International Journal of Dental Hygiene

Official Journal of the International Federation of Dental Hygienists

ORIGINAL ARTICLE

HM Hammad
MM Hammad
IN Abdelhadi
MS Khalifeh

Effects of topically applied agents on intra-oral wound healing in a rat model: a clinical and histomorphometric study*

Authors' affiliations:
Huda M. Hammad, Department of Oral Medicine and Surgery, Faculty of Dentistry, Jordan University of Science and Technology, Irbid, Jordan
Mohammad M. Hammad, *Ibrahim N. Abdelhadi*, Department of Preventive Dentistry, Faculty of Dentistry, Jordan University of Science and Technology, Irbid, Jordan
Ibrahim N. Abdelhadi, Private practice, Amman, Jordan
Mohammad S. Khalifeh, Department of Veterinary Basic Medical Science, Faculty of Veterinary Medicine, Jordan University of Science and Technology, Irbid, Jordan

Correspondence to:
Huda M. Hammad
Department of Oral Medicine and Surgery
Faculty of Dentistry
Jordan University of Science and Technology
P.O. Box 3030
Irbid 22110
Jordan
Tel.: ++ 962 2 7201000 ext. 23570
Fax: ++ 962 2 7095115
E-mail: hmhammad@just.edu.jo, hmhammad@yahoo.com
*This study was supported by Grant no. 130/2007, Jordan University of Science and Technology, Irbid, Jordan.

Dates:
Accepted 25 May 2009

To cite this article:
Int J Dent Hygiene **9**, 2011; 9–16
DOI: 10.1111/j.1601-5037.2009.00410.x
Hammad HM, Hammad MM, Abdelhadi IN, Khalifeh MS. Effects of topically applied agents on intra-oral wound healing in a rat model: a clinical and histomorphometric study.

© 2009 The Authors.
Journal compilation © 2009 Blackwell Munksgaard

Abstract: *Objectives:* Topically applied chlorhexidine and hyaluronan have many studies supporting their use to enhance oral wound healing. Allantoin is widely used topically to promote epithelial proliferation and wound healing, with very little scientific evidence to support such uses. This study investigated and compared the influence of these agents on the healing of intra-oral excisional wounds with large epithelial and connective tissue defects. *Methods:* Excisional wounds, 3 mm in diameter, were made at the centre of the palate of 125 Wistar male albino rats. Five animals constituted the baseline group at time 0. The remaining animals were divided into four experimental and one control groups, in which chlorhexidine digluconate gel 0.2% (Perio.Kin®), hyaluronan gel (Gengigel®), allantoin 0.5% in vehicle gel, vehicle gel alone and nothing were applied daily to the wounds. The wound areas were measured photographically and the epithelialization rates were determined histologically at 0, 3, 7, 14 and 21 days post-surgery. *Results:* The mean wound area and mean distance between the epithelial margins decreased significantly with time in all experimental and control groups ($P < 0.05$). A significant rate of wound area reduction was observed following the use of Perio.Kin® and Gengigel® at 7 and 14 days. Perio.Kin® showed a significant rate of wound epithelialization at 7 days. Allantoin did not positively or negatively affect wound healing. *Conclusions:* None of the tested agents had a negative effect on the rate of wound healing when applied on an excisional wound with epithelial and connective tissue defect. Positive results were achieved with Perio.Kin® and Gengigel®.

Key words: allantoin; chlorhexidine; hyaluronan; oral wound healing

Introduction

Many agents have been investigated in the quest for an agent that promotes oral wound healing and reduces post-operative complications. With the difficulties in post-operative plaque control after oral surgical procedures, topical antimicrobial agents are recommended to enhance wound healing by reducing the accumulation of plaque, in addition to reducing post-operative pain and swelling (1, 2).

Maintenance of a high level of oral hygiene and plaque control have been found to be important factors that determine the success of various oral surgical procedures, including periodontal (2, 3) and implant procedures (4). This is especially important in certain wound types which heal by secondary intention, such as those created at palatal donor sites used for free soft tissue grafts, where a significant amount of tissue loss occurs. These are often associated with post-operative discomfort, pain and sometimes delayed healing (5). In such wounds, the topical use of antimicrobial agents is especially recommended and widely used (6). Chlorhexidine is a cationic bisbiguanide with broad antimicrobial activity (7). Its ability to reduce plaque formation in a variety of concentrations has made it the gold standard antiplaque agent, with immense literature on its use and efficacy in plaque control (8).

In addition to antimicrobials, the use of some biomaterials has been introduced as an alternative approach to enhance wound healing. Hyaluronan or hyaluronic acid is one such candidate (9, 10). Hyaluronan is a member of the glycosaminoglycan constituents of the extracellular matrices of many tissues (11). It has a multifunctional role in the healing of wounds (9, 10). By virtue of its biocompatibility and non-toxicity, it is used in many biomedical fields, such as ophthalmology, dermatology and rheumatology (12). It is recommended as a very promising candidate to mediate periodontal tissue regeneration and wound healing (10). In patients with gingivitis, hyaluronan induces tissue repair and healing when topically applied (13, 14). Additionally, it is reported to be successful in maintenance following implant surgical procedures (15) and in the management of recurrent aphthous ulceration (16).

Another agent to which wound healing properties are attributed, but nevertheless inadequately investigated, is allantoin, a final product of uric acid metabolism in most organisms including some bacteria, plants and animals (17). Allantoin is thought to be the active ingredient in comfrey (*Symphytum officinale*), also known as knitbone and bruisewort, an herb which has a long history of use in the treatment of bruises, sprains and fractures in the form of poultices (18). Comfrey is also claimed to be of benefit in treating cuts and wounds and gastrointestinal ulcers (19). Most of these effects have been reported anecdotally and most medical literature regarding comfrey is limited to its potential liver toxicity with internal use (18). Allantoin used currently in the cosmeceutical industry is synthetic (20). Manufacturers list several beneficial effects for allantoin as an active ingredient in over-the-counter cosmetics, including keratolytic, moisturizing, soothing and anti-irritant properties. It is also advocated for its ability to promote the renewal of epidermal cells as well as accelerate wounds healing (21). Controlled studies confirming the efficacy of allantoin in wound healing are very limited (22). Allantoin is also widely used in oral products such as mouthwashes and toothpastes, and according to the US Food and Drug Administration (FDA), it is safe for use as an oral wound healing agent but there are inadequate data to establish general recognition of its effectiveness as such (23). The aim of this study was to investigate and compare the influence of topically applied chlorhexidine, hyaluronan and allantoin on the healing process of intra-oral excisional wounds with large epithelial and connective tissue defects that heal by secondary intention.

Materials and methods

One hundred and twenty five male Wistar albino rats, 12–16 weeks old, weighing 300–350 g were used. The study protocol was approved by the Jordan University of Science and Technology (JUST) Deanship of Scientific Research and the JUST Animal Care and Use Committee which follows the guidelines of the National Institute of Health, USA (24). Animal maintenance and treatment were carried out at the animal house facility at the Biomedical Research Center, JUST. The rats were anaesthetized using a regimen consisting of atropine (0.02–0.05 mg kg^{-1}), ketamine hydrochloride (40–87 mg kg^{-1}) and xylazine hydrochloride (5–13 mg kg^{-1}) administered intramuscularly. Animals were later killed using an anaesthetic overdose. The animals were monitored for weight loss and feeding behaviour throughout the experiment to ensure that they were not affected by the palatal wounds.

After anaesthesia, a circular, 3 mm diameter excisional wound was made in the centre of the palatal mucosa, using a disposable punch biopsy tool (Kai Medical, Kai Industries Co., Ltd., Seki City, Japan). Mucoperiosteal specimens were removed by sharp dissection exposing a circular area of bare bone left for secondary healing (25).

Five animals were killed immediately and provided the baseline group at time 0. The remaining 120 animals were randomly divided into five groups. Each of four experimental groups received a daily application of one of the tested agents

listed below, and in one control group, nothing was applied. At 24 h postoperatively, mild anaesthesia (i.m. injection containing 0.1 ml of 10% ketamine hydrochloride and 0.1 ml of 20% xylazine hydrochloride) was used to apply agents on the wounds every day. Without touching the wound, 1 ml of the tested agent was delivered directly to the wound using a syringe with a blunt cannula. After 2 h of agent application, animals were fed a standard diet of pellets and water *ad libitum*.

The tested agents included the following:

1 Perio.Kin® gel (Laboratorios Kin, S. A., Barcelona, Spain) contains chlorhexidine 0.2% and excipients.
2 Gengigel® gel (Ricerfarma, Milano, Italy) contains hyaluronic acid 0.2%, xylitol and excipients.
3 Allantoin in a vehicle gel: allantoin powder [International Specialty Products (ISP), Wayne, NJ, USA] was added to a vehicle gel, mentioned below, to a final concentration of 0.5%.
4 Vehicle gel without active ingredient (placebo) kindly prepared by the laboratory of the Jordanian Pharmaceutical Manufacturing Company, Jordanian Pharmaceutical manufacturing Co., Ltd., naor, Jordan. Each gram contained carbopol 980, 3 mg; benzyl alcohol, 10 mg; propylene glycol, 50 mg; triethanolamine, 5 mg and H_2O, 932 mg.

Six animals from each group were killed at 3, 7, 14 and 21 days (6). After death of the animals, maxillae were separated and every wound was evaluated clinically using photography and histologically. The palatal specimens were photographed at a constant distance and magnification using a Fujifilm Finepix S5700 Digital Camera (Fujifilm Corp., Tokyo, Japan). A constant distance between the camera and the specimen was maintained by placing the camera in a box especially made for this purpose. To ensure maximum accuracy, a scientific ruler was photographed with the specimens. The digital photographs were transferred to a computer and the mean wound surface area (MWSA) was calculated for each animal using AutoCAD® 2007 software Autodesk Inc., San Rafael, CA, USA where the ruler in the photograph was used as a scale reference.

After taking the photographs, specimens were directly transferred into 10% formalin for fixation for at least 24 h. Specimens were then decalcified in 10% formic acid for 2 weeks (6), and processed for histological evaluation. For each wound, five serial sections, 5 μm apart, were cut perpendicular to the palatal midline at the widest diameter of the wound and stained with haematoxylin and eosin. Sections were examined using light microscopy at a magnification of ×40 and the distance between the epithelial margins in each section was measured with the aid of a calibrated ocular micrometre. The mean wound width (MWW) was calculated for each group at every time point.

Statistical analyses were performed using Student's *t*-test for both photographic and histomorphometric evaluation. The difference between the groups was considered significant at a value of $P < 0.05$.

Results

Clinical and gross observations

Clinical examination of the wounds (Fig. 1) showed gradual healing over time in all groups. Slow wound healing was observed at 3 and 7 days postoperatively (Fig. 1b and c). Macroscopically, bone was covered with a serofibrinous layer. The

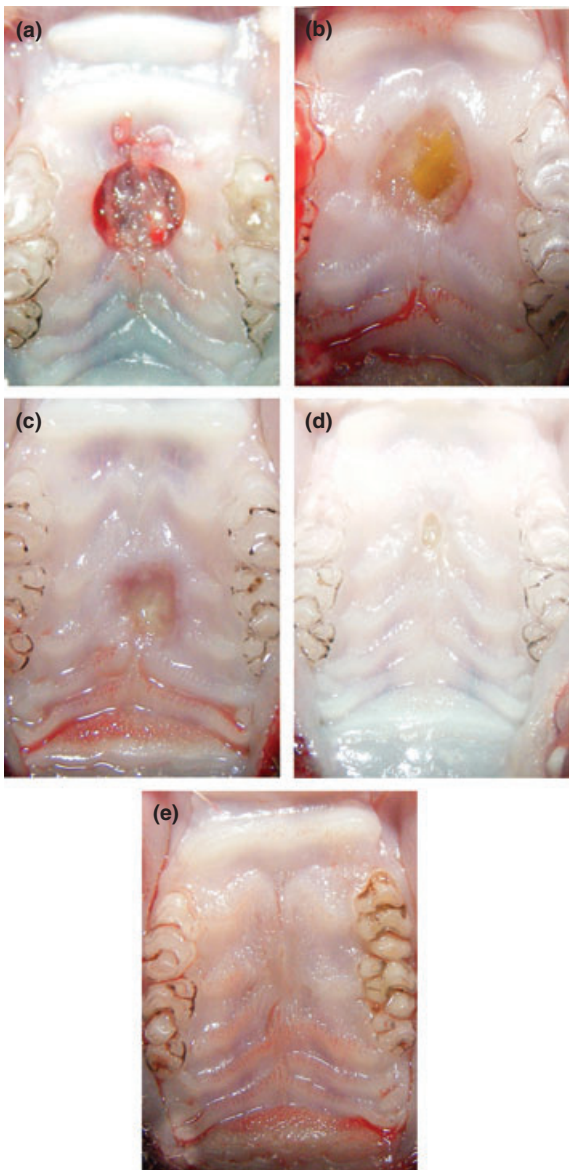

Fig. 1. Clinical photographs of the palatal wounds showing gradual healing taken at days 0 (a), 3 (b), 7 (c), 14 (d) and 21 (e) from the control group.

margins of the wound became irregular and started to migrate towards the centre of the wound. The area of fibrin covering the base of the wound decreased rapidly from day 7 to day 14. At 14 days post-operation, the defect was largely covered with epithelium (Fig. 1d). At the end of the experiment (day 21), most of the defects were healed with a minimal central depression (Fig. 1e).

Digital image observations

There were no significant statistical differences between the control and placebo groups clinically or histologically. Therefore, data from both groups were combined and presented as a control plus (control+) group. The MWSA measurements for all groups at different time points are shown in the chart in Fig. 2. The MWSA decreased significantly with time in all experimental and control groups.

At day 3, all groups showed significant reduction of the MWSA compared with the baseline group at day 0. However, there were no significant differences among the groups at this time point. At the next time point tested (day 7), all groups showed variable reduction trends of the MWSA in reference to the earlier time point, but the reduction was only significant in the Perio.Kin®-treated group ($P < 0.05$). At this time point (day 7); the MWSAs were significantly smaller in both the Perio.Kin® and Gengigel® groups compared with the control+ and allantoin groups ($P < 0.05$). Fourteen days after treatment application, the MWSAs continued to significantly decrease in all groups ($P < 0.05$) except the Gengigel® group, in which the reduction was not significant with reference to the earlier time point (day 7). Nevertheless, the MWSAs at day 14 were significantly lower in both the Perio.Kin® and Gengigel® groups compared with the control+ and allantoin groups ($P < 0.05$). At the last time point tested (day 21), the MWSAs in the Perio.Kin® and Gengigel® groups were very low, while the control+ and allantoin groups still had small residual lesions, although the MWSAs were significantly reduced compared with day 14 ($P < 0.05$).

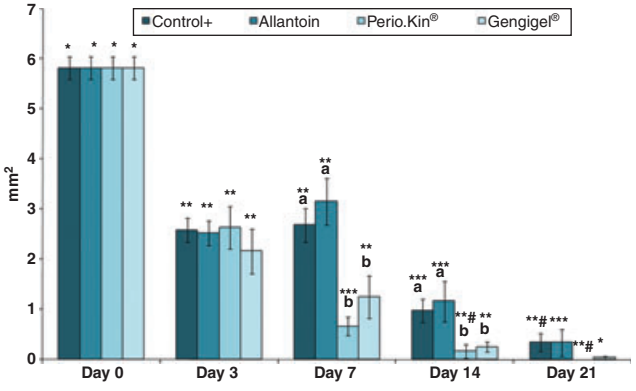

Fig. 2. The mean wound surface areas (±SEM) in square millimetres evaluated at four time points. The introduced wounds in rat palates were photographed at each time point and then evaluated using AutoCAD® 2007 software in each experimental group. For the control+ group ($n = 12$), for each of the other groups; chlorhexidine digluconate gel 0.2% (Perio.Kin®), hyaluronan gel (Gengigel®), allantoin 0.5% in vehicle gel ($n = 6$). Comparison is represented within each time point by letters and across times by symbols; different letters or symbols indicate a significant difference ($P < 0.05$).

Histological observations

The histopathological features of the ulcers were essentially the same for the control group and the tested agents (Fig. 3). The freshly created ulcers at the beginning of the experiment consisted of a defect in epithelium, with a denuded bony base. At the third day, a necrotic base was seen, consisting of a serofibrinous coagulum and cellular debris, with inflamed granulation tissue underneath and the epithelium at the margins proliferating towards the centre. With time, there was gradual proliferation of epithelium to close the defect, with complete healing in some cases at the 21-day examination, while in other cases, only small defects were still observed.

The MWW measurements for all groups at the different time points are shown in the chart in Fig. 4. The MWW decreased significantly with time in all experimental and control groups. Figure 5 shows variation in the MWW at day 7 of wound healing in the different groups. However, there were no significant differences among the groups at all time points tested except at day 7, where the MWW for the Perio.Kin® group was significantly smaller than that of all the other groups ($P < 0.05$). At day 3, only the Gengigel® group showed a significant reduction in the MWW compared with the baseline group at day 0 ($P < 0.05$). At day 7, the reduction in the MWW between days 3 and 7 was significant only for the Perio.Kin® group ($P < 0.05$). At day 14 of the experiment, the MWW reduction was significant only for the Gengigel® and allantoin groups. At the end of the experiment (day 21), the reduction in the MWW from day 14 became significant in the control+ group and continued to decrease significantly in the allantoin group ($P < 0.05$).

Discussion

The palatal excisional wound in the rat used as a model in this study represented a reproducible wound that could be followed clinically and histologically. Many studies used this model to investigate intra-oral wound healing or factors that

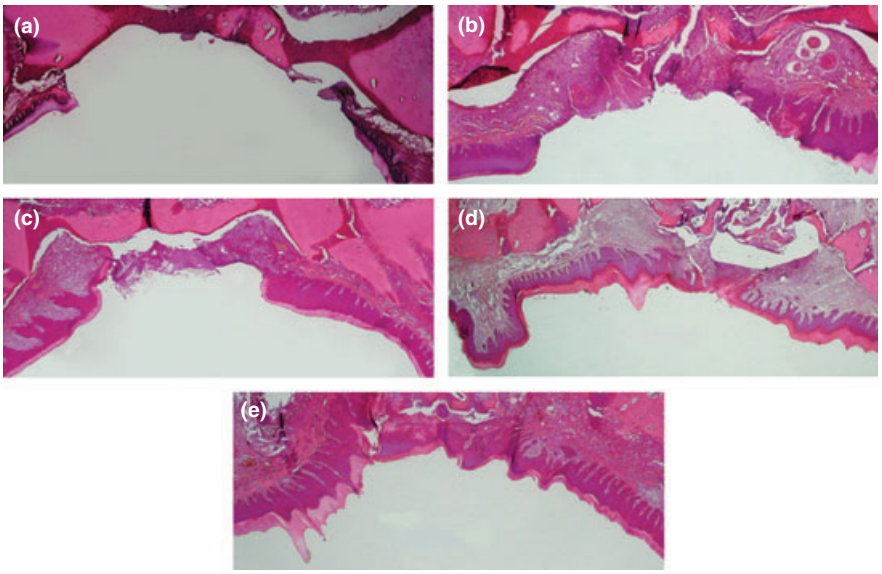

Fig. 3. Low power photomicrograph of the rat palate showing gradual healing of the excisional wounds in the control group at days 0 (a), 3 (b), 7 (c), 14 (d) and 21 (e) (haematoxylin and eosin stain, original magnification 40×).

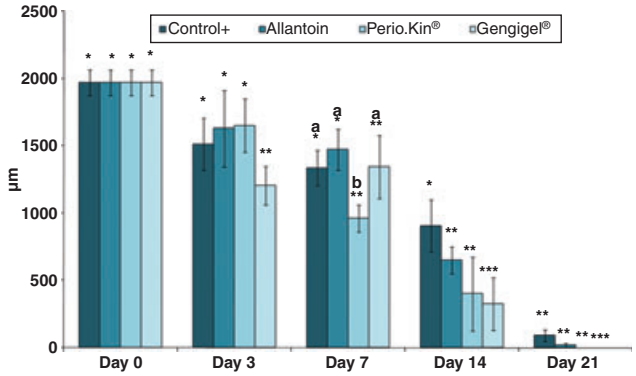

Fig. 4. The mean wound width (±SEM) in micrometres evaluated at four time points. The distance between the epithelial wound margins were measured microscopically using a calibrated ocular micrometre. For the control+ group (n = 12), for each of the other groups; chlorhexidine digluconate gel 0.2% (Perio.Kin®), hyaluronan gel (Gengigel®), allantoin 0.5% in vehicle gel (n = 6). Comparison is represented within each time point by letters and across times by symbols; different letters or symbols indicate a significant difference ($P < 0.05$).

might affect it (6, 25–27). In this study, standardized digital photographs were magnified by computer and the boundaries of the wound were determined on the magnified image, then the surface areas were calculated using AutoCAD® 2007 software. This method introduced by this study for wound surface area calculation reduced human error factors which might occur with other methods. For example, Kahnberg and Thilander (25) used tracing paper in their study to calculate the surface area, while Kozlovsky *et al.* (6) used a special marker to determine the wound margins immediately on the specimen before taking the photograph, and then calculated the surface area using a digitalizer. Use of a marker on the margins of an initially small, 5 mm in diameter wound, which becomes even smaller with time, is likely to increase the possibility of error.

The histomorphometric evaluation was similar to that used by Kozlovsky *et al.* (6) where the distance between the epithelial margins was measured. The main drawback of this method of evaluation is that it assesses only one dimension of the wound and lacks the ability to evaluate the changes that occur in the depth or length of the wound. The photographic and histomorphometric results of this experiment revealed that the dimensions of the wounds decreased significantly over time. These findings were consistent with those reported in the study by Kahnberg and Thilander (25), in which clinical healing of 3 mm-in-diameter excisional wounds in rats increased over time and became complete in most of the animals at 21 days.

The main concept in using chlorhexidine to promote wound healing is that chlorhexidine reduces the bacterial load on the wound. The clinical significance of a critical level of the bacterial load in impairing wound healing has long been established (28). Invasive wound infection interferes with the normal wound healing process (29, 30).The body's defence against infection through activation of inflammatory cells and mediators injures the granulation and the surrounding normal tissues by a variety of interactions (29, 30). Toxins and fibrinolytic enzymes produced by certain bacteria further impairs the healing process (30). Moreover, surrounding oedema and inflammation isolated the affected tissues and formation of bacterial biofilms protects bacteria from antimicrobials (30). These factors may collectively impair therapeutic intervention and wound healing. Conversely, a small bacterial load may increase the rate of wound healing by accentuating the inflammatory reaction that is a prerequisite for tissue repair (31).

It is important to note here that studies on chlorhexidine have shown considerable contradiction on its effect on wound healing. A number of *in vitro* studies showed that

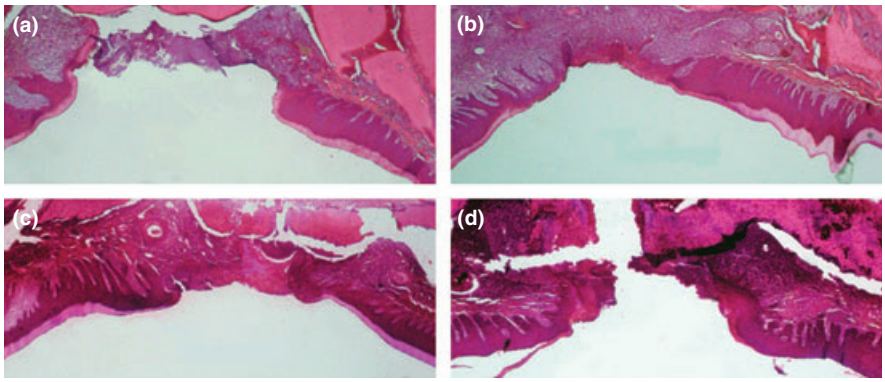

Fig. 5. Low power photomicrograph of the palatal wounds at day 7 showing variation of wound healing in the different groups. (a) Control group, (b) Perio.Kin® group, (c) Gengigel® group and (d) Allantoin group (haematoxylin and eosin stain, original magnification 40×).

chlorhexidine negatively affected fibroblast and keratinocyte proliferation in a concentration- and time-dependent manner (32–35). However, only some *in vivo* studies (36, 37) indicated a negative effect of chlorhexidine on wound healing, while a considerable number of studies indicated the value of chlorhexidine rinses after various oral surgical procedures and a positive effect of chlorhexidine on wound healing (1, 6, 38–44). The results of this study confirmed the positive *in vivo* effect of chlorhexidine. The contradiction between *in vivo* and *in vitro* results may be explained by the different cellular, molecular and environmental interactions affecting the tissue *in vivo* as opposed to those *in vitro*. For instance, in the oral cavity, chlorhexidine binds mostly to bacteria, and an additional amount of the applied chlorhexidine is precipitated by serum proteins (45, 46). Therefore, the remaining amount of chlorhexidine molecules available to bind to and harm host cells in the wound is significantly reduced (6).

In this study, Perio.Kin® (chlorhexidine gel 0.2%) resulted in significant wound improvement at the clinical and histological levels. This is in partial agreement with the observation made by Kozlovsky *et al.* (6) who found that chlorhexidine enhanced wound healing significantly at the histological level but not at the clinical level. The positive clinical result in this study may be due to the different methods used to calculate the surface area of the wound.

A similar histomorphometric study on palatal excisional wound healing in rats revealed that wound healing was disturbed by a chlorhexidine concentration of 0.5% and that wound healing was only slightly delayed with a chlorhexidine concentration of 0.1% and 0.2% (36). However, one of the drawbacks of that study was that all the animals in the control and experimental groups were receiving a systemic antibiotic during the experiment, which must have reduced the bacterial influence on wound healing; as the bacterial load might positively (31) or negatively (29, 30) interfere with the normal wound healing process. Accordingly, the positive effects of chlorhexidine compared with the control group might have been reduced or masked by the use of the systemic antibiotic.

In this study, hyaluronic acid gel (Gengigel®) significantly improved wound healing at the clinical level. This result was expected in view of the multiple functions attributed to hyaluronan during wound healing (9, 10) and consistent with the results of some studies on oral tissue healing after gingival therapy (13, 14), implant surgery (15) and management of aphthous ulceration (16). The histomorphometric evaluation, however, did not reveal significant improvement in wound healing. A possible explanation of this difference may be that the clinical evaluation included the measurement of two dimensions of the wound, while the histomorphometric evaluation included only the measurement of one dimension. Accordingly, some improvement in the other dimensions may have been missed in the histological evaluation.

Chlorhexidine gel (Perio.Kin®) caused significant improvement at both the clinical and histological levels, while hyaluronic acid (Gengigel®) caused improvement only at the clinical level. Nevertheless, there was no significant difference at the clinical level between the groups receiving Perio.Kin® and Gengigel®. It seems that Perio.Kin® is slightly better than Gengigel®, and this may be attributed to the substantival or persistence effect of chlorhexidine (47). As there was only one daily application of either agent in this study, it is reasonable to see a better effect of Perio.Kin®, while multiple daily applications of Gengigel® may increase its effect. Therefore, further studies in this area are recommended.

Allantoin did neither improve wound healing nor negatively affect it in this study. However, in view of the widespread use of comfrey, a major source of allantoin in alternative medicine, the widespread use of allantoin in the cosmeceutical industry, the many anecdotal claims of its value in tissue regeneration, the lack of adequate scientific evidence of that (18) and the fact that only one concentration of allantoin was tested

in this experiment, we believe that further studies with various concentrations and wound types are warranted before any solid conclusions can be made.

Conclusions

Significant effects on wound healing were achieved with Perio.Kin® and Gengigel® where Perio.Kin® improved wound healing clinically and histologically and Gengigel® improved wound healing clinically. It seems warranted to use either of these agents following oral surgical and periodontal procedures. Allantoin did not have any positive effect on the rate of wound healing. A single concentration of allantoin was tested in this study. Therefore, it may be valuable to re-evaluate the effect of allantoin on intraoral wound healing either at different concentrations or preparations. Nevertheless, none of the tested agents had a negative effect on the rate of wound healing when applied on an excisional wound with epithelial and connective tissue defect.

References

1 Bakaéen GS, Strahan JD. Effects of a 1% chlorhexidine gel during the healing phase after inverse bevel mucogingival flap surgery. *J Clin Periodontol* 1980; **7:** 20–5.

2 Zambon JJ, Ciancio SG, Mather ML, Charles CH. The effect of an antimicrobial mouthrinse on early healing of gingival flap surgery wounds. *J Periodontol* 1989; **60:** 31–4.

3 Nyman S, Rosling B, Lindhe J. Effect of professional tooth cleaning on healing after periodontal surgery. *J Clin Periodontol* 1975; **2:** 80–6.

4 Lambert PM, Morris HF, Ochi S. The influence of 0.12% chlorhexidine digluconate rinses on the incidence of infectious complications and implant success. *Oral Maxillofac Surg* 1997; **55(12 Suppl 5):** 25–30.

5 Farnoush A. Techniques for the protection and coverage of the donor sites in free soft tissue grafts. *J Periodontol* 1978; **49:** 403–5.

6 Kozlovsky A, Artzi Z, Hirshberg A, Israeli-Tobias C, Reich L. Effect of local antimicrobial agents on excisional palatal wound healing: a clinical and histomorphometric study in rats. *J Clin Periodontol* 2007; **34:** 164–71.

7 Denton GW. Chlorhexidine. In: Block SS, ed. *Disinfection, Sterilization and Preservation*. Philadelphia, Lea and Febiger, 1991, 274–289.

8 Jones CG. Chlorhexidine: is it still the gold standard? *Periodontol 2000* 1997; **15:** 55–62.

9 Chen WY, Abatangelo G. Functions of hyaluronan in wound repair. *Wound Repair Regen* 1999; **7:** 79–89.

10 Moseley R, Waddington RJ, Embery G. Hyaluronan and its potential role in periodontal healing. *Dent Update* 2002; **29:** 144–8.

11 Rahemtulla F. Proteoglycans of oral tissues. *Crit Rev Oral Biol Med* 1992; **3:** 135–62.

12 Goa KL, Benfield P. Hyaluronic acid. A review of its pharmacology and use as a surgical aid in ophthalmology, and its therapeutic potential in joint disease and wound healing. *Drugs* 1994; **47:** 536–66.

13 Jentsch H, Pomowski R, Kundt G, Göcke R. Treatment of gingivitis with hyaluronan. *J Clin Periodontol* 2003; **30:** 159–64.

14 Pistorius A, Martin M, Willershausen B, Rockmann P. The clinical application of hyaluronic acid in gingivitis therapy. *Quintessence Int* 2005; **36:** 531–8.

15 de Araújo Nobre M, Cintra N, Maló P. Peri-implant maintenance of immediate function implants: a pilot study comparing hyaluronic acid and chlorhexidine. *Int J Dent Hyg* 2007; **5:** 87–94.

16 Nolan A, Baillie C, Badminton J, Rudralingham M, Seymour RA. The efficacy of topical hyaluronic acid in the management of recurrent aphthous ulceration. *J Oral Pathol Med* 2006; **35:** 461–5.

17 Kim K, Park J, Rhee S. Structural and functional basis for (*S*)-allantoin formation in the ureide pathway. *J Biol Chem* 2007; **282:** 23457–64.

18 MacKay D, Miller AL. Nutritional support for wound healing. *Altern Med Rev* 2003; **8:** 359–77.

19 Foster S, Tyler VE. *Tyler's Honest Herbal: A Sensible Guide to the Use of Herbs and Related Remedies*. New York, The Haworth Herbal Press, 1999.

20 Draelos ZD. Cosmeceutical botanicals: part 1. In: Dover JS, ed. *Procedures in Cosmetic Dermatology, Cosmeceuticals*. Philadelphia, Elsevier Saunders, 2005, 71–78.

21 Akema FineChemicals. Allantoin CTFA. Available at: http://www.akema.it/allantoin.htm. Accessed March 24, 2009.

22 Margraf HW, Covey TH Jr. A trial of silver-zinc-allantoinate in the treatment of leg ulcers. *Arch Surg* 1977; **112:** 699–704.

23 Department of Health and Human Services, U.S. Food and Drug Administration (FDA). Code of Federal Regulations, Title 21, Volume 5: Food and Drugs, Part 310: New Drugs. Available at: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=310.534. Accessed March 24, 2009.

24 Institute of Laboratory Animal Resources, Commission on Life Sciences. *Guide for the Care and Use of Laboratory Animals*. Washington, DC, National Academy Press, National Research Council, 1996.

25 Kahnberg KE, Thilander H. Healing of experimental excisional wounds in the rat palate. (I) Histological study of the interphase in wound healing after sharp dissection. *Int J Oral Surg* 1982; **11:** 44–51.

26 Bodner L, Dayan D, Pinto Y, Hammel I. Characteristics of palatal wound healing in desalivated rats. *Arch Oral Biol* 1993; **38:** 17–21.

27 Bodner L, Dayan D. Epithelium and connective tissue regeneration during palatal wound healing in desalivated rats: a comparative study. *Comp Biochem Physiol A Physiol* 1995; **111:** 415–9.

28 Robson MC, Heggers JP. *Quantitative Bacteriology and Inflammatory Mediators in Soft Tissue. Soft and Hard Tissue Repair*. New York, Praeger, 1984.

29 Penhallow K. A review of studies that examine the impact of infection on the normal wound healing process. *J Wound Care* 2005; **14:** 123–6.

30 Tenenhaus M, Bhavsar D, Rennekampff HO. Diagnosis and surgical management in wound bacterial burden. In: Granick MS, Gamelli RL, eds. *Surgical Wound Healing and Management*. New York, Informa Healthcare USA, Inc, 2007, 29–37.

31 Häkkinen L, Uitto VJ, Larjava H. Cell biology of gingival wound healing. *Periodontol 2000* 2000; **24:** 127–52.

32 Damour O, Hua SZ, Lasne F, Villain M, Rousselle P, Collombel C. Cytotoxicity evaluation of antiseptics and antibiotics on cultured human fibroblasts and keratinocytes. *Burns* 1992; **18:** 479–85.

33 Pucher JJ, Daniel JC. The effects of chlorhexidine digluconate on human fibroblasts in vitro. *J Periodontol* 1992; **63:** 526–32.

34 Mariotti AJ, Rumpf DA. Chlorhexidine-induced changes to human gingival fibroblast collagen and non-collagen protein production. *J Periodontol* 1999; **70:** 1443–8.
35 Hidalgo E, Dominguez C. Mechanisms underlying chlorhexidine-induced cytotoxicity. *Toxicol In Vitro* 2001; **15:** 271–6.
36 Bassetti C, Kallenberger A. Influence of chlorhexidine rinsing on the healing of oral mucosa and osseous lesions. *J Clin Periodontol* 1980; **7:** 443–56.
37 Salami A, Imosemi I, Owoeye O. A comparison of the effect of chlorhexidine, tap water and normal saline on healing wounds. *Int J Morphol* 2006; **24:** 673–6.
38 Asboe-Jörgensen V, Attström R, Lang NP, Löe H. Effect of a chlorhexidine dressing on the healing after periodontal surgery. *J Periodontol* 1974; **45:** 13–7.
39 Hamp SE, Rosling B, Lindhe J. Effect of chlorhexidine on gingival wound healing in the dog. A histometric study. *J Clin Periodontol* 1975; **2:** 143–52.
40 Othman S, Haugen E, Gjermo P. The effect of chlorhexidine supplementation in a periodontal dressing. *Acta Odontol Scand* 1989; **47:** 361–6.
41 Lang N, Schild U, Brägger U. Effect of chlorhexidine (0.12%) rinses on periodontal tissue healing after tooth extraction. (I). Clinical parameters. *J Clin Periodontol* 1994; **21:** 415–21.
42 Metin M, Tek M, Sener I. Comparison of two chlorhexidine rinse protocols on the incidence of alveolar osteitis following the surgical removal of impacted third molars. *J Contemp Dent Pract* 2006; **7:** 79–86.
43 Torres-Lagares D, Infante-Cossio P, Gutierrez-Perez J, Romero-Ruiz M, Garcia-Calderon M, Serrera-Figallo M. Intra-alveolar chlorhexidine gel for the prevention of dry socket in mandibular third molar surgery. A pilot study. *Med Oral Patol Oral Cir Bucal* 2006; **11:** E179–84.
44 Hita-Iglesias P, Torres-Lagares D, Flores-Ruiz R, Magallanes-Abad N, Basallote-Gonzalez M, Gutierrez-Perez JL. Effectiveness of chlorhexidine gel versus chlorhexidine rinse in reducing alveolar osteitis in mandibular third molar surgery. *J Oral Maxillofac Surg* 2008; **66:** 441–5.
45 Schiott CR, Löe H, Jensen SB, Kilian M, Davies RM, Glavind K. The effect of chlorhexidine mouthrinses on the human oral flora. *J Periodontal Res* 1970; **5:** 84–9.
46 Hjeljord LG, Rolla G, Bonesvoll P. Chlorhexidine-protein interactions. *J Periodontal Res Suppl* 1973; **12:** 11–6.
47 Kornman KS. The role of supragingival plaque in the prevention and treatment of periodontal diseases. A review of current concepts. *J Periodontal Res Suppl* 1986; **2l:** 5–22.

Copyright of International Journal of Dental Hygiene is the property of Wiley-Blackwell and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.