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EFFECTS OF PERIODONTAL DRESSINGS ON FIBROBLASTS AND GINGIVAL WOUND HEALING IN DOGS

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In the present study the effects of different commercially available periodontal dressings (Peripac, Barricaid, Fittydent, Reso-Pack and Myzotect-tincture) on fibroblast (V-79-379A) proliferation and survival were tested *in vitro*. Barricaid, Fittydent and Reso-Pack periodontal dressings have only small inhibitory effects on cell proliferation (83.3 ± 9%, 71.6 ± 8.7% and 87.3 ± 4.5% of control after 48 h, respectively) in comparison with the great inhibitory effect of Myzotect-tincture (2.9 ± 0.1%) and Peripac (33.7 ± 11.4%) ($p < 0.001$). Barricaid was the only dressing where 41% of cells survived after exposure, while the other four dressings killed all the cells in 6 days. In addition, the healing of artificially created gingival wounds covered by Barricaid and Reso-Pack was followed for 7 days in 12 Beagle dogs. Histological evaluation of gingival tissue demonstrated that wounds covered by Reso-Pack showed the best epithelisation and vascularity and the least inflammatory reaction in first 4 days. Later the observed parameters were similar with those of wounds covered by Barricaid or without pack. The present results indicate that Peripac periodontal dressing and Myzotect-tincture showed the highest cytotoxicity to fibroblasts *in vitro*. From the histological observations in Beagle dogs Reso-Pack has been found to be the most suitable dressing, followed by Barricaid.

Key words: Periodontal dressings, cytotoxicity, fibroblasts, gingival healing, dogs

Gingival enlargement is characterised as various degrees of attached gingival overgrowth. Removal of excess gingival tissue by conventional gingivectomy creates an extensive gingival wound. Therefore, the role of periodontal dressings on gingival healing has been of considerable interest to periodontists. However, the value of periodontal dressings and their effects on periodontal

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wound healing have been questioned by some authors. Stahl et al. (1969) demonstrated that the placement of standard periodontal dressing and oral adhesive bandage after gingivectomy had little influence on the healing process. In addition, several other studies with periodontal dressings applied after flap operation have confirmed such conclusions (Jones and Cassingham, 1979; Allen and Caffesse, 1983; Cheshire et al., 1996). Furthermore, the possibility that bacterial plaque may be found between the dressing and the tissue surface also questions the effectiveness of periodontal packs. However, reduction of postoperative pain and facilitation of healing process have been achieved with the incorporation of antiseptics (Ashoe-Jorgensen et al., 1974; Addy and Douglas, 1975; Bay and Langebaek, 1978) and antibacterial agents (Romanow, 1964; Heaney et al., 1972; Breloff and Caffesse, 1983) in periodontal dressings. In addition, chlorhexidine mouthrinse is a widely used adjunct in periodontal therapy due to its antibacterial effects. Alleyn et al. (1991) described that chlorhexidine significantly inhibits fibroblast growth.

Various *in vitro* cytotoxicity tests with different dressings on different cell lines have been performed. Mouse fibroblasts (L 929, 3T3 cells), Hela cells, human epithelial cells, human gingival fibroblasts and leukocytes have been used (Hildebrand and De Rensis, 1974; Rivera-Hidalgo et al., 1977; Haugen and Hensten-Pettersen, 1978; Gilbert et al., 1994; Alpar et al., 1999). Soluble material leached from some formed dressings has been found toxic to cells in culture (Hildebrand and De Rensis, 1974; Rivera-Hidalgo et al., 1977) and dilution of soluble extracts from periodontal dressings decreased this toxic effect (Eber et al., 1989; Alpar et al., 1999). However, caution must be expressed in extrapolating such data from the *in vitro* environment to the *in vivo* situation. If the dressing adheres to the underlying wound tissue, high concentrations of toxic material are solubilised in the relatively stationary tissue fluids at the pack-tissue junction. Saliva flow cannot dilute soluble toxic substances, so the cellular toxicity of such dressings would be enhanced adjacent to the dressing (Rivera-Hidalgo et al., 1977).

Three categories of the most common periodontal dressings on the dental market are classified as solid and non-soluble, soft and non-soluble, and soft and soluble materials. The aim of our *in vitro* study was to test possible cytotoxic effects of four different periodontal dressings on fibroblast V-79 cell line. The effects of solid and non-soluble (Peripac), soft and non-soluble (Barricaid), and soft and soluble (Fittydent, Reso-Pack and Myzotect-tincture, which is used under the Reso-Pack dressing) periodontal dressings on cell proliferation and cell survival were studied. Barricaid and Reso-Pack showed the most favourable results in *in vitro* testing; therefore, these two dressings were evaluated also clinically and histologically in Beagle dogs.

Materials and methods

Periodontal dressings

Peripac (De Trey, Zurich, Switzerland) is a paste of calcium sulphate, zinc oxide and zinc sulphate with an acrylic type resin and a glycol solvent. It sets when exposed to air or moisture through loss of glycol. It is a solid and non-soluble pack.

Barricaid (The L.D. Coulk Co., Milford, DE, USA) periodontal dressing is a light-activated material available in a syringe for direct application. The gel is based upon a polyether urethane dimethacrylate resin, silanised silica, VLC photo-initiator, accelerator, stabiliser and colorant. After light curing Barricaid stays soft and non-soluble.

Fittydent (Fittydent International GmbH, Pinkafeld, Austria) is a paste containing carboxymethylcellulose, polyvinyl acetate, alcohol, Commiphora myrrh, petrolatum and PEG-90M. The dressing adheres to the wound and remains soft after application. It dissolves on its own after approximately 8 h.

Reso-Pack (Meyer-Haake GmbH, Oberursel, Germany) is a hydrophilic periodontal dressing very similar to Fittydent and is composed of carboxymethylcellulose, polyvinyl acetate, ethyl alcohol, myrrh, white petrolatum (Vaseline) and polyethylene oxide resin. It is a soft dressing that adheres to the wound surface and dissolves in approximately 30 h.

The highly viscous Myzotect-tincture (Meyer-Haake GmbH, Oberursel, Germany) as an astringent and antiseptic solution based on Commiphora myrrh, styrax benzoin, aloe barbadensis, PEG 300, colophonium and hydroxypropylcellulose was also tested. The tincture effectively adheres to the oral mucosa and the manufacturer recommends the use of Myzotect-tincture under the Reso-Pack dressing.

Effect of periodontal dressings on fibroblasts proliferation

Chinese hamster diploid lung fibroblasts (V-79-379 A) were grown in Eagle's MEM (minimal essential medium, Gibco, Paisley, Scotland), supplemented with 10% FCS (fetal calf serum, Gibco, Paisley, Scotland), penicillin (100 U/ml) and streptomycin (100 μg/ml) in a CO_2 incubator at 37 °C.

In all *in vitro* experiments, freshly prepared equally sized specimens, shaped as small spheres (volume: about 17 mm^3), were made and placed into the bottom near the edge of each Petri dish. In the experiments with Myzotect-tincture a volume of about 17 mm^3 was added. After the periodontal dressings had been added to the cell culture, the pH of the growth medium was not changed throughout the experiment. Extracts of four periodontal dressings and Myzotect-tincture were made after 24-h incubation in culture medium and separately tested for cytotoxicity.

Freshly prepared specimens of Peripac, Barricaid, Fittydent and Reso-Pack and Myzotect-tincture were placed into the Petri dish. Barricaid paste was exposed to UV light for 40 sec. Then the cells were added (2×10^5 cells/50-mm dish, in triplicates) and maintained in a CO_2 incubator for 24 and/or 48 h.

In the case of extracts, the cells were seeded (2×10^5 cells/50-mm dish, in triplicates) and after 3 h the medium was changed with extracts and maintained in CO_2 incubator for 48 h.

The growth properties of cell cultures are determined by recording the increase in the number of cells at specific time intervals. After 24 and 48 h the cells were counted using a haemocytometer. Percent inhibition of cell growth (% of control) was expressed as cell count per dish of treated culture against that of control culture, after 24 and 48 h.

Effect of periodontal dressings on fibroblasts survival

The colony forming assay measures the reproductive capacity of single cells, where each viable cell will divide and give rise to a colony. Freshly prepared specimens of four periodontal dressings and Myzotect-tincture were first placed into the Petri dish. Barricaid dressing was cured by UV light for 40 sec. Then cells were added (300–400 cells/50-mm dish in triplicates) and maintained in a CO_2 incubator for 6 more days. In the case of extracts, cells were seeded (300–400 cells/50-mm dish, in triplicates), after 3 h the medium was changed for extracts, and the cells were maintained in CO_2 incubator for 6 days. The colonies were fixed with methanol, stained with 10% Giemsa solution (Kemika, Zagreb, Croatia), and the colonies were counted. The cytotoxic effect was expressed as surviving fraction (SF) and was calculated according to Olah et al. (1978) as follows (Equation 1):

$$SF = \frac{\text{plating efficiency of treated culture}}{\text{plating efficiency of untreated culture}}$$

Effect of periodontal dressings on wound healing

Twelve female, 3.5-year-old Beagle dogs were included in the clinical study. One week prior to the experiment, supragingival scaling and polishing was performed on dogs. A wound was created on the buccal side of canines on the right and left side in maxilla and mandible with a diamond bur-middle coarse (Nº 850, L 018 Dendia, AG, Austria), using a water-cooled machine (Kavo Mondial, Germany) with a rotation speed of 300,000 rpm (Super-Torque, 630 B, Kavo, Germany). The wound area was approximately 50 mm^2.

At baseline, the entire wound area in the upper and lower jaw in 4 animals were covered with periodontal dressing Barricaid (test sites), in 4 dogs with Reso-Pack (test sites), while in other 4 animals the wound areas received no pack

(control sites). In order to ensure the retention of Barricaid dressing silk thread was placed around the tooth, bound by composite resin on the buccal side of the crown. All procedures were performed under anaesthesia. As premedication, 3 mg Heptanon (Pliva, Zagreb, Croatia) and 0.3 mg Combelen (Bayer AG, Leverkusen, Germany) were injected, prolonged by 80–120 mg Propofol (Zeneca, Macclesfield, Cheshire, UK). During the experiment the animals were fed with a soft diet. The experimental protocol was approved by the Veterinary Administration of the Republic of Slovenia (No. 323-02-72/00-2).

Mucoperiosteal samples of tissues were prepared on the mid-wound area with a size of approximately 3×6 mm from first to seventh day after injury. Samples were fixed in 10% neutral buffered formalin for 24 h at 4 °C, then dehydrated with ethanol and embedded in paraffin. The samples were coded for blind evaluation and oriented to give longitudinal sections through the test area in bucco-lingual direction. Five-µm thick sections were cut and mounted on silanised slides. After deparaffinisation, the sections were washed with PBS. Step-serial sections were stained with both Harris haematoxylin and eosin (H.-E.), Masson trichrome or Goldner trichrome. Stained sections were used to detail the healing response with regard to surface epithelisation of the margin and reformation of crevicular epithelium, degree of inflammation and connective tissue behaviour at the wound surface.

For the determination of vascularity, the endothelial cells were stained immunohistochemically by CD31 antibody (DAKO, Denmark). Before immunostaining, enzymatic predigestion with proteolytic enzyme was performed. Morphometric analysis of micro vessels at the wound site was performed on semi-automatic image analysis system (IBAS-1000 Kontron, Germany). Twenty computer-analysed fields were required to evaluate the entire area under 400-fold magnification. Micro vessels per area were counted.

Statistical analysis

Data were analysed by ANOVA followed by Tukey's honesty significant-difference method. A level of $p < 0.05$ was chosen for statistical significance.

Results

Effect of periodontal dressings on cell proliferation

Table 1 represents the effects of solid-pure Peripac, Barricaid, Fittydent, Reso-Pack and Myzotect-tincture solution and their extracts on cell proliferation. Inhibition of growth rate in comparison to control after 24 and 48 h is indicated. Percent of cell growth showed that Barricaid had the least inhibition of cell proliferation.

Table 1

Effect of periodontal dressings and Myzotect-tincture on cell proliferation

	Peripac	Barricaid	Fittydent	Reso-Pack	Myzotect
Solid 24 h	41.8 ± 14.4^{a}	76.7 ± 10.9^{b}	75.1 ± 14.3^{c}	66.2 ± 5.5^{d}	2.6 ± 3.3
Solid 48 h	$33.7 \pm 11.4^{*}$	83.3 ± 9.0^{e}	71.6 ± 8.7^{f}	$87.3 \pm 4.5^{*}$	$2.9 \pm 0.1^{*}$
Extract 48 h	$40.7 \pm 7.1^{*}$	83.4 ± 7.7	71.4 ± 8.3	$68.5 \pm 5.9^{*}$	$5.6 \pm 2.4^{*}$

The increase in cell number was determined by cell counting after 24 and 48-h exposure to periodontal dressings or 48-h exposure to extracts. The cytotoxic effect was expressed as % of control in relation to the exposure time. Values represent the means ± SD of four experiments each in triplicates. Statistically significant differences: between a) and b) $p < 0.001$; between c) and d) $p < 0.001$; between e) and f) $p < 0.02$; *between periodontal dressings (Peripac, Reso-Pack) and Myzotect-tincture after 48 h with solid material or their extracts $p < 0.001$

Fittydent showed slightly higher inhibition of cell proliferation at 24 h and statistically significant inhibition was observed after 48 h ($p < 0.02$) as compared to Barricaid. Reso-Pack showed smaller inhibitory effect but only after 48 h. The inhibitory effect of Peripac on cell proliferation was substantial while Myzotect-tincture exhibited almost complete inhibition of cell proliferation.

In the case of the extracts, significantly the highest effect was observed in the case of Peripac periodontal dressing and Myzotect-tincture in comparison with the other dressings ($p < 0.001$). Extracts of Reso-Pack periodontal dressing showed higher inhibition of growth rate than solid-pure material after 48 h ($p < 0.001$).

Effect of periodontal dressings on cell survival

The cytocidal effects of Peripac, Barricaid, Fittydent, Reso-Pack and Myzotect-tincture and their extracts on V-79 cells are shown in Table 2. It can be observed that Peripac, Fittydent, Reso-Pack and Myzotect-tincture have drastic cytotoxic effects on cell survival (SF = 0) after 6-day exposure to dressing. Barricaid was the only dressing where 41% of cells survived ($SF = 0.41 \pm 0.09$).

Table 2

Effect of periodontal dressings and Myzotect-tincture on cell survival

	Peripac	Barricaid	Fittydent	Reso-Pack	Myzotect
Solid	0.00	0.41 ± 0.09	0.00	0.00	0.00
Extract	0.49 ± 0.09	0.6 ± 0.13	0.03 ± 0.01	0.14 ± 0.04	0.00

The cells were incubated with periodontal dressings and their extracts for 6 days. The colonies were fixed, stained and counted and cytotoxic effect was expressed as surviving fraction (SF), calculated by Equation 1. Values represent the means ± SD of three or four experiments, each in triplicates

The extracts showed less pronounced cytotoxic effects than solid-pure materials. Barricaid and Peripac extracts exhibited the lowest cytotoxicity (SF = 0.6 ± 0.13 and 0.49 ± 0.09, respectively) and Myzotect-tincture or Fittydent the highest cytotoxicity (SF = 0 or 0.03 ± 0.01) on fibroblasts.

Clinical and histological findings

On the first day after experimental injury, a small difference was observed clinically between pack and no-pack areas. Nevertheless, wounds with Reso-Pack dressing showed the best condition compared with Barricaid and control sites. Under Reso-Pack some small red islands bleeding on touch were observed but under Barricaid and in wounds without dressing there were a lot of them. Almost the same condition was observed three days after gingival injury. On the fourth day after injury an approximately 3-mm wide inflamed gingival margin was observed at the control sites. Under Barricaid periodontal dressing the gingival margin was inflamed with bleeding islands all over the wounds. Only slightly inflamed gingival margin was observed under Reso-Pack. On the seventh day after injury inflamed gingival margin was detected in all experimental sites.

Twenty-four hours after surgery the histological picture of wounds of all three experimental groups was similar (Fig. 1). Epithelisation at the wound edge was absent and the wounds were covered by a coagulum. The connective tissue beneath the injury was slightly damaged. A slight acute inflammatory reaction developed at the gingival margin with the dilatation of micro vessels, and disrupted collagen was observed under the surface of the injury. Two and three days after the injury a coagulum still covered the wound and epithelium was proliferating from the wound edges. There was no inflammation in the underlying connective tissue.

After 4 days the coagulum had disappeared but a thin fibrin cloth was still present. Epithelial regeneration from the wound edge that bridges the gap had proceeded. Epithelisation was not complete and without rete pegs. Reformation of the crevicular epithelium was more advanced in wounds covered with Reso-Pack. The connective tissue showed no differences among wounds of the three experimental groups. Regarding epithelisation, the healing response of wounds with Reso-Pack is slightly better (Fig. 2C) in comparison with the healing of wounds with Barricaid or with the control site (Figs 2A and 2B). Seven days after injury, wounds of all three experimental groups were covered by a non-keratinised epithelium with developing rete pegs.

Morphometric analyses of vascularity after immunostaining showed that on the first day after injury the number of vessels per mm^2 was the highest in wounds covered with Reso-Pack ($p < 0.01$) and decreased three days after injury (Fig. 3). The vascularity of wounds without pack showed the same trend as that of wounds with Reso-Pack, while the vascularity of wounds with Barricaid was enhanced with the progression of healing. Four days after injury the vascularity in wounds of all three experimental groups was enhanced and was almost of the same level as seven days after the injury (Fig. 3).

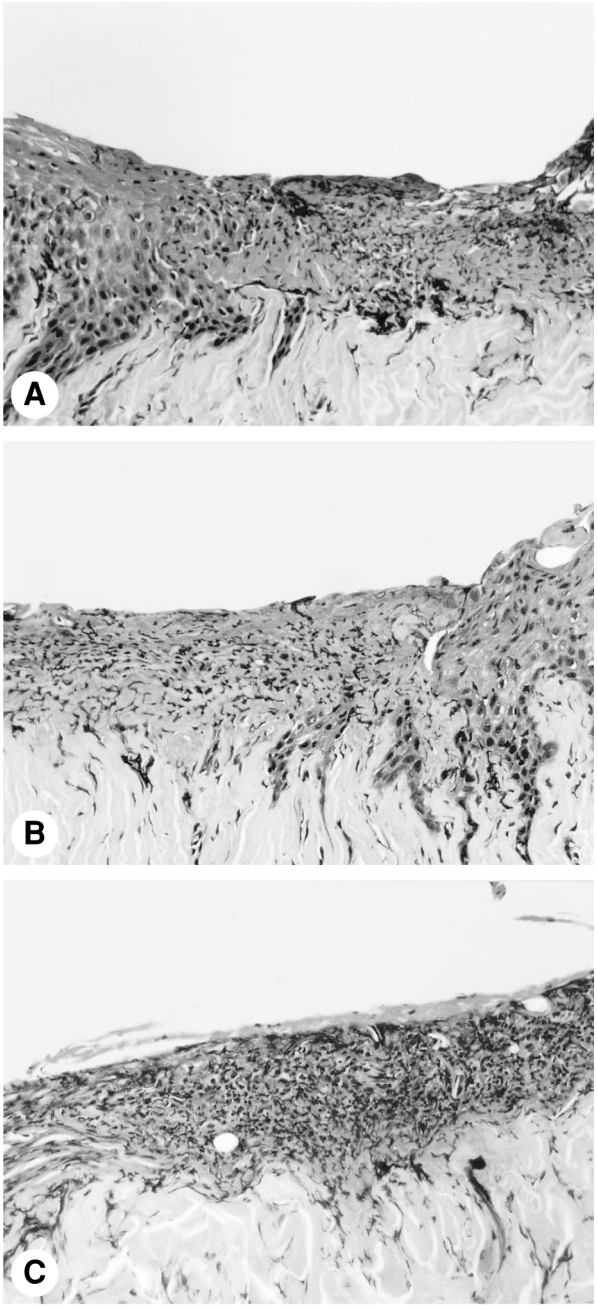

Fig. 1. Histological picture of the gingiva on the first day after the experimental injury (haematoxylin and eosin, original magnification: × 116). Wounds were similar and covered by fibrin cloth. A slight acute inflammatory reaction developed at the gingival margin. Epithelisation at the wound edge was absent. A: wound without pack, B: wound with Barricaid, C: wound with Reso-Pack

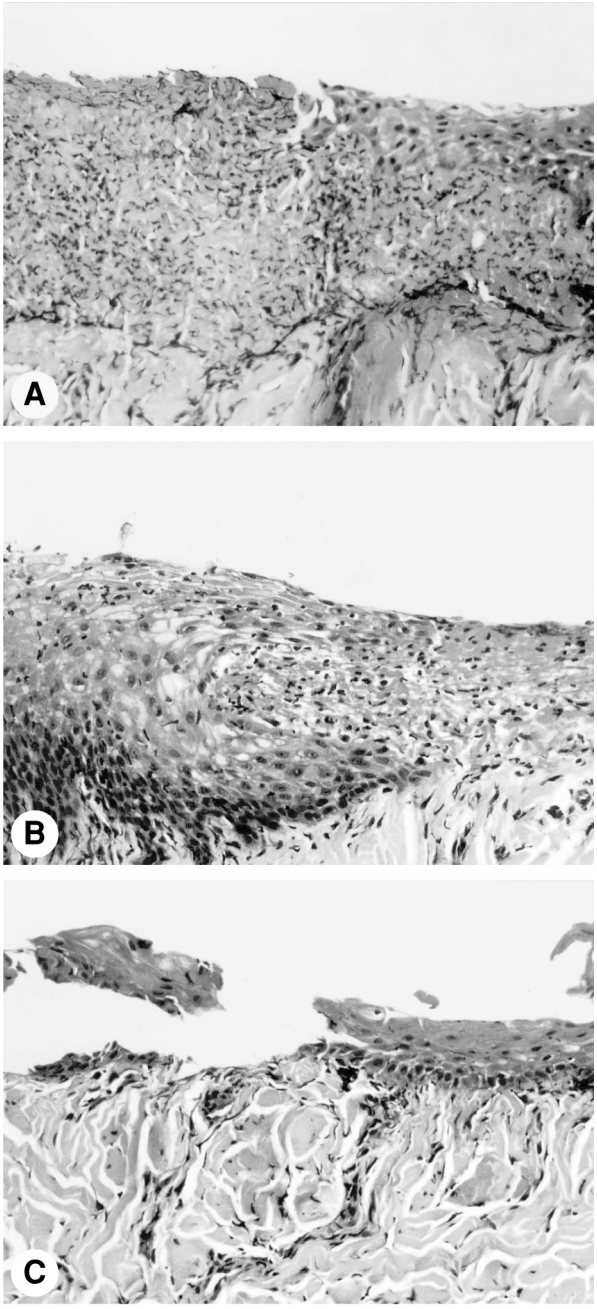

Fig. 2. Histological picture of the gingiva four days after the injury (haematoxylin and eosin, original magnification: × 116). The fibrin cloth has disappeared and epithelial regeneration from the wound edge has proceeded. The epithelium is thin, non-keratinised and without rete pegs. A: wound without pack, B: wound with Barricaid, C: wound with Reso-Pack

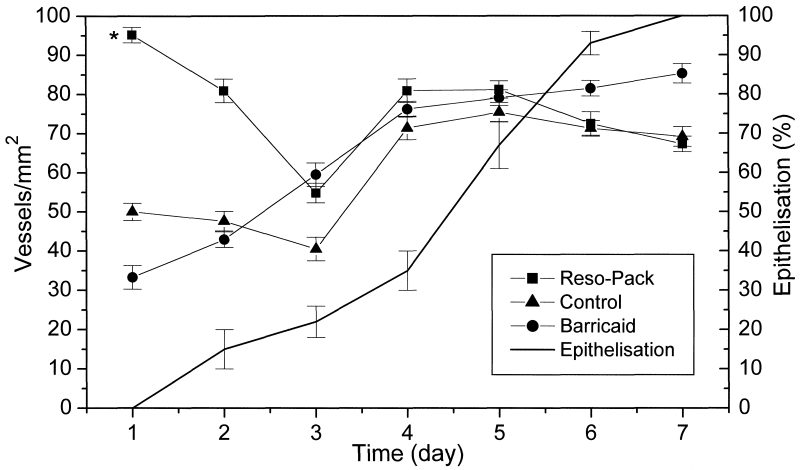

Fig. 3. Number of vessels per mm^2 in wounds with Barricaid, Reso-Pack and in control areas without pack during the time of healing. Percent of wound epithelisation was shown for all test and control sites. *Statistically significant level: $p < 0.01$

Discussion

Periodontal dressings should protect the periodontal wound against mechanical injuries during mastication and against bacterial invasion into the tissue, with the aim to decrease pain and facilitate healing. Therefore the pack should adhere to the surface of the wound, preventing the formation of bacterial plaque.

Secondly, the pack should be soft to prevent tissue damage and it should have no cytotoxic and other detrimental effects on the underlying tissues. It was found that eugenol-containing dressings showed delayed healing (Kozan and Mantell, 1978), more allergic reactions (Barkin et al., 1984) and more inhibition of fibroblast proliferation (Eber et al., 1989) compared to non-eugenol containing dressings. In the present study the cytotoxic effects of four different non-eugenol periodontal dressings and a viscous tincture on V-79 fibroblast cell culture were studied.

The use of different animal and human cell culture *in vitro* models for testing the cytotoxic effects of periodontal dressings may give information about biological effects *in vivo* (Eber et al., 1989). Several primary cell cultures and established cell lines showed different response to various periodontal dressings (Alpar et al., 1999). Diverse viability assays are now available to measure the cytotoxicity of different substances in mammalian cell systems. Methods of reproductive assay (growth rate determination, *in vitro* colony formation) are important to investigate the response of proliferating cells in culture to chemical or physical materials. It has been suggested that the *in vitro* colony formation assay is the most relevant method for estimation of cytotoxicity (Cook and Mitchell, 1989).

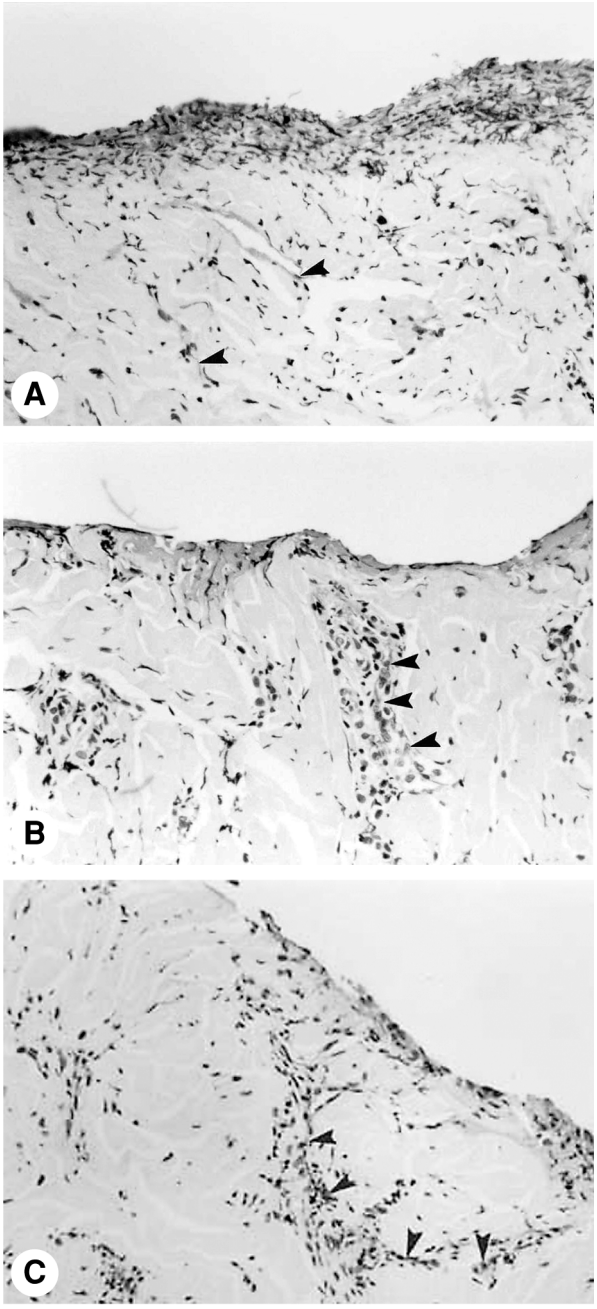

Fig. 4. Micro vessels in wounds (arrows) illustrated immunohistochemically in all three experimental groups on the first day after surgery (original magnification: × 400).
A: wound without pack, B: wound with Barricaid, C: wound with Reso-Pack

In the present study the cytotoxic effects of four different periodontal dressings and tincture were tested on cell proliferation and survival. Peripac paste had a drastic toxic effect on cell survival (no cell colony was formed after treatment) and cell proliferation (growth rate was reduced to 33.7 ± 11.4% of control after 48 h). This is in accordance with the findings of Haugen and Hensten-Pettersen (1978) who also tested the cytotoxic effect of freshly prepared samples of Peripac on cultured human epithelial cells. After 24-h incubation total cell lysis was observed. Another Peripac treatment on 3T3 mouse fibroblasts resulted in no viable cells after 24 h as reported by Alpar et al. (1999). In our study Barricaid dressing showed the lowest inhibitory effect on cell proliferation (growth rate was reduced to 83.3 ± 9% of control after 48 h) and cell survival (SF = 0.41 ± 0.09) with fully cured material. Gilbert et al. (1994) and Alpar et al. (1999) also found no cytotoxic alterations on fibroblasts and HeLa cells with fully cured dressing. In contrast, direct contact of uncured material caused growth inhibition and cell death. We confirmed a greater effect on fibroblasts with uncured dressing compared to fully cured material (data not shown).

The values of the third periodontal dressing Fittydent, included in the current study, were between those of Barricaid and Peripac. Fittydent is a soluble paste which adheres to the underlying wound surface. Therefore, the cellular toxicity of such dressing in clinical situation would be much greater according to the data of Rivera-Hidalgo et al. (1977). It was shown that Fittydent has rather small effect on cell proliferation (75.1 ± 14.3% of control after 24 h and 71.6 ± 8.7 of control after 48 h) but it exerted a drastic effect on cell survival (no cell colony was formed after the treatment). In this case, the cytocidal effect of dressing on the single cell in the colony forming ability test was strong (300 cells/ 50-mm Petri dish) at the beginning of the experiment.

The periodontal dressing Reso-Pac, very similar to Fittydent, is a soft and soluble hydrophilic periodontal dressing and the manufacturer recommends the use of Myzotect-tincture under the dressing. However, Myzotect-tincture, either pure or its extract, showed a drastic growth inhibitory effect on fibroblasts *in vitro*. Pure Reso-Pac dressing also had a detrimental effect on cell survival. On the other hand, its extract was less effective.

It was pointed out that clinically toxic materials leached from dressings should be diluted by the continuous salivary and tissue fluid flow, so irritation to the wounded tissue would be diminished. The study with the soluble extracts from periodontal dressings on leukocytes *in vitro* showed that a 10-fold dilution of extract decreased their toxic effect (Rivera-Hidalgo et al., 1977). The present results, where the cytotoxic effects of Peripac, Barricaid, Fittydent, and Reso-Pack periodontal dressings and Myzotect-tincture were tested on cell proliferation and cell survival of fibroblast V-79 cell line *in vitro*, indicate that Barricaid showed the most favourable results, followed by Reso-Pack, Peripac, Fittydent, and Myzotect-tincture.

However, *in vivo* conditions are markedly different from *in vitro* conditions, and a study with extracts would be much more similar to clinical circumstances. Therefore, an *in vivo* study was done on Beagle dogs with Barricaid and Reso-Pack periodontal dressings. Histological examination was used for evaluating wound healing by the comparison of packed versus non-packed sites. Analysis of vascularity showed that on the first day after surgery the number of vessels was the highest in wounds with Reso-Pack followed by no-pack and by Barricaid (Fig. 4). Obviously, this may have been due to the effect of Reso-Pack periodontal dressing on the tissue in the first hours. Next day the vascularity of wounds under Reso-Pack periodontal dressing was decreased as well as in the control sites. From the third to the fourth day after surgery the vascularity in wounds covered with Reso-Pack and in the control sites increased and was almost on the same level as in the Barricaid group. A decrease of vascularity from the fifth to the seventh day after surgery was then observed in Reso-Pack and control sites. In Barricaid test sites vascularity increased with the duration of healing. A possible explanation would be the effect of plaque under the dressing that irritates the tissue. Epithelisation of test and control sites was approximately 60% on the fifth day and almost complete on the seventh day after surgery (Fig. 3).

The present results indicate that Peripac periodontal dressing and Myzotect-tincture showed the highest cytotoxicity to fibroblasts *in vitro*. From the clinical and histological observations we can conclude that Reso-Pack is the most suitable periodontal dressing for clinical use in comparison to Barricaid or no pack areas.

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