The effect of periodontal dressings on intact mucous membrane and on wound healing

A methodological study

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Biological screening of dental materials which are intended for use in contact with the oral mucosa comprises a mucous membrane irritation test. The aim of the present investigation was to study the effect of 3 periodontal dressings on intact oral mucosa and to assess their effect on the epithelial proliferation during wound healing.

A total of 87 rats were used, 24 for studying reactions in intact mucosa and 63 for a wound healing study. A wound was inflicted on each animal in the palate behind the incisors. The dressing covered the wound area and the surrounding mucosa and was secured by an appliance fixed to the maxillary incisors. ^{3}H-labelled thymidine was injected prior to sacrifice after observation periods of 1, 3 and 5 days. Histological assessment and autoradiographic evaluation of mitotic activity were performed. The dressings caused no reaction in the intact mucosa. In the wound healing study the mitotic activity at day 1 was lower in test than in control animals indicating a toxic effect of all materials. No differences between the 3 dressings were found. After 5 days, all wounds were covered by epithelium. Neither at 3 nor at 5 days were any differences between test and control animals seen.

Key-words: Dental materials; periodontics; rats; histopathology; autoradiography

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A rapid healing of the tissues after gingival surgery has been considered important and the application of a periodontal dressing over the operated area has been mentioned as a means to achieve this result (12). Attempts to incorporate special substances in the dressing materials in order to promote the rate of healing have not been successful (13, 16). However, an injurious effect from the material and a retardation of wound healing cannot be disregarded. Eugenol has been mentioned as a substance that may cause epithelial degeneration and necrosis and should therefore be avoided in contact with the mucous membrane (10).

In a clinical study where 3 different dressing materials, including a zinc ox-

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ide/eugenol composition, were used, no significant differences in wound healing were found (3). Wound healing and the degree of epithelization was assessed by visual inspection only. It is possible that more sensitive methods for evaluation would reveal differences.

Biological assessment of all dental materials which during their intended use are temporarily or permanently placed adjacent to the oral mucosa, should include a mucous membrane irritation test (17, 18, 19). However, no simple, suitable mucous membrane test for dressing materials is available. Therefore a new technique was tried. An appliance designed by Kvam (11) for orthodontic tooth movement in rats was modified to serve as a holding apparatus for the dressing material (Fig. 1).

The aims of the present study were:

1. To perform the proposed mucous membrane irritation test (19) using 3 commercial, clinically acceptable periodontal dressings.
2. To assess the effect of the 3 dressing materials on epithelial proliferation during wound healing.

MATERIAL AND METHODS

Periodontal dressings

The dressing materials Coe-pak® (Coe Laboratories, Inc., Chicago, Ill. USA), Peripac® (De Trey Frères S.A., Zürich, Switzerland) and Ward's Wondrpak® (Westward Dental Products Co., San Francisco, Ca. USA) were selected for the study. A description of the dressing materials has been published previously (4).

Operative procedure

A total of 87 female albino rats (Mol: WIST) weighing 180–200 g were used in the study. The operative procedure was performed in intraperitoneal barbiturate anesthesia (0,05 ml Hypnorm® Vet Mekos pr 100 g body weight). A perforated metal plate 6 x 4 mm was soldered to an appliance made from orthodontic steel band (11). The test material was placed on the metal plate and the steel band was fixed to the maxillary incisors with composite resin material (Fig. 1). To increase the stability of the appliance a retention groove was prepared on the distal surface of the incisors before insertion. When in place, the metal plate extended over a part of the palate, keeping the dressing in contact with the mucosa behind the teeth (Fig. 1).

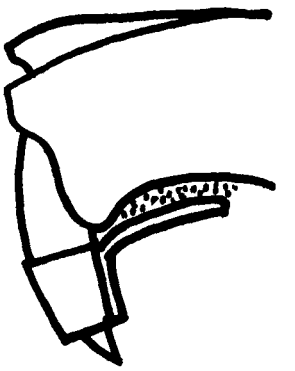

Fig. 1. Diagram of the appliance constructed for the retention of the dressing. The steel band is fixed to the maxillary incisors with composite resin material and the metal plate extends over a part of the palate. The dotted area indicates the space that is filled with the periodontal dressing during the experimental period.

Mucous membrane irritation test

Twenty-four animals were used for this part of the study, 6 for each of the dressing materials and 6 controls. Plaster of Paris is recommended as control material when testing semisolid materials (19) and was used in 4 of the control animals, 2 were left untreated. Half of the animals were sacrificed after 1 day and the rest after 3 days (Table 1).

Table 1. *Distribution of animals according to dressing materials and observation periods for the two tests performed. C:Coe-pak; P:Peripac; W:Ward's Wondrpak; S:Sham operated*

Test	Dressing	Days 0	1	3	5
Mucous membrane	C	–	3	3	–
	P	–	3	3	–
	W	–	3	3	–
	S	2	2	2	–
Wound healing	C	–	5	5	5
	P	–	5	5	5
	W	–	5	5	5
	S	3	5	5	5

Wound healing study

Sixty-three animals were used. A wound was created in the palate behind the incisors with a slow running round bur Ash no 9 with a diameter of 2.3 mm, removing the epithelium and going down into the connective tissue. The periodontal dressing covered the wound and the surrounding mucosa and was kept in place by the appliance. For each of the 3 dressing materials, 15 animals were used. In addition 15 animals did not receive any dressing to cover the wound. These sham operated animals served as controls and were supplied with the appliance only, leaving an empty space under the perforated plate. As baseline control material, 3 animals were sacrificed immediately after the surgical procedure (Table 1). The remaining 60 animals were sacrificed after 1, 3 and 5 days. One hour prior to sacrifice each animal received an intraperitoneal injection of 1μCi ^{3}H-thymidine (New England Nuclear, specific activity 2.0 Ci/mmol) per g body weight.

Histological evaluation

The anterior part of the maxilla with the incisors and the foremost part of the palate was removed, fixed in 10% neutral buffered formalin and demineralized in 5,2% nitric acid. The appliance with the dressing was then removed and the tissue blocks embedded in paraffin. The biopsies were coded for blind evaluation and oriented to give longitudinal sections through the incisors and the test area in the palate behind the teeth. Several sections were placed on each glass slide and stained with hematoxylin and eosin.

Autoradiographic evaluation

From the wound healing study sections through the central part of the wound were selected for autoradiography. Kodak NTB2 emulsion and the dipping technique were used (8). The slides, after dipping and drying, were exposed for 21 days at 4°C, developed, fixed and stained with hematoxylin.

The mitotic activity in an area extending from the cut epithelial surface toward the teeth was examined. The area filled the microscopic field at a magnification of 200x and was oriented in such a manner that the main direction of the basement membrane went through the center of the field. In the chosen area, all labelled cells in the epithelium with 8 or more grains over the nucleus were counted as an evaluation of the mitotic activity (1). Each field was counted twice and the first count recorded. Occasionally the countings differed by more than 2 cells. In such cases repeated countings were performed the next day. As an ex-

pression of the mitotic activity, the mean value of 6 sections was calculated for each animal. Student's t-test was used for evaluation of statistical significance.

RESULTS

Mucous membrane irritation test

Visual inspection and colour photography of the site of application before sacrifice (19) had to be omitted as the appliance could only be removed from the teeth after demineralization. No tissue changes could be found in the epithelium or underlying connective tissue in any of the locations examined microscopically. Compared to untreated animals, a slight depression of the tissue indicated where the periodontal dressing or plaster of Paris had rested against the mucous membrane. This slight change could be noticed in most specimens irrespective of the dressing used.

Wound healing study

Histological evaluation

At the first observation period, 24 hours after the trauma, the wound was covered by a coagulum. The area exhibited signs of inflammation with dilated vessels and cell accumulation between the disrupted collagen and muscle bundles. From the wound margin migration of epithelial cells from the basal and deeper spinous layers had started, separating the coagulum and the inflamed connective tissue (Fig. 2). The place on the mucous membrane where the bur had cut through the keratin layers and gone into the tissue could easily be distinguished in all specimens (Fig. 2). No differences could be found between the various experimental series or the controls.

Three days after the trauma a coagulum still covered the wound, whereas the epithelium proliferating from the wound edges had almost met in the center of the wound (Fig. 3). The epithelium was only a few cell layers thick, non-keratinized

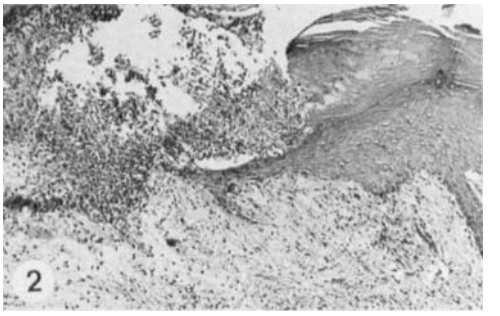

Fig. 2. At day 1 the wound is covered by a coagulum, and the area exhibits signs of inflammation. Migration of epithelial cells from the wound margin has started. The bur has cut through the keratin layers into the mucosa. X 115.

Fig. 3. Three days after the operation almost completed epithelial covering is found. The epithelium is thin, nonkeratinized and without rete pegs. The center of the wound is still covered by a coagulum. X 115.

Fig. 4. The coagulum has disappeared after 5 days. Rete pegs are developing in the epithelium. X 115.

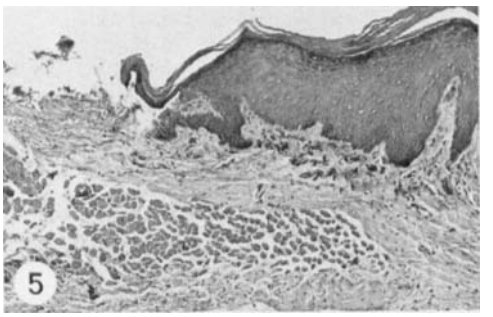

Fig. 5. Animals serving as control of the operative procedure were sacrificed at day 0. The control animal exhibits complete removal of the epithelium and the wound goes well into the connective tissue. X 115.

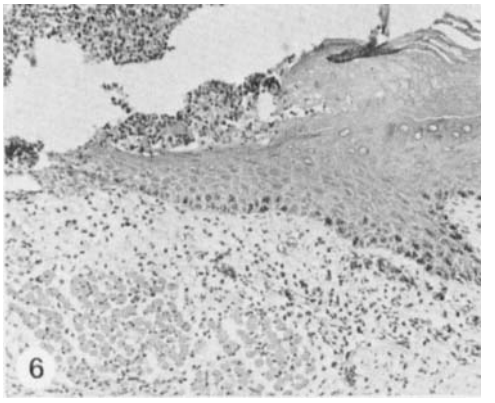

Fig. 6. The section is from a sham operated animal at day 1. The autoradiograph demonstrates labelled cells in the basal layers of the epithelium close to the wound. X 180.

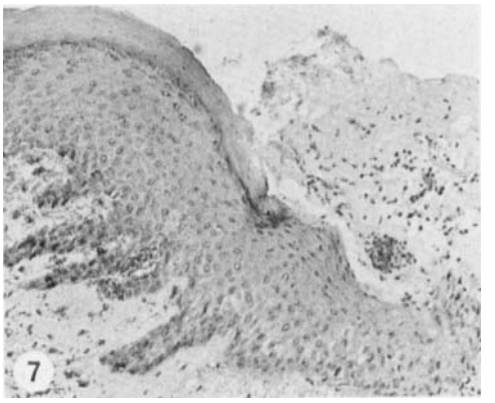

Fig. 7. In test animals the first labelled cells are found at a distance from the wound edge and labelling is more sparse in this area compared to sham operated control animals (Fig. 6). X 180.

and without rete pegs (Fig. 3). The inflammation in the underlying connective tissue was marked, and in most specimens a heavy infiltration of inflammatory cells was seen extending deeply into the connective tissue almost reaching the bone. Osteoclasts could be found at the bone surface at this observation time.

After 5 days the coagulum had disappeared and all wounds were covered by a thin layer of epithelium (Fig. 4). Development of rete pegs had started. The underlying connective tissue was still infiltrated by inflammatory cells, but to a varying extent in different specimens.

The sections from the animals sacrificed at day 0, exhibited complete removal of the epithelium and showed wounds that entered well into the connective tissue (Fig. 5). In one specimen a slight defect on the bone surface indicated that the bur had touched the bone.

Autoradiographic evaluations

The numbers of labelled cells in the chosen counting area in all animals are given in Table 2. In all groups the highest rate of labelling was found at day 1. However, in the control animals labelled cells were found close to the wound (Fig. 6), whereas in the test animals the first labelled cells were found at a distance from the wound edge and labelling was more sparse in this area (Fig. 7). No differences between animals with different dressings could be found, but all test groups showed statistically lower labelling values than did the control animals at day 1 (Table 2).

At day 3 the mitotic activity in all groups was lower than at day 1 (Table 2). No differences could be found between the values from the test and the control areas. The number of labelled cells at day 5 was less than half the number at day 3 (Table 2), and no differences between any of the control or test groups were observed.

Table 2. *Mean number of labelled cells and range at each observation time for C:Coe-pak; P:Peri-pac; W:Ward's Wondrpak and S:Sham operated*

Dressing	Day 1		Day 3		Day 5	
	x̄	Range	x̄	Range	x̄	Range
C	51	39–56	39	33–47	16	10–19
P	51	49–56	41	39–44	15	10–17
W	51	34–61	41	40–42	16	10–19
S	72*	70–77	41	39–42	15	13–16

* Significantly different from C, P and W ($p < 0.01$)

DISCUSSION

The test animals in the present study were rats, whereas the proposed animals for the mucous membrane test are rabbits or guinea pigs weighing 800 g (19). These older and larger animals have been chosen to have a palate of a size where test and control materials can be placed in the same animal. The young rats used here with the small and simple appliance (11) had the advantage of being cheaper even though the number of animals required was increased, but test and control sites had to be found in separate animals.

To pass the mucous membrane irritation test it is required that the material does not cause any reaction more severe than that caused by the control material Plaster of Paris (19). In the present study all dressing materials passed this test. The result is not surprising as these dressings have been used clinically for years and have served the purpose after periodontal surgery. However, the present findings differ somewhat from those of Kozam & Mantell (10).

Their test material was 100% eugenol applied on the labial mucosa of rats for 1 min and this resulted in necrosis. The eugenol containing pack did not provoke more irration than the other materials in the present test. The different constituents of a dressing material are probably of minor importance compared to what has actually leached out of the final dressing and at what rate, since it has been shown that a slow but continuous release of eugenol does take place (5). The diluting effect of saliva and tissue fluid must also be taken into consideration. The present results do not substantiate the claim that eugenol must be omitted from all materials that may come in contact with oral mucous membranes (10). The sensitizing potential of a eugenol containing dressing can, however, be a more relevant reason for restricted use of eugenol in these materials (5).

The wound healing study showed that healing including epithelial covering followed the usual pattern both in test and control animals (2). The autoradiographic evaluation of mitotic activity indicated an adverse effect on the epithelial cells of all dressing materials after 1 day. The finding that the distance from the wound edge to the first labelled cell was greater in test than in control animals, shows that the toxic effect was strongest close to the material. This observation is in accordance with the results from a bone implantation test where the distance between the material and the bone surface was shown to be important for the degree of reaction. Whether a coagulum, bone fragments or connective tissue filled the space was of little consequence (6). In two of the test groups one animal displayed much lower mitotic activity than the others (Table 2). Whether this

was a result of the surgical trauma the previous day, is not known.

The highest mitotic activity was found at day 1 in all groups. This corresponds with results from former studies on wound healing in rats and rhesus monkeys (15, 2). At day 3 the number of labelled cells was decreased in all groups, but at this observation time, no differences could be found between test and control animals. The higher cell proliferation recorded in control animals at day 1, had not resulted in a faster covering of the wound as could be judged by histological evaluation.

In a study by Henning (7) it was pointed out that mitotic activity in rats reached a peak between 24–28 hours after wounding. In the present experiments no observation was made in the interval between 1 day and 3 days. The delayed activity at 24 hours in the test animals may have been compensated by increased activity before the 3-day observation time.

In rats complete epithelial covering of small wounds (1 mm in diameter) within 5 days with mild to moderate inflammation in the connective tissue has been reported (14). Larger wounds (3 mm in diameter) showed extensive inflammation and alveolar bone resorption. The epithelization was incomplete in many specimens (14). The wounds created in the present study were of intermediate size and in another location of the palate. Though the depth was not standardized, the surgical procedure did not remove all tissue above the bone surface as in the study of Stahl (14). A heavy inflammation was, however, observed in some specimens at day 5, although all wounds were covered by epithelium. At day 3, osteoclastic activity was noted at the bone surface in a few animals. The present findings are in accordance with Klingsberg & Butcher (9) who found that removal of epithelium was nearly as severe a periodontal insult as removal of the entire mucoperiosteum. In their study osteoclastic activity occurred between 1 day and 4 days, and bone loss was evident. But unaffected by connective tissue alterations and bone loss the healing proceeded by lateral proliferation of the epithelium (9).

It may be concluded that epithelial proliferation proved to be a more sensitive method to evaluate local toxicity from dental materials than the proposed mucous membrane irritation test. A clear, short term toxic effect from all dressing materials was demonstrated after 1 day. However, the method could not be used to rank the 3 dressings according to their injurious effect on living cells like the reported bone implantation test (6). Implantation tests offer the advantage of a closed system to study the toxic effect of the materials, but the present test is more relevant to the clinical situation. A potential toxic effect of a dressing will always be modified by a number of factors such as the diluting effect of saliva and the presence of bacteria and plaque formation. The results from the present study support the clinical finding of no differences in healing patterns under the 3 periodontal dressings (3).

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