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Periodontal repair in dogs: examiner reproducibility in the supraalveolar periodontal defect model

Ki-Tae Koo[1], Giuseppe Polimeni[1], Jasim M. Albandar[2] and Ulf M. E. Wikesjö[1]

[1]Laboratory for Applied Periodontal and Craniofacial Regeneration; [2]Department of Periodontology, Temple University School of Dentistry, Philadelphia, PA, USA

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Abstract

Background: Histometric assessments are routinely used to evaluate biologic events ascertained in histologic sections acquired from animal and human studies. The objective of this study was to evaluate the intra- and inter-examiner reproducibility of histometric assessments in the supraalveolar periodontal defect model.

Methods: Histometric analysis using incandescent and polarized light microscopy, an attached digital camera system, and a PC-based image analysis system including a custom program for the supraalveolar periodontal defect model was performed on histologic sections acquired from one jaw quadrant in each of 12 dogs. The animals had received an experimental protocol including implantation of a coral biomaterial and guided tissue regeneration (GTR) barrier devices, and were evaluated following a 4-week healing interval. Histometric parameters were recorded and repeated within a 3-month interval by two examiners following brief training. Intra- and inter-examiner reproducibility was assessed using the intra-class correlation coefficient (ICC).

Results: Most parameters showed high intra-examiner ICCs. Parameters including defect height, connective tissue repair, bone regeneration (height/area), formation of a junctional epithelium, positioning of the GTR device, ankylosis, root resorption, and defect area yielded an ICC$\geqslant$0.9. The ICCs for bone density and biomaterial density were somewhat lower (0.8 and 0.7, respectively). The inter-examiner reproducibility was somewhat lower compared to the intra-examiner reproducibility. Nevertheless, the ICCs were generally high (ICC range: 0.6–0.9).

Conclusions: Histometric evaluations in the supraalveolar periodontal defect model yield highly reproducible results, in particular when a single examiner performs the histometric measurements, even when the examiner was exposed to limited training.

Key words: animal models; bone; histometrics; periodontal regeneration; tissue engineering; wound healing

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Clinical methods of evaluating periodontal regeneration include recordings of surrogate parameters such as probing depths, clinical attachment levels, probing bone levels, comparisons of radiographic registrations pre- to post-therapy, and entry/re-entry surgical procedures to assess changes in alveolar morphology. Any of these methods may not reliably document regeneration of the periodontal attachment. The inability of periodontal probing to assess the coronal level of connective tissue attachment has been amply demonstrated (Listgarten et al. 1976, Armitage et al. 1977, van der Velden & de Vries 1978). Also, bone fill or bone formation determined by sounding, entry/re-entry procedures or by radiographic analysis may not capture regeneration of cementum and the periodontal ligament.

Only histologic evaluations may disclose the genuine nature of healing following periodontal regenerative procedures. Thus, preclinical models primarily using canines and non-human primates have been developed to evaluate the biologic potential and application of candidate therapies prior to clinical introduction. Clinical biopsies are also sometimes used to ascertain the nature of healing following periodontal

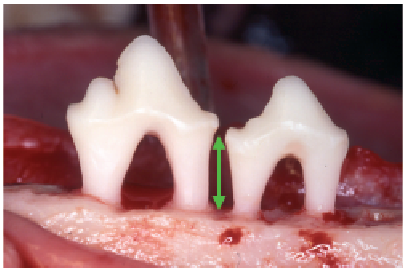

Fig. 1. The critical size, supraalveolar periodontal defect model. The defect height from the reduced alveolar bone to the cemento-enamel junction approximates 6 mm.

reconstructive surgery as part of the evaluation of novel therapies. Histometric assessments are used as part of the overall histopathologic evaluation. For periodontal regenerative protocols, the histometric evaluation is used to quantitate regeneration of alveolar bone, cementum, a functionally oriented periodontal ligament, formation of a junctional epithelium, and the position of any device and biomaterial implanted in conjunction with the surgical procedure.

The critical size, supraalveolar, periodontal defect model was first presented in 1990 (Fig. 1; Wikesjö & Nilvéus 1990, Wikesjö et al. 1990) and has been used extensively to evaluate biologic and environmental factors influencing periodontal wound healing/regeneration as well as candidate regenerative protocols, including root surface conditioning, devices for guided tissue regeneration (GTR), bone biomaterials, and extracellular matrix, growth and differentiation factors (for a review see Wikesjö et al. 1994, Wikesjö & Selvig 1999). The osteogenic potential in this defect model following sham surgery amounts to less than 20% of the defect height and a 4- or 8-week healing interval, suggesting that bone formation may be completed within 4 weeks. Similarly, cementum regeneration amounts to less than 10% of the defect height following a 4- or 8-week healing interval. This challenging model system has been proven valuable for qualitative and quantitative evaluation of candidate protocols for periodontal regeneration. The supraalveolar periodontal defect model particularly lends itself to histometric evaluations of regeneration of alveolar bone, cementum, a functionally oriented periodontal ligament, formation of a junctional epithelium, position of any implanted device and position/amount of any residual biomaterial used in conjunction with the regenerative procedure, as well as qualitative and quantitative evaluations of root resorption and ankylosis. The objective of this study was to present the intra- and inter-examiner reproducibility of histometric assessments in the supraalveolar periodontal defect model.

Materials and Methods

Histologic sections

Histologic sections from a preclinical study (Koo et al. 2003) evaluating the osteoconductive potential of a bone biomaterial in presence or absence of conditions for GTR were used. In brief, routine, critical size, 6-mm supraalveolar periodontal defects around the third and fourth premolar teeth were created in 12 young adult Beagle dogs. Five animals received a granular coral biomaterial. Seven animals received the coral biomaterial in combination with an expanded polytetrafluoroethylene (ePTFE) membrane (GORE-TEX® Regenerative Material, W.L. Gore & Associates Inc., Flagstaff, AZ, USA) for GTR. The animals were euthanized following a 4-week healing interval at which time tissue blocks of the experimental sites were collected and processed for histometric analysis.

Histometric analysis

Two masked examiners (K.-T. K. and G. P.) independently performed the histometric analysis using incandescent and polarized light microscopy (BX 60, Olympus America Inc., Melville, NY, USA), a microscope digital camera system (DP10, Olympus America), and a PC-based image analysis system (Image-Pro Plus™, Media Cybernetic, Silver Springs, MD, USA) customized for the supraalveolar periodontal defect model. The most central stained section for the mesial and distal root of the third and fourth premolar teeth was identified by the size of the root canal. This section and the immediate stained step serial section on either side were subject to analysis. Thus, three consecutive step serial sections, representing 0.2 mm of the mid-portion of the mesial and distal root for each premolar tooth, were used. The following are the histometric parameters for the buccal and the lingual tooth surfaces for each section (Fig. 2):

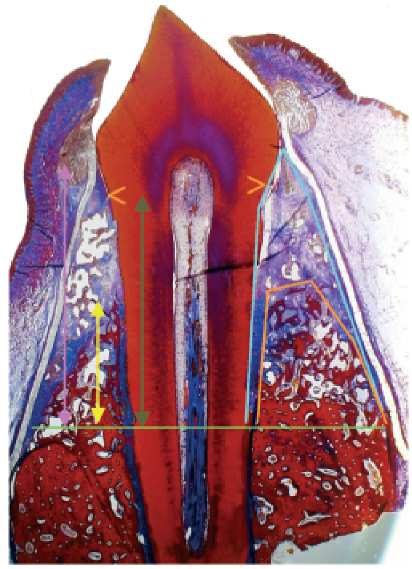

Fig. 2. Examples of histometric parameters evaluated in the critical size, supraalveolar periodontal defect model in this study. The light green line represents the base of the defect and the orange arrowheads, the cemento-enamel junction. The defect height (dark green arrow), bone regeneration height (yellow arrow), membrane height (purple arrow), defect area (light blue lines), and bone regeneration area (orange lines) are shown.

- Defect height: distance between the apical extension of root planing and the cemento-enamel junction.
- Defect area: area under the ePTFE membrane circumscribed by the planed root, the width of the alveolar bone at the apical extension of the root planing, and the membrane.
- Membrane height: distance between the apical extension of the root planing and the most coronal aspect of the ePTFE membrane.
- Junctional epithelium: distance between the apical and coronal aspect of a junctional epithelium along the planed root.
- Connective tissue repair: distance between the apical extension of the root planing and the apical extension of the junctional epithelium along the planed root.
- Cementum regeneration: distance between the apical extension of the root planing and the coronal extension of a continuous layer of new cementum or a cementum-like deposit on the planed root.
- Bone regeneration (height): distance between the apical extension of the root planing and the coronal

extension of alveolar bone formation along the planed root.
- Bone regeneration (area): area represented by new alveolar bone along the planed root.
- Bone regeneration (density): ratio of mineralized bone matrix to total bone area.
- Biomaterial density: ratio of residual biomaterial to total bone area.
- Root resorption: combined linear heights of distinct resorption lacunae on the planed root.
- Ankylosis: combined linear heights of ankylotic unions between new alveolar bone and the planed root.

Table 1. The intra-class correlation coefficients of repeated measurements of histometric parameters made by one examiner (intra-examiner reproducibility) or two examiners (inter-examiner reproducibility)

Parameter	Intra-examiner	Inter-examiner
Defect height	0.985	0.908
Defect area	0.991	0.986
Membrane height	0.981	0.963
Junctional epithelium	0.976	0.761
Connective tissue repair	0.975	0.844
Bone regeneration (height)	0.882	0.834
Bone regeneration (area)	0.941	0.926
Bone regeneration (density)	0.771	0.731
Biomaterial density	0.661	0.605
Root resorption	0.994	0.892
Ankylosis	0.983	0.766

Intra- and inter-examiner reproducibility

One inexperienced examiner (K.-T. K.) was briefly trained. This examiner performed two separate repeated histometric evaluations of all 12 parameters 3 months apart. The intra-examiner reproducibility was assessed by the intra-class correlation coefficient (ICC) (Shrout & Fleiss 1979).

A second inexperienced examiner (G. P.) was trained with the first examiner. The second examiner performed a third histometric analysis utilizing the same program and histologic sections. The inter-examiner reproducibility was assessed by the ICC.

Results

The intra-examiner evaluation showed high reproducibility for most parameters. Measurement of defect height and area, connective tissue repair, bone regeneration area, junctional epithelium, membrane height, ankylosis, and root resorption exhibited an ICC greater than or equal to 0.94. The intra-examiner reproducibility for bone regeneration height and density, and biomaterial density was somewhat lower (0.88, 0.77, 0.66, respectively; Table 1).

The inter-examiner reproducibility was slightly, but consistently lower than the intra-examiner reproducibility for all histometric parameters assessed (Table 1). The assessment of defect height and area, membrane height, and bone regeneration area yielded ICCs greater than or equal to 0.93. The assessment of connective tissue repair, bone regeneration height, and root resorption resulted in ICCs ranging between 0.83 and 0.89. The ICCs for the measurement of junctional epithelium, bone regeneration density, biomaterial density, and ankylosis ranged from 0.61 to 0.77. On the other hand, only a small number of specimens showed cementum regeneration, and the calculation of the correlation coefficients for this parameter was therefore not performed.

Discussion

ICCs are commonly used measures of raters' agreement and consistency in assessing continuous measurements. The ICC measures the proportion of a measurement variance that is attributable to a class characteristic (McGraw & Wong 1996). In this study of repeated measurements, the class variable was the examiner. The measurement errors of histometric assessments, made by one or two examiners, in the critical size, supraalveolar, periodontal defect model were evaluated. For repeated measurements made by the same examiner, nine of 11 (82%) histometric parameters yielded ICCs greater than or equal to 0.88. This suggests that the single examiner had excellent reproducibility in assessing most of the histometric parameters evaluated. For the remaining parameters, the examiner showed somewhat lower, nevertheless adequate, reproducibility.

When measurements made by two independent examiners were evaluated, more than half of the parameters assessed (seven of 11; 64%) yielded correlation coefficients greater than or equal to 0.83, also suggesting excellent consistency. Notably, the correlations were somewhat higher in repeated measurements made by one examiner than in measurements made by two examiners, and this was consistent for all the studied parameters. This finding is in accordance with other studies showing higher intra-examiner than inter-examiner reproducibility of repeated measurements of clinical periodontal parameters (Kingman & Albandar 2002).

In the critical size, supraalveolar, periodontal defect model, a number of histologic outcomes are examined following treatments. Many of these outcomes are continuous-scale variables, and the more important of these include defect height and area, and newly formed alveolar bone, height and area. Histometric analysis of the latter variables is performed regularly in studies of novel periodontal and implant therapies. This study showed that, when a single examiner performed the measurement of these parameters, between 1% and 6% of the total variation was due to examiner random measurement error. Furthermore, a measurement error component of about 1–17% of the total variation was due to differences between examiners. This suggests that assessments made by one examiner are quite adequate for the analysis of data. The use of more than one examiner to perform the histometric examination may introduce an additional, and perhaps larger, error component.

Other study outcomes examined in this model demonstrated a range of measurement reproducibilities; some variables showing higher measurement consistency than others. Insofar, measurement of newly regenerated bone and biomaterial density exhibited the highest level of measurement errors. The estimated error component was 23–34% for a single examiner and 27–40% for two examiners. On the other hand, measurement of junctional epithelium and ankylosis showed low intra-examiner measurement errors, but a higher inter-examiner error component ranging between 23% and 27%. For some of the histometric variables, the measurement errors may have been attenuated by the skewness of the variables, since some specimens did not reveal presence of the outcome variable being assessed. This was particularly evident for the measurement of cementum regeneration, which was scored as absent in a high percentage of the specimens. For this reason, the consistency of measuring cementum regeneration in this study material was not assessable.

Conclusion

Histometric evaluations in the supraalveolar periodontal defect model yield highly reproducible results, in particular when a single examiner performs the histometric measurements, even when the examiner was exposed to limited training.

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Address:
Ki-Tae Koo
Laboratory for Applied Periodontal and Craniofacial Regeneration
Temple University School of Dentistry
Philadelphia, PA
USA