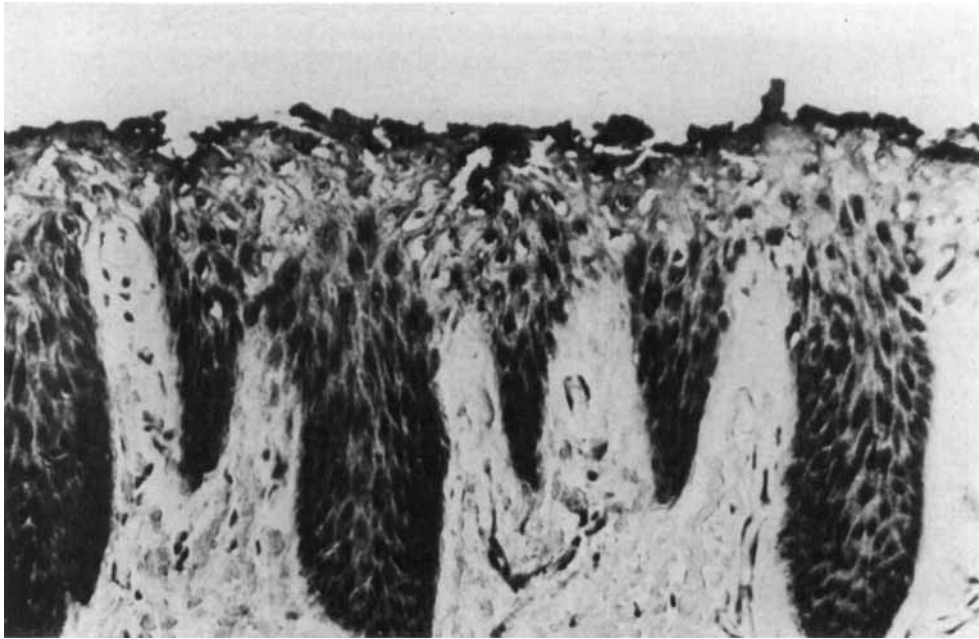


Fig. 7. Gingival tissue exposed to tooth brushing. The epithelium damage includes more than half the thickness of the epithelium.



Fig. 8. Gingival tissue exposed to tooth brushing. The tissue damage includes both the epithelium and the subepithelial connective tissue.

the positive rank sum is approximately equal to the negative. If  $T = \text{rank sum}$ , consequently  $T_+ \approx T_-$ . The 0-hypothesis is rejected if some of the rank sums is too small.

Table I. *Histological evaluation of gingival reaction to tooth brushing with polyten (Po—N) and nylon (N—S) brushes*

Dog No	Time	Tooth	Brush	Region I	Region II
<i>Manual brushing</i>					
1	15 s.	1—1	N—S	3	3
1	15 s.	—3	PO—N	2	3
2	10 s.	1+1	N—S	3	1
2	10 s.	1—1	Po—N	1	1
3	60 s.	3+	N—S	3	3
3	60 s.	+3	Po—N	3	3
4	15 s.	1+1	N—S	3	3
4	15 s.	1—1	Po—N	1	1
5	60 s.	3+	N—S	3	3
5	60 s.	1+1	Po—N	1	0
6	30 s.	3—	N—S	3	2
6	30 s.	—3	Po—N	1	2
7	40 s.	—3	N—S	3	3
7	40 s.	3—	Po—N	2	3
<i>Mechanical brushing</i>					
8	10 s.	+3	N—S	1	1
8	10 s.	—3	Po—N	0	0
9	10 s.	3—	N—S	1	3
9	10 s.	—3	Po—N	3	2
10	15 s.	—3	N—S	3	1
10	15 s.	3—	Po—N	0	3
11	10 s.	3+	N—S	1	1
11	10 s.	+3	Po—N	1	0
12	30 s.	—3	N—S	1	2
12	30 s.	3—	Po—N	0	1
13	15 s.	3—	N—S	1	2
13	15 s.	—3	Po—N	0	1
14	30 s.	3—	N—S	1	3
14	30 s.	—3	Po—N	1	2
15	30 s.	1—1	N—S	1	0
15	30 s.	1+1	Po—N	1	0

Table II. *Result of the statistical test with Wilcoxon's matched-pair signed rank test*

Dog No	Amount of Nylon brushes (N—S)	Amount of Polyten brushes (Po—N)	Difference	Rank
1	6	5	+1	+10.5
2	4	2	+2	+ 5
3	6	6	0	—
4	6	2	+4	+ 2
5	6	1	+5	+ 1
6	5	3	+2	+ 5
7	6	5	+1	+10.5
8	2	0	+2	+ 5
9	4	5	—1	—10.5
10	4	3	+1	+10.5
11	2	1	+1	+10.5
12	3	1	+2	+ 5
13	3	1	+2	+ 5
14	4	3	+1	+10.5
15	1	1	0	—

$N = 15 - 2 = 13$

$T = -10.5$

$T_+ = +80.5$

RESULTS

The microscopic examination showed that the gingiva exhibited damages already after a short time, both at manual as well as mechanical brushing. The tissue reaction to the tooth-brushing was manifested in the form of inter- and intracellular damages, ruptures and desquamation.

The results of the microscopical evaluation of the different morphological parameters are tabulated in Table I. The points presented are mean values of the grading of the tissue damages in terms of a 0—3 point scale made by the two independent observers.

The results of the statistical analysis of all the dogs and their injuries in regions I and II is accounted for in Table II.

The smallest absolute T-value was 10.5. For  $N = 13$  the significant value for  $\alpha = 0.05$  is 17. The zero hypothesis that the brushes have the same damaging effect on the tissue can be rejected under the assumption that the fault risk amounts to 5 %. Based on the present material and the method adapted for this investigation the conclusion is that the two different types of brushes most likely cause different damages on the gingival tissue. The polytene brush appeared to cause less tissue damage than the nylon brush.

#### DISCUSSION

Even if the present results relate to histological changes in the gingival tissue of dogs certain conclusions can nevertheless be drawn concerning the corresponding situation in man. The morphology of the gingival tissue of dogs is similar to that of man.

The result of the present investigation showed that the developed experimental model can be used for a comparative histological evaluation of the effect on the gingiva at tooth brushing. It also showed that tooth brushing under certain conditions can cause tissue damages dependent on among other factors the bristle material. The polytene bristle (Po—N) thereby appeared to cause less damage than the nylon bristle (N—S). The reason why the polytene bristles caused less damage than the nylon bristles cannot be stated with certainty. Differences in physical properties as well as in the shape, size, number and thickness of the bristles used can provide a contributory cause for the abovementioned observation. The difference in shape, size, number and thickness of the bristle material in the two types of tooth brushes used in this study

was well documented with the scanning electron microscope technique.

The importance of these factors will be further analyzed in a planned investigation. The influence of the brushing time and the brushing pressure used will thereby also be considered.

*Acknowledgements.* The authors are indebted to professor Sune Rosell and his staff at the Department of Pharmacology, Karolinska Institutet, Stockholm, for generous aid in carrying out this investigation.

The investigation was supported by grants from Mölnlycke AB.

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