J Periodont Res 2004; 39; 111–119
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JOURNAL OF PERIODONTAL RESEARCH

Effects of a new ultrasonic scaler on fibroblast attachment to root surfaces: a scanning electron microscopy analysis

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Kishida M, Sato S, Ito K. Effects of a new ultrasonic scaler on fibroblast attachment to root surfaces: a scanning electron microscopy analysis. J Periodont Res 2004; 39; 111–119. © Blackwell Munksgaard, 2004

Objective: A study was conducted to examine the effectiveness of scaling and root planing using a new ultrasonic scaler (Vector™).

Methods: Eighty extracted teeth affected by periodontal disease were sorted into four groups of 20, each of which was subjected to one the following procedures: use of the Vector™, Vector™ with polish, Enac® and a Gracey scaler. The time spent on cleaning was measured. Half of the sample teeth were examined at random for surface roughness, and the surface texture was evaluated by means of the Remaining Calculus Index (RCI) and the Roughness and Loss of Tooth Substance Index (RLTSI). The remaining samples were incubated in dishes with a suspension of fibroblasts. After measuring the number of attached cells, the attachment of fibroblasts was observed by scanning electron microscopy.

Results: The RLTSI values in the Vector™ and Vector™ with polish groups were significantly lower than those in the Enac® and Gracey groups, whereas the number of attached cells in the Vector™ with polish group was larger than in the Enac® group. Cell attachment in the Vector™ and Vector™ with polish groups proved to be better than in the Enac® and Gracey groups.

Conclusion: Since use of the Vector™ with polish was able to provide scaling and root planing with minimal damage and tight attachment of fibroblasts, it is suggested that this may be a useful instrument for scaling and root planing.

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Key words: fibroblast; surface roughness; ultrasonic scaler

Accepted for publication September 30, 2003

Scaling and root planing are no less indispensable treatments than brushing guidance in initial periodontal therapy. The instruments currently used for scaling and root planing include hand scalers, ultrasonic scalers, and other kinds of instruments. Although hand scalers are the most frequently used, considerable time and skill is required to be able to operate them. If the periodontal pockets are more than 4 mm deep, it is doubtful whether hand scalers can reach the root surfaces, and it is understood that they cannot completely remove the cause of the problem (1, 2). Taking these factors into account, ultrasonic scalers have come to be widely used in recent years because of their simplicity of use. However, it is difficult to achieve a smooth root surface by scaling and root planing with conventional ultrasonic scalers, and there is also the problem of some calculus being left unscaled (3–6). It is has also been reported that bacterial plaque adheres easily to the rough root surfaces after treatment with an ultrasonic scaler (7). A study comparing hand scalers and ultrasonic scalers found that after 12 strokes of scaling, the loss of tooth structure caused by hand scalers was 108.9 μm, and that caused by ultrasonic scalers was 11.6 μm (8). Recent *in vitro*

research suggests that endotoxins existing on exposed root surfaces do not extend deep into the cementum, but loosely adhere to the surface (9–13). This being the case, the recent, commonly accepted idea about scaling and root planing is that excessive removal of cementum in order to remove the endotoxins is unnecessary (14). Taking into consideration the thin layer of cementum, a high risk exists that instrumentation by hand scalers can lead to excessive removal of cementum, which has been reported to cause hypersensitivity or pulpitis (15). On the other hand, some studies have reported that ultrasonic scalers are effective in removing the endotoxins that exist on exposed root surfaces (16–18). As described above, ultrasonic scalers have a variety of merits as well as many points that need improvement.

Recently, a new type of ultrasonic scaler (Vector^{TM}), which can be used with a fluid polish in scaling and root planing, was developed, and has been used for removal of biofilms, gentle polishing, removal of subgingival calculus, cleaning of periodontal pockets, preservation of cementum and removal of endotoxins. Furthermore, a recent study reported that scaling and root planing with the Vector^{TM} can reduce the amount of pain felt by patients compared with conventional ultrasonic and hand scalers (19). However, the effect on periodontally affected root surfaces after scaling and root planing with the Vector^{TM} has not yet been studied.

The aim of this study was to perform scaling and root planing with the Vector^{TM} both with and without fluid polish, conventional ultrasonic scalers and hand scalers, in order to compare the effect on the periodontally affected root surface after scaling and root planing, and to examine the degree of attachment of fibroblasts to the operating surfaces.

Materials and methods

Teeth used

The experimental teeth used for this study were extracted human teeth affected by periodontal disease but with no previous history of periodontal treatment, single-rooted teeth with a similar deposition of calculus to the naked eye, and teeth expediently extracted during orthodontic treatment. After extraction, the teeth were washed in normal saline solution and frozen at -20 ± 4°C. Teeth that had undergone root canal treatment, or had apical lesions or caries, were excluded.

After the frozen teeth were defrosted at room temperature, small specimens of $5 \times 5 \times 1$ mm, from the cemento-enamel junction to the root apex, were created using a water-cooled dental turbine. The calculus adhering to the specimens from the teeth affected by periodontal disease were evaluated with a 60× stereomicroscope (SZH, Olympus, Tokyo, Japan) using a 10×10 micrometer attached to an ocular lens (1). Eighty of the specimens that were 50% covered with adhering calculus were selected as specimens of roots affected by periodontal disease. In addition, 20 of the teeth expediently extracted during orthodontic treatment were used as specimens of healthy roots (7). The specimens created from these teeth were put in 2% hypochlorous acid solution and the soft tissue was removed.

Scaling and root planing were performed under constant pressure on the specimens of roots affected by periodontal disease as well as those of the healthy roots until they become smooth upon visual inspection and palpation using a probe (20) using the following methods: a new ultrasonic scaler (Vector^{TM}, Dürr Dental, Bietigheim-Bissingen, Germany); a new ultrasonic scaler used together with microfine hydroxyapatite fluid polish; a conventional ultrasonic scaler (ENAC® type5, Osada, Tokyo, Japan) and a hand scaler (Gracey–curette scaler 5/6, Hu-Friedy, Chicago, IL, USA). The power level was set at medium for both the Vector^{TM} and the Enac®, and probe-type tips were used. The specimens were classified into four groups (Vector^{TM}, Vector^{TM} with polish, Enac® and Gracey), with 20 specimens in each. To set the pressure, scaling and root planing were performed at 40 ***g*** (0.39N) for the ultrasonic scalers and 500 ***g*** (4.90N) for the hand scalers with the samples mounted on a Force Gauge (DPX-5T, Imada, Tokyo, Japan) (8, 21). Specimens of the healthy roots were rinsed in phosphate-buffered saline and classified as the control group.

Measurement of the time spent scaling and root planing

The length of time spent scaling and root planing for each instrument was measured by an observing staff member, then the average time for each group was taken.

Evaluation of surface texture

Arithmetical mean deviation of the profile (Ra: JIS B 0601, 1994) and 10 point height of irregularities (Rz: JIS B 0601, 1994) of 10 specimens out of each group were measured by means of the surface roughness and shape measurement system (Surfcom1400A, Tokyo Seimitu, Tokyo, Japan). The measurement was performed with a 0.25-mm cutoff and 1.25-mm measurement length. Each specimen was measured five times at 0.5-mm intervals lengthwise and widthwise. Then the average for each specimen was taken.

After the surface roughness had been measured, the specimens were fixed for 1 h in 1% glutaraldehyde in

Table 1. Roughness and Loss of Tooth Substance Index (RLTSI) (20)

0	Smooth and even root surface without marks from the instrumentation and with no loss of tooth substance
1	Slightly roughened or corrugated local areas confined to the cementum
2	Definitely corrugated local areas where the cementum may be completely removed, although most of the cementum is still present
3	Considerable loss of tooth substance with instrumentation marks into the dentin. The cementum is completely removed in large areas, or it has a considerable number of lesions from the instrumentation

Table 2. Remaining Calculus Index (RCI) (20)

0	No calculus remaining on the root surface
1	Small patches of extraneous material probably consisted of calculus
2	Define patches of calculus confined to smaller areas
3	Considerable amounts of remaining calculus appearing as one or a few voluminous patches or as several smaller patches scattered on the treated surface

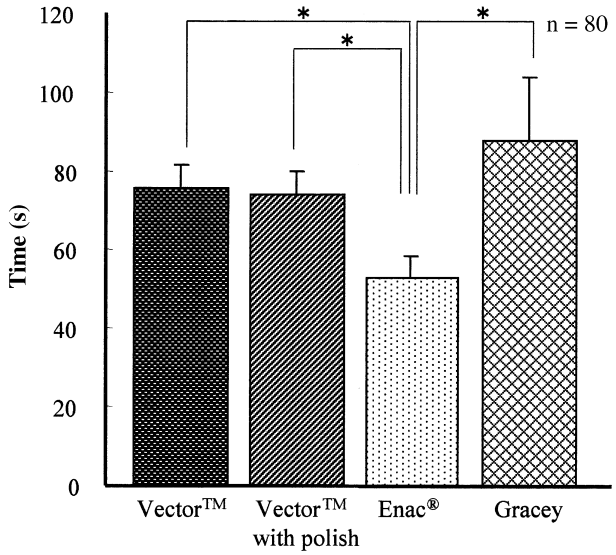

Fig. 1. Mean time(s) required to clean the test surface with each instrument. The time taken for the Enac® was significantly shorter than for the other groups. *p-value < 0.05.

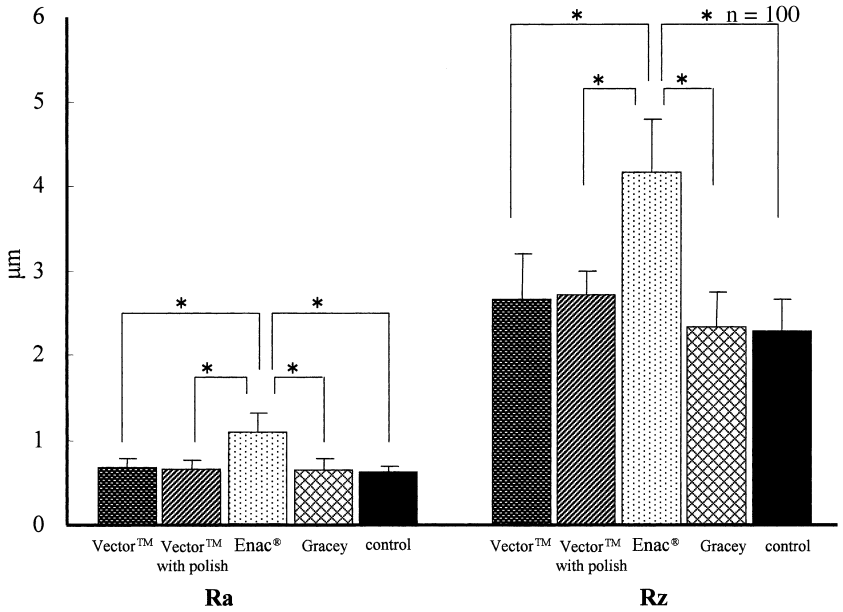

Fig. 2. Mean Ra (JIS B 0601, 1994) and Rz (JIS B 0601, 1994) values for each group. Mean Ra and Rz values for the Enac® were significantly higher than for the other groups. *p-value < 0.05.

phosphate-buffered saline solution, and rinsed in phosphate-buffered saline. The specimens were then post-fixed for 1 h in 1% osmium tetroxide solution in phosphate-buffered saline, rinsed in phosphate-buffered saline, dehydrated in an ethanol ascending series, substituted with isoamyl acetate, processed with a Critical Point Dryer (HCP-2, Hitachi, Tokyo, Japan), and gold-coated with an Ion Coater (JFC-1100, JEOL, Tokyo, Japan). Finally, they were observed using a scanning electron microscope (S-4300, Hitachi, Japan).

Scanning electron microscopy photographs magnified 100- or 500-fold, excluding the control group, were randomly selected and evaluated according to the Roughness and Loss of Tooth Substance Index (RLTSI) (20) (Table 1).

Evaluation of remaining calculus

Scanning electron microscopy photographs magnified 100- or 500-fold, excluding the control group, were randomly selected and evaluated according to the Remaining Calculus Index (RCI) (20) (Table 2).

Fibroblasts and their culture medium/cell culture

MRC-5 fibroblasts (The Japan Health Sciences Foundation, Tokyo, Japan) originated from human lung were used. The culture medium was α-minimal essential medium (α-MEM), which contained 10% fetal bovine serum (Biosource International, Camirillo, CA, USA) and antibiotics [penicillin/streptomycin solution, Sigma-Aldrich Co. (St Louis, MO, USA); final concentration 50 U/ml penicillin and 50 µg/ml streptomycin]. The fibroblasts were cultured under the following conditions: 37°C, 5% CO_2, 95% air. After four or five transfers, they were used for the experiments when the doubling time became constant.

Ten remaining specimens out of each group were sterilized using ethylene oxide gas. After sterilization, they were put into 24-hole dishes (Falcon 3047, Becton, Dickinson & Co., Franklin Lakes, NJ, USA) and prepared so that

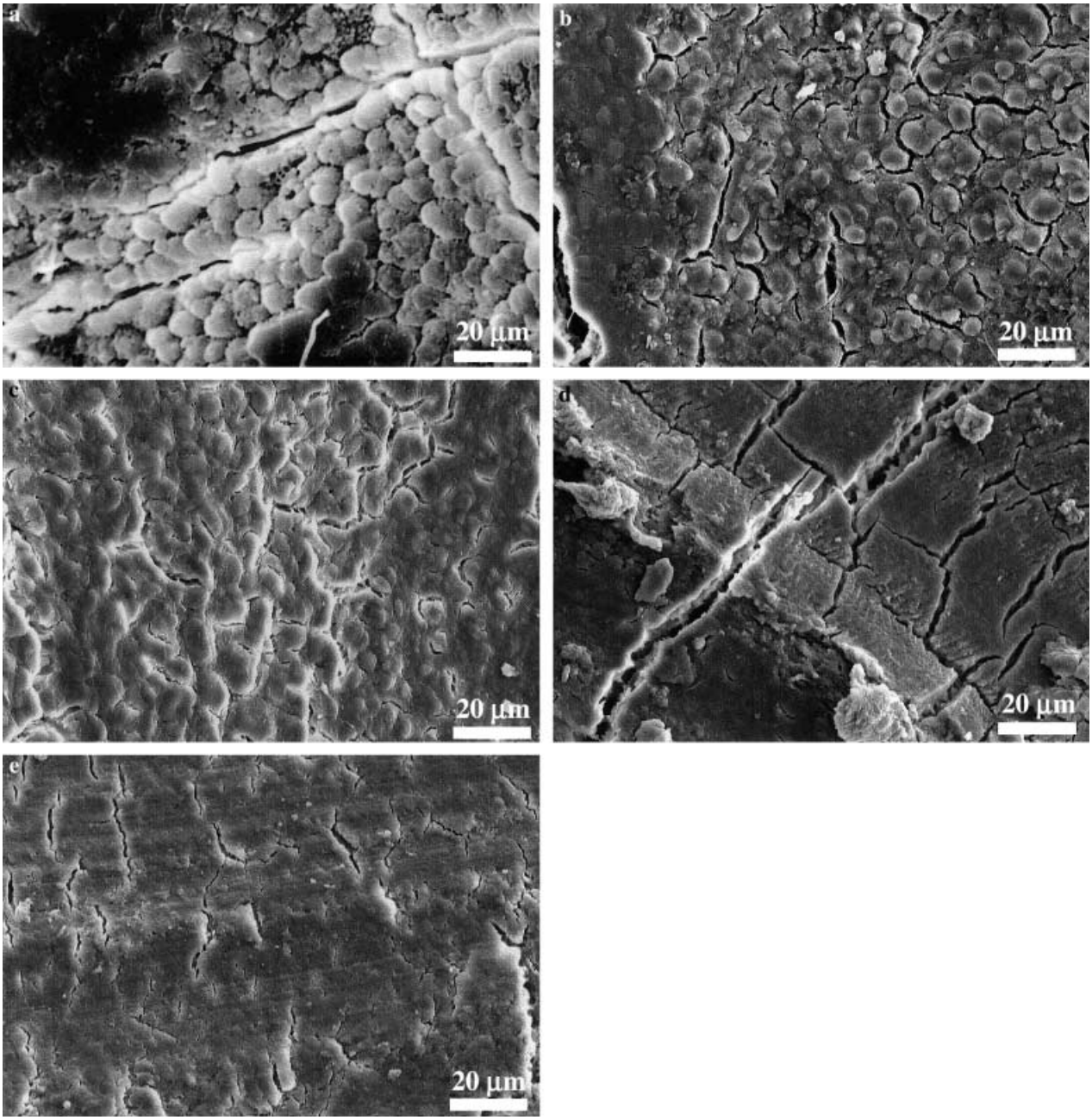

Fig. 3. (a) Scanning electron microscopy photomicrograph of a control surface (original magnification ×1000). The control group is completely covered with dome-shaped cementum. (b) Scanning electron microscopy photomicrograph of the Vector™-treated root surface (original magnification ×1000). The dome-shaped cementum structure is observed in many regions. (c) Scanning electron microscopy photomicrograph of the Vector™ with polish-treated root surface (original magnification ×1000). The dome-shaped cementum structure is present in many regions, while no remaining particles of polish are observed. (d) Scanning electron microscopy photomicrograph of the Enac®-treated root surface (original magnification ×1000). There are irregularities and defects. (e) Scanning electron microscopy photomicrograph of a Gracey-treated root surface (original magnification ×1000). There is an overall smear layer, and many linear injuries are confirmed.

each group of cells would be placed at 1.0×10^4 cells/ml in culture solution. A 24-h culture was performed on each group of cells, half at a time, under the following conditions: 37 ± 0.5°C, 5% CO_2, 95% air, pH 7.4.

Observation of the state of attached cells

Ten specimens from each group, where the cultured cells attached, were washed twice in 0.2 M phosphate-buffered saline (pH 7.2). They were then fixed using the procedures mentioned above, and stained with 0.1% toluidine blue solution. The number of attached cells was measured at five random points on the specimen surface using a 60×

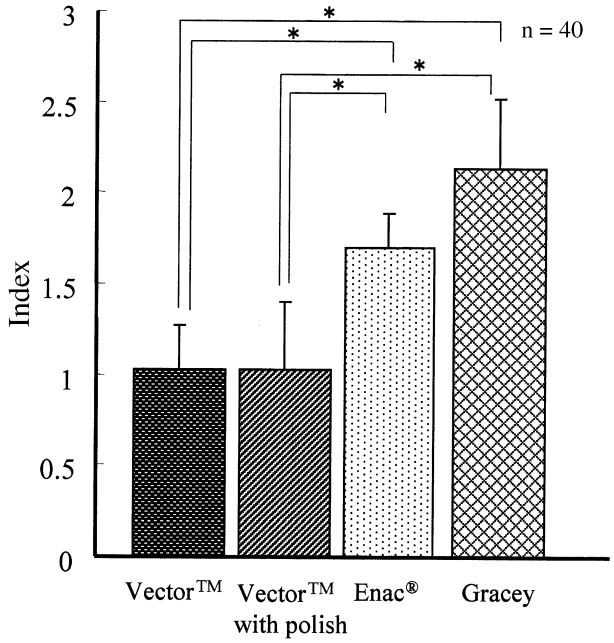

Fig. 4. Roughness and Loss of Tooth Substance Index values for each instrument tested. The Remaining Calculus Index values for the Vector™ and Vector™ with polish groups were significantly lower than those for the Enac® and Gracey groups. *p-value < 0.05.

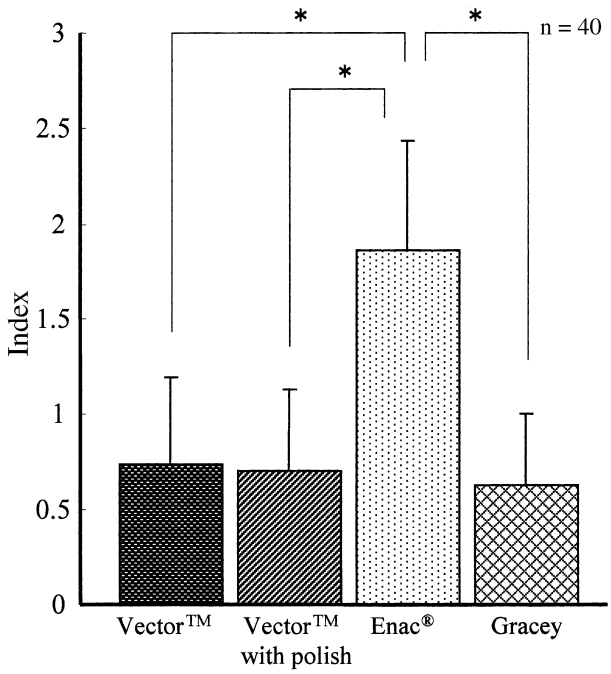

Fig. 5. Remaining Calculus Index (RCI) scores for each instrument tested. The RCI values for the Enac® group were significantly higher than for the other groups. *p-value < 0.05.

stereomicroscope and a 10×10 micrometer attached to an ocular lens. The number of cells appearing on the 16 grids of the diagonal line were counted and converted to the number per 1 mm^2. The average of the five points was deemed to be the number of attached cells per specimen after a 24-h culture (22).

Specimens were dehydrated according to the above procedure, critical point-dried and gold-coated, and the state of the cells was observed by scanning electron microscopy.

In this study, all measurements and evaluations were done independently by three examiners. None of these examiners took part in the experimental procedures or in the observation procedures.

Statistical analysis

Statistical analysis was based completely on a one-way factorial ANOVA with the Scheffe test. A risk rate of under 5% was evaluated as being a significant difference.

Results

Measurement of the time spent scaling and root planing

It was possible to treat the Enac® group in a significantly shorter period of time compared with the other groups. There was no significant difference among the Vector™, Vector™ with polish and Gracey groups (Fig. 1).

Measurement of surface roughness

Though measured values of roughness differed depending on each method of scaling and root planing, there was no significant difference among the Vector™, Vector™ with polish and Gracey groups. Surface roughness in the Enac® group was significantly different from that in the other groups.

Although there was a significant difference between the control and Enac® groups, the control group showed no significant difference from the other groups (Fig. 2).

Evaluation of root surface texture

The root surface texture in the control group was governed by the fact that the root surfaces were completely covered with dome-shaped cementum (Fig. 3a). The root surface texture in the Vector™ group was smooth with little overall injury. Under high magnification, the dome-shaped cementum structure was observed in many regions (Fig. 3b). The root surface texture of

the Vector™ with polish group revealed the same smoothness with little overall injury, as in the Vector™ group. Under high magnification, the dome-shaped cementum structure was present in many regions, while no remaining particles of polish were observed (Fig. 3c). The root surface texture of the Enac® group showed a rough overall appearance, and, under higher magnification, irregularities and defects, which were considered to have been caused by instrumentation, were found in some areas (Fig. 3d). The root surfaces of the Gracey group were covered with an overall smear layer, and many linear injuries, which were considered to have been caused by instrumentation, were confirmed (Fig. 3e).

Evaluation of the root surface texture with RLTSI revealed that the values for the Vector™ and Vector™ with polish groups were significantly lower than those for the Enac® and Gracey groups. No significant difference was observed between the Enac® and Gracey groups (Fig. 4).

Evaluation of remaining calculus

Evaluation of the remaining calculus with RCI showed that the values for the Enac® group were significantly higher than those of the other groups. There was no significant difference among the Vector™, Vector™ with polish and Gracey groups (Fig. 5).

Measurement of the number of attached cells

Statistical analysis showed that the number of attached cells in the control, Vector™ with polish and Gracey groups was significantly higher than that in the Enac® group. The number of attached cells in the Vector™ group was not significantly different from that in any of the other groups (Fig. 6).

Morphological observation of attached cells

In the control, Vector™ and Vector™ with polish groups, cell attachment to cemental prominences on root surfaces was observed, extending long, wide and planiform cytoplasmic projections and extending numerous filopodia toward the root surfaces (Figs 7a–c). In the Enac® group, cells were observed to attach to the smooth surfaces, but not to irregularities and defects, which were considered to have been caused by instrumentation. Short cytoplasmic projections and a few filopodia were observed (Fig. 7d). In the Gracey group, cells attached to the smear layer and extended very long cytoplasmic projections, while the filopodia were expanded, but very short (Fig. 7e).

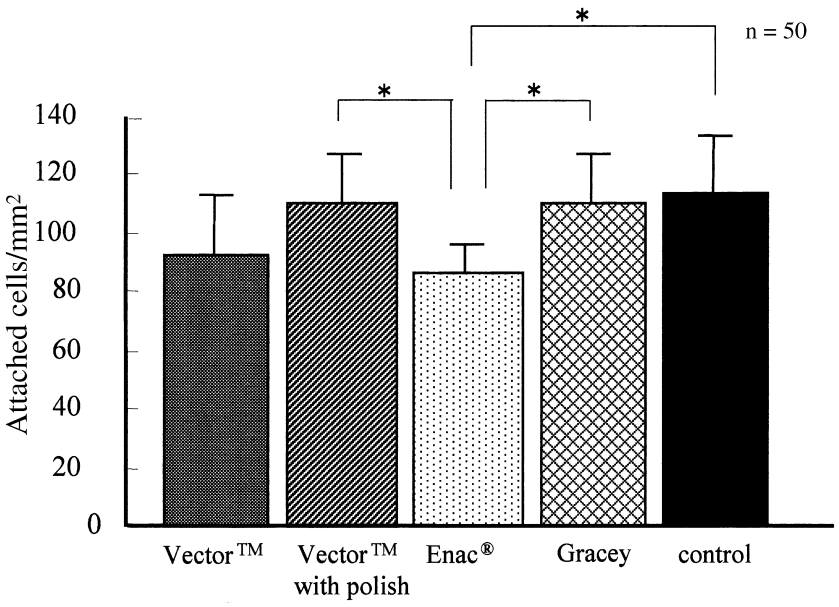

Fig. 6. The number of attached cells. The number of attached cells in the control, Gracey and Vector™ with polish groups was significantly higher than in the Enac® group. **p*-value < 0.05.

Discussion

The Vector™ used in this experiment is a piezoelectric ultrasonic scaler that can be used with microfine hydroxyapatite fluid polish. It has the characteristic that the ring at the tip applied to the surface oscillates like a hula hoop, and when the tip is placed horizontally on a root surface, it moves in an exactly vertical direction (19).

In this study, whether polish was used or not, scaling and root planing using the Vector™ produced a significantly smoother surface than that produced using the Enac®. The results of RLTSI evaluation were consistent with the results of the scanning electron microscopy observations (20), showing that the Vector™ and Vector™ with polish groups had a significantly smaller value than the Enac® and Gracey groups. In other words, it was considered that the new vibration communicating system of the Vector™ caused less loss of cementum and less injury from instrumentation by employing gentler operation on the root surface. The polish failed to enhance the effects of the Vector™ on the roughness and texture of the treated root surfaces.

The time spent scaling and root planing with the Vector™, whether polish was used or not, was significantly longer than the time needed for the Enac®, and even equal to the time spent using the Gracey. Therefore, the Vector™ cannot count ease and simplicity of operation as one of its merits. The RCI value for the Enac® group was significantly higher than for the other groups, possibly due to the difference in scaling and root planing time. Further studies will be needed to examine the effect of each instrumentation on root surfaces within the same treatment time.

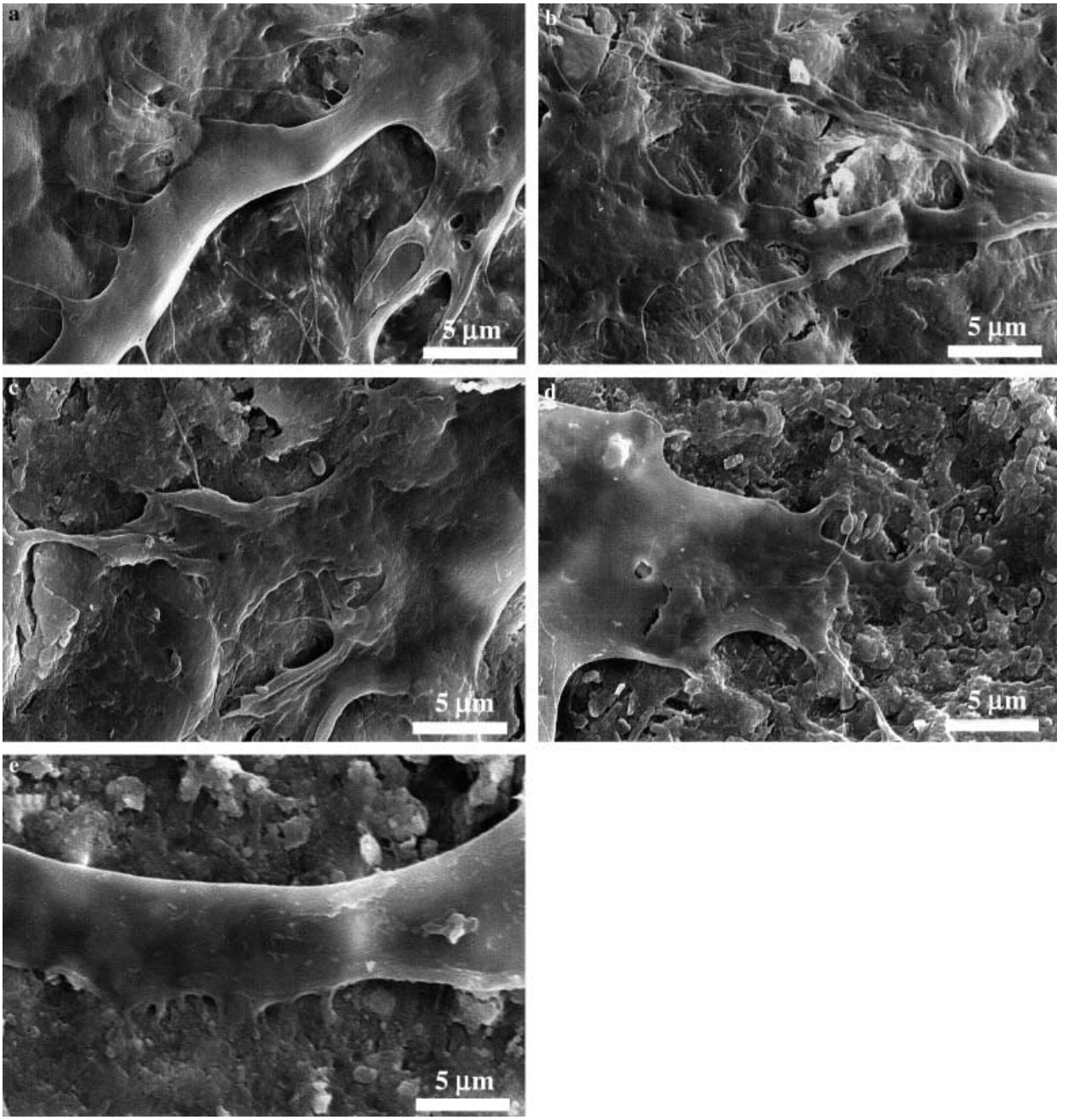

Fig. 7. (a) Scanning electron microscopy photomicrograph of cell attachment on control surface (original magnification ×5000). Cell attachment is observed, extending long, wide and planiform cytoplasmic projections and numerous filopodia. (b) Scanning electron microscopy photomicrograph of cell attachment to root surface treated with the Vector™ (original magnification ×5000). Cell attachment is observed, extending long, wide and planiform cytoplasmic projections and numerous filopodia. (c) Scanning electron microscopy photomicrograph of cell attachment to root surface treated with the Vector™ with polish (original magnification ×5000). Cell attachment is observed, extending long, wide and planiform cytoplasmic projections and numerous filopodia. (d) Scanning electron microscopy photomicrograph of cell attachment to root surface treated with the Enac® (original magnification ×5000). Cells extend very long cytoplasmic projections, while the filopodia are very short.

Even though various textures of root surface were observed for each operation, it was unclear whether any biologically acceptable root surfaces were actually achieved. Therefore we cultured fibroblasts on these surfaces and examined their state of attachment (22–24). Since the hypermineralized layer on the surface of roots affected by periodontal disease and the layer containing endotoxins in the Enac® group could not be fully removed, fibroblasts

were prevented from attaching to the surfaces in a living state and consequently died. Therefore the number of attached cells was significantly smaller. The fibroblasts' poor attachment was due to poor extension of their cytoplasmic projections and filopodia. Furthermore, since no fibroblasts were observed on the defects and rough areas, the root surface texture in the Enac® group was considered to be unfavorable for attachment and growth of fibroblasts. In the Gracey group there was a lot more tooth substance loss than in the other groups, including the contaminant layer of roots affected by periodontal disease, which was fully removed. Therefore, a larger number of cells were attached; however, the filopodia were found to be shorter than those in the Vector™ and Vector™ with polish groups. This is probably because the texture of the root surfaces in the Gracey group had some effect on cell attachment. The number of attached cells in the Vector™ group showed no significant difference compared with the Enac®. However, the Vector™ group showed a healthy attachment condition, where the attached cells extended cytoplasmic projections and filopodia. This was probably because scaling and root planing achieved smoother surfaces without injury compared with the Enac® group, and because removal of contaminants such as endotoxins on the specimen surfaces was better than in the Enac® group. Although there was no difference in the attachment capacity of the cells between the Vector™ with polish and Vector™ groups, the number of attached cells was significantly larger in the Vector™ with polish group. Though both groups showed the same surface roughness, whether polish was used or not, the use of polish caused significantly more cell attachment. Nyman *et al*. concluded that excessive removal of cementum is not necessary and that the polishing procedure suffices for the removal of contaminants (13). The results of this study also indicate that the use of polish works effectively to remove endotoxins on root surfaces. Future research in this area should include a quantitative study on how the use of polish affects the removal of endotoxins.

Conclusion

This study has shown that scaling and root planing using the Vector™ is less effective in removing large masses of calculus quickly than the Enac®. However, the Vector™ is capable of producing smooth root surfaces with preservation of more cementum, and can achieve a level of smoothness similar to a healthy root surface or that produced by hand scaling. When used in conjunction with polish, the Vector™ results in the presence of a significantly larger number of attached cells on the root surface than those observed when the Enac® is used. These results suggest that scaling and root planing using the Vector™ together with polish may be effective for scaling and root planing.

Acknowledgments

We thank Dr David B. Douglas for his excellent assistance with manuscript preparation. This work was supported in part by a Grant from the Ministry of Education, Culture, Sports, Science and Technology to promote multidisciplinary research projects and by the Sato Found on Nihon University School of Dentistry (2001, 2002).

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