Haemostatic effect and tissue reactions of methods and agents used for haemorrhage control in apical surgery

S. S. Jensen[1,2], P. M. Yazdi[3], E. Hjørting-Hansen[1,4], D. D. Bosshardt[1] & T. von Arx[1]

[1]Department of Oral Surgery and Stomatology, School of Dental Medicine, University of Bern, Bern, Switzerland; [2]Department of Oral & Maxillofacial Surgery, Copenhagen University Hospital, Glostrup, Denmark; [3]Department of Oral & Maxillofacial Surgery, School of Dental Medicine, University of Århus, Århus, Denmark; and [4]Department of Oral & Maxillofacial Surgery, School of Dentistry, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark

Summary

Jensen SS, Yazdi PM, Hjørting-Hansen E, Bosshardt DD, von Arx T. Haemostatic effect and tissue reactions of methods and agents used for haemorrhage control in apical surgery. *International Endodontic Journal*, **43**, 57–63, 2010.

Aim To compare the haemostatic effect and tissue reactions of different agents and methods used for haemorrhage control in apical surgery.

Methodology Six standardized bone defects were prepared in the calvaria of six Burgundy rabbits. Five haemostatic modalities were tested for their haemostatic effect and tissue reactions, and were compared with untreated control defects: Expasyl™ + Stasis®, Expasyl™ + Stasis® + freshening of the bone defect with a bur, Spongostan®, Spongostan® + epinephrine, and electro cauterization. The haemostatic effect was analysed visually and compared using Wilcoxon's signed rank test. Two groups of three animals were evaluated histologically for hard and soft tissue reactions related to the different haemostatic measures, after 3 and 12 weeks of healing respectively.

Results Expasyl™ + Stasis® and electro cauterization proved most effective in reducing bleeding ($P < 0.05$), but were accompanied by unfavourable tissue reactions, as indicated by the presence of necrotic bone, inflammatory cells and the absence of bone repair. These adverse tissue reactions did not recover substantially over time. However, adverse reactions were not observed when the superficial layer of bone had been removed with a rotary instrument. In contrast, Spongostan® + epinephrine showed only a moderate haemostatic effect, but elicited also only mild adverse tissue reactions.

Conclusions Haemostasis in experimental bone defects is most effectively accomplished by using Expasyl™ + Stasis® or electro cauterization. However, the bone defects should be freshened with a rotary instrument before suturing so as not to compromise healing.

Introduction

Haemorrhage control is important in apical surgery to facilitate inspection of the root-end surface and to allow placement and setting of the root-end filling. Usually, one or more local agents are needed to achieve sufficient haemostasis. These agents should be either removed completely or should be fully biocompatible and degrade without interfering with periapical healing.

The haemostatic effect and tissue reactions of bone wax, ferric sulphate (Stasis®, Belport Co, Camarillo, CA, USA), and an aluminium chloride-containing paste (Expasyl™, Pierre Rolland, Merignac, France) intended for application into the sulcus prior to impression-taking has been reported (von Arx *et al.* 2006). The combination of Stasis® and Expasyl™ or Expasyl™ alone proved most efficient in controlling haemorrhage. However, their use was accompanied by an inflammatory and a

Correspondence: Dr Simon Storgård Jensen, Department of Oral & Maxillofacial Surgery, Copenhagen University Hospital Glostrup, Ndr. Ringvej, DK-2600 Glostrup, Denmark (Tel.: +45 43 23 32 08; fax: +45 43 23 39 63; e-mail: simon.storgaard@jensen.mail.dk).

foreign body tissue reaction at the histological level. Based on these findings, the clinical use of Expasyl™ has been modified to include freshening the bony surface of the periapical crypt with a bur after placement and initial setting of the root-end filling material.

Resorbable gelatin-based sponges, such as Spongostan®, Spongostan®, Dental, Johnson & Johnson medical Ltd., Ascot, UK, are frequently used for haemostasis in several surgical specialties (Petersen *et al.* 1984, Finn *et al.* 1992, Schonauer *et al.* 2004). Spongostan® can be used alone, but is often combined with a vasoconstrictor to enhance the haemostatic effect (Rud *et al.* 2001). Tissue reactions to Spongostan® are generally considered to be mild (Alpaslan *et al.* 1997). However, when Spongostan® is left inside osseous defects, delayed healing has been reported (Liening *et al.* 1997, Schonauer *et al.* 2004). It is not known how the tissue reacts if the gelatin sponge is removed after haemorrhage control has been achieved.

Electro cauterization is an effective method for producing haemostasis by coagulation and vesicular clumping. Most often, electro cauterization is used to stop localized bleeding in the soft tissues, but it has also been reported to be efficient when used on oozing bone surfaces (Jensen *et al.* 2002). With this approach, no foreign substance is introduced into the bony crypt. However, concern has been raised about the influence on healing due to the thermal damage to the bone tissue (Eriksson *et al.* 1982).

The purpose of the present study was twofold:
- To compare the haemostatic effects of Expasyl™ + Stasis®, Spongostan®, Spongostan® + epinephrine and electro cauterization in standardized bone defects.

- To evaluate the tissue reactions after using Expasyl™ + Stasis® with and without freshening of the bone defect with a bur, after electro cauterization, and after using Spongostan® alone or in combination with epinephrine.

Material and methods

Study design

Approval to perform the study was granted by the authorities of the Canton of Bern, Department of Agriculture, Section Veterinary Service, Experimental Animal Studies (study number 100/06). The experimental study was conducted in six adult Burgundy rabbits, each at least 5 months old and weighing between 3 and 4.5 kg.

The surgical procedures were performed under intravenous general anaesthesia using the medication and surgical protocol presented by von Arx *et al.* (2006).

In each rabbit, six standardized monocortical bone defects were created in the calvarium. The defects were prepared using a trephine with an outer diameter of 4 mm. The depth of the defects depended on the thickness of the outer cortical bone layer. Each defect then received one of the following treatments in a randomized sequence (Fig. 1), with a randomization scheme generated using http://www.randomization.com (seed: 2604):
- Control: no haemostatic agent was placed.
- Expasyl™ and Stasis®: 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.
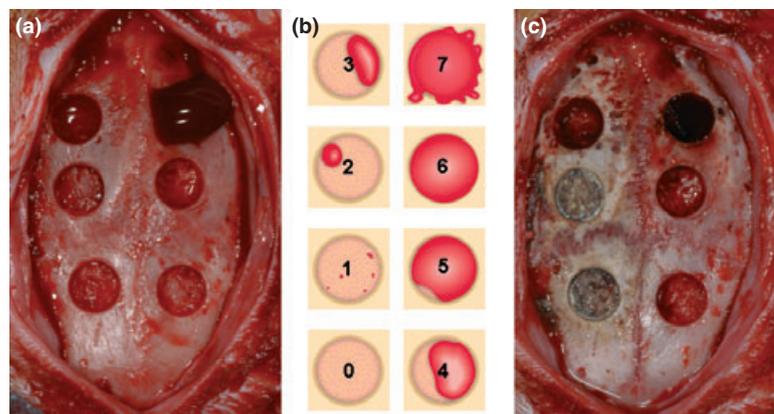

Figure 1 a) Standardized monocortical bone defects in the rabbit calvarium before application of haemostatic agents. Example of photograph used for visual assessment of initial bleeding score. b) Schematic illustrations used for visual assessment of bleeding. c) Presentation after application of haemostatic agents. Example of photograph used for visual assessment of final bleeding score.

Subsequently, a small sponge soaked with Stasis® (Belport Co, Camarillo, CA, USA) was placed for 5 s into the bone defect.

• Expasyl™ and Stasis® with freshening of the bone defect: Expasyl™ and Stasis® were applied as described above. Before primary closure, the bone defect was freshened using a small round bur (Ø: 1.2 mm) under copious saline irrigation to remove all macroscopically visible remnants of Expasyl™.

• Spongostan®: A Spongostan® sponge, 1 cm³ (Spongostan® Dental; Johnson & Johnson Medical Ltd., Ascot, UK) was compressed into the defect for 2 min using a gauze tampon, and then removed.

• Spongostan® and epinephrine: A Spongostan® sponge 1 cm³ was soaked in three drops of epinephrine 1%, compressed into the defect for 2 min using a gauze tampon, and then removed.

• Electro cauterization: Any visible bleeding within the defect was cauterized using a spatula-shaped cauterization head (straight, 2.35×19 mm) (ERBOTOM ICC, ERBE Swiss AG, Winterthur, Switzerland. Setting: Soft coagulation 60 Watt). Before primary closure, the defect was curetted using a surgical spoon.

Sacrifice

One group of three animals was allowed to heal for 3 weeks, and a second group of three animals for 12 weeks. Following each designated healing period, sacrifice was performed as previously described (von Arx *et al.* 2006). The retrieved calvarial specimens were immediately immersed in a solution of 4% formaldehyde and 1% calcium chloride.

Histological analysis

The non-decalcified specimens were embedded in methyl-methacrylate and stained with combined basic fuchsin and toluidine blue. Transversal sections with a thickness of approximately 80 μm were obtained for descriptive histology (Schenk *et al.* 1984). The histologic examination for the description of qualitative tissue reactions included absence or presence of (i) remnants of anticoagulation agents; (ii) new bone; (iii) necrotic bone; (iv) an inflammatory cell infiltrate; and (v) multinucleated giant cells.

Visual analysis of haemostatic effect

Photos were taken before application and after removal of the haemostatic agents (Fig. 1). The amount of blood per site was assessed on a scale from 0 (completely dry defect) to 7 (profuse bleeding from the defect) (von Arx *et al.* 2006). 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 application (=initial score) and after removal (=final score) of the haemostatic agents. The difference between the two means determined the mean haemostatic effect per agent (reduction of bleeding).

Statistics

The results of the visual analysis of haemostatic effect 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 pair wise comparisons were completed on the same data, the *P*-values would have needed to be adjusted to compensate for the multiple testing situation. However, because of the explorative nature of the study and the small sample size, no adjustment was carried out. Cohen's weighted *kappa* values were calculated to evaluate inter-observer variations (Fleiss & Cohen 1973).

Results

One animal in the 12-week group died immediately postoperatively because of an anaesthetic complication. An additional animal was therefore included, resulting in seven animals being included in the visual evaluation of the haemostatic effect. Another animal in the 12-week group died 7 weeks postoperatively. The calvarium of this animal was evaluated histologically and was found to demonstrate tissue reactions comparable to the two remaining animals in the 12-week group. This animal was therefore included in the qualitative histologic evaluation.

Haemostatic effect

In the visual quantification of the bleeding, there was strong agreement between the three observers (weighted kappa values: 0.71 to 0.98) (Fleiss & Cohen 1973). The initial bleeding scores, final bleeding scores, and reduction of bleeding for the individual test groups are presented in Table 1. Pair wise comparisons of the different test groups regarding final bleeding score and bleeding reduction are given in Tables 2 and 3.

No significant differences were found between the initial mean bleeding scores for the different treatment

modalities (*P* between 0.14 and 1.00). The mean final bleeding score was significantly smaller final than the 'control' for all groups except 'Spongostan®' (*P* = 0.798). 'Expasyl™ + Stasis®' and 'Expasyl™ + Stasis® + freshening' both had significantly smaller mean bleeding scores than 'Spongostan®' and 'Spongostan® + epinephrine' (*P* < 0.05). 'Electro cauterization' exhibited borderline significantly smaller final mean bleeding scores than 'Spongostan®' and 'Spongostan® + epinephrine' (*P* = 0.059 and *P* = 0.051, respectively). With regard to the mean bleeding reduction scores, all but the two 'Spongostan®' groups (*P* = 0.866 and *P* = 0.295, respectively) demonstrated significantly higher bleeding reduction than the control defects (*P* < 0.05). 'Expasyl™ + Stasis®' showed borderline significantly higher bleeding reduction than 'Spongostan®' (*P* = 0.050). 'Expasyl™ + Stasis® + freshening' reduced the bleeding significantly more than 'Spongostan®' (*P* = 0.031) and borderline significantly more than 'Spongostan® + epinephrine' (*P* = 0.051). Electro

cauterization resulted in significantly higher bleeding reduction than 'Spongostan®' (*P* = 0.034).

Histology

No attempt was made to preserve the volume of the original bone defects by covering them with a barrier membrane. Therefore, herniation of soft tissues into the defects was a frequent finding, irrespective of the haemostatic agent applied (Fig. 2).

Control sites
3 Weeks: Vivid bone formation was observed extending from the defect walls. Ongoing osteogenic activity was observed throughout the defects with woven bone trabeculae with osteoblastic seams.

12 Weeks: Maturation of the newly formed bone was generally observed (Fig. 2). However, pressure from the covering soft tissue caused surface resorption of some of the newly formed bone.

	Mean initial score (± SD)	Mean final score (± SD)	Mean reduction (± SD)
Control	3.81 (± 1.68)	3.33 (± 1.60)	0.48 (± 1.87)
Expasyl™ + Stasis®	3.24 (± 0.83)	0.43 (± 0.29)	2.81 (± 0.71)
Expasyl™ + Stasis® + freshening of defect	4.24 (± 1.74)	0.29 (± 0.21)	3.95 (± 1.84)
Electro cauterization	4.57 (± 2.12)	1.05 (± 0.90)	3.52 (± 1.74)
Spongostan®	3.62 (± 1.33)	3.10 (± 0.90)	0.52 (± 1.32)
Spongostan® + epinephrine	3.86 (± 1.59)	2.24 (± 0.92)	1.62 (± 0.93)

Table 1 Mean bleeding scores (± SD) before application and after removal of haemostatic agents, and mean reduction in bleeding scores (*n* = 7)

Table 2 P-values of pairwise comparisons of the final bleeding scores using Wilcoxon's signed rank test

	Control	Expasyl™ + Stasis®	Expasyl™ + Stasis® + freshening of defect	Electro cauterization	Spongostan®
Expasyl + Stasis®	0.036	–	–	–	–
Expasyl + Stasis® + freshening of defect	0.022	0.586	–	–	–
Electro cauterization	0.034	0.100	0.181	–	–
Spongostan®	0.798	0.022	0.016	0.059	–
Spongostan® + epinephrine	0.034	0.031	0.016	0.051	0.104

Table 3 P-values of pairwise comparisons of the scores of calculated bleeding reduction using Wilcoxon's signed rank test

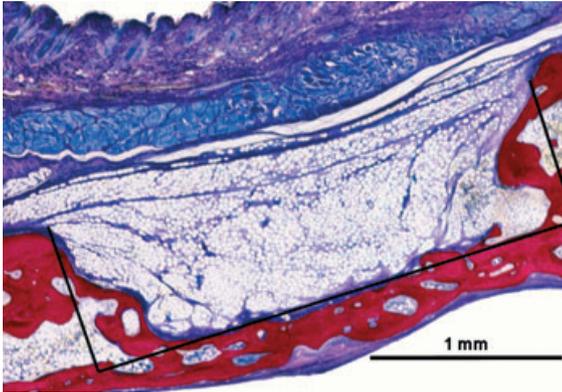|	Control	Expasyl™ + Stasis®	Expasyl™ + Stasis® + freshening of defect	Electro cauterization	Spongostan®
Expasyl™ + Stasis®	0.036	–	–	–	–
Expasyl™ + Stasis® + freshening of defect	0.031	0.106	–	–	–
Electro cauterization	0.031	0.444	0.400	–	–
Spongostan®	0.866	0.050	0.031	0.034	–
Spongostan® + epinephrine	0.295	0.141	0.051	0.150	0.204

Figure 2 Control defect after 12 weeks of healing. Most of the original defect volume (marked with lines) is occupied by soft tissue with the character of loose connective tissue dominated by epidermal fat cells. Bone formed form the defects walls has the character of mature lamellar bone.

Expasyl^{TM} and Stasis$^{®}$

3 Weeks: The entire bone surface was necrotic, without signs of repair activity. The osteocyte lacunae were empty along the surfaces of the cavities, and numerous macrophages and multinucleated giant cells were observed close to the defect walls and to remnants of Expasyl^{TM}, which were particularly evident in concavities of the defect wall.

12 Weeks: Necrotic areas could still be identified within the defect walls with areas of undermining resorption, nearly forming a sequester (Fig. 3). Remnants of Expasyl^{TM} were often observed, and were always surrounded by an extensive foreign body reaction (Fig. 3).

Expasyl^{TM} and Stasis$^{®}$ with freshening of the bone defect

3 Weeks: Moderate amounts of woven bone formation were observed close to the defect walls, particularly at sites where the bone marrow spaces were opened widely. Remnants of Expasyl^{TM} were seen rarely. However, if present, they were always accompanied by multinucleated giant cells.

12 Weeks: The amount of osseous repair was limited, but no signs of necrotic bone or foreign body reaction were present.

Spongostan$^{®}$

3 Weeks: Woven bone formation was observed along the entire surface of the bone cavities. However, the osteoblastic activity along the defect surfaces was reduced compared with the control defects.
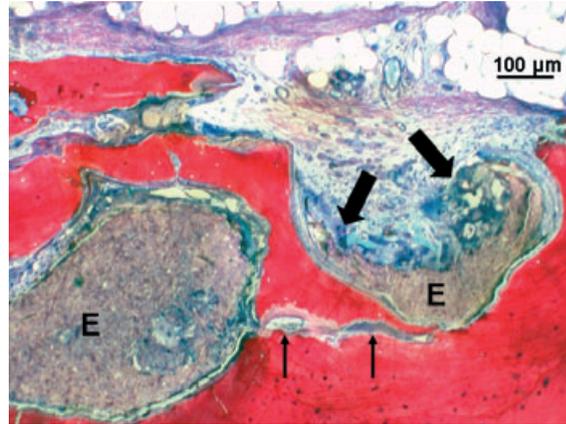

Figure 3 Defect 12 weeks after application of Expasyl^{TM} + Stasis$^{®}$ without freshening the defect with a round bur. Remnants of Expasyl^{TM} (E) are surrounded by lots of multinucleated giant cells (large arrows). Empty osteocyte lacunae can be see in a bone trabecula undermined by resorption cavities (Small arrows).

12 Weeks: The defects were dominated by maturation of the newly formed bone, with layers of parallel-fibered bone reinforcing the woven bone trabeculae.

Spongostan$^{®}$ and epinephrine

3 Weeks: Vivid new bone formation could be seen, with woven bone throughout the former bone defect (Fig. 4). Ongoing osteogenic activity, as indicated by the presence of osteoid seams and osteoblasts, was a dominating feature.

12 Weeks: Maturation of woven bone formed earlier was seen, with only sparse signs of ongoing bone formation. In general, the surfaces demonstrated lamellar bone coverage.
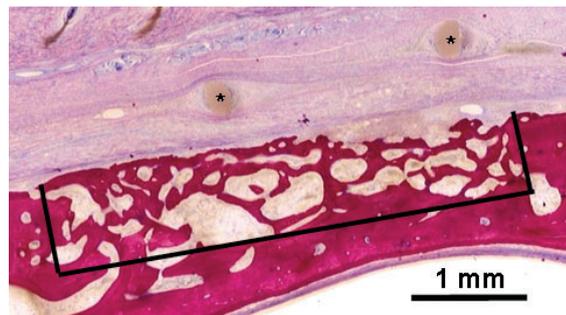

Figure 4 Defect three weeks after application of Spongostan$^{®}$. The former defect (marked with lines) is completely occupied by newly formed woven bone. The ePTFE suture used to close the periosteal layer can be recognized (asterisks).
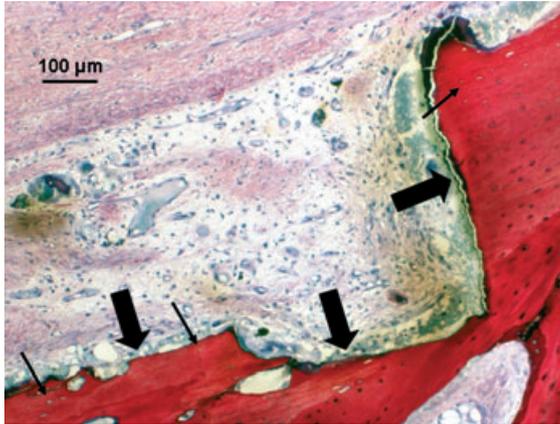
Figure 5 Defect three weeks after using electro cauterization for haemorrhage control. Multiple empty osteocyte lacunae (small arrows) can be seen close to the defect walls (large arrows). The soft tissue occupying the defect has the character of immature granulation tissue. A coagulum containing many erythrocytes is observed along the defect walls.

Electro cauterization

3 Weeks: The surfaces of the entire cavities appeared necrotic, without any signs of bone repair. The osteocyte lacunae near to the surface were empty (Fig. 5). The cell populations adjoining the surfaces consisted almost exclusively of inflammatory cells, mainly macrophages and some multinucleated giant cells. In addition, many erythrocytes were often seen on the cauterized bone surface. The opened bone marrow often revealed signs of degeneration.

12 Weeks: The bone surface appeared vital, but jagged after extensive osteoclastic activity. The size and shape of the cavities were nearly identical to the situation immediately postoperatively with very limited signs of new bone formation.

Discussion

This study evaluated the haemostatic efficacy and tissue reactions of different methods for local haemorrhage control used in apical surgery. Overall, Expasyl™ + Stasis® and electro cauterization proved most efficient in reducing bleeding, while the use of Spongostan® alone did not demonstrate any significant haemostatic effect as compared with the control defects. The addition of epinephrine to the Spongostan® had some effect on the final bleeding score, without reaching the efficacy of Expasyl™ + Stasis®.

The efficacy of Expasyl™ + Stasis® in reducing bleeding was in accordance with a previous study

using the same model (von Arx *et al.* 2006). However, a concern was the localized foreign body reaction elicited by remnants of Expasyl™ in the bone defects. Only limited documentation exists on tissue reactions to aluminium chloride in paste form, but studies which have evaluated topical application of aluminium chloride in liquid form have also reported inflammatory reactions (Barr *et al.* 1993, Kopac *et al.* 2002). The results of the present study suggest that these tissue reactions can be significantly reduced by freshening the defect with a bur. In the clinical case of using a root-end filling material that does not set during the surgical procedure [e.g. mineral trioxide aggregate (MTA)], there is a risk of flushing out the material during the freshening of the bony cavity. To avoid this, it has proved important to use a relatively small round bur to reduce the risk of touching the cut root surface and MTA filling, and to prevent direct water spray on the cut root surface.

Spongostan® is widely used in several surgical specialties to control bleeding, but most often in surgical sites where it can be left *in situ*, such as in dental extraction sockets or in donor sites after bone graft harvesting (Petersen *et al.* 1984, Finn *et al.* 1992, Blinder *et al.* 1999). In the present study, the sponge was removed after 2 min to conform with the typical protocol in apical surgery (Rud *et al.* 2001). This eliminated the compressive element, and the resulting intrinsic haemostatic effect proved to be limited. Histologic analysis revealed slightly delayed bone healing that was qualitatively comparable to the control defects. Similar findings have been reported in previous experimental and clinical studies, where the gelatin sponges were left *in situ* (Petersen *et al.* 1984, Finn *et al.* 1992). Addition of epinephrine 1% to a gelatin sponge or cotton pellet has been reported to provide sufficient haemostasis to allow undisturbed placement and setting of dentine-bonded composite resin root-end fillings (Rud *et al.* 2001, Jensen *et al.* 2002). In the present experimental setting, the addition of epinephrine to Spongostan® only marginally increased the haemostatic effect, without reaching statistical significance. No histologic difference in healing pattern was observed with the addition of epinephrine. As epinephrine is a naturally occurring circulating hormone in the organism, disturbance of healing was not to be expected.

Electro cauterization provided a haemostatic effect similar to that of Expasyl™ + Stasis®. However, bone healing was delayed when compared to control defects and to Expasyl™ + Stasis®-treated defects that were

freshened with a bur. Limited bone formation was observed after 12 weeks of healing, following initial signs of superficial necrosis. This can presumably be ascribed to thermal injury, as has previously been documented (Eriksson *et al.* 1982). In the early healing phase, an adverse tissue reaction was seen in relation to the necrotic zones. This inflammatory and foreign body reaction was not observed after 12 weeks of healing. Coagulated tissue remnants were removed with a curette before suturing. It can be speculated that the osseous healing conditions could have been improved by removing the superficial bone layer with a rotary instrument, as was observed with the freshened Expasyl™ + Stasis®-treated defects.

Conclusion

Expasyl™ + Stasis® and electro cauterization proved most efficient in the reduction of bleeding from standardized bone defects. However, the same measures were accompanied by the most pronounced adverse tissue reactions. It is recommended to thoroughly remove the superficial layer in the bone defect with a rotary instrument after application of Expasyl™ + Stasis® or electro cauterization in apical surgery to reduce these unfavourable tissue reactions. Defects treated with Spongostan® demonstrated no adverse tissue reactions but delayed bone healing. Despite the reduced haemostatic effect of Spongostan® and epinephrine compared with Expasyl™ + Stasis® and electro cauterization, this combination will often clinically ensure sufficient haemostasis for the undisturbed placement and setting of a root-end filling.

Acknowledgements

References

Alpaslan C, Alpaslan GH, Oygur T (1997) Tissue reaction to three subcutaneously implanted local hemostatic agents. *British Journal of Oral and Maxillofacial Surgery* **35**, 129–32.

von Arx T, Jensen SS, Hänni S, Schenk RK (2006) Haemostatic agents in periradicular surgery: an experimental study of their efficacy and tissue reactions. *International Endodontic Journal* **39**, 800–8.

Barr RJ, Alpern KS, Jay S (1993) Histiocytic reaction associated with topical aluminium chloride (Drysol reaction). *The Journal of Dermatologic Surgery and Oncology* **19**, 1017–21.

Blinder D, Manor Y, Martinowitz U, Taicher S (1999) Dental extractions in patients maintained on continued oral anticoagulant comparison of local hemostatic modalities. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology and Endodontics* **88**, 137–40.

Eriksson A, Albrektsson T, Grane B, McQueen D (1982) Thermal injury to bone A vital-microscopic description of heat effects. *International Journal of Oral Surgery* **11**, 115–22.

Finn MD, Schow SR, Schneiderman ED (1992) Osseous regeneration in the presence of four common hemostatic agents. *Journal of Oral and Maxillofacial Surgery* **50**, 608–12.

Fleiss JL, Cohen J (1973) The equivalence of weighted kappa and the intraclass correlation coefficient as measures of reliability. *Educational and Psychological Measurement* **33**, 613–9.

Jensen SS, Nattestad A, Egdø P, Sewerin I, Munksgaard EC, Schou S (2002) A prospective, randomized, comparative clinical study of resin composite and glass ionomer cement for retrograde root filling. *Clinical Oral Investigations* **6**, 236–43.

Kopac I, Cvetko E, Marion L (2002) Gingival inflammatory response induced by chemical retraction agents in beagle dogs. *International Journal of Prosthodontics* **15**, 14–9.

Liening DA, Lundy L, Silberberg B, Finstuen K (1997) A comparison of the biocompatibility of three absorbable hemostatic agents in the rat middle ear. *Otolaryngology Head and Neck Surgery* **16**, 454–7.

Petersen JK, Krogsgaard J, Nielsen KM, Nørgaard EB (1984) A comparison between two absorbable hemostatic agents: gelatin sponge (Spongostan) and oxidized regenerated cellulose (Surgicel). *International Journal of Oral Surgery* **13**, 406–10.

Rud J, Rud V, Munksgaard EC (2001) Periapical healing of mandibular molars after root-end sealing with dentine-bonded composite. *International Endodontic Journal* **34**, 285–92.

Schenk RK, Olah AJ, Herrmann W (1984) Preparation of calcified tissues for light microscopy. In: Dickson GR, ed. *Methods of Calcified Tissue Preparation*. Amsterdam, Netherlands: Elsevier, pp. 1–56.

Schonauer C, Tessitore E, Barbagallo G, Albanese V, Moraci A (2004) The use of local agents: bone wax, gelatin, collagen, oxidized cellulose. *European Spine Journal* **13**(Suppl. 1), S89–96.