

Assessing qualitative changes in simulated periodontal ligament and alveolar bone using a non-contact electromagnetic vibration device

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Abstract The objective of this study is to investigate the ability of a non-contact electromagnetic vibration device to assess a simulated periodontal ligament and alveolar bone conditions in experimental tooth models by applying mechanical parameters (resonant frequency, elastic modulus, and coefficient of viscosity). The non-contact electromagnetic vibration device was made up of three components: vibrator, detector, and analyzer. The experimental tooth model consisted of a cylindrical rod made of polyacetal, a tissue conditioner for soft lining material, and urethane or urethane foam to simulate the tooth, periodontal ligament, and alveolar bone, respectively. The tissue conditioner was prepared by mixing various volumes of liquid with powder. Periotest® values (PTVs) were also measured under the same conditions as those of the non-contact electromagnetic vibration device. All of the me-

chanical parameters derived from the non-contact electromagnetic vibration device significantly decreased as the proportion of liquid increased. Values for the three parameters of the urethane models were significantly larger than those of the urethane foam models. In contrast, PTVs increased significantly as the proportion of liquid increased; however, no significant difference was observed between the urethane and urethane foam models. The non-contact electromagnetic vibration device may be capable of evaluating not only periodontal ligament conditions but also bone quality. Mechanical parameters may be useful for assessing qualitative changes in the periodontal ligament and alveolar bone.

Keywords Non-contact electromagnetic vibration device · Periodontal ligament · Alveolar bone · Tooth mobility testing · In vitro study

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Introduction

A measure of tooth mobility is an important diagnostic parameter in evaluating tooth and periodontal tissue conditions, contributing greatly to treatment planning and clinical management. Conventionally, Miller's technique [1] has been used for this purpose, but because this technique is dependent on the operator's tactile sense and macroscopic confirmation, the judgment may be affected by the operator's experience and the environment of the oral cavity.

Schulte et al. [2] introduced Periotest®, a diagnostic device that measures tooth mobility objectively using contact times between an acceleration rod (probe) and the target tooth surface and displays the tooth displacement

volume as Periotest[®] values (PTVs) from -8 to $+50$. They have shown that the contact time correlates with the clinical degrees of tooth loosening. Additionally, Goellner et al. have compared the Periotest[®] method with a quantitative-metric tooth mobility measuring method by testing healthy individuals and indicated no certain correlation between the metric displacement of the tooth and PTVs [3]. These findings seem to suggest that, besides the tooth displacement, the properties of the periodontal ligament also influence the PTVs. Also, Berthold et al. [4] have reported that the Periotest[®] method can be used to detect early stages of ankylosis caused by dental trauma, especially when using the vertical PTVs. Presently, this device has been used widely in clinics because of its objective information about tooth mobility testing, cost efficiency, reproducibility, and time saving measurement [5–7].

Nevertheless, the use of Periotest[®] is limited in a narrow oral cavity. Following the instruction, the measuring method should be recommended to approach from buccal or labial sites and the loading system of the device uses a repeated uniform linear motion. Therefore, in cases of posterior teeth, the measurement might be interfered with the cheek. In other cases, such as buccal destruction of crowns, it is required to approach from the lingual direction lest the tongue may interfere with the measurement. Campbell et al. [8] described the lack of acceptance by some pre-adolescents and adolescents for the Periotest[®] device in the early post-injury period due to discomfort from the firm repetitive tapping.

The reading from Periotest[®] does not always correspond precisely to the biomechanical parameters because PTVs are strongly related to the excitation direction and position [9, 10]. Therefore, Yamane et al. [11] reported on a new non-contact electromagnetic vibration device that can analyze both tooth mobility and periodontal tissue condition using mechanical parameters (resonant frequency, elastic modulus, and coefficient of viscosity) obtained from the frequency response characteristics during tooth vibration. It vibrates the tooth with an electromagnetic force with non-contact between the device and target tooth surface. They investigated the effects of the bottom thickness of simulated periodontal ligament in an experimental tooth model on the mechanical parameters and suggested that it could be used to accurately evaluate the periodontal tissue condition. Hayashi et al. [12] indicated that this new device was capable of monitoring not only periodontal tissue condition but also implant stability using the same mechanical parameters.

In this study, it was hypothesized that the non-contact vibration device could detect qualitative changes in a simulated periodontal ligament and alveolar bone using mechanical parameters.

Materials and methods

Experimental tooth model

The experimental tooth model consisted of a 6.0×25.0 -mm cylindrical rod ($\phi \times L$) made of polyacetal, a tissue conditioner for soft lining material (Shofu Tissue Conditioner II, Shofu Inc, Kyoto, Japan; Table 1), and urethane or urethane foam (Nissin Dental Products Inc, Kyoto, Japan) to simulate the tooth, periodontal ligament, and alveolar bone, respectively. The cylindrical rod was submerged 10.0 mm into the simulated alveolar bone, and a simulated 0.5-mm-thick periodontal ligament was created between the cylindrical rod and simulated alveolar bone. A specially made jig (Nissin Dental Products Inc) was used to obtain an accurate position of cylindrical rod in the experimental tooth model as shown in Fig. 1. The simulated periodontal ligament was 0.5-mm in thickness in all of the experimental models and was prepared under a thermo-hygrostat at $23 \pm 1^\circ\text{C}$ and a relative humidity of $50 \pm 5\%$. The models were kept under these conditions for 1 h before measurement.

Conditions of simulated periodontal ligament and alveolar bone

A simulated periodontal ligament was prepared using various liquid volumes of the tissue conditioner. The standard liquid volume for the clinical use of the soft lining material according to the manufacturer's instructions is 4.0 ml of liquid with 4.8 g of powder. In this study, three different simulated periodontal ligaments were prepared by mixing 3.0, 4.0, and 5.0 ml of liquid with 4.8 g of powder. The stimulated alveolar bone was made of urethane or urethane foam.

Non-contact electromagnetic vibration device

A schematic diagram of the non-contact electromagnetic vibration device used in this study is shown in Figs. 2 and 3. This device was made up of three components: vibrator, detector, and analyzer, which modified a previous report as described by Yamane et al. [11].

Table 1 Composition of tissue conditioner II used in this study

	Manufacturer/ lot no.	Chemical composition	
		Powder	Liquid
Tissue conditioner II	Shofu/ Powder: 120867 Liquid: 120845	PEMA, other	Di- <i>n</i> -butyl sebacate, anhydrous ethanol, other

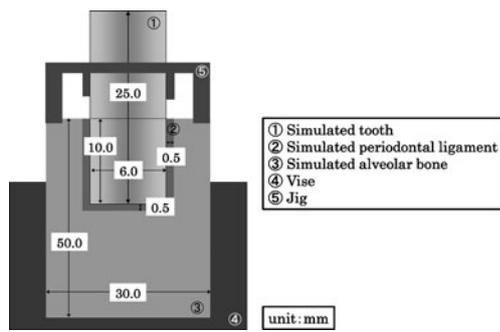


Fig. 1 Preparation of an experimental tooth model

The vibrator consisted of a magnetic disk (ϕ 4.0 mm, 0.19 g, 130 mT; Pip Fujimoto, Osaka, Japan) and the electromagnetic vibration device. The magnetic disk was attached to the lateral surface at the top of the cylindrical rod by an adhesive (cyanoacrylate; Toagosei, Tokyo, Japan). The magnetic disk receives the electrical force generated by the alternating magnetic field produced by the electromagnetic vibration device. The electromagnetic vibration device consists of a ferrite rod wound with an enamel wire (ϕ 5.0 mm) for 720 times to form a coil. The tip of the ferrite rod is conical.

The vibrations were detected with acceleration sensors that weigh 0.4 g (NP-3211; Ono Sokki, Tokyo, Japan). The acceleration sensors were attached to the top of the cylindrical rod. The output signal from the acceleration sensor was input to the fast Fourier transformation (FFT) analyzer that includes a sensor amplifier.

The frequency response characteristics of the experimental tooth model (i.e., the ratio between the output of a sweep generator and the input of an acceleration sensor) were calculated by the FFT analyzer. Measurements were made over a frequency range of 5 kHz, with a frequency resolution of 12.5 Hz and an 80-ms capture time.

Measurement method

The distance between the center of the magnetic disk and the tip of the ferrite rod was kept at 1 mm in order to apply

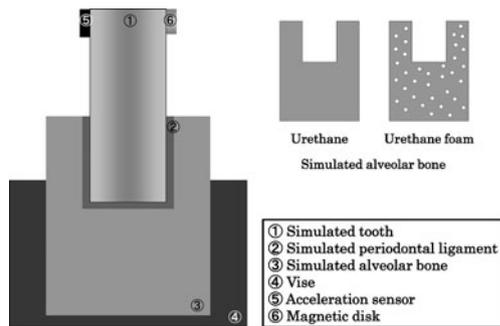


Fig. 2 Experimental tooth model

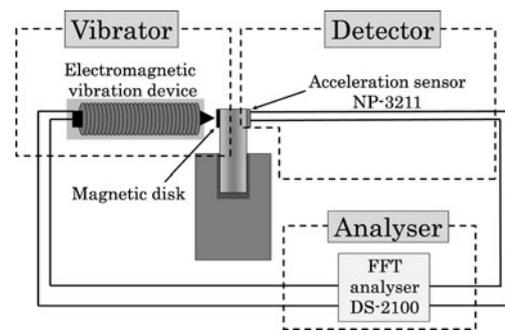


Fig. 3 Components of the experimental device

the electric force without contact. The ferrite rod was fixed at right angles to the center of the magnetic disk to ensure reproducible measurement points. The frequency response characteristics were evaluated by three repeated measurements for each experimental tooth model to reduce the measuring errors, and the mechanical parameters were calculated using the process described by Yamane et al. [11]. Each mechanical parameter was assessed from the frequency response characteristics according to the following formulas (Fig. 4):

$$\zeta = \frac{(f_2 - f_1)}{2f_n} \tag{1}$$

$$k = 4\pi^2 f_n^2 m \tag{2}$$

$$c = 2\zeta \sqrt{mk} \tag{3}$$

In these formulae, f_n is the resonant frequency (Hz), m is the mass (kg), ζ is the damping ratio (O), k is the elastic modulus (N/m^2), and c is the coefficient of viscosity ($N s/m^2$). Additionally, f_1 and f_2 are the frequencies at $1/\sqrt{2}$ times the maximum amplitude of the resonant frequency. The total mass of the polyacetal cylindrical rod, acceleration sensor, and magnetic disk (1.59×10^{-3} kg) is represented by m . The mass of the lead wire was negligible in

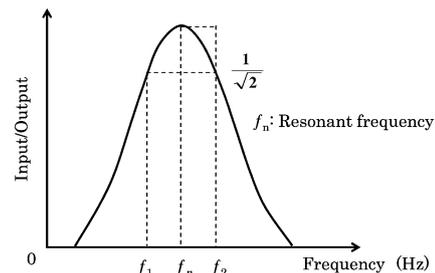


Fig. 4 Frequency response characteristics. f_1 and f_2 are the frequencies at $1/\sqrt{2}$ times of the maximum amplitude of the resonant frequency (f_n)

this study because the wire was not under tension during the measurement and its mass was small. Five experimental tooth models under each condition were made to analyze the frequency response characteristics. Data are expressed as the median with the maximum and minimum of each mechanical parameter in the five models ($n=5$).

PTVs (Periotest®; Gulden Messtechnik, Bad Bensheim, Germany) were measured under the same experimental conditions as those of the non-contact electromagnetic vibration device, but without the acceleration sensor and the magnetic disk on the simulated teeth. Three repeated Periotest® measurements were taken for each experimental tooth model according to the manufacturer's instructions.

Statistical analyses

The difference in mechanical parameters (resonant frequency, elastic modulus, and coefficient of viscosity) or PTVs among various periodontal ligament conditions were analyzed using the Kruskal–Wallis test followed by the Steel–Dwass test. Additionally, differences between the two types (urethane vs. urethane foam) of simulated alveolar bone were analyzed using the Mann–Whitney *U*-test. Differences were considered significant at $p<0.05$.

Results

Resonant frequency

The resonant frequency for each liquid volume to 4.8 g powder of tissue conditioner is shown in Fig. 5. The resonant frequency decreased curvilinearly with increasing liquid volume in both the urethane and urethane foam models. Statistically significant differences were found among the different volumes of liquid mix used (Tables 2 and 3). The median of maximum resonant frequency (1.25 kHz in urethane and 1.08 kHz in urethane foam)

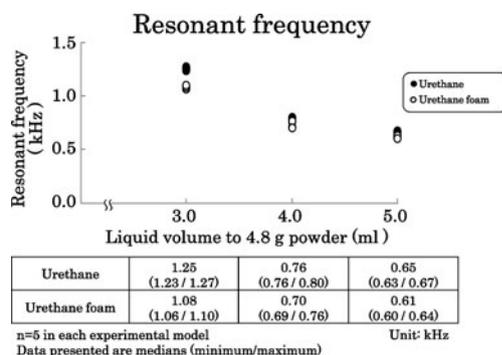


Fig. 5 Resonant frequency at different liquid volumes (closed circles—urethane, open circles—urethane foam)

occurred with 3.0 ml of liquid volume, and the median of the minimum resonant frequency (0.65 kHz in urethane and 0.61 kHz in urethane foam) was found at 5.0 ml of liquid volume. Additionally, the resonant frequency of urethane models was significantly larger than that of urethane foam models under all simulated periodontal ligament conditions (Table 4).

Elastic modulus

The elastic modulus for each liquid volume to 4.8 g powder of tissue conditioner is shown in Fig. 6. The elastic modulus decreased curvilinearly with increasing liquid volume in both the urethane and urethane foam models, similarly to the resonant frequency. There were statistically significant differences among the different volumes of liquid mix used (Tables 2 and 3). The median of maximum elastic modulus (0.98×10^5 N/m² in urethane and 0.74×10^5 N/m² in urethane foam) occurred with 3.0 ml of liquid volume, and the median of minimum elastic modulus (0.27×10^5 N/m² in urethane and 0.24×10^5 N/m² in urethane foam) was found at 5.0 ml of liquid volume. Additionally, the elastic modulus of urethane models was significantly larger than that of urethane foam models under all simulated periodontal ligament conditions (Table 4).

Coefficient of viscosity

The coefficient of viscosity for each liquid volume to 4.8 g powder of tissue conditioner is shown in Fig. 7. The coefficient of viscosity decreased linearly with increasing liquid volume in both the urethane and urethane foam models. Significant differences were also observed among the different volumes of liquid mix used (Tables 2 and 3). The median of maximum coefficient of viscosity (5.00 Ns/m² in urethane and 3.74 Ns/m² in urethane foam) occurred with 3.0 ml of liquid volume, and the median of minimum coefficient of viscosity (2.94 Ns/m² in urethane and 2.31 Ns/m² in urethane foam) was found at 5.0 ml of liquid volume. The coefficient of viscosity of the urethane models was significantly larger than that of urethane foam models under all simulated periodontal ligament conditions (Table 4).

PTVs

PTVs increased curvilinearly as the proportion of liquid increased in both the urethane and urethane foam models, unlike the situation with the non-contact electromagnetic vibration device (Fig. 8). Significant differences were observed among the different volumes of liquid mix in both the urethane and urethane foam models (Tables 2 and 3). The median of minimum PTVs (26 in urethane and 25 in

Table 2 Medians of each mechanical parameter and PTVs, and results from the Kruskal–Wallis test followed by the Steel–Dwass test between different liquid volumes to 4.8 g powder in urethane models

	Liquid volume (ml)			Kruskal–Wallis <i>p</i> -value	Steel–Dwass Between different liquid volumes
	a (3.0)	b (4.0)	c (5.0)		
Resonant frequency	1.25	0.76	0.65	< 0.01 ^a	a–b ^b , a–c ^b , b–c ^b
Elastic modulus	0.98	0.38	0.27	< 0.01 ^a	a–b ^b , a–c ^b , b–c ^b
Coefficient of viscosity	5.00	4.12	2.94	< 0.01 ^a	a–b ^b , a–c ^b , b–c ^b
Periotest® values (PTVs)	26	32	45	< 0.01 ^a	a–b ^b , a–c ^b , b–c ^b

^a Results of the Kruskal–Wallis test. Significant differences among different liquid volumes are presented (*p*<0.01)

^b Results of the Steel–Dwass test. Significant differences between different liquid volumes are presented (*p*<0.05)

urethane foam) occurred with 3.0 ml of liquid volume, and the median of maximum PTVs (45 in urethane and 47 in urethane foam) was found at 5.0 ml of liquid volume. However, no significant difference was detected between the urethane and urethane foam models in PTVs (Table 4).

Discussion

The Periotest® device developed by Schulte et al. [2] has improved the accuracy and objectivity of tooth mobility testing and contributed to diagnostic evaluation in periodontal treatment. With this device, PTVs reflect the displacement amplitude on impact loading and the properties of the periodontal ligament of the target tooth [3]. Meredith et al. [13] developed a new method for implant stability testing using the sonic resonant frequency response, and resonant frequency analysis has been recognized as a precise technique for this purpose. Their studies revealed that this method could be used to measure implant stability with greater reliability. However, periodontal tissues have both elastic and viscous properties (i.e., viscoelastic properties). Thus, measurement using PTVs or resonant frequency alone may be insufficient to assess the qualitative changes of overall periodontal tissues condition.

In 1966, Bien [14] focused on the viscoelastic property of periodontal ligament during tooth movement, particularly hydrodynamic damping, and investigated the biological responses of the periodontal ligament using both living and dead rat incisors. The results showed that the viscosity and spring constant of living rat incisors were about two times larger than those of dead rat incisors. Three biological factors were suggested to affect the damping oscillation of the tooth: the vascular system, cells and fibers, and interstitial fluid. The latter played a critical role in the viscous property of periodontal ligament.

Moreover, Kojima et al. [15] and Lee et al. [16] reported that the natural frequency of a tooth was an important parameter for the evaluation of periodontal conditions. Maeda [17], Ikeda [18], Kamimoto [19], and Hayashi [20] investigated the mechanical characteristics of periodontal tissue, analyzing the damped natural frequency and other mechanical parameters quantitatively using a contact vibration device in vitro. Their results suggested that this analysis might contribute to evaluating periodontal tissue conditions after considering the mechanical parameters and their mutual relationships.

Recently, Yamane et al. [11] focused on the viscoelastic property of periodontal tissues and developed a non-contact electromagnetic vibration device for evaluating periodontal

Table 3 Medians of each mechanical parameter and PTVs, and results from the Kruskal–Wallis test followed by the Steel–Dwass test between different liquid volumes to 4.8 g powder in urethane foam models

	Liquid volume (ml)			Kruskal–Wallis <i>p</i> -value	Steel–Dwass Between different liquid volume
	a (3.0)	b (4.0)	c (5.0)		
Resonant frequency	1.08	0.70	0.61	< 0.01 ^a	a–b ^b , a–c ^b , b–c ^b
Elastic modulus	0.74	0.31	0.24	< 0.01 ^a	a–b ^b , a–c ^b , b–c ^b
Coefficient of viscosity	3.74	3.00	2.31	< 0.01 ^a	a–b ^b , a–c ^b , b–c ^b
Periotest® values (PTVs)	25	31	47	< 0.01 ^a	a–b ^b , a–c ^b , b–c ^b

^a Results of the Kruskal–Wallis test. Significant differences among different liquid volumes are presented (*p*<0.01)

^b Results of the Steel–Dwass test. Significant differences between different liquid volumes are presented (*p*<0.05)

Table 4 Results from the Mann–Whitney *U*-test between urethane and urethane foam models

	Mann–Whitney <i>U</i> -test		
	3.0	4.0	5.0
Resonant frequency	$p=0.0090^{**}$	$p=0.0204^*$	$p=0.0212^*$
Elastic modulus	$p=0.0088^{**}$	$p=0.0204^*$	$p=0.0212^*$
Coefficient of viscosity	$p=0.0088^{**}$	$p=0.0078^{**}$	$p=0.0088^{**}$
Periotest® values (PTVs)	$p=0.5124$	$p=0.9139$	$p=0.1105$

Results of the Mann–Whitney *U*-test. Significant differences between urethane and urethane foam models are presented
 $^*p<0.05$; $^{**}p<0.01$

tissue conditions using three mechanical parameters: the resonant frequency, elastic modulus, and coefficient of viscosity. Additionally, Hayashi et al. [12] revealed that this new device can be applied to determine these mechanical parameters not only for periodontal tissue conditions but also to assess implant stability. This is based on the theoretical system reported by Kurashima [21] and Komatsu [22]. Their initial results revealed that the three mechanical parameters reflected changes in the bottom thickness of the simulated periodontal ligament and suggested that the device could assess the overall periodontal tissue condition using the mechanical parameters derived from the resonant frequency characteristics according to the theory of Yajima’s dynamic model [23].

Our study evaluated the ability of the non-contact electromagnetic vibration device to assess qualitative changes in simulated periodontal ligament and alveolar bone conditions in vitro using experimental tooth model. Cylindrically shaped rod, instead of tooth shape, was used as simulated tooth because this study is a fundamental in vitro research; therefore, it is important to make an experimental condition simpler with an uncomplicated simulated tooth shape. Tissue conditioner was used as simulated periodontal ligament. This material is suitable for artificial periodontal ligament because it has the properties of both viscosity and elasticity, such as periodontal ligament [14, 24, 25]. Bone is not a uniformly solid material, i.e., cortical bone or cancellous bone. The two types of simulated alveolar bone, urethane and urethane form, were

used to assess the qualitative changes of alveolar bone in this study because of simplified experimental bone condition and the easy procedure to make urethane into a different quality of materials.

The length of ferrite rod wound with enamel wire of the electromagnetic vibration device is shorter than that of the hand-piece of Periotest® as shown in Fig. 9; therefore, it might be easy to measure the second or third molar. However, this device is not yet applicable for routine dental controls because this research is a fundamental in vitro study using experimental tooth models. Further efforts are required to develop this device for clinical use (i.e., total device size, cost efficiency, time-saving, et al.).

Chander et al. [24] and Murata et al. [25] reported that the effect of the powder-to-liquid ratio for tissue conditioners may be altered to vary the viscoelastic properties, especially after gelation. We found that a greater proportion of liquid influenced the mechanical parameters (i.e., this could simulate the effects of the extracellular matrix including the interstitial fluid on the mechanical properties). Significant differences in all mechanical parameters were observed among the different volumes of liquid mix used (3.0–5.0 ml).

Additionally, the increased proportion of liquid must affect the repulsion and resistance of periodontal ligament, as described by Bien [14]. Thus, the decrease in the three mechanical parameters as the proportion of liquid increased suggests that this measurement method is theoretically reliable.

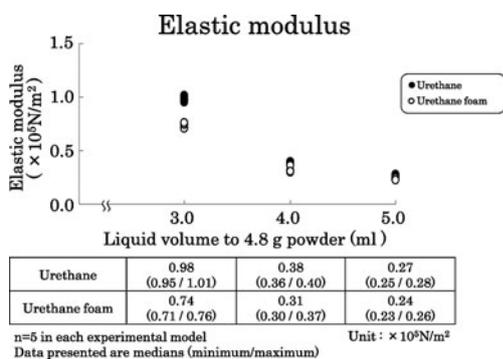


Fig. 6 Elastic modulus at different liquid volumes (closed circles—urethane, open circles—urethane foam)

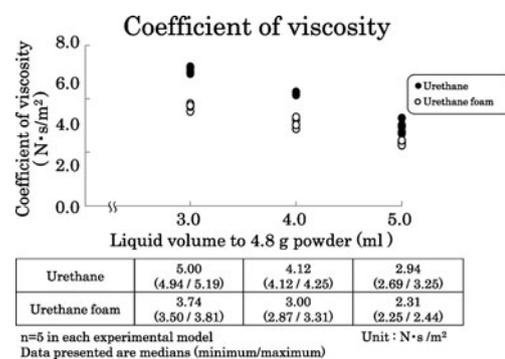


Fig. 7 Coefficient of viscosity at different liquid volumes (closed circles—urethane, open circles—urethane foam)

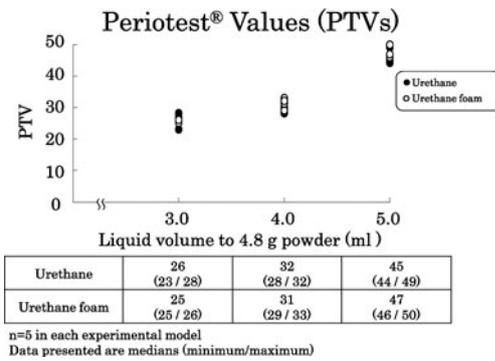


Fig. 8 Periotest® values at different liquid volumes (*closed circles*—urethane, *open circles*—urethane foam)

PTVs, which indicate, among other things, the amount of displacement volume of the simulated tooth, also detected the difference of qualitative changes in simulated periodontal ligament. This finding suggests a previous observation [3] that the properties of the periodontal ligament influence the PTVs. Increasing proportions of liquid in experimental tooth models made the tissue conditioner softer, and it may reflect the mobility of simulated teeth. Thus, these tooth models are sufficient in terms of reliability.

The three mechanical parameters showed significantly larger values in the urethane models than those of the urethane foam models. However, no significant difference in PTVs was detected. This could be because of the different valuable information between these methods. PTVs mainly describe the damping characteristics and properties of periodontal ligament; however, a non-contact electromagnetic vibration device can provide information about overall periodontal tissue condition using three mechanical parameters.



Fig. 9 Ferrite rod wound with enamel wire of electromagnetic vibration device and hand-piece of Periotest®

Both resonance frequency and elastic modulus were larger in the urethane models than the urethane foam models for all simulated periodontal ligament conditions. Generally, the force F applied to the elasticity materials is described by

$$F = kx \tag{4}$$

where k is a spring constant (the elastic modulus) and x is displacement (the expansion and contraction of the elastic material). From these relationships, when the force is constant, as for the elastic modulus, the value becomes large when displacement is small. Here, the displacement is regarded as the volumetric strain of the simulated alveolar bone. Thus, because urethane is harder than urethane foam, the displacement becomes small. As a result, Eq. 4 could theoretically lead to a larger elastic modulus of urethane than that of urethane foam [26].

Additionally, the resonance frequency f is described by

$$f = \frac{1}{2\pi} \sqrt{\frac{k}{m}} \tag{5}$$

where k is the elastic modulus and m is the mass. f becomes large with k for constant m . For these reasons, both resonance frequency and elastic modulus in urethane models are different from those of urethane foam models. The differences between urethane and urethane foam in the coefficient of viscosity were much larger than those of both the resonance frequency and the elastic modulus.

The coefficient of viscosity is described by ζ , k , and m in Eq. 3. In a result shown in Fig. 6, because the differences in k between urethane and urethane foam were small, the difference of the coefficient of viscosity may depend primarily on ζ if m is constant; ζ is a value indicating damping of the vibration. In these experimental tooth models, because the bubble of the urethane foam acted as the spring, it did not act as a damper; thus, the values of ζ could decrease. As a result, the coefficient of viscosity may decrease according to the values of ζ .

The non-contact electromagnetic vibration device could distinguish urethane foam from urethane. Thus, this device may include not only the condition of periodontal ligament but also the ability to detect bone quality.

Osteoporosis is characterized by low bone mass and micro-architectural deterioration of bone tissue [27, 28]. Recent investigations have associated radiographic evidences of changes in the mandibular cortical bone with periodontitis and linked them to osteoporosis [29]. A significant association between bone mineral density of the mandible and the peripheral skeleton in postmenopausal women has also been described [30–32]. The benchmark for the diagnosis of osteoporosis is the assessment of bone mineral density. Dual energy X-ray absorptiometry is

currently the gold standard for its assessment [33]; however, this method suffers from a lack of portability and exposure to radiation (albeit a small amount). The present study showed a strong relationship between mechanical parameters and the quality of the simulation bone material (urethane) on measurement using a non-contact electromagnetic vibration device. This device may help dentists not only to monitor the condition of the bone density in periodontitis but also to identify patients with undetected low bone mineral density.

Autogenous bone, allogeneic bone, xenogeneic bone substitutes, and alloplastic materials have all been used with the aim of achieving periodontal regeneration [34–36]. A systematic review has shown that clinical parameters are improved when intrabony and class II furcation defects are treated with these graft materials [35]. However, material-specific differences seem to exist with regard to the extension of new bone formation from the pre-existing bone in histological evaluations [37]. In such cases, a non-contact electromagnetic vibration device may be useful for monitoring bone formation.

In conclusion, mechanical parameters reflected the qualitative changes in the simulated periodontal ligament and alveolar bone. Consequently, an analysis of the three parameters might enable an objective evaluation of qualitative changes in the extracellular matrix of the periodontal ligaments and bone quality in humans. However, it is presently difficult to discriminate between the qualitative changes of simulated periodontal ligament and those of alveolar bone. Further investigations must be done to examine the difference between these three mechanical parameters for their clinical consequences. Therefore, in vitro data using other types of experimental model to examine these parameters in detail and in vivo data such as animal study have to be obtained to justify this new method before its clinical use.

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Conflicts of interest The authors declare that they have no conflicts of interest.

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