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Prognostic factors for alveolar regeneration: effect of tissue occlusion on alveolar bone regeneration with guided tissue regeneration

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Abstract

Objectives: Design criteria for guided tissue regeneration (GTR) devices include biocompatibility, cell occlusion, space-provision, tissue integration, and ease of use. The objective of this study was to evaluate the effect of cell occlusion and space-provision on alveolar bone regeneration in conjunction with GTR.

Methods: Routine, critical-size, 6 mm, supra-alveolar, periodontal defects were created in 6 young adult Beagle dogs. Space-providing ePTFE devices, with or without 300-μm laser-drilled pores were implanted to provide for GTR. Treatments were alternated between left and right jaw quadrants in subsequent animals. The gingival flaps were advanced for primary intention healing. The animals were euthanized at week 8 post surgery. The histometric analysis assessed regeneration of alveolar bone relative to space-provision by the ePTFE device.

Results: A significant relationship was observed between bone regeneration and space-provision for defect sites receiving the occlusive ($\beta = 0.194$; $p < 0.02$) and porous ($\beta = 0.229$; $p < 0.0004$) GTR devices irrespective of treatment ($p = 0.14$). The bivariate analysis showed that both space-provision and device occlusivity significantly enhanced bone regeneration. Hence, sites receiving the occlusive GTR device and sites with enhanced space-provision showed significantly greater bone regeneration compared to sites receiving the porous GTR device ($p = 0.03$) or more limited space-provision ($p = 0.0002$).

Conclusions: Cell occlusion and space-provision may significantly influence the magnitude of alveolar bone regeneration in conjunction with guided tissue regeneration.

Key words: alveolar bone; dogs; ePTFE devices; guided tissue regeneration; tissue engineering

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Scantlebury (1993) presented design criteria for guided tissue regeneration (GTR) devices including biocompatibility, cell occlusion, space-provision, tissue integration, and ease of use. Several studies have investigated the effect of space-provision on periodontal regeneration in conjunction with GTR (Haney et al. 1993, Sigurdsson et al. 1994, 1995, Trombelli et al. 1999, Polimeni et al. 2002, 2003a, b). These studies show that space-provision is a critical factor for periodontal regeneration including alveolar bone, cementum, and a functionally oriented periodontal attachment. On the other hand, absence of space-provision such as following gingival flap surgery alone when the gingival flaps collapse onto the root surface (Haney et al. 1993, Sigurdsson et al. 1994), or following GTR when the GTR device has collapsed or been compressed onto the root surface (Haney et al. 1993, Sigurdsson et al. 1994), or when non- or slowly resorbing biomaterials have been used in conjunction with GTR (Trombelli et al. 1999),

apparently compromises periodontal regeneration.

Although the concept of space-provision and cell occlusivity has been mentioned in several studies and reviews as a critical determinant for the selective re-population of the site by differentiated/undifferentiated cells from the periodontal ligament, existing evidence may not completely support this assumption. Karaki et al. (1984) evaluated the influence of space-provision for alveolar regeneration in a periodontal defect model in dogs. They used a tissue-expanding open gold mesh applied onto one defect site while the contralateral site served as a sham-surgery control. Bone formation was enhanced in defects treated with the open gold mesh compared to that in the surgical control. Zellin & Linde (1996), using a rat calvaria model comparing space-providing devices with different porosity (8, 20–25 and 100 μm diameter pore size), showed that sites receiving devices with large diameter pores exhibited increased new bone formation compared to sites receiving devices with smaller pore size. More recently, Wikesjö et al. (2003a), using occlusive and porous GTR devices in the supra-alveolar periodontal defect model, showed that periodontal regeneration including alveolar bone, cementum, and a functionally oriented periodontal attachment indeed may occur in the presence of space-provision without provisions for cell occlusion. However, the importance of cell occlusion for optimal regeneration was not determined. From the above, it appears that space-provision plays a determinant role for periodontal regeneration, however, the critical importance of cell occlusion remains unclear. The objective of this study was to evaluate the effect of cell occlusion and space-provision on alveolar bone regeneration in conjunction with GTR.

Material and Methods

Animals

The surgical and animal technical protocol has been elaborated in a previous report of this study (Wikesjö et al. 2003a). In brief, 6 young adult male Beagle dogs obtained from a USDA-approved dealer were used. Animal selection, management, and experimental protocol were approved by the Animal Care and Use Committee (W.L. Gore & Associates, Inc., Flagstaff, AZ, USA). The animals had access to standard laboratory diet and water until the beginning of the study. Oral prophylaxis was performed within 2 weeks prior to the experimental surgeries.

GTR devices

Space-providing ePTFE devices (Reinforced GORE-TEX® ePTFE, W.L. Gore & Associates Inc., Flagstaff, AZ, USA) were used. The occlusive devices had a 15–25 μm nominal pore size and were reinforced with a laminated polypropylene mesh. The porous devices exhibited the same characteristics except for laser-etched 300-μM pores at 0.8 mm (center to center) intervals allowing for penetration of the gingival connective tissue.

Surgical procedures

Food was withheld the night before surgery. The animals were pre-medicated with atropine (0.02 mg/kg i.m.), buprenorphine (0.04 mg/kg i.m.), and flunixin meglumine (0.1 mg/kg IV). A prophylactic antibiotic (cefazolin; 22 mg/kg i.v.) was administered. General anesthesia was induced with diazepam (0.2 mg/kg i.v.) and ketamine (6 mg/kg i.v.). An endotracheal tube was placed and the animals were maintained on isoflurane gas (1–2%) in 100% oxygen using positive pressure ventilation. An i.v. line was placed and the animals received a slow constant rate infusion of lactated Ringer's solution (10–20 ml/kg/h) to maintain hydration while anesthetized. Routine dental infiltration anesthesia with epinephrine was used at the surgical sites.

Critical-size, 6 mm, supraalveolar, periodontal defects were created around the third and fourth mandibular premolar teeth in right and left jaw quadrants in each animal (Wikesjö et al. 1994). The crowns of the teeth were reduced to approximately 2 mm coronal to the cemento–enamel junction and the exposed pulpal tissues were sealed (Cavit®, ESPE, Seefeld/Oberbayern, Germany). Occlusive and porous ePTFE devices were then implanted into left and right jaw quadrants in a split-mouth design. To ensure an adequate blood clot underneath the ePTFE device, autologous blood was drawn using an i.v.-catheter and aspirated blood was expelled underneath the device. The ePTFE device was fixed to the reduced alveolar bone with medical grade stainless steel tacks (FRIOS® Augmentation System, Friadent, Mannheim, Germany). The periostea were then fenestrated at the base of the gingival flaps to allow tension-free flap apposition. The flaps were advanced, and the flap margins were adapted 3–4 mm coronal to the ePTFE device and sutured (GORE-TEX™ Suture CV5).

The maxillary first, second and third premolar teeth were surgically extracted, and the maxillary fourth premolars were reduced in height and exposed pulpal tissues sealed (Cavit®) to prevent potential trauma from the maxillary teeth to the mandibular experimental sites.

Post-surgery protocol

The animals were fed a soft dog food diet. Buprenorphine (0.04 mg/kg i.v., i.m., or s.q. every 5 h) was used for analgesia the first few days. A broad-spectrum antibiotic (enrofloxacin; 2.5 mg/kg, i.m., bid) was used for infection control for 14 days. Plaque control was maintained by twice daily topical application of chlorhexidine (chlorhexidine gluconate 20%, Xttrium Laboratories, Inc., Chicago, IL, USA; 40 ml of a 2% solution) until gingival suture removal and thereafter once daily until the completion of study. Gingival sutures were removed under sedation at approximately 10 days. The animals were anesthetized and euthanized at 8 weeks when the experimental teeth including surrounding soft and hard tissues were removed *en bloc*. ePTFE devices were not removed during the healing interval.

Histological processing and evaluation

The tissue blocks were fixed in 10% buffered formalin for 3–5 days, decalcified in 5% formic acid for 8–10 weeks, trimmed, dehydrated, and embedded in paraffin. Serial sections (7 μm) were produced in a buccal-lingual plane throughout the mesial–distal extension of the teeth. Every 14th section was stained with hematoxylin for observations at 100 μm intervals.

The most central stained section of each root of the third and fourth premolar teeth was identified by the size of the root canal. This section was subjected to histometric analysis. Analysis was performed using incandescent and polarized light microscopy (BX 60, Olympus America, Inc. Melville, NY, USA), a microscope digital camera

system (DP10, Olympus America, Inc. Melville, NY, USA), and a PC-based image analysis system (Image-Pro Plus™, Media Cybernetic, Silver Springs, MD, USA) by one calibrated investigator (G.P.; intraclass correlation coefficient = 0.984). The following measurements were recorded for the buccal and the lingual tooth surfaces for each section:

- Bone regeneration (height): distance between the apical extension of the root planing and the coronal extension of alveolar bone regeneration along the planed root.
- Wound area: area circumscribed by the planed root surface, the ePTFE device, and the base of the defect at the level of the apical extension of the root planning.
- Bone width: the width of the resident bone at the apical extension of root planning.

Data analysis

The data were analyzed using univariate, bivariate, and multivariate analyses. The univariate analysis assessed the effects of treatments and wound area, separately, on bone regeneration (height). The bivariate analysis assessed the effect of treatment methods on bone regeneration (height) within various thresholds of wound area. The multivariate analysis used the Mixed Models analysis of variance (Proc Mixed in SAS V8.1, SAS Institute Inc., Cary, NC, USA), which is designed for the analysis of correlated data and modeling of random effects. The analysis assessed the relationship of alveolar bone regeneration and space-provision for defect sites receiving the occlusive or porous membranes. The correlation between the width of the alveolar bone at the base of the defect and the wound area was assessed using the Pearson correlation analysis.

Results

The univariate analysis showed that bone regeneration in animals receiving occlusive devices was significantly greater than that in animals receiving porous devices ($p = 0.007$; Table 1). Grouping the defects by wound area dimensions <3, 3–7, and $>7\,mm^2$, showed that sites with larger wound areas exhibited significantly enhanced bone regeneration compared to sites providing smaller wound areas ($p < 0.0001$; Table 2).

There were significant correlation coefficients ($r = 0.696$, $p = 0.0002$; $r = 0.742$, $p < 0.001$) and significant linear relationships ($p < 0.0004$, $p < 0.0001$, Fig. 1) between bone width and wound area for defect sites treated with the occlusive and porous devices, respectively. The relationship of bone width and wound area was not statistically different between the treatments. The analysis also showed statistically significant linear relationships between wound area and bone regeneration for the treatments ($p < 0.02$, $p < 0.0004$, Fig. 2), and no significant difference between the slopes ($p = 0.14$) (Figs 3 and 4).

The analysis showed that both wound area and device occlusivity exhibited statistically significant effects on bone regeneration. Sites receiving the occlusive device and sites with a larger wound area exhibited significantly increased bone regeneration compared to sites implanted with the porous device ($p = 0.03$) or providing smaller wound areas ($p = 0.0002$; Table 3). Comparisons of bone regeneration between the treatments were performed within different thresholds of wound area and showed that in sites with a wound area ranging from 3 to $7\,mm^2$, defects receiving the occlusive device exhibited significantly greater bone regeneration than sites receiving the porous device ($p = 0.03$, Table 4). However, in wound areas <3 or $>7\,mm^2$, although also showing greater bone regeneration in sites receiving the occlusive device rather than the porous device, the differences were not statistically significant. This may partly be due to the few sites having the upper and lower wound area thresholds.

Table 1. Mean bone regeneration (height) for animals receiving occlusive and porous ePTFE devices

Treatment	Mean (mM)	SE	*p*
occlusive	2.82	0.31	
porous	1.85	0.32	0.007

Table 2. Mean bone regeneration (height) grouped by wound area

Wound area (mm^2)	Mean (mm)	SE	*p*
<3	1.13	0.42	
3–7	2.31	0.32	0.006
>7	3.28	0.37	0.0001

Discussion

The objective of this study was to evaluate the effect of cell occlusion and space-provision on alveolar bone regeneration in conjunction with GTR. Critical-size, 6 mm, supra-alveolar, periodontal defects were created in six young adult Beagle dogs and were implanted with space-providing occlusive and porous ePTFE devices. The animals were euthanized following an 8-week healing interval for histometric analysis of the experimental sites. A significant relationship between bone regeneration and space-provision was observed for sites receiving the occlusive and porous GTR device without significant difference between treatments. Space-provision and device occlusivity both exhibited significant effects on bone regeneration. Sites receiving the occlusive GTR device and sites with enhanced space-provision showed significantly greater bone regeneration

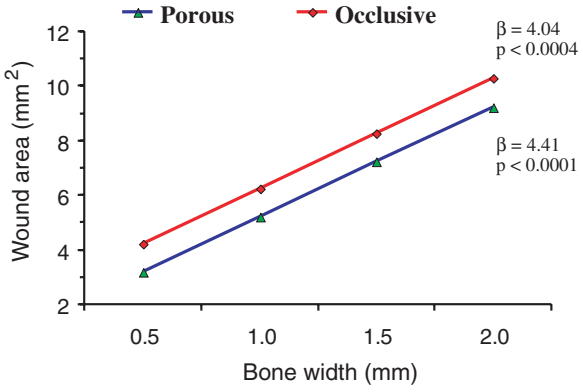

Fig. 1. Relationship between wound area and bone width in animals receiving porous and occlusive ePTFE devices. The slopes are not significantly different ($p = 0.5$).

compared to sites receiving the porous GTR device or more limited space-provision. Thus it can be concluded that cell occlusion and space-provision may significantly influence the magnitude of alveolar bone regeneration in conjunction with GTR.

The experimental model used in this study was the critical-size, supra-alveolar periodontal defect model (Wikesjö et al. 1994). This model has been shown to be highly discriminating in the evaluation of regenerative potential of alveolar bone, cementum, and periodontal attachment for various candidate protocols (Wikesjö & Selvig 1999). It has been shown that the defect morphology allows for an unbiased and highly reproducible strategy of analysis (Koo et al. 2003a, b). Alveolar bone and cementum regeneration has been shown not to exceed 15% of the defect height in sham-surgery controls over a 4- or 8-week healing interval, thus surgical controls were not deemed necessary in this study.

A significant correlation between bone width and wound area was found for both porous and occlusive GTR devices. The width of the resident bone at the base of the defect seems to efficaciously support the space provided by the regenerative device. It was

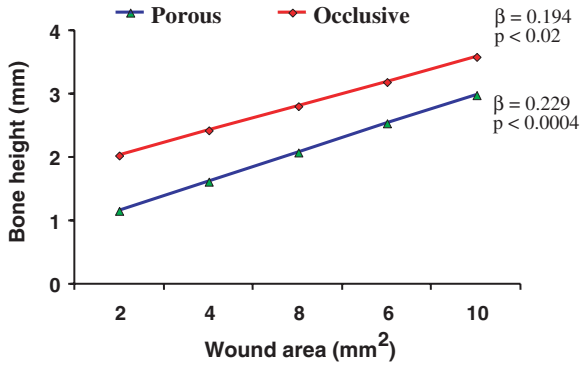

Fig. 2. Relationship between wound area and bone regeneration (height) in animals receiving porous and occlusive ePTFE devices. The slopes are not significantly different ($p = 0.14$).

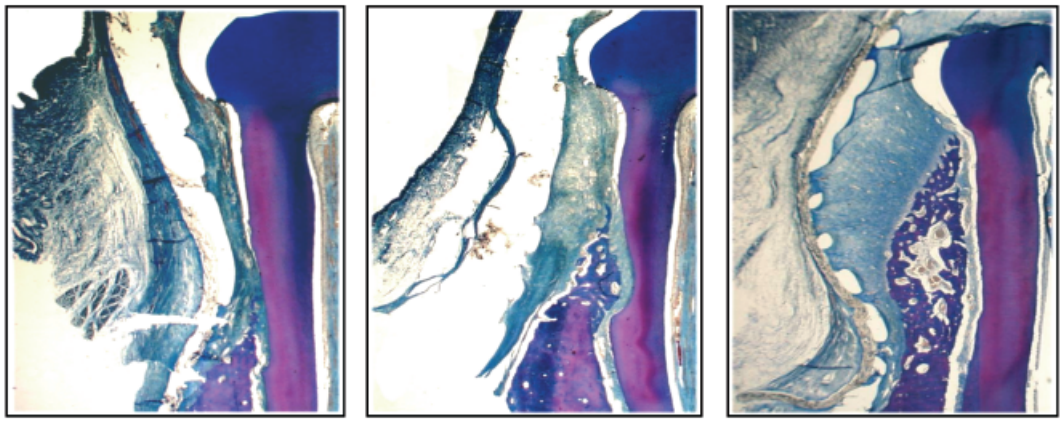

Fig. 3. Representative photomicrographs of supra-alveolar periodontal defects with space-providing occlusive ePTFE devices. The effect of space-provision can be observed in three different sites. Sites providing a small wound area resulted in limited bone formation (left and center). Sites providing a larger wound area resulted in enhanced bone formation (right).

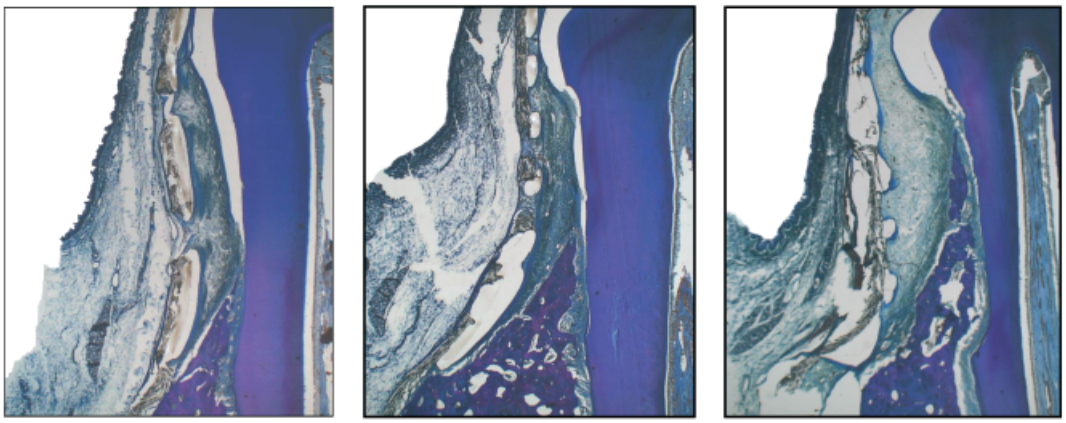

Fig. 4. Representative photomicrographs of supra-alveolar periodontal defects with space-providing porous ePTFE devices. The effect of space-provision can be observed in three different sites. Sites providing a small wound area resulted in limited bone formation (left and center). Sites providing a larger wound area resulted in enhanced bone formation (right).

Table 3. Adjusted mean bone regeneration (height) for sites receiving occlusive and porous ePTFE devices and grouped by wound area

Variable	Mean (mm)	SE	*p*
Treatment			
occlusive	2.64	0.16	0.03
porous	1.93	0.18	
Wound area (mm^2)			
<3	1.40	0.43	
3–7	2.19	0.32	0.07
>7	3.26	0.37	0.0002

Table 4. Mean bone regeneration (height) for sites receiving occlusive or porous ePTFE devices, grouped by wound area

Wound area (mm^2)	Treatment	Mean (mm)	SE	*p*
<3	porous	1.05	0.44	0.55
	occlusive	1.69	1.04	
3–7	porous	1.66	0.44	0.03
	occlusive	2.63	0.36	
>7	Porous	3.13	0.48	0.6
	occlusive	3.41	0.46	

observed that a wide alveolar crest effectively supports the device resulting in a large wound area, while a narrow alveolar crest did not appear to have a similar potential resulting in a smaller wound area in spite of the space-providing polypropylene reinforcement built into the GTR device. It was also shown that this correlation was not significantly different between the protocols. Although histological observations pointed to a passage of fibrovascular tissue through the pores, a contraction of the space provided by the porous device was not observed. Previous studies have observed that the presence of pores may allow a passage of cells/molecules/vascularity from the inner side of the device to the overlying flap (Wikesjö et al. 2003a–c). Apparently, bridging of newly formed tissue through the pores does not significantly compromise the spatial integrity of the porous GTR device. Moreover, tissue interactions over the porous surface may reduce the risk for wound failure and exposure/infection of the GTR device.

The observations herein suggest that space-provision is a critical factor for bone regeneration in periodontal sites. Different magnitudes of wound area resulted in different degrees of bone regeneration for all sites. In other words, a small wound area resulted in limited bone regeneration, while a larger wound area resulted in enhanced bone regeneration. These findings corroborate data from parallel studies using similar methodology investigating the effect of space-provision on bone regeneration in periodontal and peri-implant defects (Polimeni et al. 2002, 2003a, b).

It was also observed in this study that the relationship between space-provision and bone regeneration was significant for both the porous and the occlusive GTR devices. Alveolar bone regeneration followed similar patterns in both groups. It may be speculated that the healing process supported by these two different devices may be similar or at least be similarly influenced by space-provision.

The magnitude of newly formed bone was significantly increased for sites receiving occlusive GTR devices compared to sites receiving the porous devices when adjusted for the effect of wound area. Thus, even if space-provision appeared to be a critical factor for alveolar bone regeneration, device occlusivity appeared to provide adjunctive effects. In perspective, it may not appear legitimate to consider cell occlusion an absolute prerequisite for guided tissue regeneration. However, the observations from this study may suggest that cell occlusion under optimal circumstances for healing has the potential to maximize the magnitude of bone regeneration.

Conclusion

It can be concluded that cell occlusion and space-provision represent fundamental factors for guided tissue regeneration. Consequently, manipulation of these two parameters may enhance the outcomes and predictability of periodontal regenerative procedures.

References

Haney, J. M., Nilvéus, R. E., McMillan, P. J. & Wikesjö, U. M. E. (1993) Periodontal repair in dogs: expanded polytetrafluoroethylene barrier membranes support wound stabilization and enhance bone regeneration. *Journal of Periodontology* **64**, 883–890.

Karaki, R., Kubota, K., Hitaka, M., Yamaji, S., Kataoka, R. & Yamamoto, H. (1984) Effect of gum-expanding mesh on the osteogenesis in surgical bony defect. *Nippon Shishubyo Gakkai Kaishi* **26**, 516–522.

Koo, K-T., Polimeni, G., Albandar, J. M. & Wikesjö, U. M. E. (2003a) Histometric analysis of healing in supraalveolar periodontal defects. *Journal of Dental Research* **82**, IADR-Abstract 613.

Koo, K-T., Polimeni, G., Albandar, J. M. & Wikesjö, U. M. E. (2003b) Reproducibility of histometric measurements in periodontal defects. European Federation of Periodontology, Europerio 4, Berlin, Germany. *Journal of Clinical Periodontology* **30** (Suppl. 4), 51.

Polimeni, G., Koo, K-T., Qahash, M., Xiropaidis, A., Albandar, J. & Wikesjö, U. (2003a) Factors influencing GTR. *Journal of Clinical Periodontology* **30** (Suppl. 4), 65.

Polimeni, G., Koo, K-T., Qahash, M., Xiropaidis, A. V., Albandar, J. M. & Wikesjö, U. M. (2003b) Influence of space provision on bone regeneration in conjunction with porous and occlusive ePTFE devices. *Journal of Dental Research* **82**, IADR-Abstract 610.

Polimeni, G., Qahash, M., Xiropaidis, A. V., Albandar, J. M. & Wikesjö, U. M. E. (2002) Vertical augmentation of alveolar bone at teeth and dental implants. *Journal of Periodontology* **73**, 1242.

Scantlebury, T. V. (1993) 1982–1992: A decade of technology development for guided tissue regeneration. *Journal of Periodontology* **64**, 1129–1137.

Sigurdsson, T. J., Hardwick, R., Bogle, G. C. & Wikesjö, U. M. E. (1994) Periodontal repair in dogs: space provision by reinforced ePTFE membranes enhances bone and cementum regeneration in large supra-alveolar defects. *Journal of Periodontology* **65**, 350–356.

Sigurdsson, T. J., Tatakis, D. N., Lee, M. B. & Wikesjö, U. M. E. (1995) Periodontal regenerative potential of space providing expanded polytetrafluoroethylene membranes and recombinant human bone morphogenetic proteins. *Journal of Periodontology* **66**, 511–521.

Trombelli, L., Lee, M. B., Promsudthi, A., Guglielmoni, P. G. & Wikesjö, U. M. E. (1999) Periodontal repair in dogs: histologic observations of guided tissue regeneration with a prostaglandin E_1 analog/methacrylate composite. *Journal of Clinical Periodontology* **26**, 381–387.

Wikesjö, U. M. E., Kean, C. J. C. & Zimmerman, G. J. (1994) Periodontal repair in dogs: supraalveolar defect models for evaluation of safety and efficacy of periodontal reconstructive therapy. *Journal of Periodontology* **65**, 1151–1157.

Wikesjö, U. M. E., Xiropaidis, A. V., Thomson, R. C., Cook, A. D., Selvig, K. A. & Hardwick, W. R. (2003b) Periodontal repair in dogs: rhBMP-2 significantly enhances bone formation under provisions for guided tissue regeneration. *Journal of Clinical Periodontology* **30**, 705–714.

Wikesjö, U. M. E. & Selvig, K. A. (1999) Periodontal wound healing and regeneration. *Periodontology 2000* **19**, 21–39.

Wikesjö, U. M. E., Lim, W. H., Thomson, R. C. & Hardwick, W. R. (2003a) Periodontal repair in dogs: gingival tissue exclusion, a critical requirement for guided tissue regeneration? *Journal of Clinical Periodontology* **30**, 655–664.

Wikesjö, U. M. E., Xiropaidis, A. V., Thomson, R. C., Cook, A. D., Selvig, K. A. & Hardwick, W. R. (2003c) Periodontal repair in dogs: space-providing ePTFE devices

increase rhBMP-2/ACS induced bone formation. *Journal of Clinical Periodontology* **30**, 715–725.

Zellin, G. & Linde, A. (1996) Effects of different osteopromotive membrane porosities on experimental bone neogenesis in rats. *Biomaterials* **17**, 695–702.

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