J Periodont Res 2008; 43: 22–31

JOURNAL OF PERIODONTAL RESEARCH
doi:10.1111/j.1600-0765.2007.00990.x

Degradation of noncollagenous components by neutrophil elastase reduces the mechanical strength of rat periodontal ligament

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Ujiie Y, Shimada A, Komatsu K, Gomi K, Oida S, Arai T, Fukae M. Degradation of noncollagenous components by neutrophil elastase reduces the mechanical strength of rat periodontal ligament. J Periodont Res 2008; 43: 22–31. © 2007 The Authors. Journal compilation © 2007 Blackwell Munksgaard

Background and Objective: We have previously shown that increases in neutrophil elastase in periodontal ligament with chronic periodontitis results in degradation of the noncollagenous components. The purpose of this study was to investigate whether the destruction of noncollagenous components by treatment with elastase *in vitro* causes changes in the mechanical properties of the periodontal ligament.

Material and Methods: The transverse sections of mandibular first molars, prepared from male Wistar rats at 6 wk of age, were digested with 0–50 μg/mL of neutrophil elastase at 37°C for 4 h. Then, their mechanical properties and morphological features were examined.

Results: Digestion with elastase dose-dependently decreased the maximum shear stress and failure strain energy density of the periodontal ligament ($p < 0.05$–0.01). The histological observations after digestion revealed marked degradation of oxytalan fibers, but no marked changes of the collagen fibers, which was confirmed by the detection of very low quantities of hydroxyproline in the digest. The light and scanning electron micrographs showed that the elastase degraded the interfibrillar substances in the periodontal ligament and exposed individual collagen fibrils.

Conclusion: These results suggest that the increased neutrophil elastase observed in periodontal disease degrades the oxytalan fibers and interfibrillar substances in the periodontal ligament to decrease its mechanical strength.

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Key words: interfibrillar substances; neutrophil elastase; oxytalan fiber; periodontal ligament

Accepted for publication December 19, 2006

The periodontal ligament has important roles to play in support of the tooth during functional use and to resist externally applied forces. The periodontal ligament consists mainly of collagen fibers and noncollagenous components, including oxytalan fibers, proteoglycans and glycoproteins (1,2). It is well accepted that collagen fibers are primarily responsible for the support function of the periodontal ligament (3,4).

It is well known that several proteinases participate in the normal remodeling of the periodontal ligament and the destruction process of the periodontal tissues in chronic periodontitis (5–7). The expression and activity of several matrix metalloproteinases (MMP-1, -2, -3, -8, -9, -13 and -14) have been found to be higher in diseased, than in healthy, gingiva and periodontal ligament (6–13). It is now recognized that an imbalance between activated MMPs and their endogenous inhibitors leads to pathologic breakdown of the extracellular matrix during periodontitis. At the same time, higher neutrophil elastase activity has also been detected in gingival connective tissue, gingival crevicular fluid, saliva and water rinse samples in periodontitis than those in health (14–19). Elastase is thought to be derived from the azurophilic granules of neutrophils, which are initially attracted to infected periodontal tissues by chemoattractants from bacteria, host cells, or degraded tissue (6). Elastase not only directly induces tissue damage by degrading several extracellular matrixes but also generates peptide fragments that are chemotactic for monocytes (14). In addition, clinical improvements after nonsurgical treatment are accompanied by reductions in elastase and neutrophil activities (18). Therefore, many investigators have examined elastase as a potential diagnostic marker of periodontal disease activity (20). However, the effect of elastase on the mechanical strength of the periodontal ligament has not yet been examined.

We have detected remarkable increases of neutrophil elastase, plasminogen and MMP-9 activities in the periodontal ligaments of periodontitis patients compared with healthy periodontal ligaments (21,22). Scanning electron microscopic images reveal that the interfibrillar substances were notably degraded by treatment with neutrophil elastase, but not so extensively with plasminogen or MMP-9. Moreover, only neutrophil elastase activity was detected on a zymogram, using noncollagenous proteins extracted from porcine periodontal ligament as a substrate, and on western analysis (21,23). These results suggest that neutrophil elastase is involved in the destructive processes of noncollagenous components, including interfibrillar substances, in periodontal tissues afflicted with periodontitis.

The purpose of the present study was to investigate whether the destruction of noncollagenous components in the rat periodontal ligament by neutrophil elastase could cause changes in the mechanical properties of the periodontal ligament.

Material and methods

The present experiments were approved by the Institutional Animal Care Committee of Tsurumi University School of Dental Medicine.

Materials

Neutrophil elastase (CK828; derived from human leucocytes, activity of Meo-Suc-Ala-Ala-Pro-Val-pNA digestion: 16,000 units/mg of protein) was purchased from Elastin Products Company, Inc. (Owensville, MO, USA). Sivelestat sodium hydrate (Elaspol®), a synthetic inhibitor of neutrophil elastase with high specificity (24,25), was from Ono Pharmaceutical Co., Ltd. (Osaka, Japan).

Preparation for section of the rat mandibular first molar periodontal ligament

Fifty-two male Wistar rats (CLEA Japan, Inc., Tokyo, Japan) were purchased at 5 wk of age, fed a powdered diet (CE-2; CLEA Japan, Inc.), and given water *ad libitum* for 1 wk. They were killed by ether overdose at 6 wk of age. The left mandible was dissected free and the adhering soft tissues were removed. A transverse section of the first molar was cut at the middle level of the mesial root (Fig. 1A) using a low-speed bone saw (Isomet; Buehler, Lake Bluff, IL, USA) (26). The thickness of sections was measured and determined to be 444 ± 30 μm, using a dial thickness gauge (Peacock, Tokyo, Japan). The sections were kept in phosphate-buffered saline, pH 7.2, at 4°C until *in vitro* treatment.

Radiographic analysis

Radiographies of the transverse sections before treatment were taken in a soft-X-ray apparatus (Type EBM; Softex, Tokyo, Japan) and their radiographic images were processed in an image analyzer (Luzex 3U; Nikon, Tokyo, Japan) (Fig. 1B). We measured the perimeters of the mesial root and of the socket wall and the sectional area of the periodontal ligament (Fig. 1C). The area of the periodontal ligament facing the root cementum was calculated as the thickness of the section × the perimeter of the mesial root. The average width of the periodontal ligament was also calculated as the sectional area of the periodontal ligament/(perimeter of the mesial root + perimeter of the socket wall)/2.

In vitro treatment for section of periodontal ligament

To investigate the effect of neutrophil elastase, five groups of six specimens each were treated with 0, 10, 30 or 50 μg/mL of neutrophil elastase, or with 50 μg/mL of neutrophil elastase + 5 mM sivelestat sodium hydrate in 0.2 M Tris-HCl (pH 8.5) containing 1 M NaCl. To examine the effect of the NaCl concentration employed at the time of neutrophil elastase digestion, the other two groups of six specimens each were treated with phosphate-buffered saline and phosphate buffer (pH 7.2) containing 1 M NaCl, respectively. Each section was placed in 0.25 mL of the test solution and shaken (120 times per min) in an incubator (Bio shaker 40BR-LF; Taitec Co., Saitama, Japan) at 37°C for 4 h. The experimental period of 4 h was determined by our previous study, in which the effects of degenerative changes in the cells during incubation with phosphate-buffered saline on the mechanical properties of the periodontal ligament were insignificant (27). The concentrations of neutrophil elastase and sivelestat were determined by our preliminary and reported studies (24,25). After incubation, the specimens were washed three times with cold phosphate-buffered saline and

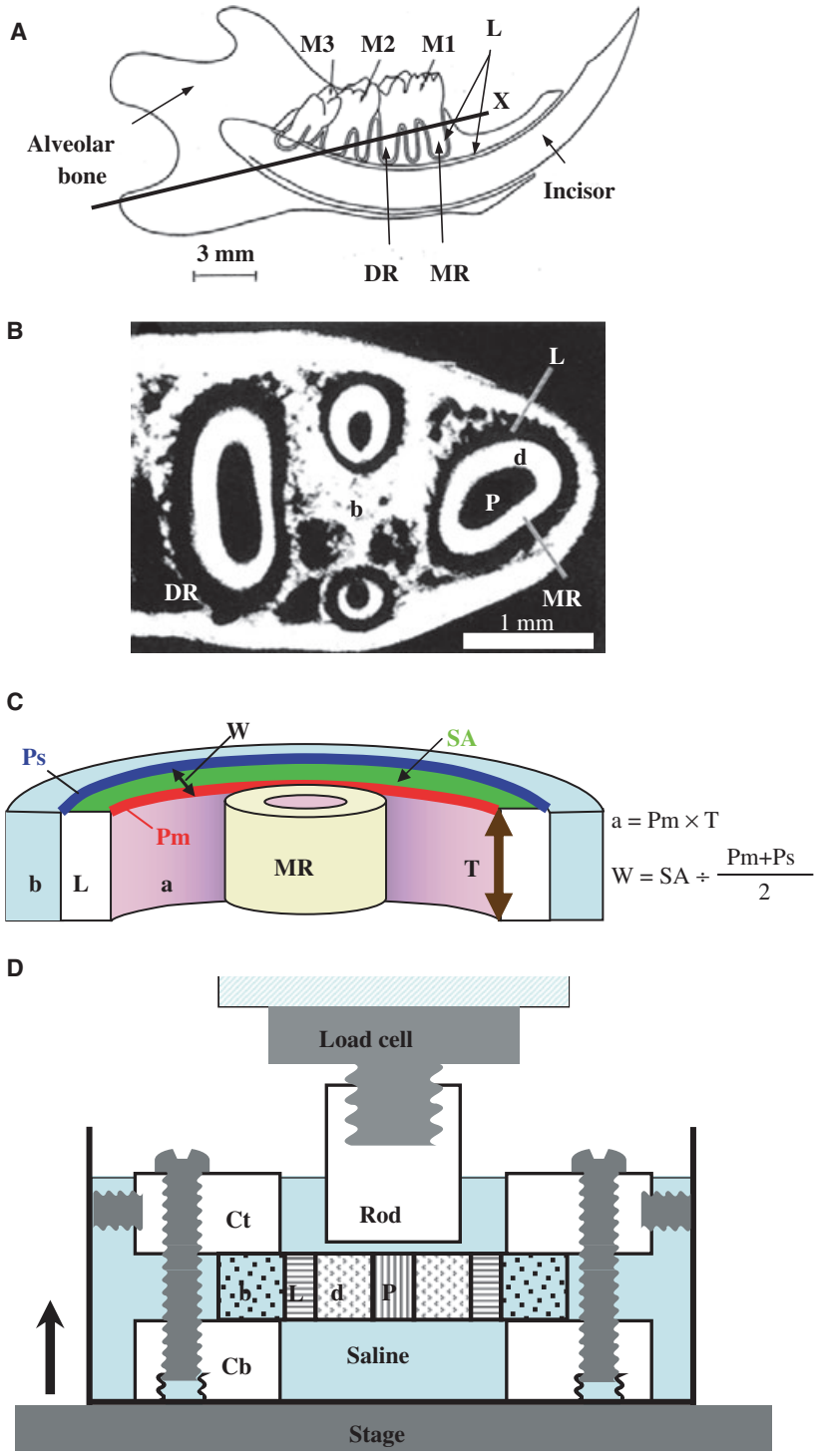

Fig. 1. (A) Diagrammatic representation of the lingual side of the rat left mandible, showing the periodontal ligament between the alveolar bone and teeth: the first, second and third molars, and an incisor. Transverse sections were cut through the axis (X) at the middle level of the mesial root and distal root of the first molar. (B) Radiograph of the transverse section of the rat mandibular first molar. (C) Hypothetical development of the periodontal ligament and socket bone of the mesial root showing concepts of calculation. We measured the perimeter of the mesial root (red line), the perimeter of the socket wall (blue line) and the sectional area of the periodontal ligament (green area), using the radiograph of transverse section and the thickness of section, using a dial thickness gauge. The area of the periodontal ligament (pink area) facing the root cementum was calculated as the perimeter of the mesial root × the thickness of the section. The average width of the periodontal ligament was also calculated as SA/(Pm + Ps)/2. (D) Diagrammatic representation of a transverse section of the molar root in the sample holder. The section fixed with screws at its bony part (b), between the acrylic and brass cylinders, was placed in saline at room temperature (23–25°C) on a stage. With upward movement (arrow) of the stage at 5 mm/min, the mesial root dentin was pushed downwards in an extrusive direction by a metal rod, causing the periodontal ligament to be deformed. The rod is connected to a load cell. a, area of the periodontal ligament; b, socket bone; Cb, brass cylinder; Ct, acrylic cylinder; d, dentin; DR, distal root; L, periodontal ligament; M1, M2, M3, first, second and third molars, respectively; MR, mesial root; P, pulp; Pm, perimeter of the mesial root; Ps, perimeter of the socket wall; SA, sectional area of the periodontal ligament; T, thickness of section; W, width of the periodontal ligament.

stored in phosphate-buffered saline at 4°C until mechanical testing.

Mechanical testing

The assembly and characteristics of the testing machine, and the method of loading, have been previously described in detail (26,28). In brief, the section fixed with screws at its bony part between two cylinders was placed in saline at room temperature (23–25°C) on a stage (Fig. 1D). With upward movement of the stage at 5 mm/min, the mesial root dentin of the first molar was pushed downwards in an extrusive direction by a metal rod attached to a load cell, causing the periodontal ligament to become deformed. The load-deformation curve was standardized into a stress–strain curve according to previous methods (28,29). From the stress–strain curve, the biomechanical measures, such as maximum shear stress (mechanical strength), maximum shear strain (extensibility), tangent modulus (elastic stiffness) and failure strain energy

density (toughness), were estimated (30,31).

Morphological examination

After mechanical testing, specimens were fixed in neutral-buffered, isotonic 10% formalin, and then processed for morphological examinations. The fixed specimens were divided between the distal and mesial roots of the first molar to observe histological features and sheared surfaces after mechanical testing in the periodontal ligament, respectively.

The specimens containing the distal roots were demineralized in 14.5% EDTA (pH 7.2), containing 15% glycerol, and then embedded in paraffin. Mesio-distal sections (6 μm thickness), parallel to the long axis of the distal root, were cut serially on a microtome (Supercut 2050; Leica Instruments, Wetzlar, Germany). Sections were stained with aldehyde fuchsin for oxytalan fibers (32) or with periodic-acid-Schiff for interfibrillar substances (33). The number and length of fragmented fibers, stained deep purple by aldehyde fuchsin, within the periodontal ligament were determined. The stained surface of cementum, ground substances and blood vessels were eliminated. Periodic-acid-Schiff-stained sections were observed by an ordinary and polarized light microscopy for collagen fibers (Laborlux 12 pol S; Leica Microscopie, Wetzlar, Germany) (34,35).

Some of the specimens containing the mesial roots that were treated with 0 or 50 μg/mL of neutrophil elastase and subjected to mechanical testing were dehydrated in a graded ethanol series, critical-point dried, mounted on stubs and coated with gold (ION COATER IB.3; Eiko, Ibaraki, Japan). Sheared surfaces of the periodontal ligament of the mesial root were examined with a scanning electron microscope (JSM-T300; JEOL, Tokyo, Japan) operated at 20 kV.

Measurement of hydroxyproline

For investigation of collagen degradation, the amount of hydroxyproline was measured in the collagen derivatives released from the specimens into the incubated test solutions treated with neutrophil elastase. The incubated test solutions were hydrolyzed in evacuated glass tubes with 6 N HCl at 110°C for 24 h, dried under vacuum, dissolved in 0.2 M sodium citrate buffer (pH 2.2), and analyzed for hydroxyproline on an automatic amino acid analyzer (JLC-500/V; JEOL Datum Ltd, Tokyo, Japan).

Statistical analyses

Scheffé's method was used to examine the difference of the mean values of biomechanical measures, and of the number and length of fragmented oxytalan fibers, among the groups treated with various concentrations of neutrophil elastase. Regression analyses were used to examine the relationship between the amount of hydroxyproline in the incubated test solution and maximum shear stress. A significant difference between the correlation coefficient and zero was examined by the Student's *t*-test. Values were taken to be significant at $p < 0.05$.

Results

Changes in the mechanical properties of the ligament

In our preliminary study, when the section of periodontal ligament was treated with extrinsic neutrophil elastase in phosphate-buffered saline (pH 7.2), neutrophil elastase digested only around the surface of the specimen and did not penetrate into the specimen within the experimental period, resulting in no significant effect on the mechanical properties (data not shown). After investigating the optimal conditions of neutrophil elastase digestion, the present experimental conditions of 0.2 M Tris-HCl buffer (pH 8.5) containing 1 M NaCl were employed, and the enzyme then induced changes in the mechanical properties of the periodontal ligament. Figure 2 shows the stress–strain curves of the mesial root periodontal ligament after treatment with various concentrations of neutrophil elastase. Each curve exhibits an initial nonlinear region, followed by a linear region and a subsequent yielding before reaching the maximum point (Fig. 2A). Increasing the concentrations of neutrophil elastase gradually decreased the slopes of the linear part of the curves and the maximum shear stress. The stress–strain curve at 50 μg/mL of neutrophil elastase + 5 mM sivelestat was similar to those at 0 and 10 μg/mL of neutrophil elastase.

The biomechanical measures were determined (Fig. 2B–E) from the stress–strain curves (Fig. 2A). As the concentration of neutrophil elastase increased, the maximum shear stress and the failure strain energy density significantly decreased (Scheffé's method, $p < 0.05$–0.01; Fig. 2B,E). However, these changes were recovered by adding the neutrophil elastase inhibitor ($p < 0.01$–0.001). The differences in the maximum shear strain and tangent modulus among each treatment were not significant (Fig. 2C,D), although the tangent modulus was considerably decreased by treatment with 50 μg/mL of neutrophil elastase.

The effects of a higher concentration of NaCl on the mechanical properties of periodontal ligament were examined (Fig. 3), as it is known that 1 M NaCl can extract a portion of the noncollagenous proteins from the periodontal ligament. The addition of NaCl to phosphate-buffered saline significantly decreased the tangent modulus and increased the maximum shear strain ($p < 0.05$). The differences in the maximum shear stress and failure strain energy density between each treatment were not significant, although the maximum shear stress was considerably decreased by the addition of NaCl.

Changes in the morphological features

The oxytalan fibers were shown as a deep purple color obtained by aldehyde fuchsin-staining on the distal side of the distal roots (Fig. 4A–C). In the control specimen (Fig. 4A), oxytalan fibers were observed to run approxi-

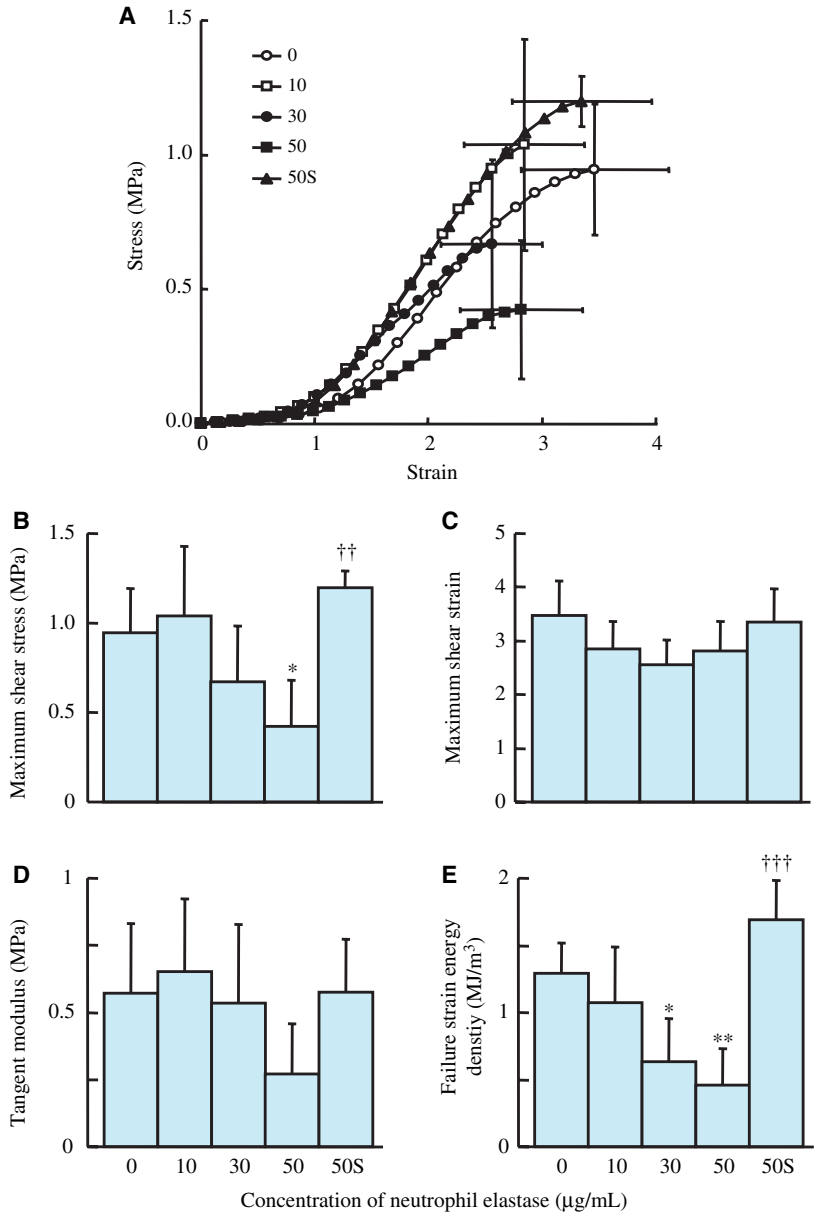

Fig. 2. (A) Stress–strain curves of the periodontal ligament obtained from transverse sections of the mesial root of the rat mandibular first molar treated with 0, 10, 30 or 50 μg/mL of neutrophil elastase, or with 50 μg/mL of neutrophil elastase + 5 mM sivelestat. The graph shows only the rising parts of the stress–strain curves. Each point represents the mean of six specimens. The end-point and the vertical and horizontal bars in each curve represent the mean ± 1 standard deviation for the maximum shear stress and the maximum shear strain, respectively. (B–E) Biomechanical measures for the periodontal ligament treated with 0, 10, 30 or 50 μg/mL of neutrophil elastase, or with 50 μg/mL of neutrophil elastase + 5 mM sivelestat. Each column and vertical bar represents the mean + 1 standard deviation of six specimens. *, $p < 0.05$; **, $p < 0.01$ [significant differences from the control value (Scheffé's method)]. ††, $p < 0.01$; †††, $p < 0.001$ [significant differences between 50 and 50S (Scheffé's method)]. 0, 10, 30 or 50 represents 0, 10, 30 or 50 μg/mL of neutrophil elastase; 50S, 50 μg/mL of neutrophil elastase + 5 mM sivelestat. [Correction added after online publication on 3 May 2007: change to Y-axis of Figure 2(C)].

mately parallel to the long axis of the root. The staining features of the control specimen were similar to those of untreated right mandibles (data not shown). When the specimens were treated with 50 μg/mL of neutrophil elastase (Fig. 4B), the long purple fibrous structure was not observed in the periodontal ligament. In the specimen treated with neutrophil elastase and its inhibitor (Fig. 4C), the staining features were similar to those of a control specimen. The number of fragmented oxytalan fibers was significantly increased by treatment with 50 μg/mL of neutrophil elastase ($p < 0.05$; Fig. 4D), resulting in a decreased length of fragmented fibers ($p < 0.05$; Fig. 4E). These changes in the number and length of fiber fragments were significantly recovered by treatment with its inhibitor ($p < 0.05$).

Changes in morphological features after treatment with 0 and 50 μg/mL of neutrophil elastase were examined by scanning electron microscopy of the sheared surfaces of the periodontal ligament adhering to the separated mesial root of specimens after mechanical testing (Fig. 5). In the specimen treated without neutrophil elastase (Fig. 5A), the surface of the sheared ligament was smooth, and the apparent fibrous structure could not be observed, although part of the noncollagenous components were removed. In the specimen treated with 50 μg/mL of neutrophil elastase (Fig. 5B), individual collagen fibrils were exposed and discernible.

Periodic-acid-Schiff-stained sections on the distal side of the distal roots of the specimen are shown in Fig. 6. In the control specimen (Fig. 6A), the cementum surface reacted strongly, the alveolar bone and dentin reacted less strongly, and the extracellular matrix reacted the weakest with periodic-acid-Schiff stain (stained purplish-red). Periodontal ligament cells, stained bluish–brown by hematoxylin, were seen in the interfibrous space. In the specimen treated with 50 μg/mL of neutrophil elastase (Fig. 6B), interfibrillar substances and cells in the interfibrous space were lost. The matrix reacted more weakly with periodic-acid-Schiff stain than the control specimen. In the specimen treated with 50 μg/mL of neutrophil elastase and its inhibitor (Fig. 6C), the morphological and staining features were similar to those of the control specimen.

When the periodic-acid-Schiff-stained sections were examined by

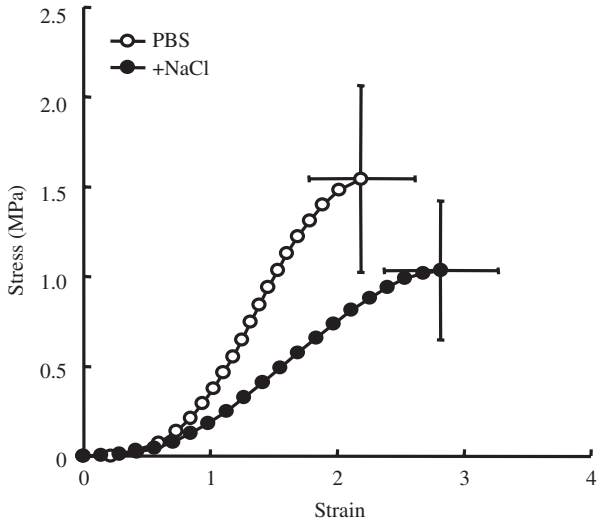

Fig. 3. Stress–strain curves of the periodontal ligament obtained from transverse sections of the mesial root of the rat mandibular first molar treated with phosphate-buffered saline, pH 7.2, or with phosphate-buffered saline (pH 7.2) containing 1 M NaCl. Each point represents the mean of six specimens. The end-point and the vertical and horizontal bars in each curve represent the mean ± 1 standard deviation for the maximum shear stress and the maximum shear strain, respectively. +NaCl, phosphate-buffered saline, pH 7.2, containing 1 M NaCl; PBS, phosphate-buffered saline, pH 7.2.

polarized light microscopy, birefringent collagen fiber bundles were observed in all specimens running obliquely across the periodontal ligament between the alveolar bone and the cementum surfaces (Fig. 6D–F). Treatment with 50 μg/mL of neutrophil elastase did not cause marked changes in the birefringence or arrangement of collagen fibers.

Measurement of degraded collagen

The amount of hydroxyproline in 0.25 mL of the test solutions after digestion with various concentrations of neutrophil elastase was 0.50 ± 0.30 μg. Such a small amount of hydroxyproline was detected without neutrophil elastase. There was no significant correlation between the hydroxyproline content in the incubated test solution and the maximum shear stress ($r = 0.339$; t-test).

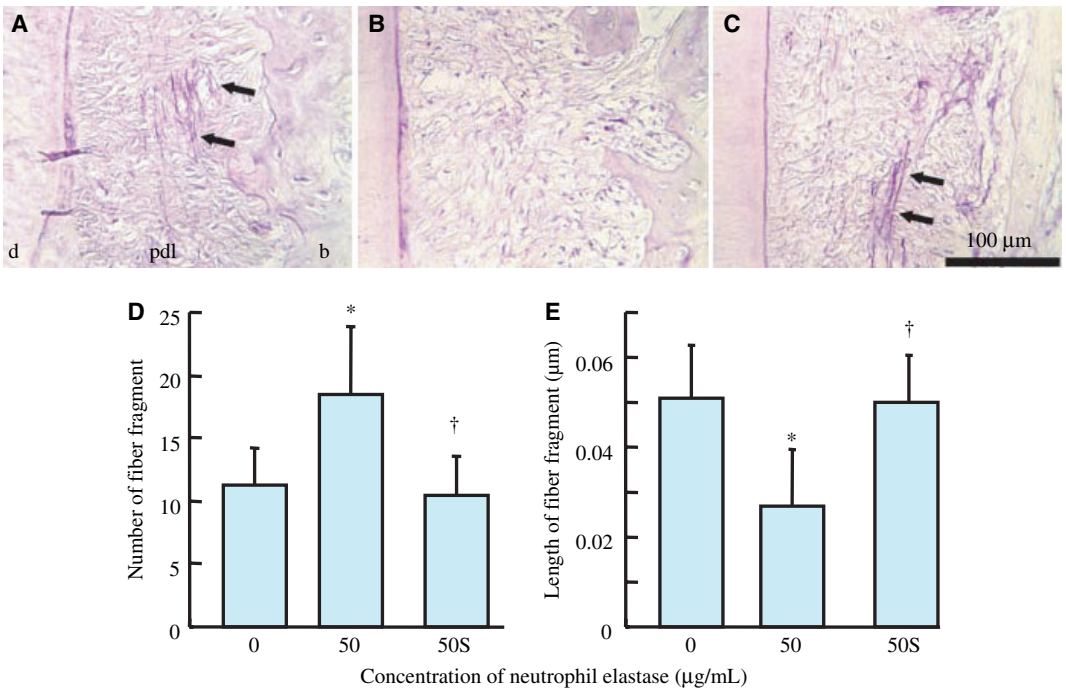

Fig. 4. Fragmentation of oxytalan fibers in periodontal ligament by neutrophil elastase. (A–C) Sagittal sections of the distal side of the distal root of the mandibular first molar. Pictures from the specimens treated with 0 μg/mL (A) or 50 μg/mL (B) of neutrophil elastase, or with 50 μg/mL of neutrophil elastase + 5 mM sivelestat (C), are shown. Sections were stained with aldehyde fuchsin-staining and observed under light microscopy. Arrows indicate the oxytalan fibers stained deep purple. The number (D) and length (E) of fragmented oxytalan fibers in the sagittal sections of the periodontal ligament. Each column and vertical bar represents the mean + 1 standard deviation of six specimens. *, $p < 0.05$ [significant differences from the control value (Scheffé's method)]. †, $p < 0.05$ [significant differences between 50 and 50S (Scheffé's method)]. b, bone; d, dentin; pdl, periodontal ligament. 0 or 50 represents 0 or 50 μg/mL of neutrophil elastase; 50S, 50 μg/mL of neutrophil elastase + 5 mM sivelestat.

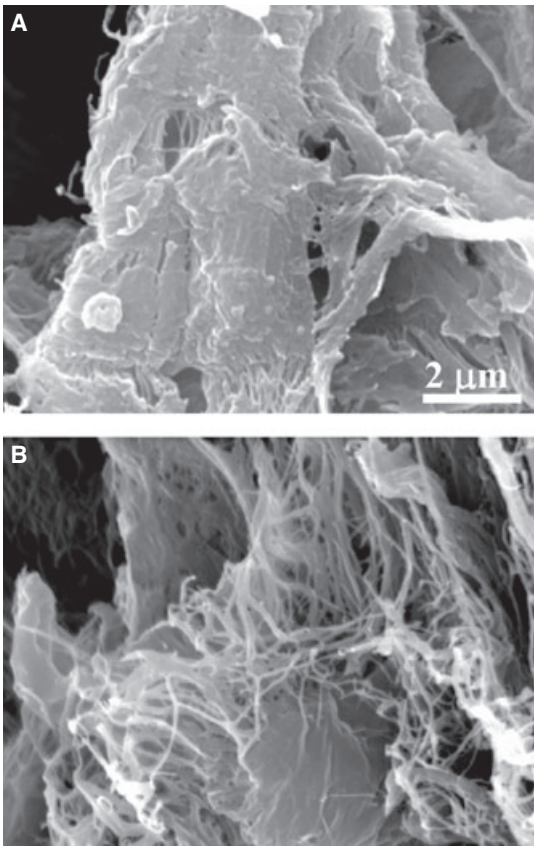

Fig. 5. Scanning electron micrographs of sheared surfaces of the periodontal ligament adhering to the mesial roots of the rat mandibular first molar after mechanical testing, obtained from specimens treated with 0 μg/mL (A) or 50 μg/mL (B) of neutrophil elastase.

Discussion

In the present study, an optimal pH for neutrophil elastase activity (36), and a higher salt concentration for the removal of salt-soluble interfibrillar substances (21,22), were employed because we observed histologically that neutrophil elastase was active only around the surface of the specimen and scarcely penetrated into the intact periodontal ligament in phosphate-buffered saline (pH 7.2) within the experimental period. In addition, the significant concentration of elastase seems to be relatively higher than that of previous studies in which elastase activities were measured from gingival crevicular fluids (15–19), although it is very difficult to compare these enzyme activities because the assay methods, substrates of elastase, expressed values and their units were different from those provided by the manufacturer of the authentic elastase used in the present study. Nevertheless, it is possible that neutrophil elastase can be active also *in vivo*, because neutrophil infiltration from peripheral blood vessels has to occur extensively *in vivo* as a result of the dense vasculature within the periodontal ligament (37,38). Under the present experimental conditions, the neutrophil elastase decreased the mechanical strength (Fig. 2B) and toughness (Fig. 2E) of the periodontal ligament in a dose-dependent manner. The elastic stiffness of the ligament (Fig. 2D) tended to be decreased by treatment with 50 μg/mL of neutrophil elastase, although the differences among each treatment were not significant. It was also confirmed that the specific inhibitor of neutrophil elastase, sivelestat, inhibited the reduction of the mechanical properties (Fig. 2) and the morphological changes (Figs 4 and 6). These results suggest that the effect on the periodontal ligament were certainly caused by neutrophil elastase. In contrast, the extensibility of the ligament (Fig. 2C) showed only minimal differences. These effects of elastase were similar to those of α-amylase (27). It has been assumed that α-amylase degraded interfibrillar substances in the ligament.

Neutrophil elastase has a broad substrate specificity, such as to elastin, proteoglycans, fibronectin, laminin and collagen types I, II, III and IV, etc (36,39). In the present study, neutrophil elastase obviously degraded the oxytalan fibers within the experimental time period (Fig. 4). The oxytalan fibers run approximately parallel to the long axis of the root, but some occasionally insert into the cementum (2), suggesting possible resistance for mechanical load to the periodontal ligament by anchoring the tooth to the alveolar bone. It has been shown that the mechanical stiffness of the engineered elastin-rich matrices are decreased by elastase digestion (40). It has also been observed that oxytalan fibers interconnect collagen fibers with cells (41) and support the blood and lymphatic vessels leading to the teeth (32). The oxytalan fibers may also be involved in the mechanical properties in the periodontal ligament directly and/or through the other components in the periodontal ligament.

Light and scanning electron micrographs showed that neutrophil elastase obviously exposed collagen fibers in the periodontal ligament by degrading the interfibrillar substances (Figs 5B and 6B). A previous report suggested that the removal of interfibrillar substances, such as proteoglycans and glycosaminoglycans, from the rat periodontal ligament, by treatment with 4% EDTA or pH 3.3 acidic conditions, markedly reduced the mechanical strength of the periodontal ligament (42). Interestingly, we showed that the higher salt concentration (1 M NaCl) in the enzyme solution was also involved in the tendency to decrease the maximum shear stress and tangent modulus (Fig. 3). In general, the solubility of a protein increases with the salt concentration (43), suggesting that removal of soluble components in the periodontal ligament by a higher salt

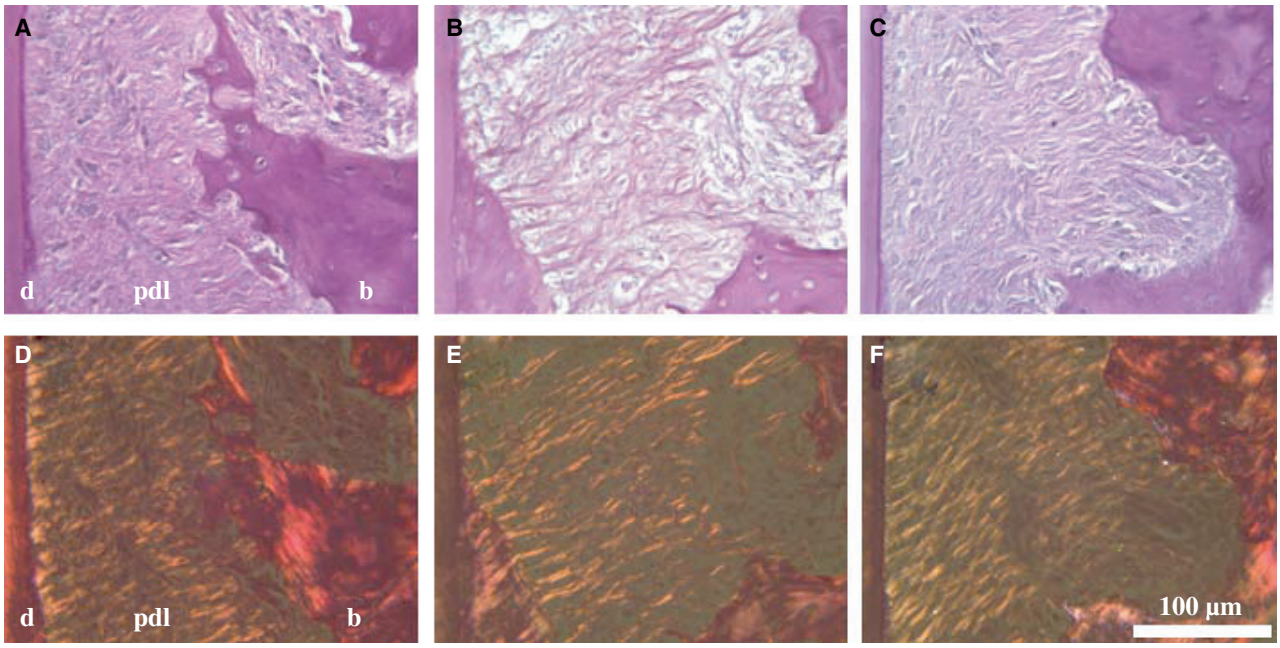

Fig. 6. Sagittal sections of the distal side of the distal root of the mandibular first molar. Pictures from the specimens treated with 0 µg/mL (A,D) or 50 µg/mL (B,E) of neutrophil elastase, or with 50 µg/mL of neutrophil elastase + 5 mM sivelestat (C,F) are shown. Sections were stained with periodic-acid-Schiff staining and observed under ordinary light (A–C) and polarized light (D–F) microscopy. b, bone; d, dentin; pdl, periodontal ligament.

concentration decreased the maximum shear stress and tangent modulus. These results support the view that the interfibrillar substances also contribute to the mechanical properties of the periodontal ligament.

In terms of collagen degradation, neutrophil elastase was able to cleave not only soluble, but also intact, helical type I collagen *in vitro* (44). In contrast, another study reported that neutrophil elastase, added alone in the absence of human fetal lung fibroblasts, did not result in the solubilization of type I collagen in three-dimensional culture (45), indicating that neutrophil elastase cannot degrade collagen under certain experimental conditions. In the present study, the treatment with neutrophil elastase did not cause obvious degradation of the collagen fiber bundles in the periodontal ligament, as observed with polarized microscopy (Fig. 6D,E), which is apparently different from the effect of bacterial collagenase (4). These observations were supported by the scant amount of hydroxyproline in the test solution after treatment with neutrophil elastase. In fact, there was no significant correlation between the amount of hydroxyproline in the incubated test solution and the maximum shear stress. These results suggest that the changes in the mechanical properties by treatment with neutrophil elastase were probably not caused by the degradation of collagen fibers in the present study.

It is evident that collagen fibers are primarily responsible for the support function of the periodontal ligament. Accordingly, MMPs may reduce the mechanical properties of periodontal ligament as a result of their degradative activity on collagen fibers. Meanwhile, in the present study, it was concluded that not only collagen fibers, but also noncollagenous components, such as oxytalan fibers and interfibrillar substances, contribute to the mechanical strength of the periodontal ligament. The increased level of neutrophil elastase in periodontitis probably degrades the noncollagenous components in the periodontal ligament, and this behavior is partly involved in the decreased mechanical strength of the periodontal ligament with periodontitis. From the characteristic changes in the morphological features of the noncollagenous components in the periodontal ligament, they appear not only to contribute to the mechanical strength of the periodontal ligament by supporting collagen fibers, but also to protect the collagen fibers from the proteinases derived from host cells or microorganisms by wrapping the collagen fibers in a protective layer. We suggest that neutrophil elastase is involved in the initial destruction of the periodontal ligament, before destroying collagen fibers in the early stages of periodontal disease and the progress of periodontal disease. Previous studies have shown that gingival tissues are also degraded by neutrophil elastase *in vitro* (46,47), and that a competitive inhibitor of elastase, an elastin peptide, protects degradation of elastic fibers by neutrophil elastase *in vivo* (48). Moreover, higher elastase activity was detected in the gingival crevicular fluid from certain patients with refractory or resistant periodontitis than in the gingival crevicular fluid from responders to conventional periodontal therapy (16). The present study suggests that the application of inhibitory agents against neutrophil elastase, such as sivelestat, in addition to conventional perio-

dontal therapy, might be useful for inhibiting the progression of periodontal disease.

Acknowledgements

The present study was supported by grants-in-aid for funding scientific research (nos. 17791335 to A.S.) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan. Pacific Edit reviewed the manuscript prior to submission.

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