# Computer Assisted Image Analysis Methods for Evaluation of Periodontal Wound Healing

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The aims of this study were to determine the accuracy of the computer assisted image analysis method and to evaluate its application for the assessment of periodontal wound healing in dogs. Histological material was analyzed with an optic microscope connected to a CCD color camera which transmitted the image to a frame grabber converting the light signals into pixels from which the measurements could be obtained. Twenty sections were read to assess the intra- and inter-examiner reproducibility for the parameters of area filled by new tissue, area of epithelium, area of bone and linear measurements of the cementum. The data were statistically analyzed using the *t*-test to test the hypothesis that there was no difference between and within examiners. No statistically significant differences were noted (with a confidence interval of 95%) for any parameter when intra-examiner reproducibility was assessed. Similar results were achieved for surface areas when the inter-examiner readings were computed. However, values of linear measurements for cementum showed statistically significant differences between recorders ($p<0.05$). Results were consistently uniform and the method demonstrated high accuracy when intra-examiner readings were evaluated.

Key Words: digital image, histometry, periodontal wound, accuracy.

## INTRODUCTION

Histological measurements of soft and hard tissues are considered the gold standard for the evaluation of periodontal wound healing. Morphometry of tissue sections has been traditionally studied to gain a better understanding of structure and function (1). Image analysis system evaluation allows the investigator to quickly and reproducibly identify and measure areas of interest. Furthermore, the image can be zoomed to allow closer examination of specific details.

With the advent of inexpensive microprocessors, high-quality cameras and large memory storage, image processing and statistical imaging analysis are both practical and cost-effective (2,3). Advances in software development make these techniques accessible and comprehensible to operators with varied experience.

The use of guided tissue regeneration proce-

dures has intensified the need to determine the types and quantity of tissues formed in healing. Regeneration is differentiated from new attachment in one basic, but very important manner: alveolar bone formation with new inserted periodontal fibers in new cementum is a prerequisite for the regeneration of periodontium (4-6). Areas of new cementum deposition without adjacent bone, and vice-versa, and areas of ankylosis, for example, do not satisfy the criteria for regeneration (7). Furthermore, recent advances in the understanding of functions and mechanisms of action of growth factors to regulate the healing process have provided evidence that these proteins may serve as therapeutic agents to enhance the healing of periodontal wounds (8-11). Sigurdsson et al. (12) found a significant enhancement in periodontal regeneration using the rhBMP-2 in dog models. They described limited root resorption and ankylosis generally limited immediately apical to the CEJ. Lynch et al. (13,14) demonstrated a synergism

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when platelet-derived growth factor and insulin-like growth factor were combined to enhance periodontal wound healing. An image analyzing system will allow measurements to determine how much of the original defect has been repaired and what type of new tissue has formed.

Previous methods available to analyze periodontal wound healing were not very accurate and were extremely time consuming. Measurements of epithelium from a photograph could be accomplished by positioning a piece of string along the contours, cutting the string, straightening and measuring it. These methods were slow and tedious but could supply useful data. Similarly, grids could be overlaid on a microscope field and the number of cells counted within grid areas. With a simple image analysis system using a stylus and digitizing table, interfaced with a computer, length and volumes can be obtained from the x and y coordinates recorded as the feature is traced. The advantage of the modern systems is that photographic rendition is unnecessary and image analysis software can allow zooming, filtering, image enhancement, panning and other techniques to be applied to the image.

Image processing is the manipulation of the digitized image to enhance and evaluate information contained in the original image. In this way, it is possible to enhance certain features in the digitized image not readily seen in the original form and multiple fields may be processed. The field of the digital image analysis is based on the assumption that images are two-dimensional representations of objects that can be interpreted by analyzing an array of discrete numerical units, or picture elements: pixels (1). These computer manipulations enhance the visual qualities of the image as it appears in the new form by varying the color range to enhance values of the individual pixels but not the relative color values of adjacent pixels (3).

When analyzing the amount of staining in tissue sections, the image analysis system must be able to mimic the many compensatory mechanisms of a trained professional by simultaneously making allowances for process variables such as section thickness variability, staining irregularities, irrelevant background staining and poor representation of the lesion (3).

An additional advantage of the computer system is the easy statistical processing of primary data (15). Data analysis and interpretation are the final stages in the process that begins with a microscopic image and ends with an analytic result data analysis that may have widely diverse objectives. Quantitative measurements allow detection and documentation of the significance of very small differences or very subtle change.

The aims of this study were to determine the accuracy of the computer assisted image analysis method and to evaluate its application for the assessment of periodontal wound healing.

## MATERIAL AND METHODS

Fifteen purebred female beagle dogs, 2-3 years old, with no periodontal disease were used in this study and maintained on a hard diet, except for the two weeks after surgery, when a soft diet was administered. Standardized periodontal defects were created in each of the left and right mandibular quadrants of the 15 dogs. These defects were created around the 2nd, 3rd and 4th premolars and 1st molar teeth under general anesthesia.

Sulcular incisions and elevation of buccal and lingual mucoperiosteal flaps were made and alveolar bone was removed around these teeth with chisels and water-cooled rotating burs. The defect involved the full circumference of the teeth including the furcation area. The defect height from the cemento-enamel junction to the bone margin was approximately 5 mm. The flaps were positioned and sutured in an apical position allowing exposure of the surgically denuded root surfaces to periodontitis-stimulating conditions for the subsequent four months. After this period, the defects were subjected to reconstructive surgery. Prior to the surgery, however, teeth in these animals were scaled and polished. Plaque control was maintained by topical application of 0.12% chlorhexidine gluconate (Peridex, Procter & Gamble, Cincinnati, OH, USA), 3 times weekly. Surgical treatment of the animal started 2 weeks after scaling and root planing.

For the surgery, each dog was sedated with *iv* ketamine HCL (25 mg/ml) followed by isofluorane gas anesthesia. The surgical area was locally infiltrated with a 2% xylocaine solution containing epinephrine (1:50,000) to reduce hemorrhage. Reference notches were placed at the bone level on the roots and extended interproximally and into the furcation area as deep as the furcation permitted.

The roots of the teeth in one of these quadrants received a combination of 1 µg each of recombinant PDGF-B and IGF-1 in methylcellulose gel (Sigma-

Aldrich, Chicago, IL, USA). A second quadrant received a placebo consisting of methylcellulose gel only. Interproximal sutures were then placed through the flaps assuring they covered 1/3 of the clinical crown of each tooth.

During the two weeks following surgery, all dogs were fed a soft diet and, during the first week, tooth brushing was suspended in order to prevent unnecessary disruption of the flap healing.

Seven days after surgery, the dogs were anesthetized with isofluorane gas for rubber cup prophylaxis. In the following weeks, the surgical sites were maintained by brushing with 0.12% chlorhexidine solution, every other day. Three months after surgery, the animals were again anesthetized and sacrificed by bleeding. The heads of the animals were perfused with 10% buffered formalin solution and then refrigerated for 1 to 2 days. The jaws were dissected free and placed in formalin for further fixation.

In order to enhance the speed of demineralization, the jaws were further sectioned into tooth blocks. Demineralization was accomplished with 10% trifluoracetic acid (TFA). Following demineralization, the tissue specimens were washed, dehydrated, infiltrated and embedded in paraffin and then sectioned in 6-micron intervals. Twelve to 20 nonserial histologic sections were made of each treated tooth, cut in a mesio-distal direction, 30 microns apart. Sections were stained with hematoxylin and eosin, Mallory's trichrome or by silver impregnation.

Statistical evaluation was performed to assess the intra-examiner and inter-examiner accuracy of the image analysis system. Twenty sections from different specimens were read by one expert examiner without calibration before the measurements. Two hours later the same examiner read all 20 sections again to evaluate the intra-examiner reproducibility. The same 20 sections were then read by the second examiner to assess the inter-examiner reproducibility. The data were analyzed using the *t*-test to test the hypothesis of no difference between and within examiners.

### Technique

The input for the image analysis system was an Olympus BH-S optical microscope. This system was set up for Kohler illumination and images were obtained with 1X and 4X objective lens.

A CCD color camera (Sony, Tokyo, Japan) was connected to the microscope and the transmitted image was processed by a Color Frame Grabber Vision Plus AT (Sony). The Frame Grabber is an imaging board that accepts a video signal, performs analog to digital conversion and stores the image. This frame image was transmitted to a microcomputer (Intel Pentium P90 computer system) that was then visualized on a high resolution screen. The image was then processed using Image Pro-Plus software (Media Cybernetics, Silver Spring, MD, USA). Once the image was acquired, manipulation of contrast and colors was performed by selecting the appropriate illumination and settings. The image was then processed by thresholding and filtering. Thresholding is one of the simplest algorithms and helps to select the area of interest from the background. A pixel property was selected and a threshold set, all pixels below the threshold were not counted, pixels above the threshold were marked. In the image analysis system, often an interactive task, the operator observes the effect of the threshold as it is applied and the threshold can be increased or decreased to exclude or include more pixels.

Another type of thresholding operation is the erosion or dilation of the pixels to separate or enlarge selected areas. This procedure can, for example, separate two cells that touch, a process which can be interactive in that single pixels may be added or subtracted until the desired separation or blending is produced.

Various filters are available which help to increase the contrast between the area of interest and the background. Background reduction and elimination of uneven illumination can greatly enhance the image. The area of interest may also be electronically defined by the user, thus eliminating extraneous information.

Calibration of the system was performed with a stage slide micrometer that assigns an actual size per pixel of 0.0203 mm. The measurements were traced electronically using the notch as the reference point. In the absence of the notch, the beginning of newly formed tissues detected by the examiners was taken as a reference point. Measurements were taken in relation to the total area filled by new tissues, area of epithelium, area of new bone and linear measurements of the cementum were obtained by subtracting the area without new cementum from the total extension of the furcation (Figure 1).

Data were automatically transferred from the image analysis system to a spread sheet (Excel). This exchange was accomplished by Dynamic Data Exchange (DDE). The Student *t*-test was used to test the hypothesis of no differences between mean values for the following parameters: area filled by new tissue, area of epithelium, area of new bone and linear measurements of the cementum, in order to determine agreement or disagreement (non-variation or variation) between and within examiners. A confidence interval of 95% was adopted.

## RESULTS

The mean values for all parameters are reported in Tables 1 and 2. There were no statistically significant differences between the intra- or inter-examiner readings for the area filled by new tissue, area of epithelium or area of new bone (Tables 1 and 2). However, there was a statistically significant difference for inter-examiner reading for the linear measurement of the cementum, but no difference for intra-examiner readings.

## DISCUSSION

Healing of periodontal wounds is a very complex process, being the result of the interaction of calcified and soft tissues: epithelium, connective tissue, cementum and bone. Recent research findings have shown that biological substances such as growth factors may enhance periodontal wound healing. These findings lead us to ask two critical questions that should be answered to assess the predictability of these therapies: the type of new tissues formed and the amount of resolution of the original defect.

Ideal evaluation of histological sections should be based on quantitative and qualitative observations. Computer assisted analysis methods permit accurate evaluation of the tissue sections and allow the investigator to quickly and reproducibly identify and measure areas of interest.

For the purpose of this study, areas of epithelium and connective tissue attachment, as well as the amount of new bone and new cementum, were quantified using areas and linear measurements. An intra-examiner and
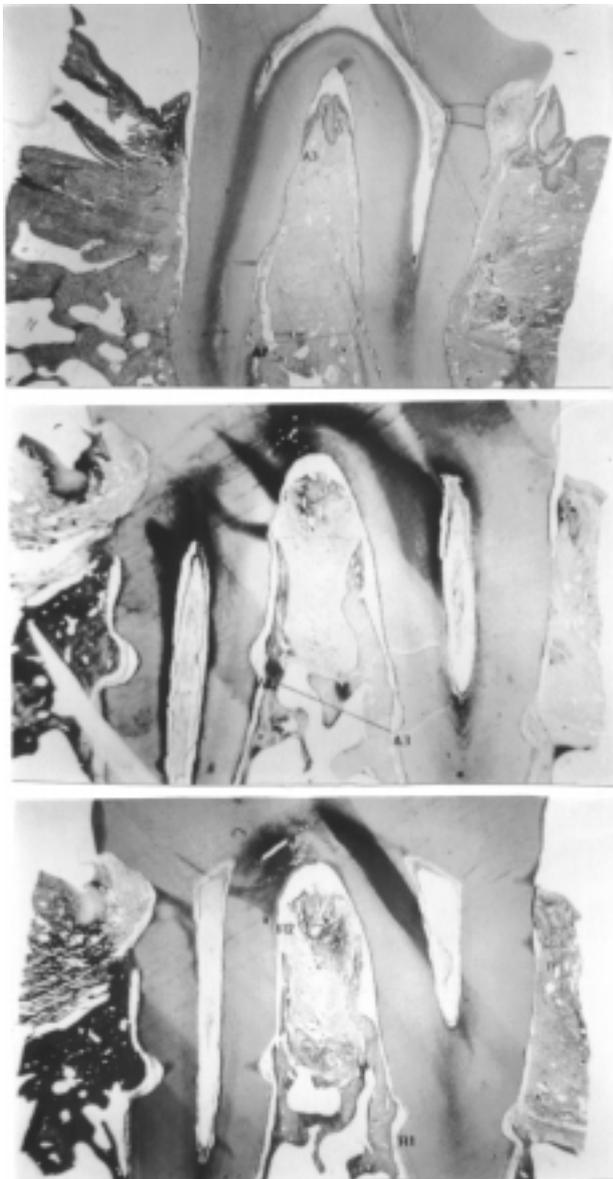


Figure 1. Digital images of histologic sections of class III furcation in dog premolar: Top, area filled by new tissues (A2) and area of epithelium (A3); Middle, area of new bone (A2); Bottom, linear measurements of the cementum (R1-R2).

Table 1. Statistical results for intra-examiner reproducibility (mean ± standard error).

|  | 1st reading | 2nd reading | p values |
| --- | --- | --- | --- |
| Area of epithelium | 0.47 ± 0.08 | 0.50 ± 0.09 | 0.98 |
| Area of new tissue | 9.19 ± 0.66 | 9.21 ± 0.70 | 0.83 |
| Area of new bone | 2.00 ± 0.37 | 1.83 ± 0.34 | 0.74 |
| Linear measurements of the cementum | 4.72 ± 0.67 | 5.2 ± 0.69 | 0.64 |

N = 20 for all parameters. Confidence interval of 95% - *t*-test.

an inter-examiner reproducibility test was performed to ensure the reliability of the measurements. It was possible to identify and quantify each tissue type since this method allows examination of specific areas by zooming. For example, if a root notch was not clearly identified in the section, increased magnification of this specific landmark allowed closer observation of the newly formed tissues.

Statistical analysis demonstrated no significant difference when the same investigator read all the sections at different times for area filled by new tissues, area of epithelium, area of bone and linear measurements of the cementum ($p > 0.05$). These results were consistently uniform and the methodology demonstrated high accuracy.

Similar results were found when inter-examiner reproducibility was tested for area filled by new tissues, area of epithelium, and area of new bone. There was a statistically significant difference, however, between the readings of the two examiners for the linear measurements of the cementum. It may be that the two different examiners were not well calibrated to identify the exact extension of the cementum particularly where areas of root resorption or ankylosis could be seen intertrimed with cementum in some sections. Another possibility is that the linear measurements could be more difficult to read than the areas since only an inter-examiner statistically significant difference for the linear measurements of the cementum was found. This hypothesis should be evaluated further.

Other methods, such as the use of photographs scanned into a digital image and highlighting of each tissue using different colors, are valuable devices to assess the volume of new tissue formed (16). However, methodologies utilizing photographs are very time consuming and do not allow the examiner to answer the critical question as to the quality of the tissue formed.

The advent of sophisticated and affordable microprocessors, cameras and image analysis of microscopic images in the medical field provides a means to quantify, in a small way, the complex, natural image processing capabilities of the human brain. Image processing is considered cost-effective, accurate, labor-saving, and reproducible (3). In the past, as reported by Jarvis (17), a single color image of 512 x 512 pixels at a brightness resolution of 8 bits or 256 levels for each of the red, green and blue components, consumed 768 Kb which exceeded the entire memory capacity of many microcomputer systems. Such disparity in memory requirements, and the early design of video digitizers (requiring triple hardware installation), inevitably limited the system configuration for natural color display and the results that could be achieved with the software.

Multicenter studies, largely based on the data generated by tracing measurements, aim to evaluate the reproducibility and prognostic value of such assessments (17). With digital image methods, double-blind or observer-blind multicenter trials are feasible (17,18). By representing an image as a series of numbers, the image can be stored permanently, simply by recording the numbers that represent the image. Numbers do not fade, change colors, or become scratched or damaged. Digital images can be transmitted to a distant site by sending the series of numbers that represents the image via modem or computer network (19).

Another advantage of this system is that this software can be used by operators with only a limited knowledge of the background theories involved. However, to appreciate and usefully implement the many applications of a image analyzer, it helps to understand the distinct vocabulary, basic algorithmic tools and limitations of image processing (3).

The development of a software that could clinically measure periodontal wound healing would be of enormous value in the regeneration field.

## ACKNOWLEDGMENT

## RESUMO

Palioto DB, Sato S, Ritman G, Mota LF, Caffessee RG. Imagem

Table 2. Statistical results for inter-examiner reproducibility (mean ± standard error).

|  | 1st examiner | 2nd examiner | p values |
|---|---|---|---|
| Area of epithelium | 0.50 ± 0.09 | 0.49 ± 0.09 | 0.98 |
| Area of new tissue | 9.44 ± 0.74 | 9.42 ± 0.76 | 0.94 |
| Area of new bone | 2.08 ± 0.40 | 1.91 ± 0.35 | 0.76 |
| Linear measurements of the cementum | 7.53 ± 0.71 | 4.96 ± 0.67 | 0.012* |

N = 20 for all parameters. *Confidence interval of 95% - *t*-test.

digitalizada para avaliação de cicatrização periodontal. Braz Dent J 2001;12(3):167-172.

O objetivo desse estudo foi determinar a precisão de um método de imagem digitalizada e avaliar sua aplicação na cicatrização periodontal. Material histológico de um estudo realizado em cães foi analisado em microscópio óptico conectado a uma câmera que adquire as imagens e transmite os sinais luminosos para um computador na forma de pixels e dessa forma, permite a realização de medidas. Vinte campos foram medidos para acessar a reprodutibilidade intra e inter examinador para os parâmetros: área preenchida por novo tecido, área de epitélio, área de osso e medidas lineares de cemento. Os números foram analisados pelo test-*t* para testar a hipótese de não diferença entre as medidas executadas pelo mesmo examinador e entre examinadores diferentes. Nenhuma diferença estatística significativa num intervalo de confiança de 95% foi encontrada quando as medidas intra-examinadores foram analisadas (p>0,05). Resultados similares foram encontrados para as medidas interexaminadores quando analisadas medidas de área. Entretanto, houve diferença estatística significativa quando as medidas lineares de cemento foram analisadas (p<0,05). Os resultados foram consistentes e uniformes e o método demonstrou ser bastante preciso quando medidas intra-examinadores foram avaliadas.

Unitermos: imagem digital, histometria, cicatrização periodontal, precisão.

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