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Effect of local antimicrobial agents on excisional palatal wound healing: a clinical and histomorphometric study in rats

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Abstract

Aim: To assess the effect of topically applied antimicrobial agents on palatal excisional wound in rats.

Materials and Methods: Excisional wounds, 5 mm in diameter, were made in the centre of the palate of 125 Wistar male rats. In four experimental groups, chlorhexidine digluconate (CHX) 0.12% solution, 1% CHX gel, phenolic compounds solution (Listerine®), amine/stannous fluoride solution (Meridol®) and saline solution as a control group were applied daily for 1 min. The wound area was measured photographically and the epithelialization rate was determined histologically at 3, 7, 14 and 21 days post-surgery.

Results: The mean wound area and mean distance between the epithelial margins decreased significantly with time ($p < 0.001$) in experimental and control groups, with the greatest wound area reduction and rate of epithelialization on day 14. A significantly superior rate of wound epithelialization ($p = 0.03$) was presented following use of 1% CHX gel and Listerine® and a comparatively inferior one when the Meridol® solution was applied.

Conclusions: Each tested antimicrobial agent when applied on an excisional wound with epithelial and connective tissue deficiency did not have a negative effect on the rate of wound closure. The best results were achieved with 1%CHX gel and Listerine®.

Key words: antimicrobial; chlorhexidine; excisional; healing; wound

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The oral cavity provides a unique environmental challenge for healing wounds produced during various periodontal and implant surgical procedures. Trauma from mastication, relatively large commensal oral flora and elevated levels of dental plaque (due to difficult post-surgical mechanical plaque control) can impair the normal sequence of the healing process. Prevention of bacterial contamination and maintenance of a high standard of plaque control are determining factors for the successful outcome of periodontal (Burke 1971, Nyman et al. 1975, 1977, Yumet & Polson 1985, Blomlöf et al. 1988, Zambon et al. 1989, Wikesjö et al. 1992, Lindhe & Echeverria 1994, Flores de Jacoby & Mengel 1995, Sanz & Harrera 1998) and implant surgery (Lambert et al. 1997, Horwitz et al. 2005).

The use of adjunctive chemotherapeutic agents after periodontal surgery reduces bacterial plaque accumulation, post-operative pain, swelling and tissue oedema, enhances wound healing and improves clinical results (Bakaeen & Strahan 1980, Yukna et al. 1986, Zambon et al. 1989). Therefore, most protocols based on the concept that immediate post-operative plaque control is hard to perform recommend post-surgical use of antimicrobial agents to support wound healing.

Among the antiseptics that provide sufficient data regarding their clinical efficacy in controlling plaque and gingi-

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vitis (Ciancio 1988, Mandel 1988) and that support wound healing are the bis-biquanide chlorhexidine digluconate (Peridex®, Corsodyl®), phenolic compounds (Listerine®), and heavy metal salts, such as stannous fluoride (Meridol®) (Bakaeen & Strahan 1980, Sanz et al. 1989, Zambon et al. 1989, Horwitz et al. 2000, Heitz et al. 2004).

After periodontal and implant placement surgery, minor discontinuity between the wound margins is present with bone covered by a well-nourished muco-periosteal flap. Thus, the applied antiseptic solution is in direct contact with the keratinized oral epithelium. However, while harvesting a palatal-free graft (Nabers 1966, Sullivan & Atkins 1968) or applying a rotated full-thickness palatal flap (Nemcovsky et al. 2000), a palatal donor area excisional wound devoid of epithelium is created, extending deep into the connective tissue and/or bone. This type of wound requires a significant amount of new tissue formation and heals with secondary intention (Farnoush 1978, Nemcovsky et al. 2000, Del Pizzo et al. 2002). In these clinical situations, post-surgical control of the recipient graft area (root surface or extraction site) by an antimicrobial agent is recommended and widely used. With the goal to create an optimal environment for wound repair at the graft recipient site or implant placement site covered by rotated palatal flap, the excisional palatal wound is exposed to the antimicrobial agents, which display both bactericidal activity and potential cytotoxicity (Pucher & Daniel 1992, Kubert et al. 1993, Mariotti & Rumpf 1999, Hidalgo & Dominguez 2001, Souza-Junior & Castro-Prado 2005).

The purpose of the present study was to assess the effect of topically applied antimicrobial agents on the closure and rate of epithelialization of palatal excisional wound healing by secondary intention.

Material and Methods

The Ethics and Institutional Animal Care and Use Committees of Tel -Aviv University approved the study protocol. The study group consisted of 125 Wistar-derived, 3-month-old male rats, each weighing an average of 280 g. Animals were fed a standard diet of pellets and water ad libitum. The animals were anaesthetized intra-peritoneally with 10% Ketamine (90mg/1kg; Kepro, Deventer, Holland) and 2% Xylazine (10mg/1kg; Medical Market, Tel Izhak, Israel), and a circular excisional wound, 5 mm in diameter, was made in the centre of the palatal mucosa, using a punch-out biopsy tool. Muco-periosteal specimens were removed by sharp dissection exposing a circular area of uncovered bone left for secondary healing (Kahnberg & Thilander 1982). Five animals were sacrificed immediately and provided the baseline group at time 0. The remaining 120 were randomly divided into five groups: four experimental groups received a daily application of the tested antimicrobial agent, and a control group, in which saline was applied. The antimicrobial agents used in the study were 0.12% chlorhexidine digluconate solution (Pharmadentix, Trima, Maabarot, Israel); 1% w/w chlorhexidine digluconate–equivalent to 5% v/w (Corsodyl Dental Gel; GlaxoSmithKline, München, Germany); phenolic compounds solution, Listerine® menthol (Warner-Lambert, Pfizer Inc., New York, USA); and 125 p.p.m. stannous fluoride and 125 p.p.m. aminefluoride 297 (ASF) solution (Meridol®), Teva, GABA International AG, Basel, Switzerland).

At 24 h post-operatively, a mild anaesthesia (Clorketam 0.1 cc/200 g and Xyl 0.05 cc/200 g) was used to apply agents on the wounds every day. Without touching the wound, 1 cc of each agent was delivered directly to the wound using a syringe with a blunt cannula, and was sustained in the area for 1 min. Water and food were provided to the animals 2 h after treatment. Six animals were sacrificed from each group using CO_2 inhalation at 3, 7, 14 and 21 days post-operatively. Animals were decapitated, and the maxillae were separated and transferred for fixation into 10% formalin for 24 h.

Photographic evaluation

The palate specimens were photographed at a constant distance and magnification (Niall et al. 1982, Bodner et al. 1992) using a Nikon F-3 camera (Nikon Corp., Tokyo, Japan). Magnifications (9×12 cm) were prepared. On each photograph (Fig. 1), the wound margins were marked and the area of the defect was measured with a Computerized Digitizer (SummaSketch; Symmagraphics; Summagraphics Corp., Austin, TX, USA).

Histologic evaluation

After 24 h of fixation in formalin, sections were decalcified in 10% formic acid for 2 weeks. Samples were then trimmed and embedded in paraffin. For each wound, five serial sections, 5 μm apart, were cut perpendicular to the palate midline and stained with haematoxylin and eosin. Sections were examined microscopically at a magnification of $\times 40$ using a standard 100-point scale or grid (100 grids = 2.5 mm). Measurements were taken with the aid of a calibrated ocular micrometer.

The distance between the epithelial margins in each slide in each time frame was measured to determine the rate of epithelialization of the wound (Fig. 2a). In addition, as epithelial cell migration begins at the periphery towards the wound centre, the distance between the palatal tooth aspect, bilaterally, was measured in each slide to indicate the palatal width (Fig. 2b).

The mean and standard deviation of the measurements recorded on the five serial sections were calculated, thus providing two parameters for each wound sample: the distance between the epithelial margins of the healing wound and the palatal width in the measured area.

The wound area measurements in experimental and control groups at different time points were compared and analysed using two-way analysis of variance (ANOVA) (day of sacrifice and type of applied agent). A positive correlation (Pearson's Correlation) between residualwound width and width of the palate was found. A multiple linear regression (dependent variable –residual wound and independent variables–palate width and time) was applied to determine the influence of palate width on the residual wound. After adjusting for palate and time, one-way ANOVA on the residual wound size (presented as the distance between the epithelial margins) was used to compare the various treatments.

Results

During the experimental period, the animals did not lose weight, indicating that the feeding behaviour was satisfactory in spite of the palatal wound.

Clinical and macroscopic observations

Clinical examination of the wound (Fig. 1) showed slow healing during the first post-operative week. Bone was covered with a fibrinous layer with patechia

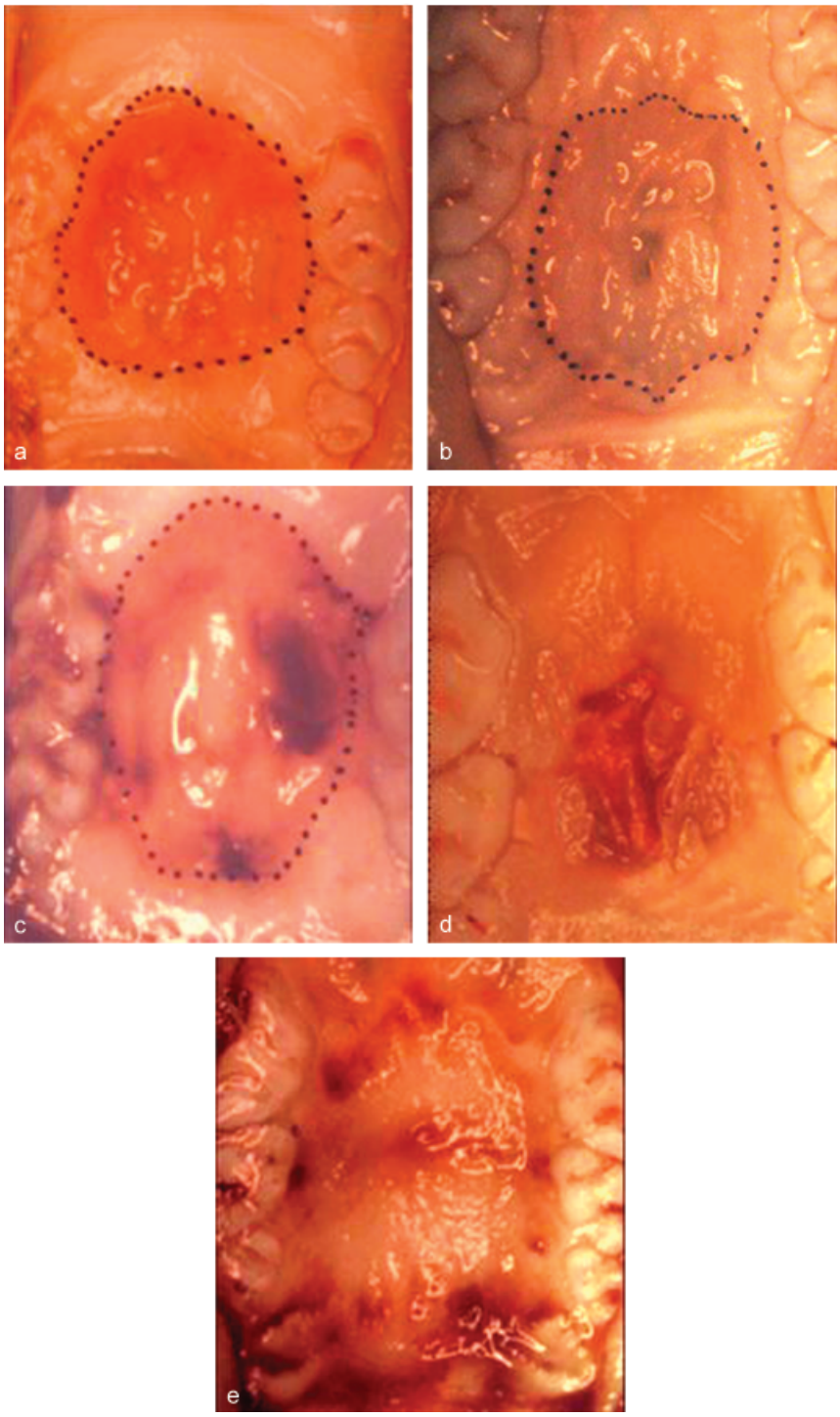

Fig. 1. Clinical photograph of the rat wound on day 0. The 5 mm in dimension excisional wound in the center of the palate (wound margins marked for digital measurements of wound area). (b) On day 3, a representative photograph of wound treated with 0.1% chlorhexidine digluconate (CHX) solution. Clear band of intact masticatory mucosa separates the wound border from the palatal surface of the maxillary teeth. (c) On day 7, slow healing with mild wound reduction. The wound was treated with Meridol®. (d) On day 14, the wound size decreased extensively; wound treated by Listerine®. (e) On day 21, a defect treated with 1% CHX gel almost completely healed with minimal residual tissue irregularity.

present on the wound margins. Between 7 and 14 days, the area of fibrin covering the base of the defect decreased rapidly. At 2 weeks post-operatively, the defect was largely covered by epithelium with only a small residual wound. On day 21, the defect almost completely healed with a minimal central depression of the original excisional defect.

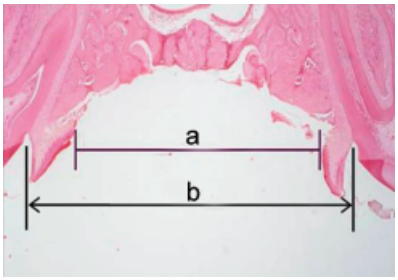

Fig. 2. Photomicrograph of wound presenting the measurements performed on each slide: (a) the distance between the epithelial margins; (b) the palatal width at the examined section cut.

Photographic observations

On day 0, immediately post-operatively, the wound surface covered $20.42 \pm 1.32\,mm^2$. Wound area measurements of all groups at the different time intervals are presented in Table 1. The mean area of the circumscribed defects decreased significantly with time ($p < 0.001$) in all experimental and control groups. Slow healing was observed during the first post-operative week. Between 7 and 14 days, wound size decreased extensively, with 30–67% reduction of wound area (Fig. 1). On day 14, the most extensive residual wound area was measured in the saline group (70%) and the smallest in the CHX gel group (33%). From 14 to 21 days, a further reduction in the mean size of the residual wound (up to 20%) was observed in all groups.

Differences between wound size in the experimental groups compared with the control group did not reach statistical significance at any time point during the experiment.

Histologic examination

Immediately post-operatively, the wound margins were sharply demarcated through a cut into the epithelium and connective tissue. The bone surface was almost exposed without reminiscences of the periosteum (Fig. 3).

On day 3 post-operatively, the sharp contour of the wound edges had become rounded, and in several of the wounds, the epithelial remnants at the margins diminished (Fig. 3) with some enlargement of the wound margins.

Table 1. Macroscopic wound area measurements (mm^2), mean and standard deviation (SD) ($n = 6$)

Substance/day	Baseline	Saline	CHX 0.1%	CHX gel 1%	Listerine®	Meridol®
0	20.42 ± 3.25					
3		20.75 ± 4.52	20.28 ± 3.66	22.18 ± 4.40	21.17 ± 1.16	23.76 ± 3.07
7		23.41 ± 6.51	18.93 ± 6.21	19.52 ± 3.25	19.98 ± 5.53	21.2 ± 5.39
14		14.1 ± 1.61	11.04 ± 4.03	6.62 ± 4.33	13 ± 3.42	12.98 ± 5.47
21		6.48 ± 6.72	5.99 ± 4.16	5.26 ± 6.10	5.54 ± 4.01	5.4 ± 4.12

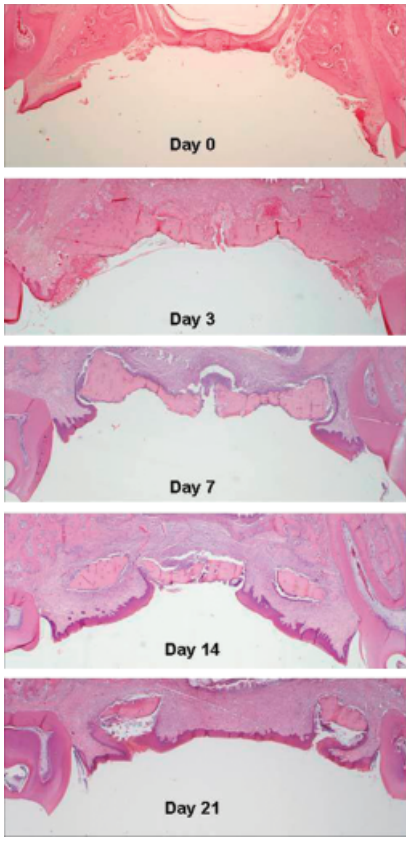

Fig. 3. Low-power photomicrographs of the rat palate presenting a full-thickness portion of an excisional wound treated with saline (heamatoxylin and eosin, original magnification × 10). Day 0 – Notice the sharp edge of the wound margins.

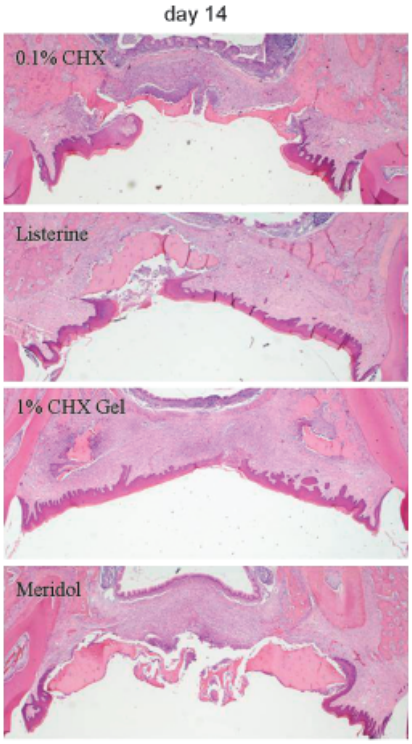

Fig. 4. Low-power photomicrograph of the wounds on day 14 following wounding. Notice the differences in the degree of epithelialization between the various groups (haematoxylin and eosin, magnification × 20).

As a result of basal cell proliferation, epithelial migration was observed in most of the specimens, which moderately increased on day 7 (Fig. 3), with rapid epithelialization on days 14 (Figs 3 and 4) and 21 (Fig. 3). The wound surface was composed of necrotic bone, debris and fibrin, with marginal epithelial cells migrating underneath while exfoliating the sequestral bone (Figs 3 and 4).

Histomorphometric examination

On days 14 and 21, the 1% CHX gel group presented complete wound epithelialization in 20% and 60% of the measured specimens, respectively, compared with 0% and 30–33% in the other tested and control groups.

The mean wound width measurements, i.e., the distance between the epithelial margins of the wound, are presented in Table 2.

The wound width decreased significantly with time in all experimental and control groups. Multiple linear regression analysis revealed a significant influence of the time ($p < 0.001$) and the width of the palate ($p = 0.05$) on the residual wound ($R^2 = 0.577$). One-way ANOVA, which was applied on the residuals of the regression [Δwound = wound – (338.55 – 6.671 × time – 0.716 × maximal width of palate)], revealed a significant difference between the groups ($p = 0.03$), with 1% CHX gel and Listerine® presenting the best rate of epithelialization (– 10.56 SE 11.05 and – 10.06 SE 6.80, respectively), followed by CHX 0.12% (– 2.61 SE 8.02), saline (6.75 SE 7.50) and Meridol® (24.69 SE 9.57) as the slowest.

Discussion

The present study was conducted to test the influence of various antimicrobial agents on the healing process of excisional wounds with large epithelial and connective tissue deficiency that heals by secondary intention. The rate of macroscopic healing as assessed by calculating the total wound area was not slowed down by the application of any of the tested antimicrobial agents as compared with the control. However, microscopic examination revealed that the rate of re-epithelialization of the wound was significantly superior following the application of both 1% CHX gel and Listerine® and slower when Meridol® was applied.

Wound healing is a complex biological process that includes a series of biological events commonly divided into three overlapping phases: inflammation, re-epithelialization and granulation tissue formation and matrix formation and tissue remodelling (Clark 1996). The use of antiseptic agents can affect the nature and quality of the inflammatory infiltrate and that of the granulation tissue component. In the present study, the rate of re-epithelialization as a criterion to indicate the influence of the antiseptic products on the healing tissue was challenged. Re-epithelialization is a major component of the wound-healing process, which is achieved through a complex interplay of diverse growth factors, cytokines and cell cycle regulators (Santoro & Gaudino 2005). Epithelial cell migration from the periphery of the wound towards the central part of the defect is necessary for

Table 2. Histomorphometric measurements

Substance/day	Parameter	3	7	14	21
Saline	a	175.74 ± 17.03	166.51 ± 23.86	133.65 ± 20.41	68.83 ± 73.97
	b	195.39 ± 12.01	182.08 ± 9.95	181.23 ± 7.83	185.0 ± 19.24
CHX 0.12%	a	182.26 ± 24.52	159.33 ± 15.96	103.00 ± 38.76	69.95 ± 46.63
	b	205.77 ± 7.05	174.81 ± 13.92	169.80 ± 16.75	188.45 ± 9.66
CHX Gel 1%	a	176.67 ± 21.61	154.80 ± 28.48	80.75 ± 74.13	58.20 ± 76.34
	b	194.80 ± 11.62	183.24 ± 16.57	186.45 ± 8.94	191.55 ± 14.94
Listerine®	a	166.90 ± 34.17	157.33 ± 19.45	150.38 ± 31.49	34.68 ± 37.76
	b	197.84 ± 9.35	183.70 ± 13.56	176.06 ± 11.27	189.17 ± 26.13
Meridol®	a	189.70 ± 20.95	169.57 ± 33.70	126.76 ± 30.37	96.45 ± 68.03
	b	204.11 ± 2.44	195.00 ± 7.01	183.63 ± 5.56	196.65 ± 10.61

a, distance between epithelium edges covering the experimental wound; b, width of the palate.
Measured in grids (100 grids = 2.5 mm), magnification × 40. Values are the mean and SD obtained from six rats, five slides in each specimen.

wound closure. Migration and proliferation of keratinocytes and the close relationship between the migratory cells and the collagen of the newly formed connective tissue determine the rate of re-epithelialization of the wound. Bacterial colonization of the wound surface results in an increased area of inflammation and granulation tissue that may prolong the repair process (Bucknall 1980, Robson et al. 1990). As oral wounds are constantly subjected to a relatively large commensal flora, it is clear that bacteria affect wound healing in the oral cavity. Elevated levels of bacteria (over 10^5) decrease epithelialization; bacterial metabolites inhibit epithelial cell migration, digest dermal proteins and polysaccharides (Robson et al. 1990, Thomson 1998, Edwards & Harding 2004) and increase production of neutrophil's proteases, cytotoxic enzymes and free radicals that damage vulnerable epithelium (McGuckin et al. 2003). Nevertheless, bacteria play an important positive role in normal wound healing by accentuating the inflammatory response, a prerequisite for granulation tissue formation and initial tissue repair (Häkkinen et al. 2000). Thus, bacteria may act directly or indirectly on epithelial and connective tissue cells depending on the type and concentration, and can either accelerate or delay oral wound modulation and healing.

Post-surgical application of antimicrobial agents for chemical plaque control has a disinfecting effect on the oral cavity due to suppressed salivary and mucosal bacterial counts. CHX is more effective in reducing mucosal bacterial count than Listerine® (Rosin et al. 2002). Meridol® contains ingredients capable of inhibiting the growth of oral bacteria in vitro (Kagermeier-Callaway et al. 2000) and is capable of reducing plaque formation (Tinanoff et al. 1980). The antibacterial activity of CHX, as measured by the vitality of the plaque bacteria, is the most powerful, followed by Meridol® and Listerine® (Brecx et al. 1992). The antibacterial action of CHX is mediated by its substantivity, which is still evident approximately 12 h after application, while the antibacterial effect of Listerine® and Meridol® occurs immediately after application and remains for a short time (Ross et al. 1989, Scheie 1989, Kubert et al. 1993, Rosin et al. 2002). We can assume that the beneficial effect of 1% CHX in gel on the rate of epithelization, as shown in the present study, was achieved due to the superior persistence of its antimicrobial activity in the mouth compared with Listerine® or Meridol®. The concentration of CHX in the 1% CHX gel is higher than that of the 0.12% CHX solution and in addition to the viscosity of the gel, results in a slower clearance of the active agent from the animal's mouth. Thus, a prolonged contact time with the wound area is achieved, further promoting a pharmacotherapeutic effect (Cosyn & Sabzevar 2005).

Chlorhexidine possesses a broad antimicrobial activity and its ability to reduce plaque formation in a variety of concentrations has made it a gold standard antiseptic agent (Jones 1997). CHX binds strongly to bacterial cell membranes, increases permeability and initiates leakage and/or precipitates intra-cellular components leading to bacterial cell death. Unfortunately, the toxic qualities of CHX are not reserved only for bacteria and can be noxious to different mammalian cells. CHX, in a concentration-and time-dependent manner, negatively affects fibroblasts and keratinocyte cell proliferation in vitro (Damour et al. 1992, Pucher & Daniel 1992, Mariotti & Rumpf 1999, Hidalgo & Dominguez 2001). In certain concentrations, CHX can be cytotoxic to human oral epithelial cells, gingival fibroblasts, red blood cells, neutrophils and macrophages. The drug, while negatively affecting the normal function of fibroblasts, can impair wound contraction by inhibiting the interaction of fibroblasts with collagen fibres (Pucher & Daniel 1992). On the basis of these in vitro studies, chlorhexidine can impair normal wound healing by disrupting the function of cells participating in the healing process. In the present study, however, CHX did not disturb the healing process. In the oral cavity, CHX binds mostly to bacteria that populate the oral cavity and a certain amount of the applied CHX is precipitated by serum proteins (Schiøtt et al. 1970, Hjeljord et al. 1973). Therefore, the amount of CHX molecules available to bind to and potentially harm host cells in the wound is significantly reduced, thus explaining the discrepancies between the present results and the in vitro studies (Damour et al. 1992, Pucher & Daniel 1992, Mariotti & Rumpf 1999, Hidalgo & Dominguez 2001).

Several animal studies examined the influence of local application of antiseptic agents on wound healing in different post-surgical situations. Lindhe et al. (1970) studied the effect of CHX on the oral mucosa of the hamster, and found that CHX did not penetrate the undamaged oral epithelium and did not histologically and histochemically induce any alterations in the basal cell layers. When pure soft lesion deprived of epithelium exists, CHX application secures ideal conditions for healing, with only minor signs of inflammation and no attachment loss (Hamp et al.

1975). The secondary intention wound is covered with a fibrin–fibronectin clot in which polymorphonuclear cells become incorporated to form a ''polyband'' layer under which epithelial cells migrate to cover the wound (Engler et al. 1966). The fibrin layer that forms immediately following tissue injury may offer a protective surface to prevent CHX from reaching the underlining epithelial and connective tissue cells. CHX application aimed at preventing biofilm accumulation and contamination of the wound should be postponed 24 h post-operatively, in which time organized fibrin layer covers the wound surface.

The present study confirmed the observation made by Bassetti and Kallenberger (1980) that application of 0.1% and 0.2% CHX solution shows undisturbed wound healing at day 7 following injury. However, in the same study, twice-daily delivery of a much higher concentration of an active agent in the form of 5 cm^3 of 0.5% CHX on open mucosal–osseous wounds resulted in a disturbed healing pattern. The present model of an experimental wound did not include direct injury to the underlining bone, whereas in the study of Bassetti and Kallenberger (1980), a hole was bored in the exposed bone that could impair bone vitality and the environment suitable for cell growth and migration. Furthermore, to eliminate the possible bacterial influence on wound healing, the animals in that study received penicillin added to the drinking water throughout the experiment. It is possible that the absence of bacterial challenge in the saliva increased the amount of CHX available to bind to host cells, which may have significantly affected the healing process (Pucher & Daniel 1992).

The plaque and inflammation inhibitory properties of the phenolic compound, Listerine®, have been confirmed (Axelsson & Lindhe 1987, DePaola et al. 1989, Brecx et al. 1992, Moran et al. 1997, Riep et al. 1999, Santos 2003). Listerine® is also significantly more effective in improving the healing of gingival flap surgery wounds than saline (Zambon et al. 1989). The tested Cool Mint Listerine® contains 21.5% ethanol, the key factor in the mouthwash-induced oral pain (Bolanowski et al. 1995). Ethanol may disturb the re-epithelialization process by disruption or inhibition of actin stress fibres that are essential for epithelial cell migration (Nusrat et al. 1992, Mohajer et al. 2002). However, ethanol and other substances, such as methylsalicylate in the Listerine®–menthol mouth rinse, confer strong anti-oxidant properties (Battino et al. 2002). This has been demonstrated in vitro when fibroblasts treated with hydrogen peroxide (H_2O_2) in the presence of Listerine® presented reduced DNA cell damage compared with control cells. The inflammation phase of the wound-healing process features intensive oxygen consumption and generates reactive oxygen species (ROS) and free radicals (FR) that lead to reduced concentrations of growth factors and proteinase inhibitors. Consequently, an imbalance between degradation and remodelling processes is produced (Battino et al. 1999), which negatively influences the healing process. Gingival epithelial cells are highly sensitive to attack by PMN-derived oxidants (Altman et al. 1992). Antioxidants are essential to remove or inactivate FR/ROS as soon as they form and to protect the epithelial cells from FR. Therefore, Listerine®, besides its benefits as an antimicrobial agent, can also act as an antioxidant to eliminate the negative effects of oxygen-free radicals on epithelial cells and support epithelalization of the wound as confirmed clearly in the present study. It is still necessary to demonstrate the in vivo antioxidant activity of Listerine®.

In contrast to the antioxidant properties of Listerine®, the stannous fluoride active ingredient of Meridol® shows a dissimilar influence in vitro, i.e., total inhibition of the peroxidase activity in human saliva (Vanden Abbeele et al. 1995), and thus may disturb the clearance of the toxic hydrogen peroxide (H_2O_2) and its derivatives from oral fluids (Hannuksela et al. 1994). The enzyme peroxidase is the main agent responsible for decomposition of hydrogen peroxide in saliva (Kraus et al. 1957). It is possible that as oral peroxidase systems protect the mammalian cells from the toxicity of H_2O_2 and its derivatives (Hänström et al. 1983, Tenovuo & Larjava 1984, Tipton et al. 1995). Since epithelial migration is highly sensitive to a high level of free radicals (Altman et al. 1992), the inhibition of peroxidase activity by Meridol® may explain the inferior rate of wound epithelialization following application of the Meridol® presented in the current study. Furthermore, the increase of superoxide release by human neutrophils as a result of the AmF and SnF_2 ingredients of Meridol® (Shapira et al. 1997) may be considered as another indirect negative influence of Meridol® on mammalian cells, which may slow down epithelial migration and wound re-epithelialization.

Conclusions

Topical application of 0.1% CHX solution, 1% CHX gel, Listerine® and Meridol® on an excisional wound with epithelial and connective tissue deficiency do not have a negative effect on the rate of wound closure. Thus, these antimicrobial agents do not impair the healing process of the donor area and may be used for post-operative infection control of the surgical sites. Nevertheless, the use of 1% CHX gel and Listerine® is beneficial due to the superior rate of wound epithelialization shown as compared with Meridol®.

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Clinical Relevance

Scientific rationale for the study: Most protocols following periodontal and implant placement surgery recommend use of antimicrobial agents to reduce bacterial plaque accumulation and support wound healing. These agents confer both bactericidal activity and potential cytotoxicity to cells participating in the healing process. Harvesting of free gingival graft from the donor area on the palate creates an excisional wound devoid of epithelium, exposing the underlining tissues to direct contact with the antimicrobial agents used post-surgically. Therefore, it is important to evaluate the influence of various antimicrobial agents on the process of wound healing by secondary intention.

Principal findings: Each tested antimicrobial agent, when applied on an excisional wound with epithelial and connective tissue deficiency, did not have a negative effect on the rate of wound closure. A significantly superior rate of wound epithelialization was shown following the use of 1% CHX gel and Listerine® and comparatively inferior when Meridol® solution was applied.

Practical implications: The findings of the present study support the use of chlorhexidine antimicrobial agents and phenolic compounds for post-operative infection control in situations of excisional wound healing.