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Replacement therapy for periodontitis: pilot radiographic evaluation in a dog model

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Abstract

Aim: The aim of this study was to radiologically evaluate the impact of replacement therapy by monitoring bone density changes and alveolar bone level in periodontal pockets in a dog model.

Material and Methods: Eight male beagle dogs with moderate periodontitis were enrolled in this split-mouth, double-blind randomized trial with ethical approval. Periodontal defects were surgically created bilaterally in the lower jaw. Four months later, the defects were randomly assigned to initial therapy (scaling and root planing) alone (control sites), or combined with multiple subgingival application of beneficial species. Intra-oral follow-up radiography was performed at this stage and 3 months later to verify the treatment effect.

Results: The bone density within periodontal pockets treated with beneficial bacteria improved significantly after 12 weeks, while this was non-significant for the control pockets, receiving a single root planing at baseline. There was a significant increase in the bone level at the end of the study for the pockets receiving beneficial bacteria. Again, no significance was noted for the control pockets.

Conclusions: This pilot study indicates the potential effect of a subgingival application of beneficial species in periodontal pockets, and illustrates the strength of standardized follow-up radiography to evaluate the effects of different treatment strategies on bone re-modelling.

Key words: alveolar bone level; jaw bone density; periodontitis; radiology; replacement therapy

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Beneficial bacteria have been extensively studied for their health-promoting effects (Parvez et al. 2006). The main field of research has been in the gastro-intestinal tract. In oral health, the beneficial effects on tooth decay have been reported for more than two decades (Herod 1991). In the past few years, probiotics have been investigated for periodontal health (Teughels et al. 2008). These studies have shown that certain gut bacteria can exert beneficial effects in the oral cavity by inhibiting pathogenic species. The concept of periodontal replacement therapy, first proven by Teughels et al. (2007), consists of applying beneficial oral bacteria subgingivally to prevent re-colonization of periodontal pockets by pathogens after scaling and root planing. The mechanism behind the concept seems to be related to bacterial interference and immune modulation. It is likely similar to the probiotic mechanisms of interaction in other parts of the alimentary tract. However, data are still sparse, and thus, more information is needed on the colonization of probiotics in the oral cavity and their possible effects on and within oral biofilms. Because of the globalization and increasing problems with antibiotic resistance, the alternative concept of probiotic therapy merits further research in the field of oral health care.

Detailed radiological analysis allows for in vivo detection of small density changes in the jaw bone (Jacobs et al. 1996). Recently, a dedicated tool has been developed and validated enabling the detection of bone density changes as small as 6% in in vitro settings (Nackaerts et al. 2006). Such subtle changes can be important to perform a controlled and standardized follow-up of bone re-modelling, on short term, which is not possible when visually evaluating two-dimensional images (Christgau et al. 1998).

Conflict of interest and source of funding statement

The authors declare that there are no conflicts of interest in this study.

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The aim of the current study was to radiologically evaluate the impact of replacement therapy by monitoring bone density changes and alveolar bone level (ABL) in periodontal pockets in a dog model. As such, not only the change in the vertical dimension of the alveolar bone but also its mineralization could be evaluated.

Material and Methods

Subjects

Eight male beagle dogs suffering from mild periodontitis with an average age of 3.1 ($\pm$ 0.4) years were used. The protocol was approved by the University's Ethical Committee for Animal Experimentation (K. U. Leuven, Belgium). Pockets were created surgically 4 months before the start of the study. With a water-cooled bur, 5 mm of alveolar bone was removed from the canines, second, third and fourth premolars. The defects extended from mid-approximal to mid-buccal. Before wound closure, the root surfaces were conditioned with heparin (15,000 IU/ml) (Aventis Pharma, Brussels, Belgium) (Wikesjő et al. 1991). All interventions were performed under general anaesthetics. Therapeutical procedures were described in detail previously (Teughels et al. 2007). None of the dogs received antibiotics before or during the course of the study.

Interventions

The pockets were randomly assigned to one of the following treatments by a clinician unfamiliar with the study design:

Rp: Subgingival scaling and root planing at baseline.

Bb: Subgingival scaling and root planing and repeated application of beneficial bacteria at baseline and weeks 1, 2 and 4.

The bacteria were described in detail by Teughels et al. (2007). Pellets of *Streptococcus sanguinis*, *Streptococcus salivarius* and *Streptococcus mitis* were locally applied, pure and unsuspended, in the designated pockets by injection with a blunt needle. Pockets around teeth numbers 37, 38, 47 and 48 were assessed in the current study. Nine pockets belonged to the Rp group. Within the Bb group, application of single bacteria was done in 12 pockets (four pockets for each bacteria), and in 11 pockets, a mixture of the three bacteria was administered.

Radiography

Radiographs were made before and after surgical pocket enhancement, at baseline (BL: 4 months post-operatively) and at week 12 (F: 7 months post-operatively). After anaesthesia, intraoral radiography was performed with a Planmeca Prostyle Intra® device (Planmeca Oy, Helsinki, Finland) using conventional F-speed film (Eastman Kodak Company, Rochester, NY, USA) and exposure protocol 70 kV, 8 mA, 0.24 s. Films were developed with an automatic film processor (XR 24 Nova®; Dürr Dental, Bietigheim-Bissingen, Germany) using fresh chemicals (Dürr Automat XR®) including automatic re-generation. The right and left premolar region of the lower jaw was radiographed. Rinn XCP® (Dentsply, York, PA, USA) film-holding instruments were adapted to contain an aluminium step wedge to standardize radiographs and to allow densitometric analysis. The wedge consisted of nine steps gradually increasing with 1.3 mm. At the time of creating the periodontal pockets, individualized impression moulds (Kerr Compound Sticks, Kerr Corporation, Paris, France) were made for each dog and attached to a bite block to standardize the geometrical conditions of the radiographs. The use of the paralleling technique, complemented with a position holder, minimized image enlargement and geometric distortion of the radiographs. The conventional films were scanned at 800 dpi (Agfa SnapScan, Agfa, Mortsel, Belgium).

Density

For density analysis, custom made software was used, converting grey values into millimetre aluminium equivalent (mm Al eq) values. The software was previously described and thoroughly tested in vitro (Nackaerts et al. 2006). The region of interest covered the entire area where pockets were created (Fig. 1b).

ABL

ABL was measured in Photoshop. The width of the step wedge was used as a correction factor for potential vertical distortions. The facing cemento-enamel junctions of teeth 37 and 38 (for measuring bone level at pocket round tooth

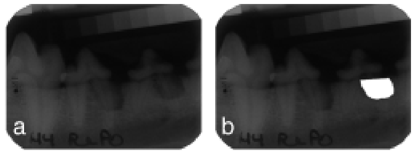

Fig. 1. (a) Original radiograph; (b) delineation of the region of interest, covering the entire pocket area.

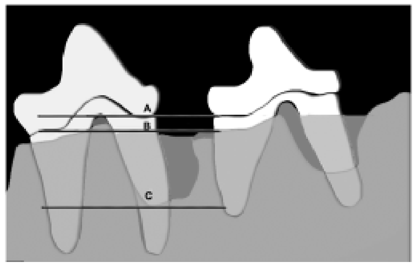

Fig. 2. Change in bone level was assessed by measuring distance BC. The distance AB was used for correcting a potential generalized change in horizontal alveolar bone level.

38) and between 36 and 37 (tooth 37) were connected (line A; Fig. 2). The same was done for teeth 47 and 48 (tooth 48), and 46 and 47 (tooth 47). Line A served as a reference for all further bone-level measures. A second line was drawn through the highest point of the inter-proximal bone crest, parallel to the first line (line B; Fig. 2). Another line, parallel to the first, was drawn contingent to the lower border of the surgically created pocket (line C; Fig. 2). The distance A to B was considered as a reference to explain the observed bone-level change over time. If it would be registered as increasing over time, a decrease in total crater depth could be attributed to this, while it should actually be qualified as bone loss. If on the other hand |AB| would remain stable, any observed change could be attributed to a change in the base of the crater. This strategy allowed a critical assessment of the observed bone level changes.

Statistical analysis

Because of a limited number of conditions and measurements, descriptive statistics were applied to attempt finding distinct healing in the Rp and Bb group. With the pocket as the unit of analysis, the Wilcoxon test for paired samples was used to detect differences in measurements at baseline and 12 weeks later.

Table 1. Wilcoxon test for density of the pocket area and radiographically evaluated alveolar bone level (ABL)

	Root planing		Beneficial bacteria	
	BL	F	BL	F
Density				
n	8	8	12	12
Median	5.85	5.80	5.40	5.70
Interquartile range	5.25–6.55	5.20–6.80	5.05–6.30	5.40–6.85
p-value		0.95		0.03
ABL				
n	8	8	12	12
Median	0.47	0.41	0.45	0.35
Interquartile range	0.46–0.50	0.32–0.51	0.39–0.48	0.32–0.45
p-value		0.25		0.04

BL, baseline; F, week 12.

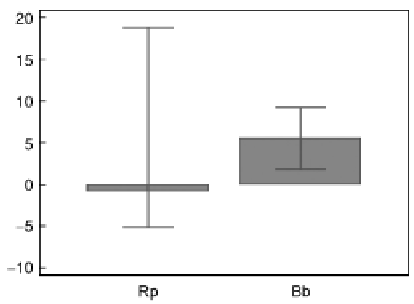

Fig. 3. Percentage of bone density change in the pocket area between baseline and 12 weeks after therapy.

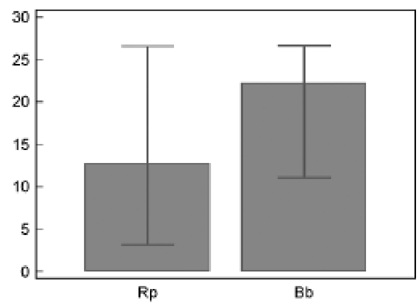

Fig. 4. Percentage change in bone level between baseline and 12 weeks after therapy.

Results

Not all radiographs could be analysed. Because of inadequate image quality caused by movement during exposure (eight sites) and the loss of radiographs during development (four sites), only 20 sites were available for analysis: out of these sites, eight pockets belonged to the Rp group and 12 to the Bb group. Out of these 12, in five the mixture of bacteria was administered, and in seven a single bacteria (two: *S. mitis*, four: *S. salivarius*, one: *S. sanguinis*). The measurements of the Bb group were pooled.

Table 1 gives an overview of the Wilcoxon test for measurements of the density of the pocket area and radiographically evaluated ABL. No significant differences in radiological measurements ($n = 8$) could be found between baseline and week 12 in the Rp group. In contrast, differences in density and ALB measurements yielded statistical significance for the Bb group ($n = 12$).

Starting from the baseline images, the percentage change in bone density and bone level was visualized for the two treatment modalities. Figures 3 and 4 show the results. Density measurements showed a decrease in density for Rp and an increase for Bb 12 weeks from baseline. In Fig. 4, the gain in bone level is shown to be higher in the Bb group.

Discussion

When evaluating the bone healing after periodontal replacement therapy, differences between the control periodontal pockets and the pockets receiving beneficial bacteria were recorded. Significant improvement in jaw bone density and alveolar bone gain was found in periodontal pockets that received beneficial bacteria adjunctive to scaling and root planing. Such significant differences were not found in periodontal pockets that received only scaling and root planing. It was previously confirmed that in pockets treated with beneficial bacteria, subgingival re-colonization of periodontopathogens was delayed and reduced, as was the degree of inflammation (Teughels et al. 2007). Based on our results, radiologically, the healing of a periodontal pocket after scaling and root planing seems better when beneficial bacteria are applied.

One should consider the data obtained in this pilot study as preliminary. It is evident that it is impossible to draw definite conclusions taking into account the shortcomings involved in the present study. If the animal (i.c. beagle dog) would be the unit of analysis, a confounding factor might be the inter-subject variation. To handle this shortcoming, a split-mouth design was chosen. Yet, it is clear that the latter is also far from ideal. Because of ethical considerations, the number of animals to be used is limited, resulting in a small sample size with less persuasive results. In addition, it cannot be excluded in such design that intra-oral translocation takes place (Hujoel & DeRouen 1992), which in turn could distort the outcome. Considering these factors, data for single and mixed application of beneficial species were pooled in the frame of this pilot study. The results can therefore only give an idea of the potential effect of the application of probiotics on bone regeneration as assessed on periapical radiographs; definite conclusions on the effect of specific species and/or combined treatment need further studies with a larger sample size enabling a more robust comparison of different therapies.

As to the knowledge of the authors, no study has ever followed up jaw bone density changes as a result of periodontal therapy over such a short time. Consequently, the differences that were found are small. It is known that to visually detect a change in bone density on consecutive radiographs, a rather large amount of change in mineralization needs to occur (Southard & Southard 1994, Christgau et al. 1998). However, thanks to computer-aided densitometric image analysis, the detection of rather subtle bone changes has become feasible (Brägger 2005). Even after 6 months, Eickholz et al. (2007) could only find non-significant differences after guided tissue regeneration in infrabony defects. Holland et al. (1998) found that 12 months after removal of silk ligatures inducing periodontal disease in a dog model, the alveolar bone had increased in height. Linear alveolar bone measurement at 9 months revealed no such difference. The pockets created in the current study are to be defined as wide, rather than deep and small. Eickholz et al. (2004) found a more pronounced healing of infrabony defects that were narrow and deep in comparison with shallow but broad defects. In addition, it is possible that bone growth at the base of the crater was underestimated, due to insufficient maturation for the bone to be visible on the periapical radiographs (Yun et al. 2005).

Radiographic assessment of jaw bone is associated with sources of error, such as exposure settings, geometry, development of films, etc. Many of these were addressed in the study set-up. The step wedge did not only offer the possibility of standardizing density (Nackaerts et al. 2007) but also enabled taking into account geometric distortion of the image. Furthermore, the stent reduced the chance of projection errors in this follow-up approach. Another factor potentially introducing errors is the use of conventional films rather than digital image plates. Borg et al. (2000) assessed the marginal bone level around implants in a dog study and found no difference in accuracy and precision between digital and conventional films. This was confirmed by Pecoraro et al. (2005). Nevertheless, Li et al. (2007) found that digital films, corrected for attenuation and visual response, did improve the measurement accuracy. In the current study, it was not feasible to use digital films, though this should be taken into account in future study set-ups. An additional benefit of digital radiography is the possibility of immediate retakes in case of image errors, although this is not applicable in patient studies. One more specific problem related to this radiographic study in a dog model includes the stabilization of the beam-aiming device. Involuntary tics could shift the aiming device during exposure. Therefore, some of the radiographs needed to be excluded for analysis.

The current study set-up enabled the in vivo evaluation of bone density and height, using standardized imaging, creating a versatile tool to monitor minute changes in the jaw bone. Despite the limitations mentioned, this made the study unique in animal research.

Conclusion

This pilot study indicates the potential effect of a subgingival application of beneficial species in periodontal pockets on bone healing and illustrates the strength of follow-up radiography to evaluate the effects of different treatment strategies on bone re-modelling.

The integrated use of jaw bone density evaluation and alveolar bone height measures is assumed ideal in animal models to test new treatment or surgical strategies.

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Clinical Relevance

Scientific rationale for the study: Because of increasing problems with antibiotic resistance, the alternative concept of probiotic therapy merits further research in the field of oral health care. The aim of the current study was to radiologically evaluate the impact of beneficial bacteria by monitoring bone density changes and ABL in periodontal pockets in a dog model.
Principal findings: Increase in density and ABL was small but significant in replacement therapy and non-significant in the control group.
Practical implications: The results encourage continued research on the effect of beneficial species on bone healing in periodontal pockets.