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Periodontal healing may be affected by aging: a histologic study in rats

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Background and Objective: Although wound healing has been reported to be impaired with aging, very little is known about its effect on periodontal tissues. Therefore, the aim of this study was to evaluate, histologically in rats, the influence of aging on a spontaneous periodontal healing model.

Material and Methods: Twenty-four male Wistar rats were used and assigned to the following groups: control ($n = 12$; 2 mo old) and aged ($n = 12$; 18 mo old). Fenestration defects ($4 \times 3 \times 1$ mm) were created bilaterally at the buccal aspect of the distal root of the first mandibular molars, and the mandibulae were retrieved 3 and 6 wk postoperatively. The percentage of bone fill and density of newly formed bone, new cementum formation (NC), and the extension of the remaining defect (ERD) were histometrically obtained.

Results: Intragroup analysis demonstrated that, except for cementum, all histological parameters significantly improved over time ($p < 0.05$). Intergroup analysis additionally showed that the defects were initially similar in size, and that at 3 wk aging negatively influenced newly formed bone (86.38 ± 2.99% and 73.06 ± 3.21%, $p < 0.001$, for groups control and aged, respectively), BF (75.84 ± 16.53% and 57.70 ± 22.28%, $p = 0.014$) and ERD (0.41 ± 0.20 mm and 1.17 ± 0.37 mm, $p < 0.001$). At 6 wk, aging negatively influenced newly formed bone (88.12 ± 2.90% and 78.19 ± 5.35%, $p < 0.001$, for groups control and aged, respectively) and ERD (0.01 ± 0.006 mm and 0.34 ± 0.18 mm, $p = 0.003$), but not BF (98.15 ± 2.43% and 87.87 ± 11.63%, $p > 0.05$). No new cementum was formed along the root surface in the above groups.

Conclusion: Within the limits of the present study, data analysis suggests that aging may impair, but not prevent, periodontal healing.

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It is commonly stated that wound healing is impaired as a function of age (1); however, the exact underlying mechanisms are unclear. *In vitro* studies have reported that aging is associated with a significant reduction in neutrophil chemotaxis and phagocytosis, and with a decline in macrophage function (2,3). Wound repair *in vivo* in aged animals is reported to present a 20–60% delay in the rate of healing as compared with young animals (4). In addition, capillary permeability, diapedesis of neutrophils, and infiltration of macrophages and T lymphocytes in the wound bed, are also delayed in the aged (5–8). Finally, old animals display a decreased level of collagenase activity (9), leading to a diminished turnover and remodeling of newly formed collagen, and also a decline in endothelial reponsiviness to specific factors, which may account for the delayed wound angiogenesis reported in aged mice (10).

With respect to the effects of increased age on periodontal tissues, histological findings, such as thinning and diminished keratinization of the epithelium, periodontal ligament with

decreased cell density and synthesized collagen, and also a decreased number of cells on the osteogenic layer of the alveolar bone, have been reported (11–13). Additionally, *in vitro* studies have demonstrated that aged periodontal ligament fibroblasts may present a reduced proliferative and mitotic capacity (14), an altered expression of cytokines under mechanical stress (15) and an altered proteoglycan expression from the cementum (16), which may be associated with the reduced ability of the periodontum to regenerate. As very limited information is available on whether aging would affect the regeneration of periodontal tissues, the present study aimed to investigate, histologically in rats, the impact of aging on the healing capacity of periodontal tissues.

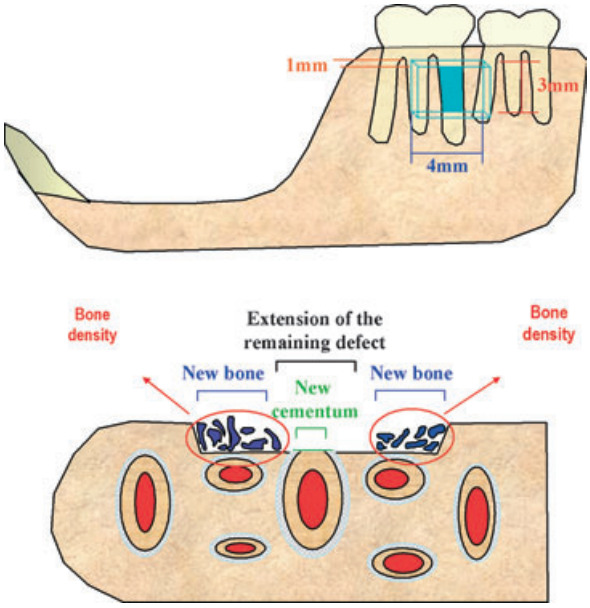

Fig. 1. Diagram showing surgical creation of the periodontal defect and the evaluated parameters.

Material and methods

Animals

Twenty-four male Wistar rats were included in the study. The animals were kept in plastic cages and had access to food and water *ad libitum*. Prior to the surgical procedures, all animals were allowed to acclimatize to the laboratory environment for a period of 5 d. The protocol was approved by the University of Campinas Institutional Animal Care and Use Committee.

Experimental design

General anesthesia was obtained by intramuscular administration of 0.5 ml/kg ketamine (Francotar, Virbac Laboratories, Roseira, Brazil). Periodontal fenestration defects (4 mm in width, 3 mm in length and ≈1 mm deep) were created at the buccal aspect of the distal root of first mandibular molars, as previously described (17) (Fig. 1). Briefly, the superficial bone was removed using a round dental bur (diameter 2 mm), at slow speed under saline irrigation, and the distal root of the first mandibular molar was denuded of its periodontal ligament, cementum and superficial dentin using a chisel. Neither antibiotics nor anti-inflammatory drugs were administered after surgery. Acetaminophen (Paracetamol; Abbot Laboratories, São Paulo, Brazil) was given for pain control. The animals were assigned to one of the following groups, according to their age: control (control) ($n = 12$; 2-mo-old animals) and aged (aged) ($n = 12$; 18-mo-old animals). The animals were killed 3 and 6 wk postoperatively, and the mandibulae were retrieved for histological evaluation.

Histomorphometric analysis

The jaws were fixed in 4% neutral formalin for 48 h, and subsequently demineralized in a solution of equal parts of 50% formic acid and 20% sodium citrate for 45 d. Paraffin serial sections (6 μm) were transversally obtained in an apico-coronal direction and stained with hematoxylin and eosin (H&E). The coronal and apical margins of the defect were identified by the presence of a denuded first molar distal root, and six sections per specimen representing the middle portion of the defect (24 μm apart) were selected and the percentage of bone fill (BF), the density of newly formed bone, new cementum formation (NC), and extension of the remaining defect (ERD) were histomorphometrically obtained by using an image analysis system (Image-Pro; Media Cybernetics, Silver Springs, MD, USA) (Fig. 1).

Statistical analysis

The hypothesis that there was no difference between the groups regarding the evaluated parameters was tested by using the parametric analysis of variance (ANOVA) (3 wk control vs. 3 wk aged vs. 6 wk control vs. 6 wk aged, $\alpha = 0.05$). Pairwise multiple comparisons were carried out by using the Tukey test ($\alpha = 0.05$) in the cases where the ANOVA detected significant differences.

Results

Histological analysis indicated newly formed bone and fibrous tissue scattered within the defect area (Figs 2 and 3). Data analysis showed no significant differences among the groups in terms of initial defect size at 3 wk (4.09 ± 0.20 mm and 4.18 ± 0.26 mm for control and aged groups, respectively, $p > 0.05$) and at 6 wk (4.13 ± 0.18 mm and 4.16 ± 0.21 mm, $p > 0.05$). Intragroup analysis demonstrated that, except for cementum, all histological parameters significantly

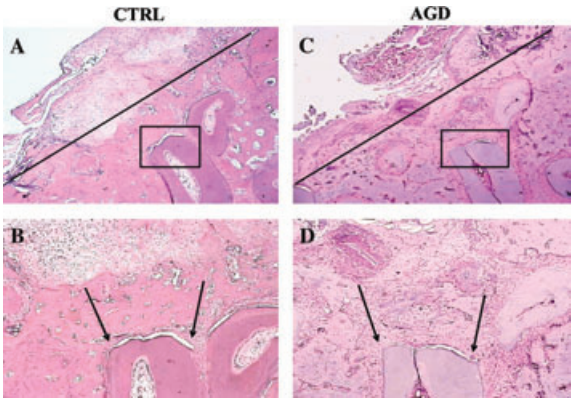

Fig. 2. Histological illustration of periodontal healing 3 wk after surgical creation of the fenestration-type defects (hematoxylin & eosin staining). The lines in Fig. 2A,C correspond to the original extension of the defects, while the arrows in Fig. 2B,D indicate the margins of the pre-existing cementum. Figure 2A,B represents the control (control) group and Fig. 2C,D represents the aged (aged) group (original magnification: Fig. 2A,C, ×5; Fig. 2B,D, ×20).

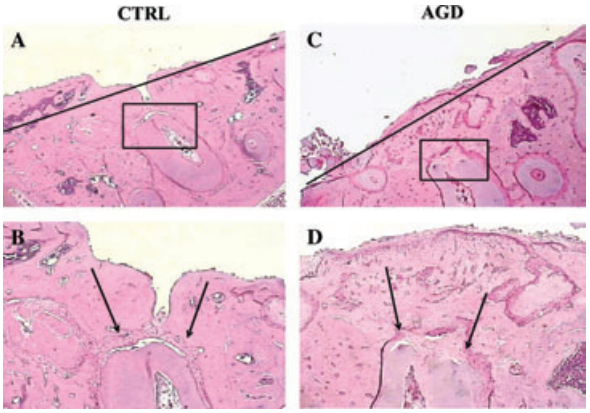

Fig. 3. Histological illustration of periodontal healing 6 wk after the surgical creation of the fenestration-type defects (hematoxylin & eosin staining). The lines in Fig. 3A,C correspond to the original extension of the defects, while the arrows in Fig. 3B,D indicate the margins of the pre-existing cementum. Figure 3A,B represents the control (control) group and Fig. 3C,D represents the aged (aged) group (original magnification: Fig. 3A,C, ×5; Fig. 3B,D, × 20).

improved over time ($p < 0.05$). Intergroup analysis additionally showed that at 3 wk aging negatively influenced newly formed bone (86.38 ± 2.99% and 73.06 ± 3.21%, $p < 0.001$, for control and aged groups, respectively), BF (75.84 ± 16.53% and 57.70 ± 22.28%, $p = 0.014$) and ERD (0.41 ± 0.20 mm and 1.17 ± 0.37 mm, $p < 0.001$). At 6 wk, aging negatively influenced newly formed bone (88.12 ± 2.90% and 78.19 ± 5.35%, $p < 0.001$, for control and aged groups, respectively) and ERD (0.01 ± 0.006 mm and 0.34 ± 0.18 mm, $p = 0.003$), with a numerical, but not statistically significant, tendency for a reduction in BF (98.15 ± 2.43% and 87.87 ± 11.63%, $p > 0.05$) (Fig. 4).

Discussion

Aged people are believed to have an impaired wound-healing ability; however, most of the evidence of this has been derived from clinical observations without adjustment for confounders other than age (18). Moreover, studies have proved difficult to interpret owing to a lack of proper control for subject comorbidy, which is a common situation among the elderly (19). As rodents are suitable models, and have been extensively used to investigate key biological processes involved in the phenomena of aging, the present study aimed to evaluate, at an experimental level, the impact of age on the healing process of periodontal tissues. Within the author's knowledge, this is the first study to demonstrate *in vivo* that aging may impair the healing process of periodontal tissues.

Historically, healing in the aged has been considered defective, and although the mechanisms involved remain to be elucidated, some of the reasons of this could be associated with an excessive inflammatory response and matrix degradation with an age-related modulation of the responses of inflammatory cells (19). In addition, delayed healing in the aged has been shown to result from overexpression of matrix metalloproteinases (MMPs) and underexpression of their natural inhibitors (tissue inhibitors of matrix metalloprotinase (TIMPs)) (20,21), suggesting that with aging, the periodontal ligament cells produce a larger amount of bone-resorbing and a smaller amount of bone-formative factors (15). In addition, the periodontal ligament undergoes progressive reduction in width, cell density, mitotic activity and organic matrix production, and is believed to decrease its capability to differentiate into other periodontal cells and regenerate the periodontium (14). Previous studies have reported a significant decrease in collagen secretion (22), a shorter proliferative life span (23), a lower replication rate (24) and reduced expression of an essential factor for cell proliferation (c-fos) (25), reflecting the decreased cell proliferative ability and the reduced regenerative capacity of the periodontal ligament fibroblasts obtained from aged donors. Previous investigations have reported, in aged periodontal ligament cells, a significant decrease in the activity of alkaline phosphatase (22) and in the expression of secreted protein, acidic and rich in cysteine (SPARC) (14), which play important roles in bone formation (especially mineralization) and regulation of the synthesis of matrix components and metalloproteinases.

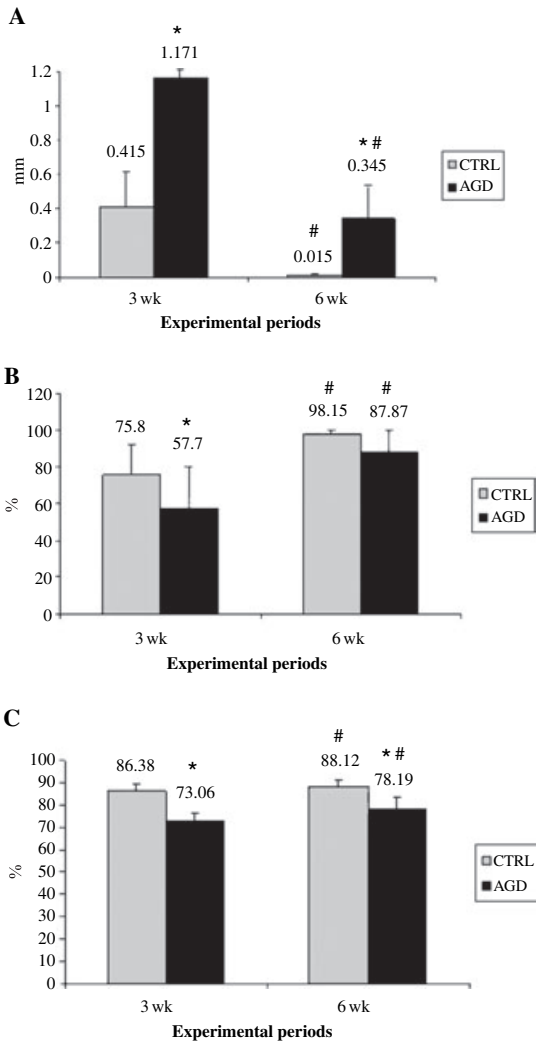

Fig. 4. Mean and standard deviation of the remaining extension of the bone defects (Fig. 4A), the bone fill (Fig. 4B) and the bone density (i.e. proportion of mineralized tissue) (Fig. 4C), 3 and 6 wk after the creation of the defects for all the experimental groups. *Statically significant intergroup analysis [analysis of variance (ANOVA) $p < 0.05$]. Statically significant intragroup analysis [analysis of variance (ANOVA) $p < 0.05$]. control, control group; aged, aged group.

Decreased bone formation during distraction osteogenesis, and femoral and humeral fractures, have all been reported and are compatible with the results of the present study (26,27). Aberrant recruitment, decreased proliferation, and/or fewer numbers of osteogenic precursors have been proposed to explain this generalized age-related decline in bone formation (28). Recent studies have found that juvenile osteoblasts produce and organize, to a greater degree than adult osteoblasts, critical cytoeskeletal and extracellular matrix proteins necessary for attachment, proliferation, and signaling (29). In addition, there may be, with age, a decrease of the replication capacity, response to growth factors and a 10-fold reduction in the number of osteoblasts, caused by changes in the phenotypic characteristics of such cells and also an absence of stimulatory factors in the cellular environment (30). Intriguingly, King *et al.* (31), found that mature rats have more osteoblasts on both alveolar bone surfaces than young animals, despite substantial losses in bone formative activity, and suggested that the age-related decrease in alveolar bone formation was a result primarily of the loss of osteoblastic activity rather than the ability to recruit new cells.

This rat periodontal defect model, as used by King's group and others (32,33), has proven to be a predictable and reliable model in studying periodontal healing without oral bacterial contamination or ingrowth of gingival epithelium. Although this model may not represent a critical-size defect for periodontal regeneration, it serves as a reasonable screening model for using to examine the wound-healing kinetics. A very interesting finding of the present study, and of others, is the fact that despite the absence of 'contamination' with dental biofilm, no new cementum was formed along the root surface in any of the above-mentioned groups. By using this model, cementum regeneration has been mostly seen if any regenerative technique or collagen carrier was applied (32,33). This observation raises the point that cementum presents a very limited self-healing capacity, even in the absence of previous exposure to the dental biofilm. Further studies should be designed to determine the real potential for cementum to regenerate, and also factors that would be involved.

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