Journal of Hard Tissue Biology 24[4] (2015) 357-374

CODEN-JHTBFF, ISSN 1341-7649

Original

The Effect of Insulin Administration and Antibacterial Irrigation with Chlorhexidine Gluconate on the Disturbance of Periodontal Tissue Caused by Food Impaction in Streptozotocin-Induced Diabetic Rats

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(Accepted for publication July 6, 2015)

Abstract:This histopathological study used streptozotocin (STZ)-induced diabetic rats to investigate insulin administration in conjunction with an antibacterial irrigation of chlorhexidine gluconate solution and its effect on the disturbance of periodontal tissue and destruction of alveolar bone induced by mechanical compression at the col due to food impaction. The experimental animals were divided into normal rats (Group N) and STZ-induced diabetes mellitus (DM) rats. Mechanical compression was done by insertion of a gutta-percha point (GP). The diabetic rats were further divided into Group (DM+Ins), in which insulin was administered, and into Group (DM+Chlo), in which 0.2% chlorhexidine gluconate solution was used for bactericidal irrigation. Histological comparisons of the destructive process and reparative changes of the periodontal tissue were performed at 1, 3, 7 and 14 days after the col compression.

The antibacterial irrigation effects caused by chlorhexidine gluconate solution were considered to be low during the early stages. However, since there was no sequestrum formation at the alveolar crest at 7 days after the col compression, reparative changes of periodontal tissue were able to progress. These results suggest that there was a constant antibacterial effect after the chlorhexidine gluconate solution irrigation. Even so, the use of only chlorhexidine gluconate solution in cases with very destructive changes or in cases with persistent mechanical compression, made it difficult to perform a successful treatment of the disturbance of the periodontal tissue. Results for Group (DM+Ins) were better than those for Group (DM+Chlo) with regard to the inhibition of bacterial contaminant at the food impaction area, alleviation, and improvement of the healing process after disturbance in the periodontal tissue. The persistent use of 0.2 % chlorhexidine gluconate solution did appear to lead to a constant effect on the bacteria. Therefore, when treating a disturbance of the periodontal tissue due to food impaction in the diabetic state, the first step should include the administration of insulin in order to improve the diabetic state.

Key words: Streptozotocin-induced diabetic rat, Gingival col, Mechanical compression, CPeriodontal tissue, Insulin administration, Chlorhexidine gluconate

Introduction

Food impaction that is often encountered clinically is one of the local factors responsible for the disturbance of the periodontal tissue. Textbooks have clinicopathologically described the sensation of the pressure of the interdental region as a dull pain, an aching over the deep portion of the jaw, periodontitis with bleeding and unusual tastes in the localized area, premature contact and hypersensitivity on percussion, gingival recession, formation of periodontal abscess, inflammation of the periodontal ligament within the alveolar socket, destruction of alveolar bone, and the induction of root surface caries, in addition to perpendicular resorption that accompanies the destruction of the alveolar bone[1-3)]. Various animals have been used in experimental and pathological studies designed to examine periodontal tissue disturbances[4-18)]. Results have shown that food impaction and mechanical compression at the col of the periodontal tissue tends to cause hyalinization necrosis, ulcer formation, inflammatory cell infiltration, and perpendicular bone resorption of the alveolar bone during the early stages at the col of the periodontal tissue[8,17-18)]. Moreover, when there was strong pressure at the col region, results showed there were characteristic destruction stages of the alveolar bone, such as marked bone resorption in the lower portion of the alveolar bone crest and rapid reduction of the alveolar bone crest[8,17-18)].

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Other clinical and basic studies have investigated the role of diabetes, which is one of the general factors of periodontal disease. These studies examined the intimate relationship between diabetes and the healing that occurs after wounds, extraction and fracture, depression of the defense function and healing delays after tissue attacks as compared to that seen in non-diabetic subjects[19-29]. Further studies have investigated the connection between diabetes and periodontal disease and reported that diabetic patients exhibited a higher incidence of periodontal disease acceleration and alveolar bone resorption as compared to non-diabetic subjects[30-43]. These studies also clarified the effects of the diabetic state on the destruction and recovery of periodontal tissue, the induction of periodontal disease, and the associated level of severity, in addition to trying to determine if poor control of blood sugar was related to the periodontal disease severity rate[30-43]. One other experimental and pathological study[44] found that traumatic occlusion in the diabetic state caused a delay in alveolar bone resorption at the compressed side as compared with the normal state, and thus, the periodontal ligament was intensely disturbed due to the persistent constriction of the periodontal ligament. Sasaki et al.[45,46] used a diabetic rat model and further showed that both normal feeding and aseptic feeding of diabetic rats led to more intense inflammatory changes along with intense movement of the bottom of the pocket of the root apex side, greater amounts of resorption of the alveolar bone, and marked destruction of periodontal tissue, as compared to the non-diabetic rat. In addition, they determined that the resistance of the periodontal tissue was poor in the diabetic state. Tokunaga et al.[47] examined the gingival col of streptozotocin (STZ) -induced diabetic rats and found that mechanical compression induced hyalinization, necrosis, inflammatory cell infiltrates and ulcer formation at the col during the early stages. They also described finding characteristic and massive bacterial deposits, delays in bone resorption at the alveolar crest, and sequester separation. Their study also indicated that exposure of alveolar bone to the ulcerated region induced the formation over time, with intense destructive changes of the periodontal tissue occurring at the lower portion of sequester in the diabetic state that induced a marked reduction in the alveolar crest in conjunction with the sequester separation. These findings indicate that the diabetic state was related to the intense destruction of the periodontal tissue and the adhesion of large amounts of bacteria, which ultimately led to a delay in the repair process. Tokunaga's study also demonstrated that irrigation with 3 % H_2O_2 (oxydol) antibacterial solution along with the application of Periocline antibacterial ointment to the bacterial adhesion at the col led to an extreme reduction in the massive bacterial deposits without sequester at the alveolar crest. This finding showed that large amounts of bacterial deposits associated with food impaction in the diabetic state are a major factor in the marked disturbance of the periodontal tissue.

Clinical practices attempt to control the diabetic state in diabetic patients, particularly type I diabetic mellitus, by administering insulin in order to maintain blood sugar values within normal ranges. However, there have only been a few studies performed that tried to examine and clarify the yet unknown effects of insulin administration on the destructive and reparative processes that occur in the periodontal tissue. A mechanical compressive experiment at the col in the diabetic state performed periodontal treatments that used chlorhexidine gluconate solution to irrigate areas with abundant and massive bacterial deposits and sequestrum formation. Therefore, chlorhexidine gluconate solution is an interesting agent that appears to lead to improvements of the tissue.

Similar to other experiments, our present histopathological study also used STZ-induced diabetic rats. After mechanical compression in the gingival col, we attempted to elucidate the effects on the destruction and repair of the periodontal tissue. The specific aim of this study was to examine the administration of insulin used to control diabetes and determine if the diabetic state was related to the bactericidal action at the col that occurs after an antibacterial irrigation with chlorhexidine hydrochloride gluconate solution.

Materials and Methods

Animal experiments were approved and performed in accordance with the guidelines of the Fukuoka Dental College Experimental Animal Committee. A total of 65 male Sprague Dawley (SD) rats that were 5 weeks of age were divided into a (Group N): non diabetic mellitus of 15 rats and a diabetes mellitus (Group DM) diabetic mellitus of 50 rats. For the preparation of a type I diabetic model rat, 125 mg/kg of STZ (Sigma, MO, USA) was injected intraperitoneally, with the diabetic state (values above + 3 and above 500 mg/dl of urine sugar) confirmed by Tes-Tape (Uriace Ga, Terumo, Co., Ltd., Tokyo, Japan) for DM examination. For the food impaction model, a gutta-percha point (GP #30) was inserted into the gingival col between the left upper 1st (M_1) and 2nd (M_2) molars of the Group DM and Group N rats. In the subsequent step, the Group DM rats were further divided into an insulin (Ins) administration group (Group DM+Ins) of 25 rats and an irrigation group that was given 0.2 % chlorhexidine gluconate solution (Chlo; Dainihon Sumitomo Pharmaceutical Co., Ltd., Japan) (Group DM+Chlo) of 25 rats at the col. After insertion of the GP at the col in Group DM+Ins, all rats then received an intramuscular injection of NPH insulin 5 IU (Novo Nordisk Pharma. Co., Ltd., Japan) with persistence for 24 hours every morning during the entire experimental period. Improvement of the diabetic state after the STZ administration was confirmed by a negative diabetic Tes-Tape evaluation. Gross observations performed after the insulin administration revealed that while the rats had a slightly smaller size, they had a good coat of fur that

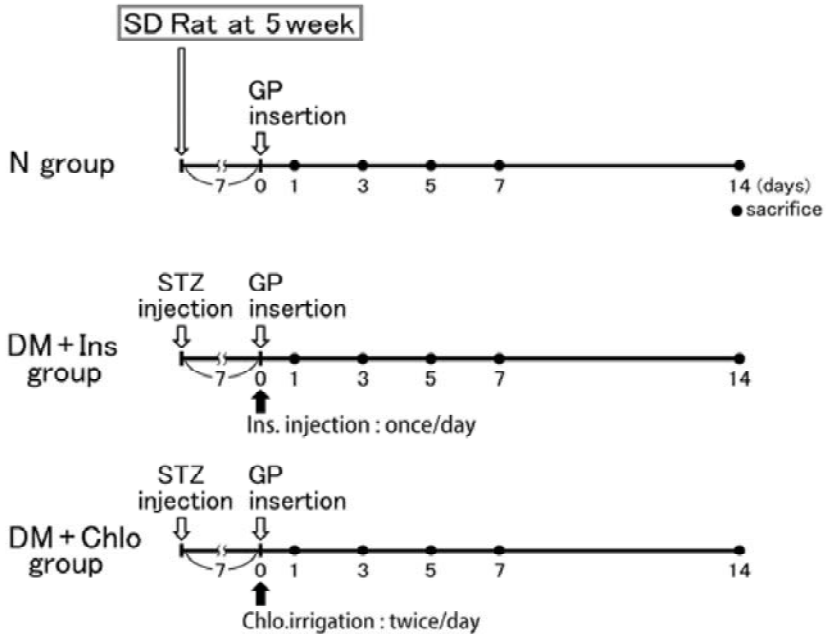

Figure1. Experimental schedule.

was the same as that observed in the Group N rats and exhibited no pathological conditions during the experimental period.

In the Group (DM+Chlo) rats, after GP insertion at the col, a 1 ml syringe that contained 2 ml of 0.2 % Chlorhexidine gluconate solution was used to wash the col twice a day (morning and evening) during the entire experimental period. After the insertion of a cotton block that was placed to prevent the solution from flowing into the throat during the treatment, the col with the GP was washed with a strong stream of the solution. Gross observations of the animals found they had a slightly smaller size with a dull coat of fur. A larger body size was observed in the Group N rats as compared to the animals in the other groups and these rats all exhibited a better coat of fur (Fig. 1). In Group N, there were no treatments done during the entire experimental period, with the animals only undergoing an insertion of a GP at the col. Periodontal tissue of the upper right molars was examined for the control of Group N, Group (DM+Ins) and Group (DM+Chlo) animals. In line with the other previous studies, all animals were examined at 1, 3, 5, 7 and 14 days after the mechanical compression similary to the other previous studies. All animals had free access to solid food and water throughout the duration of the experiment.

After the animals were sacrificed by deep general anesthesia, the maxilla was excised, immersed in 10 % formalin solution for 1 week, decalcified with 10 % formic acid for 3–4 weeks, dehydrated, and embedded in paraffin. The serial mesiodistal sections were prepared for hematoxylin and eosin staining, with the sections from Group N, Group (DM+Ins), and Group (DM+Chlo) histopathologically compared. Some specimens were smeared for Gram staining in order to examine the adhesion of the bacteria and the invasion state at the col. Specimens were prepared using a standard method of Gram staining, with each specimen smeared using Hucker's staining solution followed by Lugol's solution. After discoloration and staining by safranin solution, microscopic examinations were performed.

Results

Control group (Fig. 2a-c; Fig. 3a-c)

Group N, Group DM+Ins and Group DM+Chlo exhibited almost the same findings from 1 to 14 days after the compression at the col. There were no pathological conditions such as inflammation of the periodontal tissue and gingival pocket formation found in any of the groups, there was no food impaction or marked adhesion of bacteria at the col, and all of the gingiva showed sound papilla. The superficial surface zone was covered by stratified squamous epithelium. Gingival epithelial attachment was located at the cementoenamel junction. Inflammatory cell infiltrates were not observed in the lamina propria beneath the epithelium, the horizontal ligament between M_1 and M_2 was mesiodistally developed, and the connective tissue fiber showed an orderly arrangement.

Alveolar bone exhibited a slightly round shape at the alveolar crest, and there was very little bone resorption observed. While osteoclasts and a small amount of diffuse bone resorption were seen in the middle-upper portion of the interalveolar septum at M_2, no marked bone resorption was found. Examination of the physiological distal movement of M_1 indicated that the periodontal ligament space was slightly thinner on the mesial versus the distal side of the interalveolar septum, and the periodontal ligament fiber showed a sound state with an orderly arrangement. Thickening of

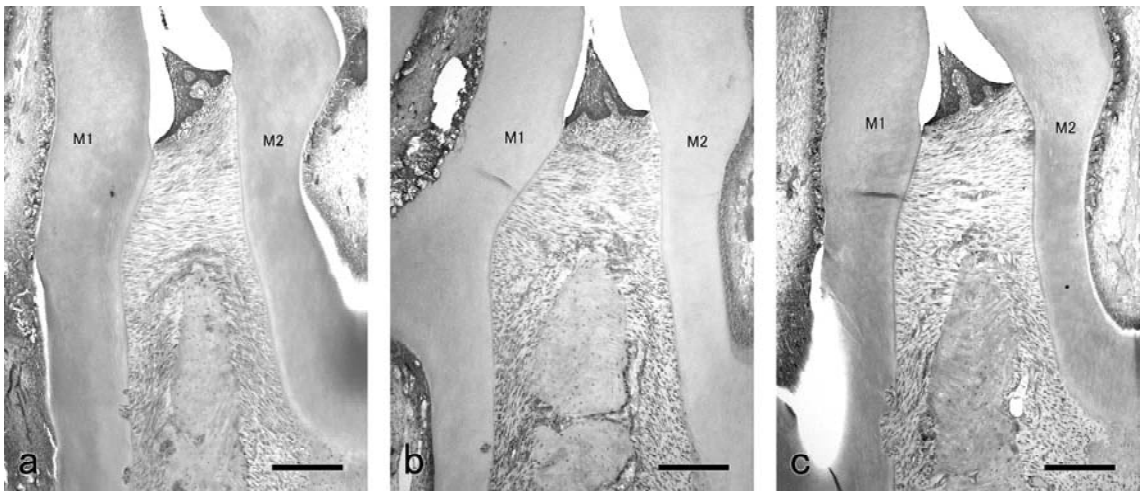

Figure 2. 1 day after treatment of non GP insertion group. a: Group N; b: Group (DM+Ins); c: Group (DM+Chlo); a~c: The col area shows healthy state without inflammation in periodontal tissue. H&E; Scale bars =200 μm.

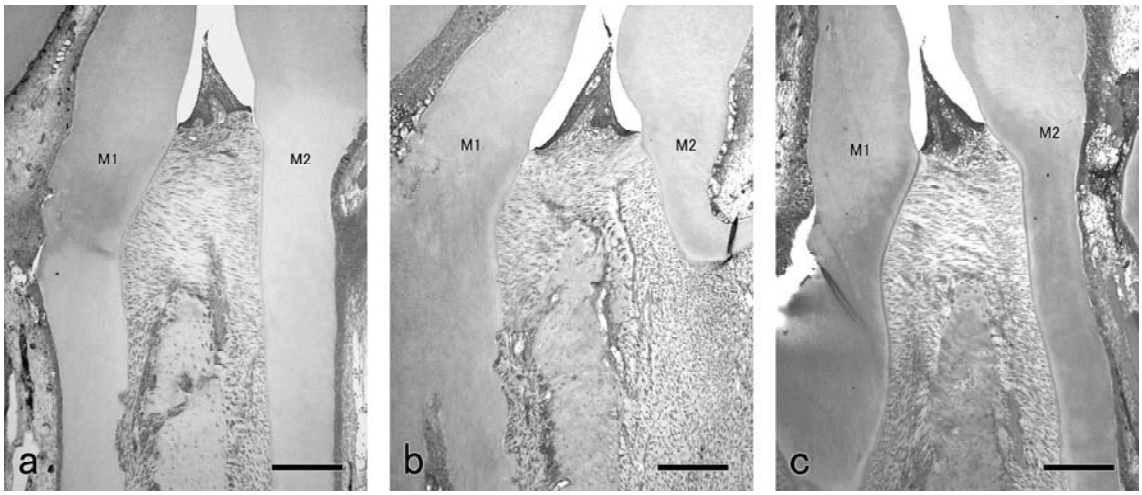

Figure 3. 7 days after treatment of non GP insertion group. a: Group N; b: Group (DM+Ins); c: Group (DM+Chlo); a~c: The col area shows healthy stategr without inflammation in periodontal tissue. H&E; Scale bars=200 μm.

the epithelial covering of the gingiva was seen over time, with some of the epithelial projections due to lateral proliferation of the epithelium at 14 days after the operation in Group N. However, there were no differences noted between Group N, Group DM+Ins and Group DM+Chlo.

Experimental groups (compression experiment with GP at the col region)

During this experiment, the GP was always retained at the col region. Histopathological sections confirmed the presence of a round GP.

Group N

Gross observation indicated that the insertion of the GP at the col was maintained. Although the gingival recession due to GP compression was seen around the col, comparatively healthy colored gingiva was seen; without inflammatory findings such as marked redness, swelling or easy bleeding. At 1 day after compression at the col region in Group N, the col region was found to be concave. The epithelium that covered the surface was destroyed. The connective tissue was strongly compressed. The horizontal interalveolar septum showed obscurity, the portion with partially slight hyaline degeneration and the portion with slight compression. There was no bone resorption by osteoclasts seen at the alveolar crest in the interalveolar septum between M_1 and M_2. The alveolar crest exhibited a rounded shape and there was obvious remarkable bone resorption by the osteoclasts in the lower portion of the alveolar crest. The periodontal ligament was free of destructive changes. Gram staining for bacteria showed the presence of a slight bacteria at the concaved col and at the superficial layer. However, there was no obvious invasion of bacteria into the deeper tissue (Fig. 4a; Fig. 5a).

At 3 days after the compression, the epithelial covering at the

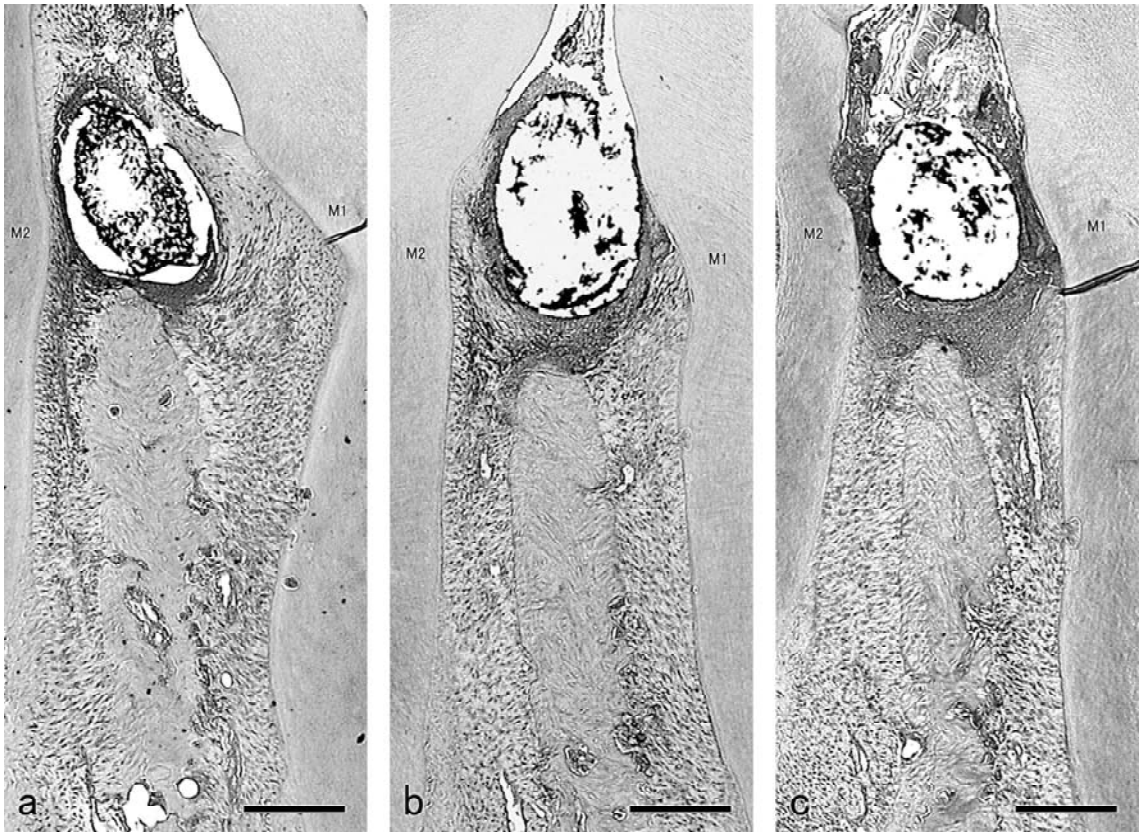

Figure 4. 1 day after col compression of the experimental group. a: Group N; b: Group (DM+Ins); c: Group (DM+Chlo); a ~ c: The col area is concave due to GP compression with ulcer formation. In both Group (DM+Ins) and Group (DM+Chlo), thick necrotic layer is formed. The inflammatory cell infiltration is present up to the alveolar crest.
H&E; Scale bars = 200μm.

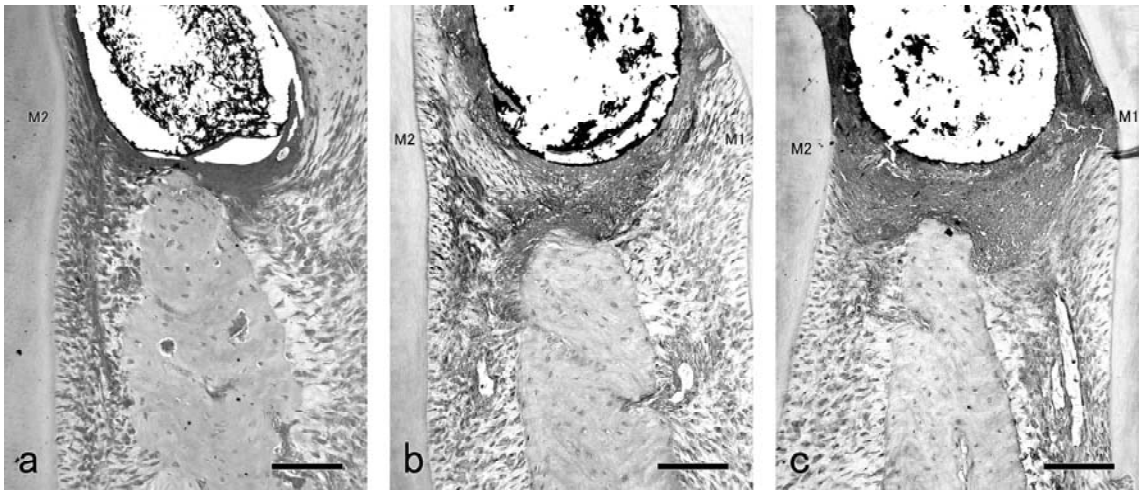

Figure 5. 1 day after col compression of the experimental group. a: Group N; b: Group (DM+Ins); c: Group (DM+Chlo); a~c: High magnification of Fig.4; H&E; Scale bars=100μm.

col and the interdental horizontal ligament of the lower portion were destroyed and had disappeared, thereby showing deep ulcer formation. The partial hyaline degeneration, necrosis and slight inflammatory cell infiltration were observed at the lower portion. The alveolar crest showed slight bone resorption without changes of periodontal ligament. Gram staining demonstrated there was a necrotic area at the col region and the presence of a few bacteria at the superficial layer of ulceration, even though there was no bacteria in the deeper tissue. These findings were the same as those seen at 1 day after the compression at the col (Fig. 6a, d;

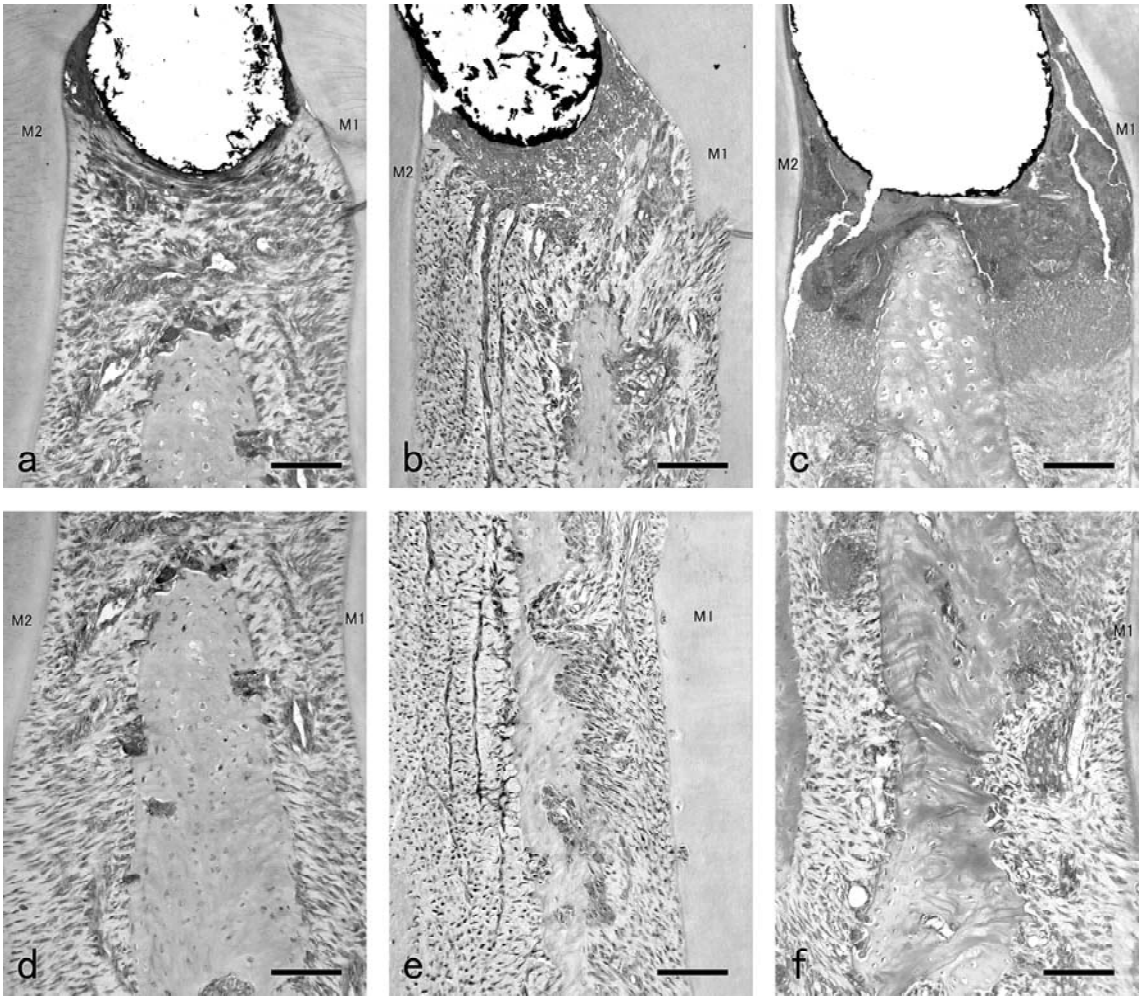

Figure 6. 3 days after col compression of the experimental group. a, d: Group N; b, e: Group (DM+Ins); c, f: Group (DM+Chlo). In Group (DM+Ins) and Group (DM+Chlo), the necrotic layer and inflammatory cell infiltration at the col are pronounced. In Group (DM+Chlo), the alveolar crest contacts with necrotic layer and inflammatory cell infiltration layer. H&E; Scale bars=100μm.

Fig. 7a).

At 5 days after the compression, the col was depressed, and the epithelial layer was distinctly seen on the superficial layer. During this period, regeneration of the epithelium occurred on the ulcer surface. The inflamed granulation tissue formed and spread to the periodontal ligament on the lower portion of the regenerated epithelium. Both the path of the connective tissue fibers and the periodontal ligament fibers were irregular. There was progression of the bone resorption in the upper portion of the alveolar bone, and there was a slight decrease of the height of the alveolar bone crest along with a narrowing of the width of the interalveolar septum. The close proximity to the ulcerated surface at alveolar crest that was observed at 3 days after the compression at the col subsequently disappeared. Bone resorption due to scattered osteoclasts was observed in the middle-lower portion of alveolar bone, which indicated there was a slightly narrower width of the entire interalveolar septum. Gram staining demonstrated that there was no bacteria in the periodontal tissue at the col.

At 7 days after the compression, the col was depressed, and there was thickening of the regenerated epithelium on the superficial layer due to stratification. Inflamed granulation tissue existed up to the lower portion of the epithelium at the alveolar crest, while the gingiva at the col was repaired. Although the alveolar crest decreased due to resorption, we observed the addition of new bone at the bone surface as compared to that seen at 5 days after the compression, in addition to a thickening of the width of the interalveolar septum. There was little bone resorption observed, with the addition of new bone found in places at locations in the middle-lower portion of the alveolar bone (Fig. 9a, d).

At 14 days after the compression, the col exhibited a slight concavity due to the compression of the GP, but regenerated epithelium covered the superficial layer. The epithelial ridge of the epithelium was irregularly elongated. The inflammatory cell infiltrates beneath the epithelium disappeared in conjunction with

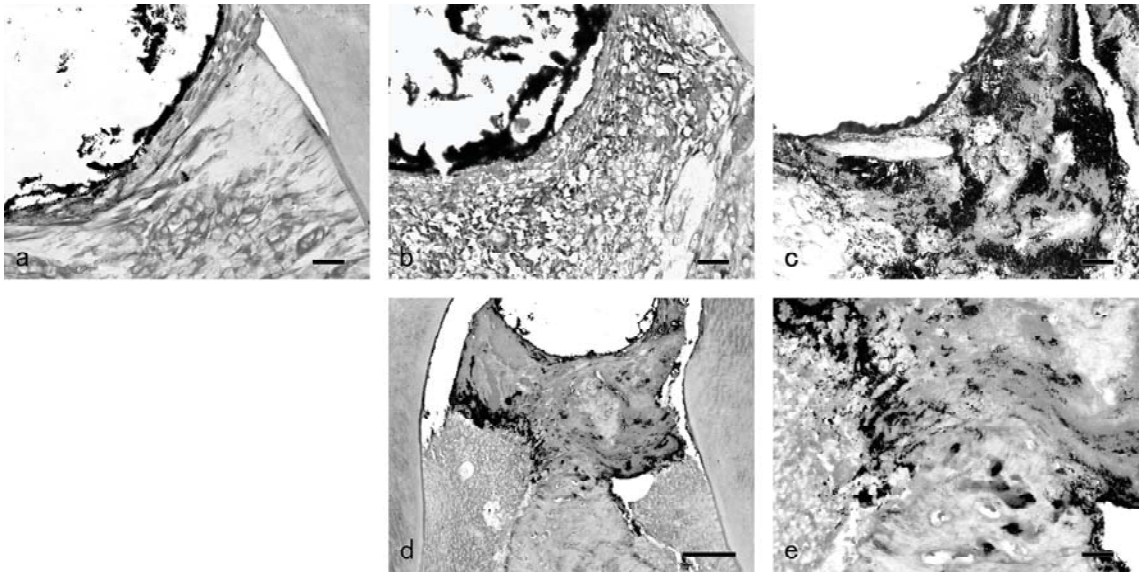

Figure 7. 3 days after col compression of the experimental group. a: Group N; b: Group (DM+Ins); c, d, e: Group (DM+Chlo). The Group N exhibits small number of bacteria, whereas in Group (DM+Ins) and Group (DM+Chlo), ulcer at the col and a large number of bacteria at the necrotic layer are observed. In particular, in Group (DM+Chlo), the alveolar crest contacts with necrotic layer and the bacteria invade into the alveolar crest. (d, e: partial high magnification of d). Gram staining; a, b, c, e Scale bars=20 μm, d . Scale bars = 100μm.

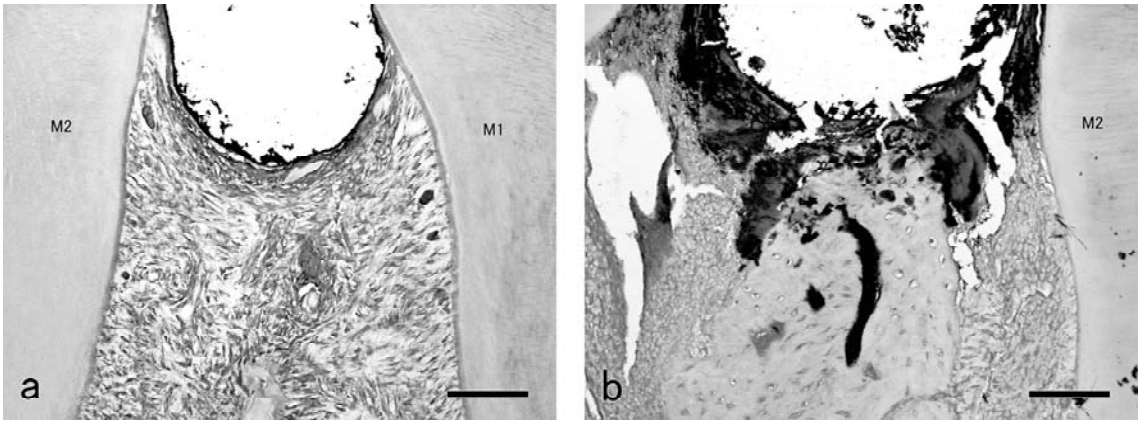

Figure 8. 5 days after col compression of the experimental group. a: Group (DM+Ins); b: Group (DM+Chlo). In Group (DM+Ins), there is a small number of bacteria at the superficial layer of the col, whereas in Group (DM+Chlo), ulcer at the col, a large number of bacteria in necrotic layer and sequestration with invasion of bacteria into alveolar crest occur. Gram staining; Scale bars =100μm.

the progression of the regeneration of the epithelium, and the connective tissue fibers became denser. The findings were consistent with the observations for the lamina propria in Group N. Although there was a slight decrease in the height of the alveolar crest, the addition of new bone was occasionally observed on the surface of the alveolar bone, and thus, the width of alveolar bone at the interalveolar septum was kept constant (Fig. 10a, d).

Group DM+Ins

Gross observations indicated that the insertion of the GP at the col was maintained. Although the gingival recession around the col was observed, inflammatory findings such as marked redness, swelling and easy bleeding were not seen; with Group N showing gingiva with comparatively healthy color.

At 1 day after compression and insulin administration, the col exhibited concavity due to the simultaneous GP compression at the col. While degeneration, necrosis and ulceration were observed at the epithelial layer at the col and the horizontal ligament between teeth, there was no marked inflammatory cell infiltration seen. The bone crest was in close proximity to the ulcer surface and there was no bone resorption by the osteoclasts. Similar to that seen in Group N, the bone crest exhibited a round contour. Osteoclasts caused bone resorption leading to an intense irregular contour in the middle-lower portion of the alveolar bone at M_1.

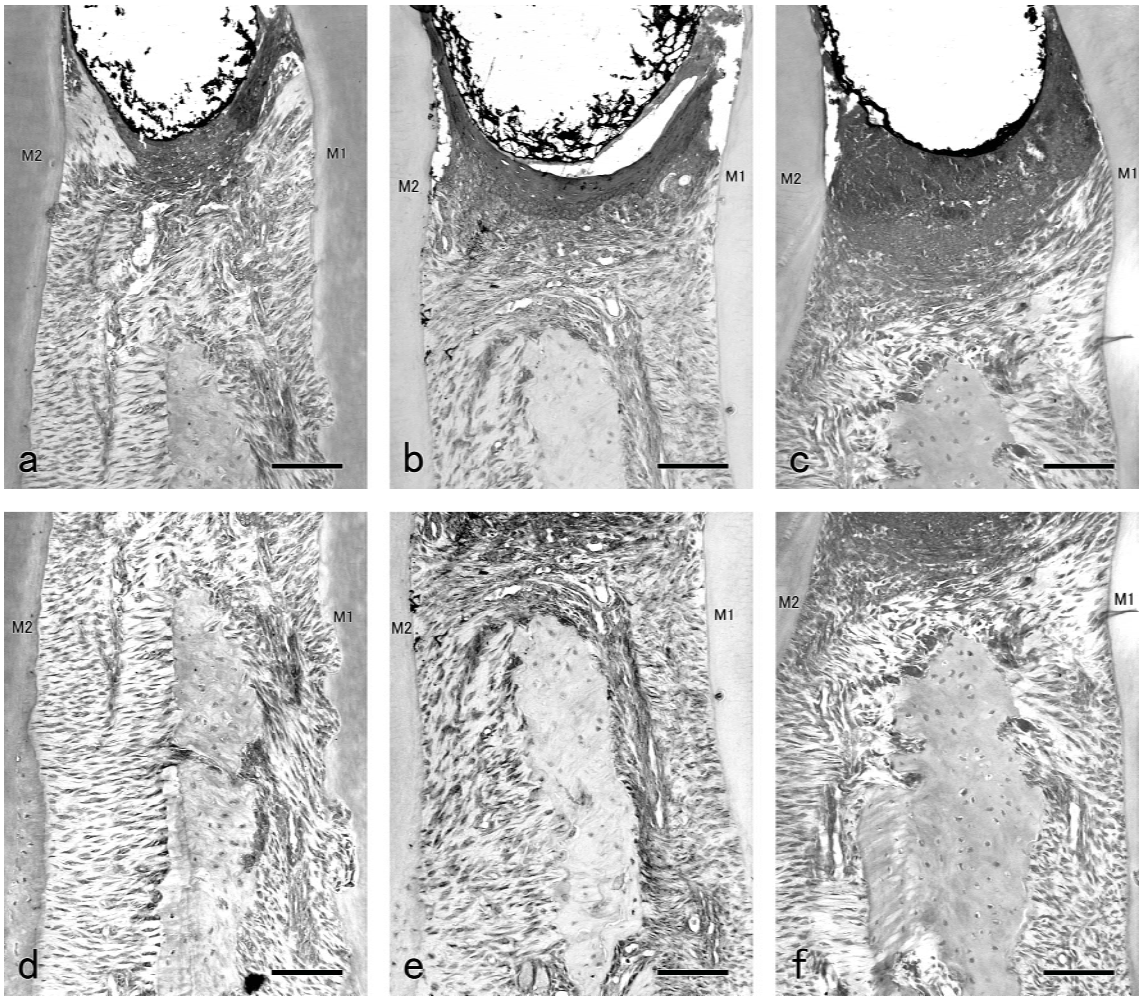

Figure 9. 7 days after col compression of the experimental group. a, d: Group N; b, e: Group (DM+Ins); c, f: Group (DM+Chlo). In Group N, Group (DM+Ins) and Group (DM+Chlo), regeneration of epithelium occurs at the col; inflammatory cell infiltration is decreased. In Group N and Group (DM+Ins), reparative changes in the lamina propria and alveolar bone as well as regeneration of epithelium occur. In Group (DM+Chlo), relatively much resorption of alveolar bone are observed. H & E; Scale bars=100μm.

Bone resorption was particularly observed at the region where there was narrowing of the periodontal ligament space. In this region, the periodontal ligament fibers were slightly indistinct. In the other regions, there was very little bone resorption observed, and the periodontal ligaments were sound. At the interdental region and the superficial layer at the col, gram staining demonstrated the presence of numerous bacteria and the bacterial mass that were scattered throughout the area. However, there was no invasion of bacteria into the alveolar crest observed (Fig. 4b; Fig. 5b).

At 3 days after the compression, the col became concave, and a degenerated necrotic layer was observed on the surface layer. In some cases a regenerating covered epithelium was also seen. Hyaline necrosis occurred, and there was an increase in the granulation tissue in the lower layer of the connective tissue. Even so, there were very few inflammatory cell infiltrates observed. Bone resorption due to numerous osteoclasts occurred at the adjacent alveolar bone crest, with an abundant irregular contour shape observed. Furthermore, there was also bone resorption due to numerous osteoclasts at various locations in the lower portion of the alveolar crest and at the middle-lower portion of M_1. An extensive irregular contour was observed in the alveolar bone due to the extensive bone resorption that occurred during this period. The periodontal ligament exhibited an appearance similar to the granulation tissue that was observed around the alveolar bone crest, with a sound state maintained in the middle-lower portion. Gram staining showed there were fewer bacteria on the necrotic and ulcerated layers of the superficial layer at the col as compared to that observed at 1 day after the compression in the col. There was no bacterial invasion into the alveolar crest found (Fig. 6b, e; Fig. 7b).

At 5 days after the compression, the GP was compressed deeply into the col, which showed an intense concave contour. During this period, at least one or more of the layers regenerated epithelium close to the GP, with granulation tissue formed under this layer. Furthermore, there was repair of the horizontal ligament between the teeth, along with recovery in the deep layer. The alveolar crest

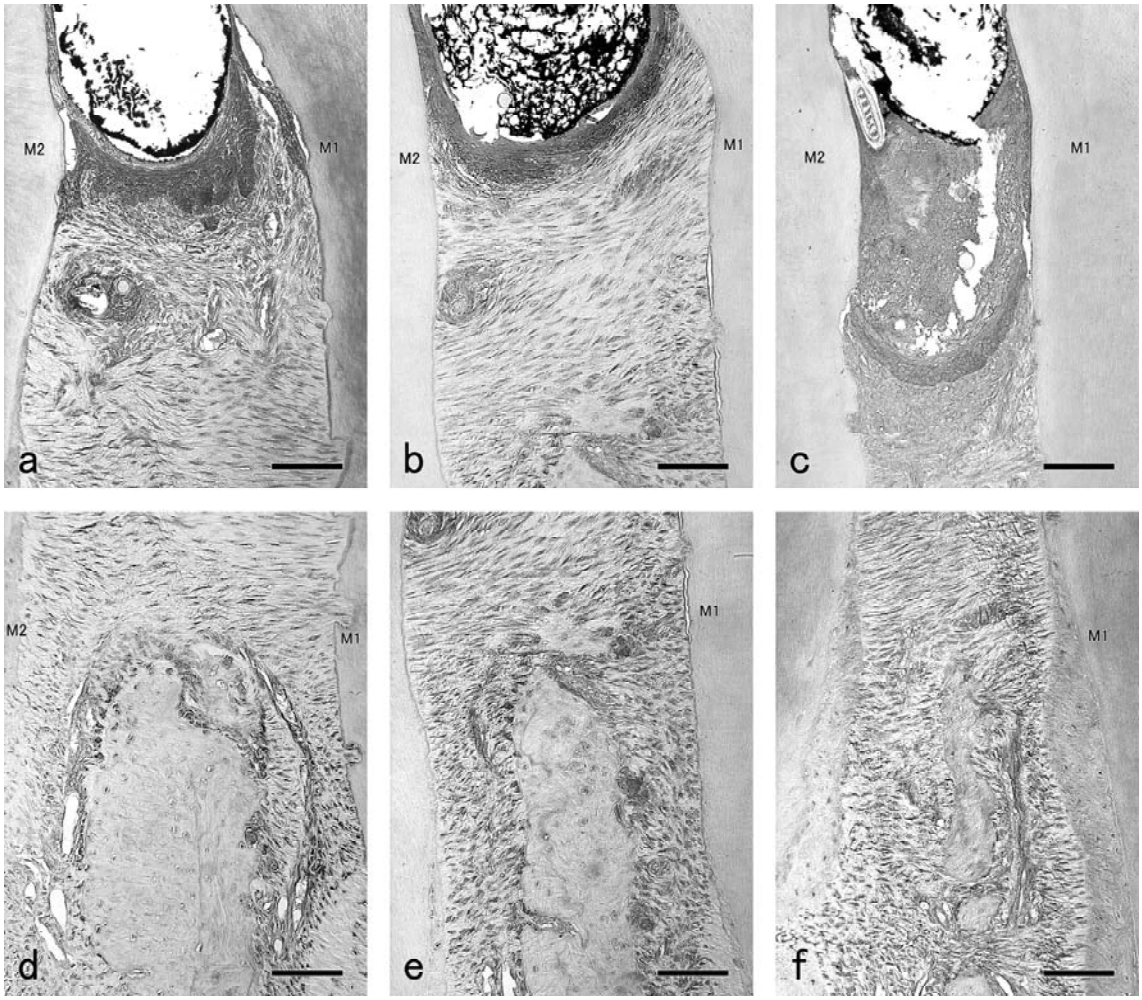

Figure 10. 14 days after col compression of the experimental group. a, d: Group N; b, e: Group (DM+Ins); c, f: Group (DM+Chlo). In Group N, Group (DM+Ins) and Group (DM+Chlo), regeneration of epithelium at the col, disappearance of inflammatory cell infiltration and progress of repair in alveolar bone are seen. The Group (DM+Chlo) exhibits more marked decrease in alveolar bone as compared with that of Group N and Group (DM+Ins.). H & E; Scale bars =100μm.

disappeared due to resorption, and there was a marked decrease in the height of the alveolar crest. The alveolar bone at M_1 exhibited an irregular contour. Although we did find a narrowing of the width of the interalveolar septum space, there were very few osteoclasts observed. Instead, we found that the osteoblasts were densely arranged throughout the entire alveolar bone and included reparative changes such as the addition of new bone. As compared to Group N, there was a widening of the periodontal ligament entirely at M_2, although at M_1 it became slightly narrower. There was elongation of the periodontal ligament fiber at M_2, whereas the path of the periodontal ligament fiber at M_1 was slightly atrophic and irregular. Gram staining detected very few bacteria throughout the entire area at the col (Fig. 8a).

At 7 days after the compression, the col was concave. However, there was an increased thickness of the regenerated epithelium due to stratification. At the lower layer of the epithelium, there was an irregular formation of granulation tissue and the horizontal ligament between the teeth. Although the alveolar crest started to recover its round shape, the entire interalveolar septum was free from any alveolar bone resorption. An intense arrangement of osteoblasts was observed and bone addition occurred. The alveolar bone was found to have a less irregular smooth surface. The periodontal ligament observed was similar to that seen at 5 days after the compression. At M_2 there was a slight widening, along with a slight elongation of the periodontal ligament fiber. The path of the periodontal ligament fiber at M_1 was slightly narrower and irregular (Fig. 9b, e).

At 14 days after the compression, the col region was still concave due to the compression of the GP. The superficial layer was covered with a relatively thick epithelial layer that was accompanied by an epithelial ridge of epithelium. The horizontal ligament between the teeth had regenerated, with the regular pathway observed beneath the epithelium. The observed repairs were similar to that seen in Group N. Although there was a decrease

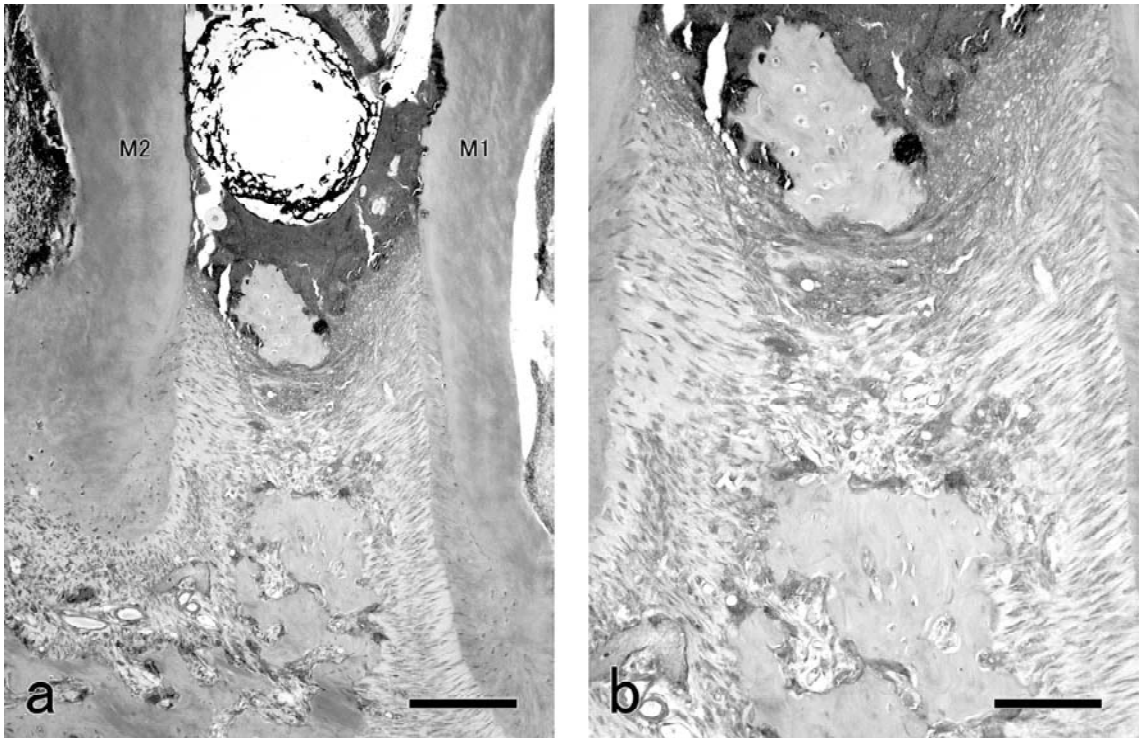

Figure 11. 14 days after col compression of the experimental group. a, b (partial high magnification of a): Group (DM+Chlo). In Group (DM+Chlo), ulcer and necrotic layer are still formed at the col in partially experimental cases. Bacteria enter into alveolar crest area. Marked bone resorption is seen at the lower portion of alveolar crest. The decrease in alveolar crest is marked. H&E; a:Scale bar=200μm,b: Scale bar=100μm.

in the height of the alveolar bone crest, the alveolar bone surface was free of any bone resorption and similar to that seen in Group N. Although smooth surfaces were observed in areas that underwent bone addition, there was very little bone resorption noted. Repairs of the periodontal ligament were similar to that for Group N at both M_1 and M_2, with a regular periodontal ligament fiber pathway observed (Fig. 10b, e).

Group DM+Chlo

Gross observation of the group administered 0.2 % chlorhexidine gluconate solution irrigation in conjunction with GP insertion at the col in diabetic rats, showed there was maintenance of the GP insertion at the col along with a comparatively healthy color of the gingiva without any inflammatory findings such as marked redness, swelling, or easy bleeding. This was found despite the fact that chronologically there was gingival recession seen around the col.

At 1 day after the compression, the col showed concavity in the STZ-induced diabetic rats. There was compression due to the GP and a bactericidal effect associated with the simultaneous irrigation with 0.2 % chlorhexidine gluconate solution. At this point there was formation of the focus of the necrosis with a thickening of the layer of degeneration, in addition to the attachment of numerous bacterial contaminants. The focus of the necrosis was at the lower layer, where an intense inflammatory cell infiltration was observed. The focus of necrosis and the inflammatory cell infiltration layer was found to be close to the alveolar crest with round contour. Neither osteoclasts nor bone resorption were observed at the alveolar crest. However, slight bone resorption due to osteoclasts often occurred in the lower portion of the alveolar crest. In the middle-lower portion of the alveolar bone, bone resorption was not observed, with a smooth surface seen at M_1. In contrast, bone resorption due to osteoclasts occurred with numerous irregular bone surfaces observed at M_2. Although the periodontal ligament exhibited a slightly irregular pathway within the region of the alveolar bone resorption, the ligament showed a regular arrangement that was similar to Group N as a whole. Gram staining demonstrated that there were large numbers of bacteria at the interdental region, on the necrotic layer at the col, and at inflammatory cell infiltration region. Very little bacterial invasion was observed in portions that were deeper than the inflammatory cell infiltration region (Fig. 4c; Fig. 5c).

At 3 days after the compression, the col region began to exhibit a deep concavity along with a thickening of the focus of necrosis at the superficial layer. Numerous bacterial contaminants adhered around the GP and there was a partial intermingling with the focus of the necrosis. The alveolar bone crest was surrounded by the focus of the necrosis, which included the bacterial contaminants.

Inflammatory cell infiltration also began to appear in the lower layer. Gram staining demonstrated the presence of a large amount of bacteria after the col compression at day 1. Bacteria invaded into the partially bony canal of the alveolar bone crest with the subsequent formation of sequestra. Invasion and proliferation of bacteria such as bacteria were confirmed by Gram staining. Numerous osteoclasts were observed throughout the middle-lower portion of alveolar bone and there was marked bone resorption. The bone surface exhibited a serrated state with numerous irregular contours. The periodontal ligament was destroyed in the alveolar crest portion. In the middle-lower portion of the alveolar bone, there was a slightly irregular path for the periodontal ligament fiber through the area in which the alveolar bone resorption occurred. In contrast, there was a normal placement of the periodontal ligament fiber and there was no destruction noted in any of the other areas (Fig. 6c, f; Fig. 7c-e).

At 5 days after the compression, there was a thickening at the focus of the necrosis that included numerous bacterial contaminants around the GP at the col, along with inflammatory cell infiltrates beneath the necrotic region. The alveolar bone crest was in close proximity to the focus of the necrosis and in contact with bacterial contaminants. Gram staining delineated not only the necrotic layer, but also showed there was a large invasion of bacteria on the bone surface, the intertrabecula, and within the small bone cavities of the partial alveolar crest. Sequestration was found in a portion of the alveolar crest. Beneath the alveolar crest, progression of bone resorption was observed and the alveolar crest exhibited a free bone state, with resorbing bone fragments. In the middle-lower portion of the alveolar bone, there were few osteoclasts and little bone resorption observed. However, osteoblasts were densely arranged in areas where the addition of new bone occurred. There was expansion of the periodontal ligament space at the alveolar crest portion due to the bone resorption, with the formation of granulation tissue observed. In the middle-lower portion of the alveolar bone, there was no marked bone resorption observed, and the periodontal ligament fiber followed a comparatively regular path (Fig. 8b).

At 7 days after the compression, some of the covering epithelial layers were closely compressed to the GP at the col, with the regeneration of the epithelium observed. In the regenerated epithelium at the superficial layer of col, granulation tissue formed in the lower layer and there was formation of horizontal ligaments between the teeth, which indicated reparative changes within the deep layer. The observed inflammatory cell infiltration was restricted to the area that was directly beneath the regenerated epithelium, becoming milder over time when compared to that seen at 5 days after the col compression. There was progression of the resorption at the alveolar crest, with the height of the alveolar crest decreasing and showing a slightly rounded contour shape. During this stage, bacterial contaminants were not found throughout the entire col. Gram staining demonstrated there were bacterial adhesions on the sequestrated free bone. In addition, bacteria was hardly observed when the epithelium regenerated or during the time of the reparative changes. In the middle-lower portion of the alveolar bone, bone resorption was not observed at M_2, with only a flat surface with newly added bone observed. Although slight bone resorption was partially observed at M_1, the area showed an irregular bone surface, and there were reparative changes observed as compared with that seen at 5 days after the compression. When compared to the Group N, the periodontal ligament was slightly broader at M_2, whereas it was slightly narrower at M_1. Furthermore, there was elongation of the periodontal ligament fiber at M_2 ,whereas an irregular path with slight atrophy was found at M_1 (Fig. 9c, f).

At 14 days after the compression, there were cases in which reparative changes were progressing and in which destructive changes were still seen at the col. In cases where there was progression of the reparative changes, the col was deeply concave due to the GP. However, regenerated epithelium was clearly formed at the superficial layer. Very little inflammatory cell infiltration was observed directly beneath the epithelium, and there was repair of the interdental horizontal ligament within the deep portion. Although the alveolar crest appeared to be low, there was very little bone resorption, with the area exhibiting a rounded contour shape. Bone resorption was not found in the middle-lower portion of the alveolar bone, and there was a rather extensive addition of new bone due to osteoblasts that occurred, with a comparatively smooth bone surface. There was a uniform width of the periodontal ligament space, and the periodontal ligament fiber was orderly arranged, which indicated recovery to the same state observed in Group N and Group (DM+Ins). There was little bone resorption observed in the decreased alveolar bone, and the periodontal ligament fibers were arranged in a comparatively ordered fashion.

In contrast, in the cases with destruction, the col exhibited a large concavity as compared to that seen at 7 days after the compression. The bacterial contaminants were seen around GP. Regenerated epithelium was not observed at the superficial layer and the formation of erosion and ulcer was observed. This showed the presence of a mechanical compression at the col. There were a marked decrease in the height of the alveolar bone. The sequestrum formation at the alveolar crest and mark bone resorption due to osteoclasts in the lower portion were also observed. However, no bone resorption and a comparatively smooth surface was noted in the lower region of the alveolar bone. The size of the periodontal ligament space was almost the same on both the M_1 and M_2 sides. A comparatively smooth arrangement was also seen for the periodontal ligament fiber (Fig. 10c, f; Fig. 11a, b).

Discussion

Experimental studies of food impaction that have used several

different food impaction methods have been performed using various experimental animals, including rats, mice, dogs and monkeys, among others[5-7,11]. Matsuura et al.[8] examined the characteristic changes on the destructive alterations of the periodontal tissue and alveolar bone after food impaction induced a separation between the tissue and the bone due to grinding of proximal surface of the molars in rats**.** Their results showed that bone was not initially resorbed on the alveolar crest, but rather, marked bone resorption occurred on the side of the lower region. Moreover, they also found that the free bone occurred on the alveolar crest, which induced a rapid chronological decrease in the alveolar crest. Osaki[17] further examined whether food impaction had a role in the mechanical compression of the col by GP. Study results showed that the disturbance of the periodontal tissue, including the alveolar bone resorption that was induced by the mechanical compression of the col by GP was similar and significantly different from the findings reported by Matsuura et al.[8]. Based on these results, we designed our present experiment to further investigate the mechanical compression procedure by using GP and then compare our findings to those reported by Tokunaga, et al.[47], who also examined periodontal tissue disturbances in STZ-induced diabetic rats.

Diabetic rats were prepared by using alloxan and STZ, which are known to induce a diabetic state. STZ injury is specific and directed at the beta cells of islets of Langerhans, thereby inducing hyperglycemia without ketosis. STZ can be used to maintain a diabetic state for a long period, with the metabolic mechanism similar to that found in human type I diabetic mellitus[45-47]. Therefore, we decided to use STZ for our diabetic model in the present study.

The intravenously administered dose of STZ varied from 40 to 120 mg/kg. Intraperitoneal injection of 125 mg/kg was also performed in the present study in order to be able to compare our results with those in the previous studies[44,47]. In our study, we confirmed the diabetic status to be >+2 (which is comparable to a value of 500 mg/dl of urine sugar) by examining the animals with Tes-Tape within one week after the initial administration. In our Group N rats, the col compression induced concavity, the appearance of hyaline necrosis at the superficial layer, no bone resorption at alveolar crest, along with an alveolar bone resorption during the early stage in the lower portion of the alveolar crest at 1 and 3 days after the col compression were observed. However, we found no bone resorption at the alveolar crest. These characteristic changes were similar to those reported in previous studies[8,17-18]. Wada[7] examined alterations of the periodontal tissue caused by food impaction, especially during the early stages, after partial slicing of the proximal surface of the rat molars. He reported that there were very few inflammatory cell infiltrates when there was no destruction of epithelium, despite the fact that there was a 3-6 hour stagnation of food after the food impaction and a presumed proliferation of bacterial cells. However, after destruction of the epithelium, he reported finding extensive inflammatory cell infiltration in the lamina propria at 12-24 hours, with the harmful effect due to the compression then extended to the periodontal ligament. In our present study, destructive changes of periodontal tissue at 1 and 3 days after the col compression due to GP in Group N resulted in the same destructive process that occurs during the early stages of food impaction. These changes were thought to be induced by the mechanical compression of the food impaction and not due to a bacterial effect. Starting at day 5 and continuing until day 14 after the col compression, we noted a progression in the reparative changes. The superficial layer at the col region exhibited epithelial regeneration, formation of granulation tissue at the lower portion of the epithelium, and a subsequent repair of the interdental horizontal ligament. Progression of bone resorption at the lower region of the alveolar crest in the alveolar bone was observed with a marked lowering of the height of the alveolar crest between 7 and 14 days after the col compression. We also found new bone addition by the osteoblasts in the superficial layer of the alveolar crest. These reparative changes were similar and without any distinct differences from those reported in previous studies[8,17-18]. Concerning the reparative phenomenon at this time point, the transition from destructive to reparative changes appeared to be induced by the rapid resorption of the alveolar bone that directly resulted from the food impaction, along with the weakening of the mechanical compression at the col. In contrast, Tokunaga et al.[47] examined the disturbance in the changes of the periodontal tissue after col compression in diabetic model rats and reported findings: (1) The adhesion of numerous bacterial masses, in addition to the appearance of hyaline necrosis, ulceration, and inflammatory cell infiltrates on the superficial layer after col compression beginning from an early stage; (2) Sequestrum formation due to the invasion of numerous bacterial masses on the alveolar crest at 3 and 5 days after the col compression; (3) Observation of sequestrum separation in addition to epithelial regeneration at the col at 7 and 14 days; (4) Persistence of marked bone resorption in the lower portion of the alveolar bone, which induced a pronounced lowering of the alveolar crest; and (5) Induction of a severe destruction of the periodontal tissue and a delay in the reparative changes as compared to the healthy state. In addition, this study also demonstrated that the diabetic state caused adhesion and proliferation of the bacterial mass at the regions where the food impaction and mechanical compression occurred. This was one of the major factors responsible for the disturbance of the periodontal tissue, especially regarding the destruction of alveolar bone due to inflammation and sequestrum formation at the col.

Based on these previous findings, the present study was designed to further investigate how insulin administration is able

to prevent the intense disturbance of the periodontal tissue induced by the diabetic state. The types of insulin vary from those that have an instantaneous effect up to others with a long-lasting action effect. The present study used a diabetic rat model that used STZ to create rats with degraded insulin secretion due to the destruction of the b cells of the pancreas. Although controlling blood sugar and urine sugar via an optimal insulin administration can be difficult to undertake, our present experiment used an intermediate-acting insulin that only required a single administration in order to achieve a stable and persistent effect for 24 hours. By using of this type of insulin, it was possible to easily improve the state of the blood sugar and urine sugar in the experimental animals. Thus, all experimental animals were given an intramuscular injection of 5 IU every morning once a day throughout the duration of the study. Kato[51-52] evaluated the effect of insulin administration on the periodontal condition of STZ-induced diabetic rats with experimental periodontitis. Results showed that there was a decrease in the high blood glucose level of the diabetic rats that was similar to the level observed in non-diabetic rats after an insulin administration. In the present experiment, the negative Tes-Tape results suggested that there was an improvement in the diabetic state (±0) after the insulin administration. In the Group (DM+Ins), insulin was administered immediately after the GP insertion in a diabetic rat. In these rats, disturbances due to mechanical compression such as concavity at the col, hyaline degeneration, necrosis, and ulceration were observed during the early stages, at 1 and 3 days after the col compression. Although there was no bone resorption by the osteoclasts found at the alveolar crest, the disturbance changes, which included bone resorption in the lower portion, were the same as those noted for Group N. Moreover, there was regeneration of the epithelial covering on the superficial layer at the col at 3 and 5 days. There was almost no inflammatory cell infiltration at the lower portion of the epithelium. At 7 and 14 days after the col compression, progression of the reparative changes for the interdental horizontal ligaments at the col was similar to that seen in Group N. In the alveolar bone, there was rapid resorption of the alveolar crest that was close to the ulcerated region during the early stage. At 5 and 7 days after the col compression, there was a decrease in the height of the alveolar crest, which led to a repairing of the alveolar crest shape. There was also a decrease in the overall bone resorption, along with an active addition of new bone that progressed with a smooth repair of the bone, similar to that observed in Group N. Tokunaga et al.[47] demonstrated that there was a large adhesion of bacterial masses at the col, intense inflammatory cell infiltration and sequestrum formation at 5 and 7 days after the col compression. In the present study, however, our findings were very different, with hardly any changes observed in the Group (DM+Ins). In other previous studies that used STZ-induced diabetic model rats[25,51-53], results showed there was delayed healing of fractures, anomalies of the microscopic structure of the osteoblast, a decrease in the ability to form an osteoid, maldevelopment of the ruffled border in osteoclasts, and disturbance of bone resorption. Moreover, previous studies have also reported that STZ-induced diabetic rats exhibit a smaller occurrence of osteoclasts than that seen in the alveolar bone of normal rats in addition to only very small amounts of bone formation.

In contrast, other reports have indicated that insulin administration to the STZ-induced diabetic rats resulted in normal amounts of bone formation, while DM caused a decrease in the resorption and addition of bone. However, after the administration of insulin, the ability to form osteoid recovered. Since the present study showed that the destruction of the periodontal tissue and the reparative process in Group(DM+Ins) was similar to that seen in Group N, this shows the effectiveness of the administration of insulin in preventing these changes. The observed reduction in the disturbance of the periodontal tissue and the promotion of wound healing demonstrates the importance of controlling the blood and urine sugars in diabetic patients. Tokunaga, et al.[47] examined the use of 3 % H_2O_2 solution (oxydol) irrigation and the application of Periocline antibacterial ointment to treat adhesions of numerous bacterial masses at the col during the early stages after mechanical compression in diabetic rats. After this treatment, there were no adhesions of the numerous bacterial masses after the col compression and sequestrum formation was not observed at 3-7 days after the col compression. In addition, in spite of the state of the diabetes, it was reported that there was a big improvement and progression of the reparative changes at 7 and 14 days after the col compression. In the present study, only insulin administration distinctly inhibited the massive adhesion of bacteria and sequestrum formation in Group (DM+Ins) despite the fact that neither bacterial irrigation nor the application of antibacterial ointment at the col were done. These findings suggest that improvement of the diabetic state due to the administration of insulin contributes to the inhibition of the proliferation of intraoral bacteria related to periodontal disease and biofilm formation, and to the recovery of the resistance of the periodontal tissue to bacteria. However, as the mechanism responsible for the insulin administration effects on the inhibition of bacterial proliferation or the acquisition of an improvement in bacterial resistance during periodontal disease remains unknown, further investigations will need to be undertaken. Irrigation to the periodontal pocket or oral gargling with chlorhexidine gluconate solution, (diluted Hibitane solution), are commonly used clinically. Low concentrations of chlorhexidine gluconate solution exhibit a bacteriostatic effect while high concentrations have an antibacterial effect. Thus, it is well established that chlorhexidine gluconate solution is an effect gargling agent that can be used after a surgical operation for periodontal disease[55-63]. However, the international

standard guidelines recommend the use of a solution with a high concentration of 0.12-0.2 % in the USA and Europe, whereas a diluted solution of less than 0.05 % is used in Japan. Therefore, differences exist between Japan and other countries. In order to elucidate the antibacterial and irrigation effects on the adhesion of bacterial masses that occur after mechanical compression during early stages in the diabetic state, the present study used a 0.2 % diluted chlorhexidine gluconate solution in accordance with the international guidelines that recommend a higher concentration. To increase the antibacterial and irrigation effects, this study performed an irrigation twice a day, once in the morning and once in the evening. In Group (DM+Chlo), comparatively thick layers of degeneration and necrosis at the col were formed during the early stages, at which time the adhesion of numerous bacterial contaminants and inflammatory cell infiltration also occurred. Bacterial staining showed there was a large amount of bacteria in the interdental region, on the necrotic layer at the col, and in the region with the inflammatory cell infiltrates. There was no bone resorption observed at the alveolar crest, which was surrounded by a necrotic layer and a layer with inflammatory cell infiltrates at 3 and 5 days after the col compression. Sequestration appeared at the time that the invasion of bacteria into the partial alveolar crest took place. The alveolar bone resorption in the lower region of the alveolar crest occurred over a comparatively wide range. The findings for the alveolar crest demonstrated the changes in the state of free bone over time. These pathological conditions were extremely similar to the findings reported by Tokunaga et al.[47] for the specific bacterial proliferation and changes in the destruction of the periodontal tissue that occurred during the early stages after the col compression. As previously noted, it is well-known that chlorhexidine gluconate solution has both bactericidal and sterilizing effects. With regard to bactericidal action for staphylococcus, it has been reported that the efficacy of chlorhexidine gluconate solution is dependent upon contact with the bacteria for a relatively long time[64,65]. In the present study the instantaneous bactericidal effect was probably quite low since the contact between the chlorhexidine gluconate solution and the bacteria in the focus of the necrosis at the col and the other contaminants was most likely very short. Tokunaga et al.[47] reported that the use of a 3 % H_2O_2 solution in conjunction with Periocline antibacterial ointment induced a marked decrease in the bacterial mass. The better bactericidal and irrigation effects observed in this previous study were very different from the results of our present study. Although there appears to be is no definite reason for these differences, it is inferred that the H_2O_2 solution is able to exhibit bactericidal effects with a relatively strong cytotoxicity, while the presence of the Periocline ointment for a relatively long period at the col most likely provided a persistent bactericidal effect. Moreover, since the 0.2 % chlorhexidine gluconate solution could not penetrate into the relatively thick necrotic layer or reach the bacteria within the inflammation, this resulted in poor bactericidal effects. The specific details responsible for these differences will need to be examined in a further study.

Starting from 7 days after the col compression, the alveolar bone showed a more intense disturbance of the periodontal tissue, and bone resorption of the alveolar bone as compared to either the Group N or Group (DM+Ins). Even so, regenerated epithelium at the col was found in a few cases. Reparative changes such as new bone addition by osteoblasts occurred in the lower portion of the alveolar bone. Although bacterial infection occurred at the alveolar crest at 5 days after the col compression, there was also concern over potential sequestrum formation. However, our present study found neither sequestrum formation nor the separation of the sequestrum over time as has been reported in the diabetic state[47]. While reparative changes were delayed as compared to Group N and Group DM+Ins, there was a weakening of the destructive changes over time, with the reparative changes finally occurring. It is believed that these changes may depend on the irrigation and the bactericidal effects associated with the 0.2% chlorhexidine gluconate solution that is used against the bacterial proliferation at the col. However, there were some cases that exhibited a very intense disturbance of the periodontal tissue. When the mechanical compression in the diabetic state persisted in these types of cases, the severity of the disturbance combined with the proliferation of bacteria resulted in no improvement in either the necrotic layer or the ulcer formation. Thus, the use of only irrigation and bactericidal action of a 0.2 % chlorhexidine gluconate solution are not enough to reduce severe cases of disturbance of the periodontal tissue. When food impaction occurs clinically, dental treatments that prevent food impaction are attempted in conjunction with removal of the contaminants that contain the focus of the necrosis at the col. In the diabetic state, however, in order to reduce the disturbance of the periodontal tissue due to food impaction, the first step that needs to be done is to improve the diabetic state by administering insulin.

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