Original

Disturbance of Periodontal Tissue Caused by Mechanical Compression into the Gingival Col of Streptozotocin-induced Diabetic Rats

Tetsuhiko Tokunaga, Norio Enoki, Kazuhiko Okamura and Kunihisa Taniguchi

Division of Pathology, Department of Morphological Biology, Fukuoka Dental College, Fukuoka, Japan
(Accepted for publication, January 20, 2014)

Abstract   FThe purpose of this study is to clarify the destructive processes of periodontal tissue, especially of alveolar bone, induced by food impaction. Streptozotocin-induced diabetic rat was used, gutter percha point (GP) was inserted into interdental gingival col space between upper M1 and M2, and the change of body weight, histopathological findings and 3D SEM structure of alveolar bone were compared between diabetic (Group DM) and control (Group N) animals (Experiment 1). Large amounts of bacterial deposits and sequester were observed at the alveolar crest, and subsequently experiment 2 was carried out to explore the relationship between bacterial infection and sequester formation. The combination of mechanical compression and daily twice cleaning with 3% oxydol and Periocline were carried out. As a result of experiment 1, ulceration with partial exposure of alveolar crest, inflammatory infiltrates and hyaline degeneration occurred in the col, and bone resorption was scarcely observed at 1d in Group N. Bone resorption of alveolar bone progressed at 3d and 5d, decreasing the height of alveolar crest and the width of alveolar bone. Reepithelization of ulcer surface was observed at 7d and 14d, and concurrently bone resorption regressed and new bone formation suggested reparative changes. SEM observation confirmed these changes of alveolar bone. On the contrary, massive bacterial deposits were observed, and bone resorption was scanty in the upper region and slight in the middle to lower region of alveolar bone at 1 day of Group DM. Massive bacterial deposits with partial exposure of alveolar bone were observed at 3d and 5d, and sequester was isolated owing to intense undermining bone resorption. Regeneration of epithelium was seen beneath the sequester at 14d, showing the phenomenon of foreign body exclusion. The light microscopic changes of Group DM were consistent with SEM findings. The total number of osteoclasts was fewer at 1d and larger at 3d and 5d in Group DM. Osteoclasts at the upper region of alveolar bone increased from 1d to 5d in Group N, although the number of osteoclasts did not show a significant change in Group DM. At the middle to lower portion of alveolar bone, the number of osteoclast was significantly fewer at 1d, larger at 3d and 5d in Group DM. In experiment 2, the col with ulceration and necrosis did not show bacterial deposits or sequester, suggesting the intimate relation between bacterial infection and sequestration. The present study suggests that food impaction and subsequent mechanical compression to the col could induce various damages of periodontal tissue, including sequester formation, and elaborate plaque control at the initial stage of food impaction could prevent sequester formation and protect the height of alveolar bone.

Key words: Streptozotocin-induced diabetic rat, Gingival col, Mechanical compression, Periodontal tissue, Alveolar bone resorption

Introduction

Food impaction, one of disturbance factor to periodontal tissues, is frequently encountered phenomena and induces damages of periodontal tissue, including resorption of alveolar bone. Causes for periodontal diseases are known to be various factors, dental plaque (biofilm), calculus, inappropriate prosthetics, mouth breathing (xerostomia), malalignment of teeth, bruxism, food impaction, et al. as local factors, and malnutrition, endocrinosis, hereditary factors, allergic diseases, circulatory disturbances,

metabolic disturbances[1].

The combination of local and systemic factors would make the destructive changes by periodontal disease much more intense at diabetic state, although dental plaque (biofilm) is the major etiology of periodontal disease. Consequently the influence of diabetic state on periodontal disease induce by food impaction would be one of great interest to be elucidated.

Biodefense function is known to be lowered and retard healing at diabetic state, on the basis of clinical and basic researches on the healing process after wound, tooth extraction, bone fracture and the others[2-12]. Recently the association between diabetic state and periodontal diseases has been established, for example, the

Correspondence to: Dr. Kunihisa Taniguchi, Division of Pathology, Department of Morphological Biology, Fukuoka Dental College, 2-15-1, Tamura, Sawara-ku, Fukuoka, 814-0193, Japan; Tel +81-92-801-0411;E-mail: taniguk1@college.fdcnet.ac.jp

occurrence of periodontal disease is higher and accelerates resorption of alveolar bone, and inadequate control of blood sugar level was related to the severity of periodontal diseases[13-26]. However, tissue reaction to injury is not fully clarified in oral tissues under diabetic state.

Numerous experimental studies are available on food impaction, and experimental data on rat[27-29] showed a series of characteristic tissue reactions, in which the col was intensely impaired by compression, the absorption of alveolar bone crest was delayed at earlier stage, and marked bone resorption started on the surface of alveolar bone beneath the alveolar crest. On the contrary, very few literatures are available on the influence of food impaction on periodontal tissue at the diabetic state. Morooka[30] made experimental research on occlusal trauma in rat periodontal tissue at diabetic state, and reported that the delay of bone resorption, narrowing of periodontal space with intense impairment of periodontal ligament, marked attrition and tooth facture.

The present study was planned to elucidate destructive processes of periodontal tissue by mechanical compression to the col, and made experimental diabetic rat model, and compared tissue destruction of periodontal tissue between diabetic and healthy rat histopathologically. The three dimensional structure of destructive processes was also examined on the alveolar bone with scanning electron microscopy.

Massive bacterial deposits were observed at the col of diabetic rat, followed by the sequester formation at the alveolar crest. Furthermore, the positive relation of sequester formation and bacterial deposition was examined and validated with additional experiment comparing tissue reaction of diabetic rats with or without disinfection of periodontal tissue.

Materials and Methods

Animal experiment was performed under the permission of Fukuoka Dental College Experimental Animal Committee.

Eighty eight of 5 week Sprague Dawley rat were divided into normal (Group N) and diabetes mellitus (Group DM) groups. For the preparation of type I diabetic model rat, 125 mg/kg of streptozotocin (STZ, Sigma, MO, USA) was intraperitoneally injected, and diabetic status was confirmed as >+3 (compatible with 500mg/dl of urine sugar value) with tes-tape for DM examination. Solid food and water was freely supplied to all animals.

Experiment 1

Guttapercha point(GP#30) was inserted into the gingival col region between left upper 1st (M1) and 2nd (M2) molars of diabetic and control rats, as a food impaction model. Histopathological and scanning electron microscopic observation was carried out for 8 rats of Group N and Group DM at 1d, 3d, 5d, 7d and 14d
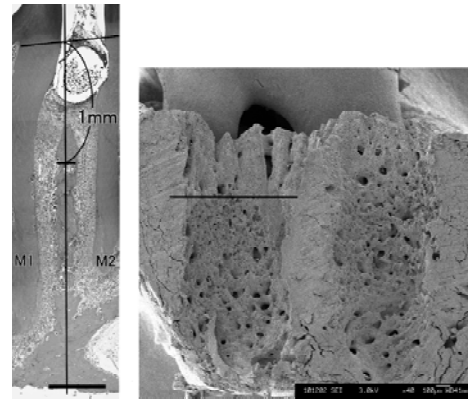

Figure 1. The 1-mm lower part from the enamel-cement boundary of a left H-E image is equivalent to the area to a black line part including the precise and smooth field which is the alveolar bone upper part of the interalveolar septum of a right Scanning electron microscope image.

after mechanical compression respectively. Body weight was measured at each time point. At sacrifice, animals were killed by deep general anesthesia, maxilla was excised, immersed in 10% formalin solution for 1 week, decalcified with 10% formic acid for 1 weeks, dehydrated and embedded in paraffin. Serial mesio-distal sections were prepared for HE examination.

Scanning Electron Microscopy

Excised maxilla was treated with protease P "Amano" 3G (Amano enzyme Co) for 2 weeks, periodontal soft tissues were digested and removed completely. Maxilla was sectioned at the distal root of M1 bucco-palatally, mesial side of alveolar bone between M1 and M2 was exposed, dehydrated in alcohol, received critical point drying, sputter coated by gold, and observed with scanning electron microscope JSM-6330F (JEOL Ltd, Japan)

Measurement of Osteoclast Number

The number of osteoclasts were counted at the interalveolar septum. Three different sections of HE preparation were used for the measurement (N=9 both for Group N and DM). Measured area was upper area and middle-lower area of alveolar socket, these two areas were standardized by determining upper area as from the level connecting cement-enamel junctions of M1 and M2 to the level lowering 1mm apically. The measurement was carried out 1d, 3d, 5d and 7d on the samples of Group DM and Group N, and the 14d samples were exclude owing to massive loss of alveolar crest (Fig. 1) .The number of osteoclasts at each time point was statistically compared between Group N and Group DM with student's t-test.

Experiment 2

This experiment was added to disclose the relation between sequester of alveolar crest and bacterial colonies formed at col
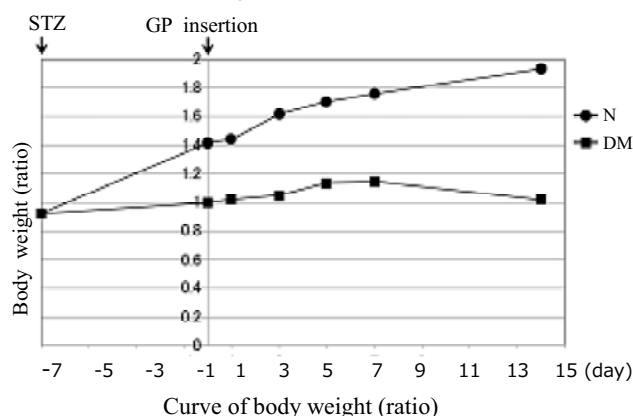
STZ   GP insertion

Figure 2: The growth curve of body weight during the experimental period. The weight difference of normal group and diabetes mellitus group showed the tendency which becomes large temporally.

region. After the insertion of gutter percha point (GP) to animals of Group DM, col region was washed with 3% oxydol (Kenei Pharmaceutical Co. Ltd, Osaka, Japan) and subsequently treated with PERIOCLINE (Sunstar Inc., Osaka, Japan) twice (morning and evening) a day (without removing inserted gutter percha) throughout the whole experimental period. Four rats were used for this experiment at one time point, and two time points (3d and 5d) were selected after the result of experiment 1. Strategies for observation and analysis were the same as the light microscopic approach of experiment 1.

Results
Body weight

The body weight of 5 w old rat ranged 130 to 150 g. The body weight of diabetic rats (Group DM) was normalized to the body weight of Group N rats at the initiation of GP insertion (Fig. 2). The diabetic rat showed hyperphagia, polydipsia, pollakiuria, lusterless body hair and smaller size, in comparison with those of Group N.

The group N showed steady increase of body weight. On the contrary, the weight gain of Group DM was scarce accompanying diarrhea, although diabetic rats showed hyperphagia and polydipsia.

HistopathologicalFindings (H-E staining)
Group N (Figs. 3-6)

Gutter percha point existed without dropping-off during the experimental period, and round shaped remnant of GP was confirmed between M1 and M2 histopathologically.

The col region was compressed and showed concave contour 1d after the compression. The surface epithelium was lost and formed ulceration, followed by intense inflammatory infiltrates. The lamina propria underneath showed the destruction of connective tissue fibers, accompanied by hyaline necrosis partially.

Osteoclastic bone resorption was scarcely observed at the upper region of interalveolar septum. On the contrary, marked bone resorption with many osteoclasts was observed at the middle-lower portion. Inflammatory infiltrates of periodontal ligament were confined to the upper region.

The col structure was destroyed and lost, forming deep ulcer and the alveolar crest was partly exposed 3d after compression. Bone resorption was not observed and the adjoining periodontal ligament was partly destroyed with the deposit of necrotic tissue and fibrin on the surface. The inflammatory infiltrates spread downward, inducing partial destruction and dissociation of periodontal ligament. At the middle-lower portion of alveolar socket, inflammatory infiltrates and the number of osteoclasts were decreased, reducing bone resorption, in comparison with the same region 1d after compression.

Reepithelization occurred beneath GP 5 d after compression, although the col itself maintained concave contour due to the compression. Inflamed granulation tissue was formed under the regenerated epithelium and spread to periodontal ligament, subsequently the collagen fibers of the gingiva and periodontal ligament travelled irregularly. Bone resorption of alveolar crest progressed and the height of alveolar bone was slightly decreased with the narrowing of alveolar crest. Osteoclastic bone resorption was scatterely distributed at the middle-lower region of alveolar bone, followed by slight decrease of the width of alveolar bone.

The thickening of covering epithelia was seen along with stratification at the col 7 d after compression, and the concave contour of col was maintained. The repair of gingival tissue progressed, although inflamed granulation tissue existed from subepithelial tissue to the alveolar crest. Although the height of alveolar bone lowered, bone was added on the surface of upper portion with the thickening of interalvelar septum. Bone resorption was scarcely observed and addition of new bone was found at the middle-lower portion.

The col was slightly depressed and concurrently stratification and thickening of epithelium further progressed 14 d after compression. The rete pegs were elongated, inflammatory infiltrates were decreased, and the connective tissue fibers were increased, approaching the normal state of gingival lamina propria. Although the height of alveolar crest was slightly lost, bone resorption was not observed throughout the surface of alveolar bone. New bone formation was occasionally observed, and consequently the width of interalveolar septum was kept constant.

Group DM (Figs.7-10)

The covering epithelia was completely lost and ulcerated beneath GP 1 d after compression, and hyaline necrosis occurred by compression at the lamina propria. Large amount of bacterial colonies were found scatteringly among hyaline materials. Periodontal ligament adjoining the alveolar crest also showed
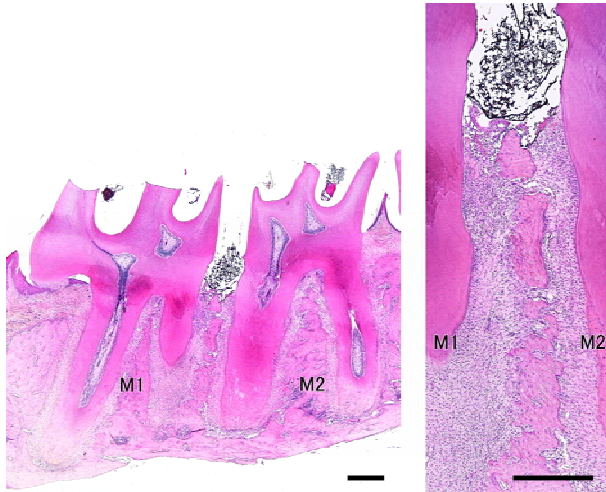
Figure 3. 3 day after col compression of the normal group. The col area is broken and disappeared by ulcer formation and the top of alveolar bone has exposed. H-E staining (Scale bar = 500μm).
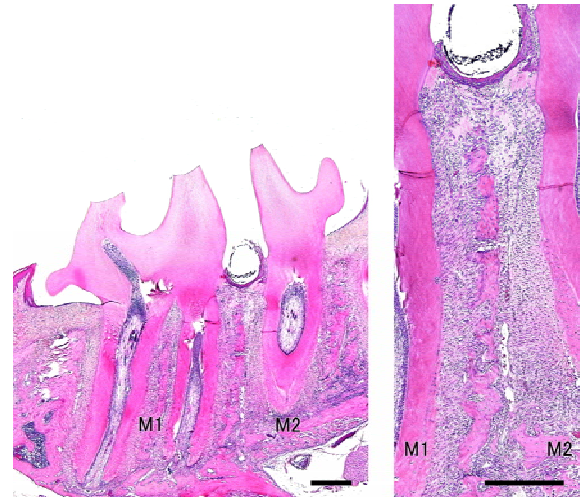

Figure 4. 5 day after col compression of the normal group. Covering of epithelium is seen in the surface of the col area depressed. At the upper portion of the alveolar bone, bone resorption progressed, and the width of interalveolar septum became thin. H-E staining (Scale bar = 500μm).
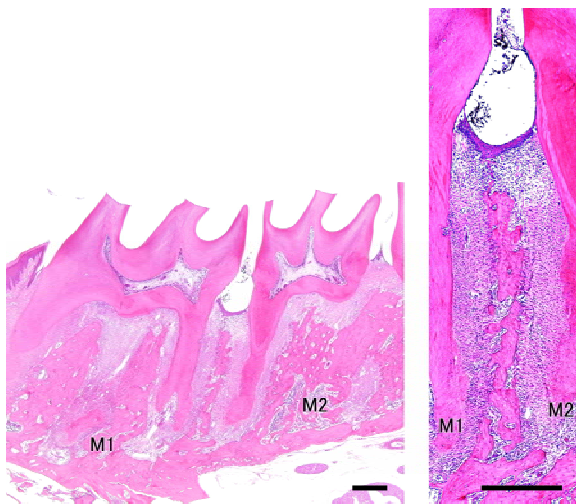

Figure 5. 7 day after col compression of the normal group. Covering of epithelium is seen in the surface of the col area. Inflammatory granulation tissue is formed under an epithelium and top of the alveolar bone is further lowered by bone resorption. H-E staining (Scale bar = 500 μm).
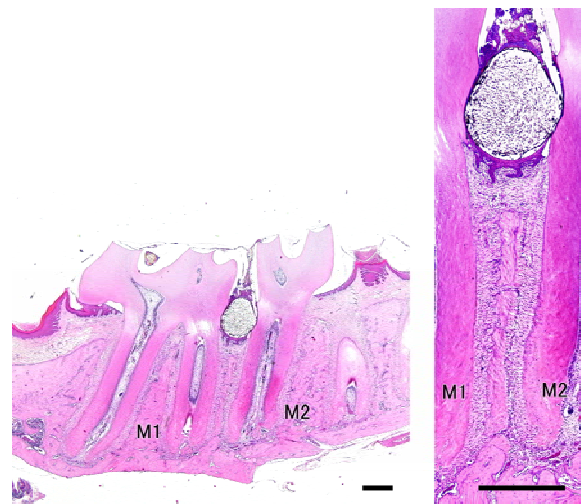

Figure 6. 14 day after col compression of the normal group. Inflammatory cell infiltration becomes slight and repatative change is progressing in the col area. Mostly, bone resorption is not seen at by the alveolar bone, and addition of the neonatal bone is also progressing. H-E staining (Scale bar = 500μm).

hyaline necrosis and bacterial colonies of varied sizes. These destructive changes of col and periodontal ligament were more marked in Group DM than in Group N. Periodontal ligament at deeper portion was hardly impaired, although slight inflammatory infiltrates were present. The alveolar crest was generally intact, slight bone resorption was observed at the middle-lower portion of alveolar bone, and the number of osteoclasts was fewer than that in group N.

The col was markedly destructed and ulcerated 3 d after compression, surface necrotic area was thickly formed with deposition of large amount of bacterial colonies. The alveolar crest

was partly exposed or covered with necrotic tissue, followed by deposit of bacteria and sequester formation. Periodontal ligament surrounding the alveolar crest received intense inflammatory infiltrates, and was partially dissolved. Osteoclastic activity was not observed around the alveolar crest, but marked underneath. Large number of osteoclasts appeared at the middle-lower portion of alveolar bone, accompanying intense bone resorption.

The col was ulcerated 5 d after compression and sequester at the top of alveolar crest was isolated by the undermining bone resorption, and was occasionally exposed from the ulcer bottom. Inflammatory infiltrates were intense around sequester and spread
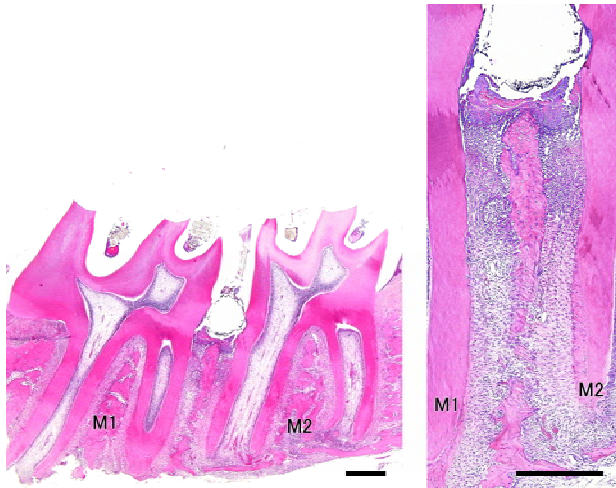
Figure 7. 3 day after col compression of the diabetes mellitus group. Ulcer arises in a col area and the further necrosis is seen in the surface. Remarkable inflammatory cell infiltration is seen in the periodontal membrane. The top of the alveolar bone is in contact with the deep ulcer part, and in the upper portion, alveolar bone absorption was not seen but has arisen in middle-lower portion. (Scale bar =500μm).
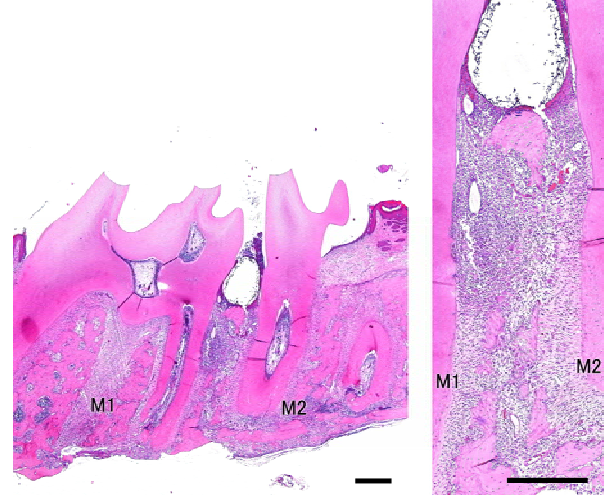

Figure 8. 5 day after col compression of the diabetes mellitus group. Ulcer is formed and destroyed and remarkable adhesion of a bacteria mass is seen in the col area. Remarkable inflammatory cell infiltration is seen in a periodontal membrane, absorption arises from the side of the alveolar bone in the middle-lower portion, and the top of the alveolar bone is presenting the state of the isolation bone. H-E staining; (Scale bar = 500μm).
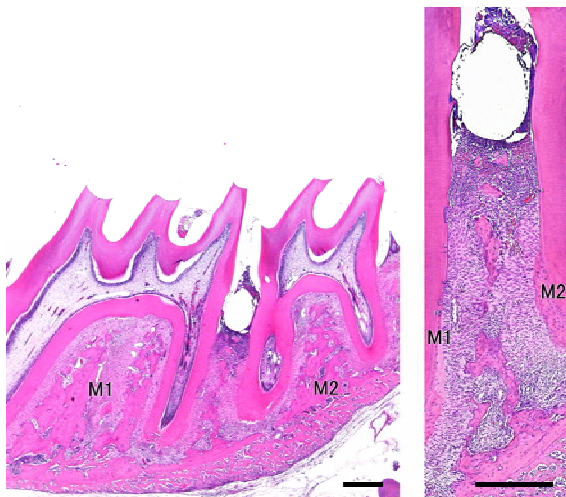

Figure 9. 7 day after col compression of the diabetes mellitus group. Ulcer is formed and destroyed and adhesion of a bacteria mass is seen in the col area. Inflammatory cell infiltration is seen widely in a periodontal membrane. Bone resorption arises in the alveolar bone on the whole, and the top of the alveolar bone is lowerd. H-E staining; Scale bar (500μm).
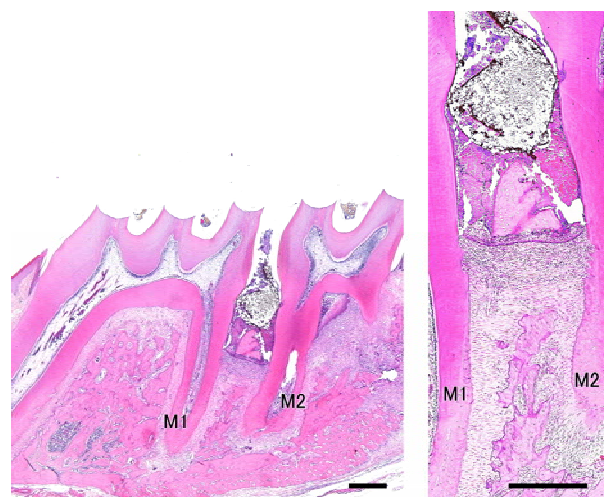

Figure 10. 14 day after col compression of the diabetes mellitus group. GP, many pieces of food, and bacteria mass are seen by the col area. Regeneration of an epithelium arises on a col surface and slight inflammatory cell infiltration is seen under the epithelium. Sequestration is produced, and exposed to the ulcer part, and sequestrum exclusion mechanism has arisen in the upper part of the alveolar bone. H-E staining; Scale bar (500μm).

deep into the periodontal ligament. Bone resorption by osteoclasts progressed at the middle-lower portion, and interstitial space in periodontal ligament widened.

Subsequently the width of interalveolar septum was narrowed at several places.

The col was ulcerated with massive deposition of bacterial colonies and intense inflammatory infiltrates 7 d after compression. The upper portion of alveolar bone became sequester, and boroad

bone resorption progressed under the alveolar crest, making large bone defect. The alveolar bone lost its original height and narrowed its width. The enlargement of interstitial space was occasionally observed at the middle-lower portion, the overall width of interalveolar septum narrowed, although the number of osteoclasts was decreased compared with 5 d after compression.

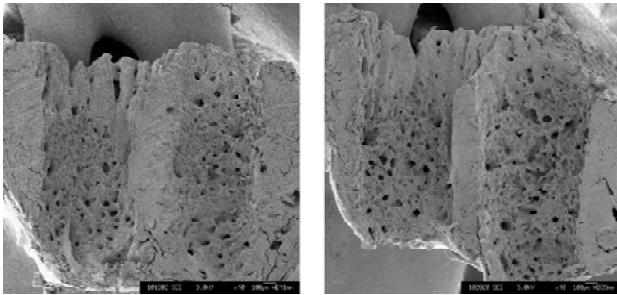Bacterial deposits and food impaction were observed at GP

Figure 11. 1 day after col compression of Scanning electron micro-scope image of the interalveolar septum. The normal group(left) and the diabetes mellitus group(right).
Group N:The upper portion of the alveolar bone has a smooth and precise bone surface, and has rough surface by bone resorption in the lower portion.
Group DM:Bone destruction is not seen by the alveolar bone upper portion like a normal group. Bone destruction is mainly advancing in the middle-lower portion. The interstitial space in periodontal ligament shows a large and uneven shapes.
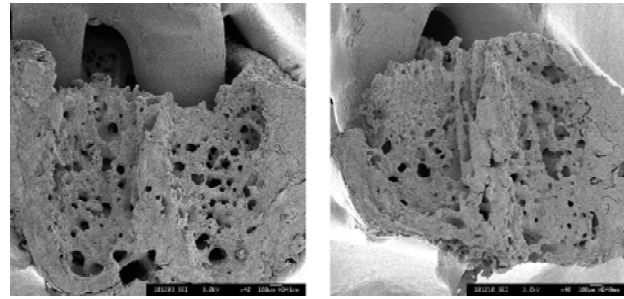

Figure 12. 5day after col compression of Scanning electron microscope image of the interalveolar septum. The normal group(left) and the diabetes mellitus group(right).
Group N:The smooth surface of the upper portion of the alveolar bone has mostly disappeared, and the top of the alveolar bone had lowered. The bone defect has arisen remarkably in the lower part of the upper portion and the interstitial space in periodontal ligament shows a large and uneven shapes.
Group DM:The top of the alveolar bone has maintained, and a difference with a normal group is seen. The bone destruction has advanced in the middle-lower portion.
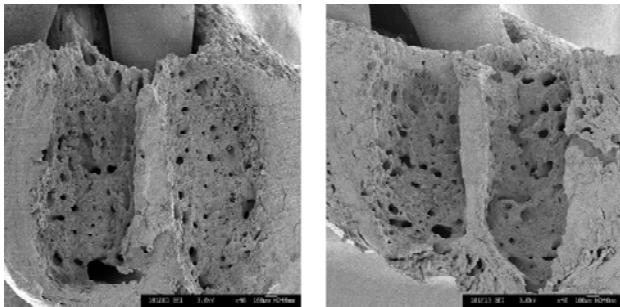

Figure 13. 7day after col compression of Scanning electron microscope image of the interalveolar septum. The normal group(left) and the diabetes mellitus group(right).
Group N:The bone resorption from the mesial side is seen remarkably.
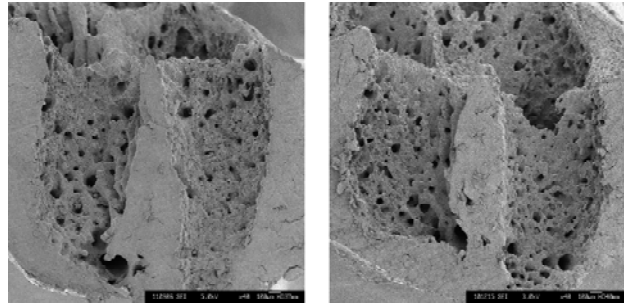Group DM:The bone destruction has advanced in the lower portion of the top of the alveolar bone.

Figure 14. 14day after col compression of Scanning electron micro-scope image of the interalveolar septum. The normal group(left) and the diabetes mellitus group(right).
Group N:The top of the alveolar bone has lowered by bone destruction in the upper and lower portion, but small granular neonatal bone is seen in some places of the bone surface.
Group DM:The upper portion of the alveolar bone is destructed, and the top of the alveolar bone has lowered. Trabecular of the alveolar bone has a precise and small granular surface in some places.
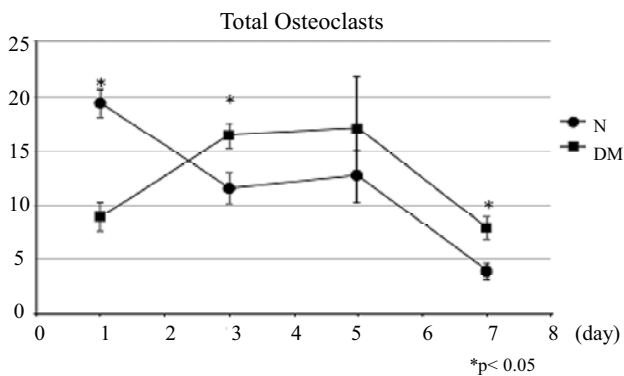

Figure 15. The total number of osteoclast was fewer in diabetes mel-litus group than in normal group 1 day after compression, and in-creased 3 day and 5 day, showing higher value than in normal group.

granulation tissue was formed. Occasionally the col surface was reepithelized under the sequester, showing outward exclusion of sequester as foreign body. The height of alveolar bone was decreased markedly, although inflammatory infiltrates were mild and bone resorption was slight and new bone formation was detected partially.

SEM Finding (Figs. 11-14)

Mesial aspect of alveolar bone between upper M1 and M2 was observed with SEM after the compression of col by GP insertion.

Group N

The alveolar crest consisted of relatively smooth surface of

and surrounding tissue of the col space 14 d after compression. In most instance, sequester at the alveolar crest disappeared and

compact bone, and took round outline as observed in light microscopy. This compact bone of alveolar crest was 500-600mm in width at maximum, and bone trabeculae and many interstitial space for vasculatures and nerve bundles existed underneath at the middle-lower portion, taking appearance of uneven structure. The border between compact bone and uneven trabecular structure was sharp. These SEM findings were consistent with LM findings.

The smooth surface of compact bone at the crest narrowed 3 d after compression, and round outline was occasionally impaired. Bone defect was observed at the middle-lower portion, the interstitial space was enlarged, and bone trabeculae became further uneven. Alveolar bone received resorption sidewise as in light microscopic observation.

The alveolar crest was widely destructed 5 d after compression and almost disappeared, lowering the height of alveolar bone. The smooth and compact area of the crest was scarcely present, lost round contour and became saw-tooth appearance. Broad bone defect occurred with many large interstitial spaces, making uneven appearance at the mesial aspect of alveolar bone.

The smooth and compact bone surface was completely lost 7 d after compression, with deep bone defect and saw-tooth appearance. Bone defect was observed at the middle-lower portion, showing broad bone destruction.

Broad bone defect was observed from the alveolar crest to the middle-lower portion with many interstitial spaces and rough surface 14d after compression, likewise in 7 d after. New bone formation with relatively flat surface was partly formed, and the number of interstitial spaces decreased, suggesting reparative change.

Group DM

The bone surface was compact and smooth at the alveolar crest 1 d after compression, taking the round appearance in contour. Many interstitial spaces existed at the middle-lower portion as in control. Thin bone trabeculae took rough surface appearance.

The compact and smooth bone surface was preserved with the round contour at the top of alveolar crest 3d after compression. Marked bone destruction occurred sidewise immediately beneath the alveolar crest. Mesial bone defect was enlarged, large interstitial spaces were observed occasionally, taking rough surface appearance.

Smooth and destructive surface were intermingled at the alveolar crest 5d after compression, subsequently showing rough surface. The majority of interstitial spaces was large at the middle-lower portion, making quite uneven surface.

Compact and smooth surface was preserved at the crest 7 d after compression, unlike in Group N. Bone defect occurred immediately beneath the smooth alveolar crest. Relatively smooth surface and large interstitial spaces or bone defects coexisted at the middle-lower portion.

Round-shaped contour was lost at the alveolar crest 14 d after compression, taking rough surface and lowering the height of alveolar bone. Relatively smooth bone surface was intermingled with large interstitial spaces showing rough surface at the middle-lower portion.

The Number of Osteoclasts in Group N and Group DM (Measurement of 0steoclasts *p < 0.05)

After the confirmation of characteristic resorption of alveolar bone after GP compression, osteoclasts were measured at the interalveolar bone surface.

The number of osteoclasts at the upper portion and at the middle-lower portion was calculated, and these two data as well as summated data (the whole number of osteoclasts distributed in the entire alveolar socket) were compared chronologically.

The total number of osteoclast was maximal 1 d after compression in Group N, and decreased hereafter. On the contrary, the total number of osteoclast was fewer in Group DM than in Group N 1 d after compression, and increased 3d and 5d, showing higher value than in Group N. The number was decreased at 7d, still maintained higher value than the osteoclastic number in Group N (Fig.15).

The number of osteoclasts in the upper portion was generally few both in Group N and Group DM. The number tended to increase until 5d, and then slightly decreased at 7 d in Group N. The number kept almost constant during the whole time point in Group DM (Fig. 16).

The number of osteoclasts in the middle-lower portion was higher than in the upper portion. The number was maximal at 1d, decreased with time and almost disappeared at 7 d in Group N. The number was significantly lower at 1 d in Group DM than in Group N, but became higher at 3 d and 5 d. The number finally decreased at 7 d, still keeping higher value than at the same day in Group N. (Fig. 17)

Experiment 2
Histopathological Findings of Group DM (H-E staining) (Figs.18, 19)

The gingival col was depressed concavely by the GP insertion at 3 d after compression, and covering epithelium and lamina propria were ulcerated. Bacterial colonies were scarcely noted around GP. Ulcerated surface was covered with necrotic tissue and fibrin clot with hemorrhage, under which inflammatory infiltrates were observed. Alveolar crest was in contact with ulcer bottom, and the adjoining periodontal ligament was mildly infiltrated with inflammatory cells. The alveolar crest was free from bone resorption, and maintained smooth and round outline, instead undermining bone resorption was observed with the appearance of numerous osteoclasts. Scanty number of osteoclasts appeared at the middle-lower portion of alveolar socket, and
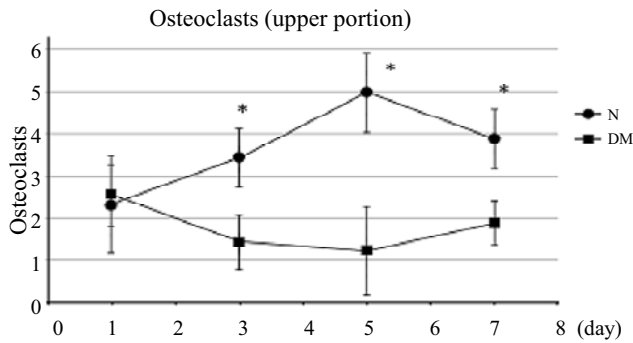
Figure 16. The number of osteoclasts in the upper portion tended to increase until 5 day, and then slightly decreased at 7 day in normal group
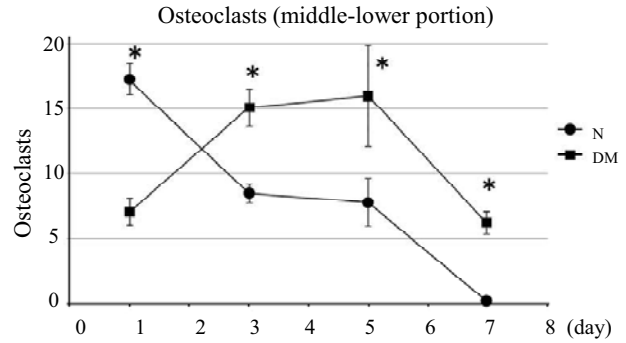

Figure 17. The number of osteoclasts in the middle-lower portion was higher than in the upper portion. The number was maximal at 1 day, decreased with time and almost disappeared at 7 day in normal group.
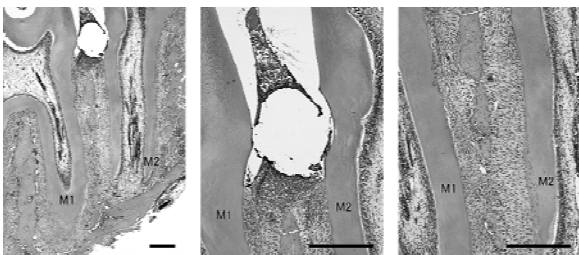

Figure 18. 3 day after col compression of the diabetes mellitus group on experimental 2.
Piece of food, bacterial adherence, ulcer formation, inflammatory cell infiltration, hyaline necrosis, etc. were seen in the col area. H-E staining (Scale bar = 500mm)
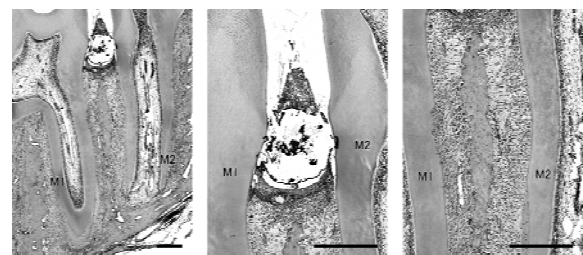

Figure 19. 5 day after col compression of the diabetes mellitus group on experimental 2.
Piece of food, ulcer formation, necrosis in the surface area and inflammatory cell infiltration under the surface area were seen in the col area. Slight bone resorption was seen at the top of the alveolar bone, and it was also seen diffusely at the middle-lower area. Neither exposure of an alveolar bone nor sequestrum formation was seen. H-E staining (Scale bar = 500μm)

periodontal ligament received minimal injury.

The col was followed by GP and small amount of food, and no bacterial colonies were formed at 5 d after compression. The col was depressed and ulcerated as at 3 d. Ulcer surface consisted of necrotic tissue, fibrin deposit and inflammatory infiltrates. Granulation tissue was frequently intervened between ulcer bottom and alveolar crest. In case such granulation tissue was formed, osteoclastic bone resorption was observed at the alveolar crest, and the smooth and round contour of crest was impaired and became irregular. No sequester was noted. Focal bone resorption was observed immediately beneath the alveolar crest, decreasing the width of interalveolar septum. Osteoclasts were hardly observed at the middle-lower portion.

Discussion

Experimental research on food impaction has been performed with use of various animal species including rat, dog, monkey, et al., in which a variety of impaction methods were employed[27,29,31-39]. Groove or pit formation at the contact surface of tooth, the combination of such deletion of tooth and mechanical dissocation, the insertion of various materials (gum, silk string, cotton string, gutter percha point) into the col were used. Osaki[28] reported that disturbance of periodontal tissue including alveolar bone

resorption induced by mechanical compression of the col by GP was similar to characteristic changes by the combined tooth grinding and mechanical dissociation[27], showing no substantial differences. Consequently mechanical compression by GP was selected in the present study.

Alloxan, streptozotocin (STZ) are known as medicines inducing diabetic state. Liver and kidney damages are less in STZ than in alloxan. STZ injury is specific to beta cells of islets of Langerhans, and induces hyperglycemia without ketosis. STZ is frequently used for diabetic model in this study, because STZ maintains diabetic state for a long period, and metabolic mechanism is similar to human type I diabetic mellitus[40-42]. The dose of STZ varied from 45 to 120 mg/kg. The present study followed Morooka's study (125 mg/kg)[30].

In the present study of col compression simulating food impaction, Group N and Group DM showed both similarity and difference in chronological morphological changes.

As similarities, ulceration, hyaline degeneration and inflammatory infiltrates were observed at the col from early stage. Bone resorption was not detected at the alveolar crest, which was in close proximity to ulcer bottom and occasionally exposed to

the surface. Bone resorption started immediately beneath the alveolar crest, undermining lateral surface of alveolar socket. These light microscopic findings were consistent with scanning electron microscopic findings. Bone resorption progressed chronologically at middle-lower portion, and the width of interalveolar septum became thin.

Marked lowering of the level of alveolar crest was observed at 14d after compression, however, the number of osteoclasts decreased, bone resorption weakened and reparative change as new bone addition was observed.

The destructive and reparative changes in the Group N and Group DM at the col were generally consistent with previous studies of food impaction or mechanical compression[28,30,43,44]. Sawakuma[29] used germ-free rats and compared destructive processes of alveolar bone between two groups of 100-150 mm and 250-300 mm   grinding to contact point of M1 and M2. Group with wider interdental space (250-300 mm) showed marked hyaline necrosis at the col and intense vertical bone defect at the lower portion of alveolar bone, and the alveolar crest was finally isolated free. These findings were compatible with Matsuura's experimental report on food impaction [27]. Group with narrower interdental space (100-150 mm) showed gradual horizontal bone defect. The findings with wider space were speculated to occur by the stronger compression, which force prevented the appearance of osteoclast at the alveolar crest and induced the osteoclastic differentiation at the middle-lower portion avoiding the direct effect of strong compression. The result of the present study was similar to the finding of Group with wider space, suggesting the existence of strong compression at the col. Reparative changes after 7d, including regeneration of epithelium, decrease of inflammatory infiltrates and osteoclasts, and addition of new bone, were also consistent with previous reports on food impaction or mechanical compression. The sequential destructive to reparative changes at the col and alveolar bone were tissue reaction specific to injury by mechanical compression, in which tissue pressure and damages tended to be quenched with time.

As differences between Group N and Group DM, more intense tissue damages occurred and reparative changes were weaker in Group DM. Ulceration and large amount of bacterial deposits existed and lasted long at the col, sequester was formed at the alveolar crest surrounded by intense inflammatory infiltrates and damage in periodontal ligament. Very few reports on food impaction at diabetic states were known, and similar destructive changes were not reported in the previous reports on food impactions at diabetic and healthy animals. Consequently the above changes were thought to be characteristic on the food impaction at diabetic state. The decrease of tissue response specific to diabetic state, such as retardation of wound healing and bone recovery, and metabolic changes of osteoblasts and osteoclasts was consistently preserved at the tissue injury by food impaction

and mechanical compression in the present study, and consider to be specific injurious change caused by systemic diabetic disorders. Intense compression at the col failed prompt bone resorption at the alveolar crest, subsequently the aveolar crest exposed from ulcer surface, inducing bacterial infection to the crest. Consequently sequester was thought to be formed at the crest.

The measurement of Osteoclasts

Based upon the finding that bone resorption differed in interalveolar septum between Group N and Group DM, the measurement and statistical analysis of osteoclasts were performed to examine the effect of diabetic state on the behavior of osteoclast and bone resorption. The total number of osteoclasts was maximal at 1d in Group N and at 3d in Group DM, suggesting slight retardation of bone resorption in Group DM. Group N received earlier bone resorption and subsequent reparative change. These findings were consistent with light microscopic findings. The number of osteoclasts at upper portion of alveolar septum was significantly larger in Group N, which suggested retardation of bone resorption in Group DM probably due to the lowering of bone resorption to injury. This lower tissue reactivity would induce the isolation and sequester formation at alveolar crest. Numerous osteoclasts appeared at 1d in Group N, and osteoclasts reduced to minimal level at 7d, suggesting that bone resorption made completion at short time. On the contrary, osteoclasts appeared more slowly in Group DM and bone resorption continued to be active at 7d, showing the retardation of bone resorption. In other words, adaptation to compression was retarded in Group DM, resulting in long-lasting disturbance by tissue compression in the col, alveolar bone, and periodontal ligament.

Kaneko et al.[45] reported both morphological and functional changes of the osteoclasts in streptozotocin-induced diabetic rat. Kobayashi[8] made ultrastructural examination on healing of bloodless femur fracture on STZ-induced diabetic rat, and speculated that retardation of healing would be due to metabolic disturbance of bone, such as ultrastructural change of osteoblasts and osteoclasts and following disturbance of extracellular matrix production, calcification or remodeling. Summing up these discussions, tissue reaction to compression at the col was retarded in diabetic state than in control, and concurrently resorption of alveolar bone was also retarded in relation to functional impairment of osteoclasts. Bacterial infection doubled the number of tumor necrosis factor-a-expressing cells and increased apoptotic cells adjacent to bone 10-fold, and subsequently the number of osteoclasts was reduced drastically.

The decrease of migrating and phagocytic activity of leukocytes in diabetic patients, and the abnormality of migration of polymorphonuclear leukocyte or macrophages in an experimental study[46] were reported. Nishikata[7] reported that the retarding tissue repair was due to the decrease of tissue reactivity, and the exudates

and the amount of granulation tissue formation was scant, suggesting the effect of diabetic state on inflammatory and reparatory.

In this study, large amount of bacterial deposits and sequester formation at the alveolar crest were characteristic changes specific to Group DM. On the contrary, sequester was not formed throughout the whole period, although the alveolar crest was in close proximity with ulcer surface at the early phase. Sequester of Group DM was heavily infected with bacterial colonies, and consequently could hardly be resorbed and isolated by surrounding inflamed granulation tissue, bringing persistent inflammation, and impaired healing[12,47,48]. Subsequently experiment 2 was carried out to examine the relation between sequester formation and large amount of bacterial deposition, where the effect of disinfection to the gingival col was challenged. Consequently, sequester was not formed at 3 d and 5 d, at which days sequester formation was evident at Group DM without disinfection. Although ulceration at the col and slight resorption of alveolar crest and lowering in the height of alveolar bone suggested injurious influence of mechanical compression, inflammatory infiltrates were slight and confined to the ulcer surface, the granulation tissue was formed under the ulcer surface, and specific changes such as the exposure of alveolar crest and the formation of sequester were not observed. Namely, disinfection improved destructive changes specific to diabetic state, suggesting the improvement in the severity of bacterial infection was related to the rescue from tissue destruction specific to diabetic animal. Massive bacterial deposits tended to occur at the col, and could be a major factor inducing intense destructive changes including sequester formation. Exposure of alveolar crest and subsequent sequester formation would be induced by the retarded bone resorption at the alveolar crest as a principal factor. The formation of dental plaque is associated with saliva, namely secretion rate, buffer capacity or antibacterial effects. Diabetic state is known to reduce saliva secretion[49,50], and similar phenomenon would occur and contribute to massive bacterial deposits at the col in the present study. The effect of diabetic state to salivary function, including self-detergent capacity, buffer capacity or antibacterial effects, should be clarified hereafter.

The present study showed clinical disinfection and elaborate plaque control at the initial stage of food impaction was important to reduce destructive changes by food impaction (mechanical compression) at diabetic state. Local administration of antibacterial agent was considered to be effective in the treatment of periodontal diseases under diabetic condition[51,52]. Consequently oral care and early treatment considering food impaction, biological state of the gingival col would be required to maintain and improve oral health at diabetic patients.

References

1. Akiyoshi M. Periodontal diseases. In: Oral Pathology I, edited by Ishikawa G and Akiyosi M. Kyoto: Nagasue Kyoto Press, 1989
2. Rosen RG and Enquist IF. The healing wound in experimental diabetes. Surgery 50: 525-528, 1961
3. Abbey LM, Cohen M and Shklar G. The effect of streptozotocin-induced diabetes on the healing of artificially produced tongue wounds in rats. Oral Surg Oral Med Oral Pathol 33: 672-683, 1972
4. Prakash A, Kapur M and Maini BS. Wound healing in experimental diabetes: histological, histochemical and biochemical studies. Indian J Med Res 61: 1200-1206, 1973
5. Prakash A, Pandit PN and Sharman LK. Studies in wound healing in experimental diabetes. Int Surg 59: 25-28, 1974
6. Goodson WH and Hung TK. Studies of wound healing in experimental diabetes mellitus. J Surg Res 22: 221-227, 1977
7. Nishikata S. Experimental studies on the adaptability of inflammatory response in diabetic rats induced by streptozotosin. J Jpn Stomatol Soc 38: 550-563, 1989
8. Kobayashi T. Morphological studies on the healing process of fracture in rats with diabetes mellitus induced by streptozotocin. Jpn J Oral Biol 32: 600-644, 1990
9. Oda M. Morphological Studies on Bone Metabolism in Streptozotocin induced Diabetic Rats. Jpn J Oral Biol 35: 157-185, 1993
10. Lassila V and Virtanen P. Impact of streptozotocin-induced diabetes on rat blood and alveolar bone affected by occlusal stress. Scand J Dent Res 97: 216-221, 1989
11. Giglio MJ, Giannunzio G, Olmedo D and Guglielmotti MB. Histomorphometric study of bone healing around laminar implants in experimental diabetes. Implant Dent 9: 143-149, 2000
12. deMolon RS, Morais-Camilo JA, Verzola MH, Faeda RS, Pepato MT and Marcantonio E Jr. Impact of diabetes mellitus and metabolic control on bone healing around osseointegrated implants: Removal torque and histomorphometric analysis in rats. Clin Oral Implants Res 24: 831-837, 2013
13. Tervonen T and Karjalainen K. Periodontal disease related to diabetic status. A pilot study of the response to periodontal therapy in type 1 diabetes. J Clin Periodontol 24: 505-510, 1997
14. Nishimura F, Kono T, Fujimoto C, Iwamoto Y and Murayama Y. Negative effects of chronic inflammatory periodontal disease on diabetes mellitus. J Int Acad Periodontol 2: 49-55, 2000
15. Nelson RG, Shlossman M, Budding LM, Pettitt DJ, Saad MF, Genco RJ and Knowler WC. Periodontal disease and NIDDM in Pima Indians. Diabetes Care 13: 836-840, 1990

16. Al-Shammari KF, Al-Ansari JM, Moussa NM, Ben-Nakhi A, Al-Arouj M and Wang HL. Association of periodontal disease severity with diabetes duration and diabetic complications in patients with type 1 diabetes mellitus. J Int Acad Periodontol 8: 109-114, 2006

17. Cerda J, Vázquez de la Torre C, Malacara JM and Nava LE. Periodontal disease in non-insulin dependent diabetes mellitus (NIDDM). The effect of age and time since diagnosis. J Periodontol 65: 991-995, 1994

18. Firatli E. The relationship between clinical periodontal status and insulin-dependent diabetes mellitus. Results after 5 years. J Periodontol 68: 136-140, 1997

19. Taylor GW, Burt BA, Becker MP, Genco RJ, Shlossman M, Knowler WC and Pettitt DJ. Non-insulin dependent diabetes mellitus and alveolar bone loss progression over 2 years. J Periodontol 69: 76-83, 1998

20. Taylor GW, Burt BA, Becker MP, Genco RJ and Shlossman M. Glycemic control and alveolar bone loss progression in type 2 diabetes. Ann Periodontol 3: 30-39, 1998

21. Tsai C, Hayes C and Taylor GW. Glycemic control of type 2 diabetes and severe periodontal disease in the US adult population. Commun Dent Oral Epidemiol 30: 182-192, 2002

22. Tervonen T, Karjalainen K, Knuuttila M and Huumonen S. Alveolar bone loss in type 1 diabetic subjects. J Clin Periodontol 27: 567-571, 2000

23. Seppälä B, Seppälä M and Ainamo J. A longitudinal study on insulin-dependent diabetes mellitus and periodontal disease. J Clin Periodontol 20: 161-165, 1993

24. Engebretson SP, Hey-Hadavi J, Ehrhardt FJ, Hsu D, Celenti RS, Grbic JT and Lamster IB. Gingival crevicular fluid levels of interleukin-1 beta and glycemic control in patients with chronic periodontitis and type 2 diabetes. J Periodontol 75: 1203-1208, 2004

25. Khader YS, Dauod AS, El-Qaderi SS, Alkafajei A and Batayha WQ. Periodontal status of diabetics compared with nondiabetics: a meta-analysis. J Diab Compl 20: 59-68, 2006

26. Meenawat A, Punn K, Srivastava V, Meenawat AS, Dolas RS and Govila V. Periodontal disease and type I diabetes mellitus: Associations with glycemic control and complications. J Indian Soc Periodontol 17: 597-600, 2013

27. Matsuura T. Histopathological and scanning electron microscopical studies on the change of the periodontal tissue after food impaction in rat molar teeth. Kyushu Shika Gakkai Zasshi 42: 733-752, 1988

28. Osaki H. Changes of periodontal tissues during mechanical impaction and removal of the impacted materials in the interdental papilla of the rat molars. J Fukuoka Dent Coll 19: 147-170, 1992

29. Sawakuma B. Experimental pathological study on changes in the periodontal tissue after food impaction with interproximal space in rat molar teeth. J Fukuoka Dent Coll 31: 71-92, 2004

30. Morooka M. Effect of occlusal trauma on periodontal tissue in streptozotocin-induced diabetic rat. J Fukuoka Dent Coll 25: 283-302, 1998.

31. Prichard JF. A technique for treating infrabony pockets based on alveolar process morphology. Dent Clin North Am 4: 85-105, 1960

32. Larato DC. Relationship of food impaction to interproximal intrabony lesions. J Periodontol 42: 237-238, 1971

33. Carranza FA Jr, Simes RJ, Mayo J and Cabrini RL. Histometric evaluation of periodontal bone loss in rats. I. The effect of marginal irritation, systemic irradiation and trauma from occlusion. J Periodontal Res 6: 65-72, 1971

34. Abe K, Kobayashi S, Asano K, Kato H and Ishikawa J. Clinical study of local factors causing vertical bone resorption. J Jpn Soc Periodontol 17: 274-279, 1975

35. Irokawa T, Ishida E, Fukuoka Y, Maita E and Yanagawa S. A study on the effects of continuous mechanical irritation to the periodontal tissues of germfree and conventional rats. J Jpn Soc Periodontol 19: 333-340, 1977.

36. Yamada S and Sato T. Histopathological study of the food impaction in rats. J Jpn Soc Periodontol 20: 234-245, 1978

37. Schroeder HE and Lindhe J. Conditions and pathological features of rapidly destructive, experimental periodontitis in dogs. J Periodontol 51: 6-19, 1980

38. Wada H. Histopathological study on the experimental formation of the food impaction in rat. J Jpn Soc Periodontol 22: 319-340, 1980

39. Inoue M, Shiozawa I, Hashimoto Y and Matsui Y. Periodontal Tissue Destruction by Experimentally Induced Food Impaction. Nihon Hotetsu Shika Gakkai Zasshi 26: 849-855, 1982

40. Junod A, Lambert AE, Orci L, Pictet R, Gonet AE and Renold AE. Studies of the diabetogenic action of streptozotocin. Proc Soc Exp Biol Med 126: 201-205, 1967

41. Junod A, Lambert AE, Stauffacher W and Renold AE. Diabetogenic action of streptozotocin: relationship of dose to metabolic response. J Clin Invest 48: 2129-2139, 1969

42. Rerup CC. Drugs producing diabetes through damage of the insulin secreting cells. Pharmacol Rev 22: 485-518, 1970

43. Sasaki S. The effect of topical stimulation on periodontal structure in streptozotocin induced diabetic rats. 1. The experiment in conventional rats [in Japanese]. J Jpn Soc Periodontol 30: 399-413, 1988

44. Sasaki S. The effect of topical stimulation on periodontal structure in streptozotocin induced diabetic rats. 2. The experiment in germfree rats. J Jpn Soc Periodontol 30: 414-427, 1988

45. Kaneko H, Sasaki T, Saitoh T and Higashi S. Ultrastructural studies of osteoclast in rats. Showa Shigakkai Zasshi 10: 216-227, 1990

46. Tsukamoto Y, Takayama A, Mori C, Morikawa Y and Fujimoto H. Abnormal chemotaxis of polymorphonuclear leucocytes and macrophages in rats with experimentally induced diabetes. J Oral Biosci 28: 132-142, 1986

47. Albrecht M, Banoczy J and Tamas G Jr. Dental and oral symptoms of diabetes mellitus. Commun Dent Oral Epidemiol 16: 378-380, 1988

48. de Oliveira Diniz CK, Correa MG, Casati MZ, Nociti FH Jr, Ruiz KG, Bovi Ambrosano GM and Sallum EA. Diabetes mellitus may increase bone loss after occlusal trauma and experimental periodontitis. J Periodontol 83: 1297-1303, 2012

49. Siudikiene J, Machiulskiene V, Nyvad B, Tenovuo J and Nedzelskiene I. Dental caries and salivary status in children with type 1 diabetes mellitus, related to the metabolic control of the disease. Eur J Oral Sci 114: 8-14, 2006

50. Romero AC, Ibuki FK and Nogueira FN. Sialic acid reduction in the saliva of streptozotocin induced diabetic rats. Arch Oral Biol 57: 1189-1193, 2012

51. Iwamoto Y, Nishimura F, Nakagawa M, Sugimoto H, Shikata K, Makino H, Fukuda T, Tsuji T, Iwamoto M and Murayama Y. The effect of antimicrobial periodontal treatment on circulating tumor necrosis factor-alpha and glycated hemoglobin level in patients with type 2 diabetes. J Periodontol 72: 774-778, 2001

52. Martorelli de Lima AF, Cury CC, Palioto DB, Duro AM, da Silva RC and Wolff LF. Therapy with adjunctive doxycycline local delivery in patients with type 1 diabetes mellitus and periodontitis. J Clin Periodontol 31: 648-653, 2004