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Haemostatic agents used in periradicular surgery: an experimental study of their efficacy and tissue reactions

T. von Arx[1], S. S. Jensen[1,2], S. Hänni[3] & R. K. Schenk[1]

[1]Department of Oral Surgery and Stomatology, School of Dental Medicine, University of Berne, Berne, Switzerland; [2]Department of Oral and Maxillofacial Surgery, Copenhagen University Hospital, Glostrup, Denmark; and [3]Department of Restorative Dentistry, School of Dental Medicine, University of Berne, Berne, Switzerland

Abstract

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Aim To evaluate the haemostatic efficacy and the histologic tissue responses after the application of different haemostatic agents used in periradicular surgery.

Methodology The study was conducted in the calvarium of six rabbits. Standardized bone defects (diameter 4 mm) were trephined, and different haemostatic agents were applied and compared with control defects: bone wax (left for 10 min), Stasis® (ferric sulphate, left for 5 s), Expasyl™ (aluminium chloride, left for 2 min and left permanently *in situ*), and a combination of Expasyl™ (2 min) and Stasis® (5 s). The sites were photographed before the application and after the removal of the haemostatic agents. Three independent examiners judged the initial and final bleeding (on the photographs) using a bleeding score for each site and treatment. The results were compared using Wilcoxon's signed rank test. For the histologic analysis, three animals were killed after 3 weeks and three animals after 12 weeks. Transverse, nondecalcified sections were stained with combined basic fuchsin and toluidine blue for descriptive histology.

Results The most efficient haemorrhage control was provided by Expasyl™ in combination with Stasis® and by Expasyl™ alone, whereas bone wax had the weakest bleeding reduction effect. The histologic analysis after 3 weeks demonstrated an inflammatory and foreign body tissue response towards all haemostatic agents. At 12 weeks, this tissue response was less pronounced but still present in sites treated with bone wax or Expasyl™. In general, the inflammatory tissue reactions were limited to the bone defects, and never extended into the surrounding tissues.

Conclusions Expasyl™ alone or in combination with Stasis® appeared to be the most efficient of tested agents to control the bleeding within the bony defects created in a rabbit calvarium model.

Keywords: aluminium chloride, bone wax, ferric sulphate, haemorrhage control, periradicular surgery.

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Introduction

One of the objectives of periradicular surgery following root-end resection is to hermetically seal the root canal system, thereby enabling healing by forming a barrier between the irritants within the confines of the affected root and the tissues surrounding the root. Haemorrhage control is an essential step in periradicular surgery, allowing adequate intra-operative diagnostic evaluation of the root-end, of the resected surface, and of the root-end preparation (Witherspoon & Gutmann 1996). In addition, it is a prerequisite for placement and setting of most root-end filling materials. Inadequate visibility due to copious bleeding at the surgical

Correspondence: Dr T. von Arx, Department of Oral Surgery and Stomatology, School of Dental Medicine, University of Berne, Freiburgstrasse 7, CH-3010 Berne, Switzerland (Tel.: ++41 31 632 25 66; fax: ++41 31 632 98 84; e-mail: thomas.vonarx@zmk.unibe.ch).

site can be frustrating and time-consuming to control (Kim & Rethnam 1997).

Various agents and techniques have been promoted for haemorrhage control during periradicular surgery. Locally applied haemostatic agents can be classified by their mechanism of action: mechanical, vasoconstrictive, intrinsic and extrinsic. A number of studies have described the tissue reactions of haemostatic agents (Ibarrola *et al.* 1985, Alberius *et al.* 1987, Haasch *et al.* 1989, Finn *et al.* 1992, Solheim *et al.* 1992, Jeansonne *et al.* 1993, Lemon *et al.* 1993, Allison 1994). Other studies have evaluated the systemic aspects following the use of haemostatic agents or have evaluated the clinical efficacy of haemostasis in periradicular surgery (Vickers *et al.* 2002, Vy *et al.* 2004).

While bone wax is relatively easy to use, and is generally considered as both a haemostatic agent and a debris collector in periradicular surgery, wax residues may produce severe tissue reactions. As an alternative haemostatic agent, the authors have used ferric sulphate. Although the application of this haemostatic solution is very simple, oozing of blood may occur prematurely, and repetitive application results in a creamy substance making working within the bony crypt more difficult than easier. In 2001, the principle author started to use a paste containing aluminium chloride that clinically appeared to be very efficient to control bleeding in periradicular surgery. In certain situations, aluminium chloride and ferric sulphate were combined synergistically to control recurrent bleeding.

The objective of this study was to assess the haemostatic effect and to evaluate the tissue responses after application of these mentioned haemostatic agents in the standardized bone defects in the calvarium of rabbits.

Material and methods

Study design

The study protocol was approved by the authorities of the Canton of Berne (Department of Agriculture, Section Veterinary Service, Experimental Animal Studies, study number 51/03). The experimental study was conducted in six adult Burgundy rabbits, each at least 5 months old and weighing between 4 and 5 kg. Histologic analysis was conducted after healing periods of 3 weeks (three animals) and 12 weeks (three animals).

Medication of animals

All surgery was performed under intravenous general anaesthesia. The animals were premedicated with ketamine, 65 mg kg^{-1} (Narketan®; Vétoquinol, Berne, Switzerland) and xylazine, 4 mg kg^{-1} (Xylapan®; Vétoquinol), mixed and injected intramuscularly into the hind leg. Subsequently, a cannula was placed into the lateral ear vein and general anaesthesia was maintained with an intravenous infusion of ketamine and xylazine (double quantity of premedication dosage) in 100 mL physiologic saline. Each animal was given 100 000 IU benzylpenicilline intramuscularly (Duplocillin LA®; Intervet BV, Boxmeer, the Netherlands). Postoperatively, the animals were given analgesics for 3 days (Novalgin®; Aventis, Zurich, Switzerland; 50 mg kg^{-1}, once a day intramuscularly).

Surgical protocol

The animals were shaved on the top of the head between the eyes and the ears. The skin was disinfected using an iodine-polyvinylpyrrolidone solution (Betadine®; Mundipharma, Basel, Switzerland). After the subcutaneous administration of a local anaesthetic (1 mL Ultracain DS®; Aventis Pharma, Frankfurt a.M., Germany), a midline incision was made and the skin and periosteum were reflected to expose the vault of the skull. Using a bone trephine, circular bone defects (diameter 4 mm, depth 1.5 mm) were drilled into the outer cortex (tabula externa). It was attempted to avoid perforating the inner cortex (tabula interna) and thereby contacting the 'dura mater', but avoiding contact was not always possible. A total of six bony defects were created, three on each side of the sagittal suture. After the removal of the outer cortical bone plate, the six defects received one of the following treatments in a randomized sequence (concealed envelopes) (Fig. 1a):

- Control site: no haemostatic agent was placed.
- Bone wax site: bone wax (Johnson & Johnson AG, Spreitenbach, Switzerland) was placed into the bone defect with a spatula, flush with the adjacent outer cortex; after 10 min the bone wax was removed with a dental curette.
- Expasyl™ site (temporary): Expasyl™ (Pierre Rolland, Merignac, France) was placed into the bone defect with a spatula, flush with the adjacent outer cortex; after 2 min the paste was removed with a dental curette.

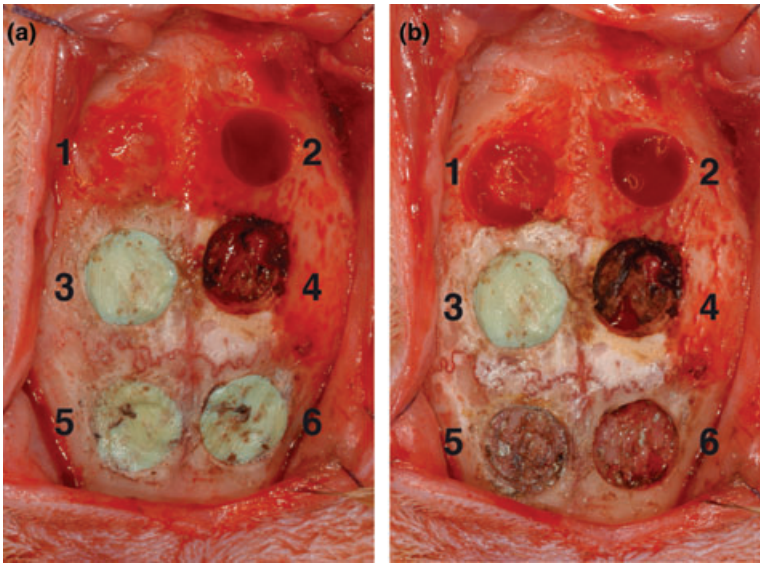

Figure 1 (a) Circular bone defects in the rabbit calvarium following placement of haemostatic agents: site 1, bone wax for 10 min; site 2, control site; site 3, expasyl™ left *in situ*; site 4, Stasis® for 5 s; site 5, Expasyl™ for 2 min and Stasis® for 5 s; site 6, Expasyl™ for 2 min. (b) Assessment of bleeding after removal of the haemostatic agents.

- Expasyl™ site (permanent): Expasyl™ was placed into the bone defect with a spatula, flush with the adjacent outer cortex; the material was left *in situ* throughout the study period.
- Stasis® site: a small foam pellet (3 × 4 × 3 mm) saturated with Stasis® (Belport Co., Camarillo, CA, USA) was placed for 5 s into the bone defect; then the sponge was removed.
- Expasyl™ and Stasis® site: Expasyl™ was placed into the bone defect with a spatula, flush with the adjacent outer cortex; after 2 min the paste was removed with a dental curette. Subsequently, a small sponge soaked with Stasis® was placed for 5 s into the bone defect, and then the sponge was removed.

Following the removal of the test agents with the dental curette (Fig. 1b), no additional bone freshening with drills was performed. The sites were rinsed with saline and wound closure was accomplished in a two-layer technique. The periosteum (galea aponeurotica) was closed using expanded polytetrafluoroethylene (ePTFE) – suturing material (Gore-Tex® Suture CV-5; W.L. Gore & Associates Inc., Flagstaff, AZ, USA). This suture material was chosen to avoid tissue reactions, as ePTFE is an inert and biocompatible material. The skin was closed with single interrupted sutures (Vicryl® 5-0; Ethicon, Johnson & Johnson, Brussels, Belgium).

Sacrifice

Following premedication with ketamine, 65 mg kg^{-1} (Narketan®; Vétoquinol) and xylazine, 4 mg kg^{-1} (Xylapan®; Vétoquinol), a cannula was placed into the lateral ear vein. Death was induced with 1.4 mL kg^{-1} pentobarbital (Nembutal®; Abbott Laboratories, Chicago, IL, USA). After a rectangular skin incision, the calvarium was removed with an oscillating autopsy saw. The retrieved specimens were immediately immersed in a solution of 4% formaldehyde and 1% calcium.

Histological analysis

The nondecalcified specimens were embedded in methyl-methacrylate and stained with combined basic fuchsin and toluidine blue. Transverse sections with a thickness of approximately 80 µm were obtained for descriptive histology (Schenk *et al.* 1984).

Visual analysis of haemorrhage control

Photos were taken before and after the application of the haemostatic agents. The amount of blood per site was assessed on a scale from 0 (completely dry defect) to 7 (profuse bleeding from the defect) (Fig. 2). Three evaluators independently examined the photos and determined the bleeding score per site. A mean bleeding score was calculated per treatment for the different sites before (=initial score) and after (=final score) the application of the haemostatic agents. The difference between the two scores determined the mean haemostatic effect per agent (reduction of bleeding).

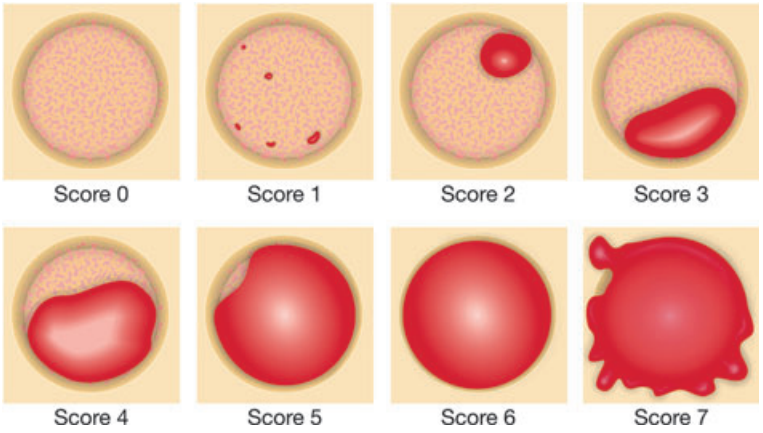

Figure 2 Schematic illustrations of bleeding scores used for visual determination of haemorrhage.

Table 1 Mean bleeding scores (±standard errors of the mean) and mean bleeding reduction (±standard errors of the mean) per treatment ($n = 6$)

Treatment[a]	Initial bleeding score	Final bleeding score	Calculated bleeding reduction[b]
Control	4.22 (±0.82)	5.50 (±0.66)	−1.28 (±0.62)
Bone wax	4.00 (±0.41)	2.72 (±0.57)	1.28 (±0.43)
Stasis®	5.28 (±0.64)	3.28 (±0.57)	2.00 (±0.69)
Expasyl™	4.11 (±0.32)	0.78 (±0.39)	3.50 (±0.41)
Expasyl™ + Stasis®	5.34 (±0.73)	0.56 (±0.25)	4.78 (±0.69)

[a]One treatment (Expasyl™ left *in situ*) not applicable to bleeding assessment.
[b]Positive values represent decrease of haemorrhage and negative values represent increase of haemorrhage.

Table 2 Pairwise comparisons of initial bleeding scores using Wilcoxon's signed rank test ($n = 6$, exact two-sided *P*-values)

Treatment	Control	Bone wax	Stasis®	Expasyl™
Bone wax	1.00	–	–	–
Stasis®	0.56	0.12	–	–
Expasyl™	1.00	0.41	0.12	–
Expasyl™ + Stasis®	0.31	0.31	1.00	0.12

P-value adjustment method: none.

Table 3 Pairwise comparisons of final bleeding scores using Wilcoxon's signed rank test ($n = 6$, exact two-sided *P*-values)

Treatment	Control	Bone wax	Stasis®	Expasyl™
Bone wax	0.094	–	–	–
Stasis®	0.125	0.656	–	–
Expasyl™	0.031	0.125	0.031	–
Expasyl™ + Stasis®	0.031	0.062	0.031	0.750

P-value adjustment method: none.

Statistics

The results were compared using Wilcoxon's signed rank test for paired samples. Exact two-sided *P*-values were computed to detect differences between the various treatment options. As pairwise comparisons were done on the same data, the *P*-values were adjusted to compensate the multiple testing situation. However, due to the explorative nature of the study and the small sample size, no adjustment was carried out. With regard to the interobserver variation, Cohen's *kappa* values were computed.

Results

All animals healed uneventfully and were killed as scheduled. The visual analysis of the haemorrhage control showed the highest effect for the combined Expasyl™ and Stasis® application (Table 1). All initial and final bleeding scores as well as the calculated bleeding reduction per treatment are summarized in Table 1. No differences were found for the initial bleeding scores per treatment (Table 2). With regard to the final bleeding scores, Expasyl™ and Expasyl™ in combination with Stasis® performed better than the control or Stasis® alone (Table 3). Bleeding reduction was more pronounced for Expasyl™, Stasis® and for Expasyl™ in combination with Stasis® compared with the control, as well as for Expasyl™ and Expasyl™ in combination with Stasis® compared with bone wax (Table 4). The calculated *kappa* values of the pairwise comparisons (three examiners) were 0.56, 0.51 and 0.62. These values indicated fair to strong concordance between the three

Table 4 Pairwise comparisons of calculated bleeding reduction using Wilcoxon's signed rank test ($n = 6$, exact two-sided *P*-values)

Treatment	Control	Bone wax	Stasis®	Expasyl™
Bone wax	0.094	–	–	–
Stasis®	0.031	0.438	–	–
Expasyl™	0.031	0.031	0.188	–
Expasyl™ + Stasis®	0.031	0.031	0.094	0.188

P-value adjustment method: none.

observers. As there were eight possible scores, one might interpret a difference of one as agreement. In this case, *kappa* values increased to 1.00, 0.92 and 0.95 showing that a difference of two or more occurred very rarely. The histological analysis is reported separately for each treatment option.

Control sites/3 weeks

Two of the three defects were bicortical. In those, woven bone formation could be observed on the defect walls without bridging the defects. The third defect showed woven bone formation to the level of the original cortex. In one of the sections, a small area with foreign body reaction was observed in the soft tissue covering the defect. Otherwise, the soft tissue presented without inflammatory reactions.

Control sites/12 weeks

Almost complete osseous healing with woven bone, reinforced with parallel-fibred bone was observed in all defects. In between the bone trabeculae, both fatty and haematopoietic bone marrow could be observed. One minor area of chronic infection could be seen in the top of one of the defects.

Bone wax sites/3 weeks

Bone formation was limited or nonexistent in all three defects. At the bottom of the defects large empty vacuoles, representing bone wax remnants (dissolved during the embedding procedure) could be observed surrounded by a soft tissue with chronic inflammatory changes and many foreign body giant cells.

Bone wax sites/12 weeks

A little more bone formation was seen after 3 weeks, but a slight to severe foreign body and chronic inflammatory reaction consistently surrounded bone wax remnants (Fig. 3).

Expasyl™ sites (temporary)/3 weeks

Sparse woven bone formation could be observed in two of the defects, none in the third. Varying amounts of foreign material were seen in the defects, and all showed abundant chronic inflammation including giant cells and phagocytes containing Expasyl™ remnants.

Expasyl™ sites (temporary)/12 weeks

No or very little bone formation could be seen (Fig. 4). The inflammatory reaction was reduced. There was a small amount of residual foreign material and an

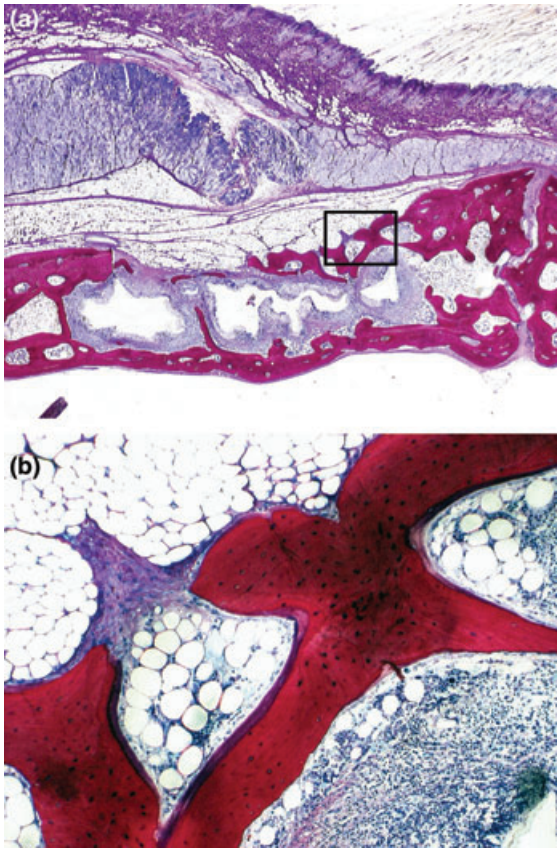

Figure 3 Histology after 12 weeks of defect treated with bone wax for 10 min (basic fuchsin and toluidine blue). A severe foreign body reaction with chronic inflammation is found around bone wax remnants in the centre of the bone (a, original magnification ×10). The enlargement shows the severe inflammatory response on the right side of the picture (b, original magnification ×90).

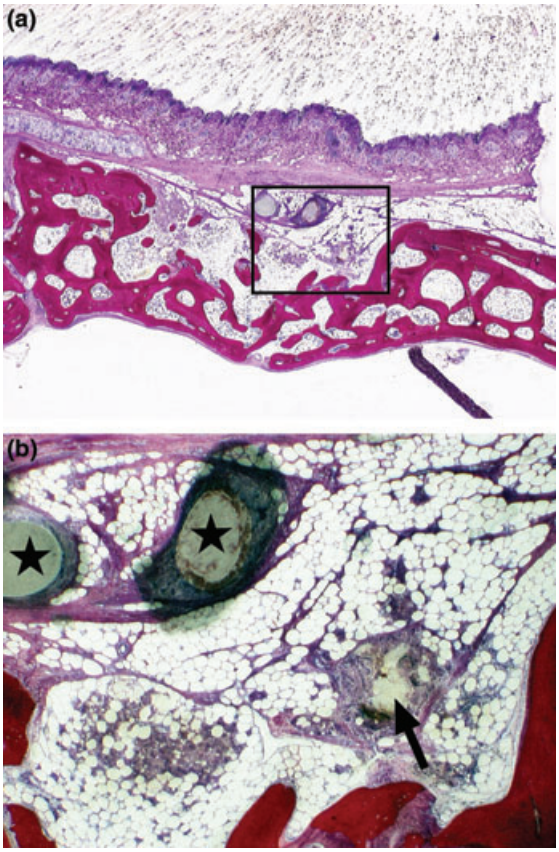

Figure 4 Histology after 12 weeks of defect treated with Expasyl™ for 2 min (basic fuchsin and toluidine blue). New bone formation is limited to the lower portion of the original defect (a, original magnification ×10). The enlargement shows an inflammatory reaction around Expasyl™ remnants (arrow), and in the upper left the ePTFE-suture (asterisk) (b, original magnification ×90).

increasing volume fraction of fatty bone marrow. A moderate amount of phagocytes and foreign body giant cells were identified.

Expasyl™ sites (permanent)/3 weeks

A more or less dense mass of foreign material occupied the main part of all three defects. No bone formation could be observed in any of the defects. Pronounced inflammation and foreign body reaction in the overlying soft tissue was a uniform finding.

Expasyl™ sites (permanent)/12 weeks

There was a limited bone formation on the defect walls. The amount of foreign material was clearly reduced in comparison with the 3-week specimens. Most of the defects were occupied by chronic inflammatory tissue containing giant cells and phagocytes, with Expasyl™ remnants in the cytoplasm.

Stasis® sites/3 weeks

Three unicortical defects showed 0%, 50% and almost 100% bone fill respectively. Areas with brown/yellow discoloration containing a variable amount of foreign material and foreign body giant cells were uniformly found. The overlying soft tissue showed slight to severe chronic inflammation.

Stasis® sites/12 weeks

All three defects showed almost complete osseous regeneration with woven bone, reinforced with parallel-fibred bone (Fig. 5). Apart from one small nidus of chronic inflammation, and one small area of discoloration (as seen in the 3-week specimens), the bone marrow was mature and free of inflammatory reactions.

Expasyl™ and Stasis® sites/3 weeks

The defects were dominated by the presence of chronic inflammation, with multiple multinucleated giant cells around remnants of the materials. Almost no new bone formation could be observed on the defect walls.

Expasyl™ and Stasis® sites/12 weeks

Moderate amounts of new bone formation could be seen. Mature fatty and haematopoietic bone marrow could be seen between areas with remnants of foreign material, chronic inflammation and multinucleated cells.

In general, the observations above were restricted to the defect sites. No chronic or acute tissue reactions could be observed in the marrow spaces surrounding the defects.

Discussion

This experimental study evaluated the immediate haemostatic effect of five different treatment options, and analysed histologically the tissue reactions to them. Both aspects are of clinical interest for surgical management of root structures and associated tissue lesions during periradicular surgery. While effective

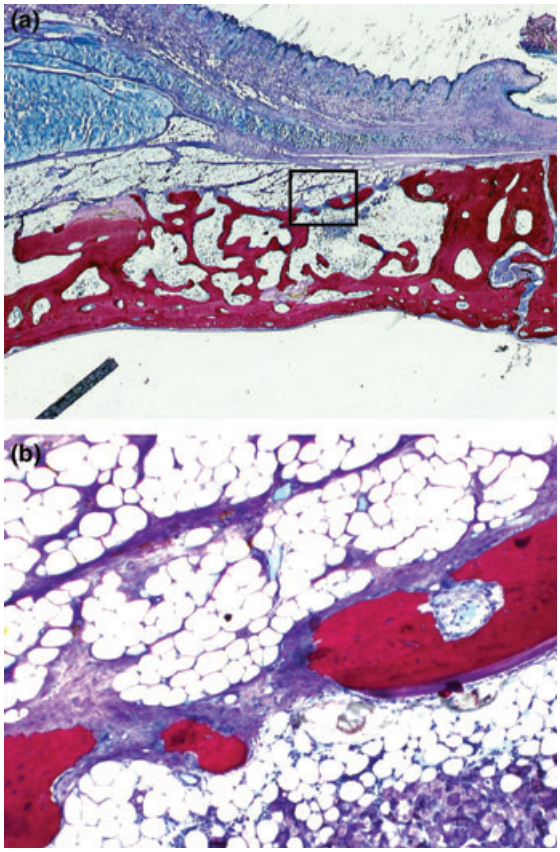

Figure 5 Histology after 12 weeks of defect treated with Stasis® for 5 s (basic fuchsin and toluidine blue). New bone formation is almost complete (a, original magnification ×10). Apart from a small area of chronic inflammation (lower right corner), the enlargement demonstrates mature bone marrow (b, original magnification ×90).

haemostasis is important during surgery for intraoperative diagnostic evaluation and root-end treatment, adverse tissue reactions to the agents applied for haemostasis might negatively influence the healing of the surgical site.

Two human studies have evaluated the haemostatic efficacy during periradicular surgery (Vickers *et al.* 2002, Vy *et al.* 2004). Vickers *et al.* reported adequate haemostasis in all 17 cases when racemic-epinephrine cotton pellets were used, and adequate haemostasis was achieved in 15 of 16 cases following the application of 20% ferric sulphate. Vy *et al.* reported complete haemostasis in 39 of 42 cases in which collagen sponges saturated with racemic epinephrine were applied. In contrast, haemorrhage control was not achieved in five of six cases that were treated with collagen sponges saturated with saline.

In the present study, the degree of bleeding was judged before and after the application of the haemostatic agents by three examiners independently using a score from 0 (no bleeding) to 7 (profuse bleeding) and schematic illustrations. Mean scores of bleeding reduction and the mean final bleeding scores showed that Expasyl™ alone or in combination with Stasis® was efficient in achieving good haemostasis of the surgical site.

Expasyl™, a paste containing aluminium chloride and kaolin, has been advocated for gingival retraction to ensure separation of the marginal gingiva and drying of the sulcus before impression-taking and insertion of restorations (Pescatore 2002, Shannon 2002). However, it has been shown that aluminium chloride elicits inflammatory tissue reactions. In a clinical comparative study on gingival retraction, aluminium chloride (25%) showed slower healing and more inflammatory reactions compared with a Nd:YAG-laser treatment (Abdel Gabbar & Aboulazm 1995). A dermatologic report on four cases demonstrated that aluminium chloride could cause a proliferative histiocytic reaction when used as a topical cauterizing agent (Barr *et al.* 1993). In an experimental study in eight beagle dogs evaluating four different retraction agents, racestyptine containing 25% aluminium chloride showed the most aggressive inflammatory infiltrate in gingival connective tissue (Kopac *et al.* 2002). In the present study, Expasyl™, alone or in combination with Stasis®, demonstrated a typical foreign body reaction including giant cells and inflammatory tissue response after 3 and 12 weeks. In contrast to control sites, new bone formation was minimal and clearly delayed in sites treated with Expasyl™. Although Expasyl™ is hydrophilic and easily washed out with saline, there may be a risk of leaving behind residues in the cancellous bone. It is therefore recommended to clean the surgical site with a bone curette and to freshen the walls of the bony crypt with a round bur before wound closure. In the present study, no attempts were made to completely remove the Expasyl™ employing such procedures. As no chronic or acute tissue reactions could be observed in the marrow spaces in the vicinity of the defects, it can be speculated that the adverse effects of Expasyl™ could be avoided by freshening the bony crypt as mentioned above.

In the present study, ferric sulphate (Stasis®) was found to be less effective than aluminium chloride (Expasyl™) in controlling the bleeding. Vickers *et al.* (2002) reported in their clinical study that in one-third

of the cases where ferric sulphate was used, some oozing of blood occurred in the bony crypt, requiring suctioning to maintain the dryness of the root-end preparation. Ferric sulphate has been used for more than a century in medicine. Ferric sulphate acts by agglutination of blood proteins resulting in plugs that occlude the capillary orifices (Lemon *et al.* 1993). When adequately curetted and irrigated from the surgical site prior to closure, ferric sulphate appears not to cause persistent inflammation or delay osseous repair (Jeansonne *et al.* 1993). In contrast, when ferric sulphate was left *in situ* for maximum exposure, an intense inflammatory response including foreign body reaction and delayed osseous healing were reported histologically after 18 and 46 days (Lemon *et al.* 1993). Interestingly, similar findings were seen in the present study for the shorter healing group of 3 weeks, whereas after 12 weeks, sites treated with ferric sulphate showed osseous regeneration and were free of any inflammatory reaction.

Bone wax containing purified beeswax, paraffin wax and isopropyl palmitate as a softening agent, has been recommended as an effective haemostatic agent in periradicular surgery since 1970 (Selden 1970). However, several reports have shown that bone wax residues are not resorbed and produce a foreign body giant cell reaction and inhibit bone reformation (Ibarrola *et al.* 1985, Alberius *et al.* 1987, Finn *et al.* 1992, Solheim *et al.* 1992, Allison 1994). From a clinical point of view, bone wax (after it was removed from the site) did not provide sufficient reduction in bleeding in the present study. Taking into consideration its adverse effects on tissue healing, it should no longer be used for haemostasis control in periradicular surgery.

Conclusions

- This explorative study in the rabbit calvarium clinically and histologically assessed the effect of various haemostatic agents.
- The key findings were: the visual analysis of pre- and post-application photographs demonstrated excellent bleeding reduction within trephined bony defects using Expasyl™ (aluminium chloride) alone or in combination with Stasis® (ferric sulphate). Histologic analysis showed a marked inflammatory tissue response towards Expasyl™ and bone wax within the immediate site of application, but no adverse tissue reactions were seen in the vicinity of the bone defects.
- Although not assessed directly in the study it is recommended that before wound closure of sites treated with such haemostatic agents, the bony crypt must be curetted to remove any foreign material, or preferably, freshened using rotary instruments.
- A future study should be performed to evaluate whether complete removal of Expasyl™ would prevent an inflammatory foreign body reaction and would allow for complete bone regeneration.

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