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Blood Flow in Denture-Supporting Maxillary Mucosa in Response to Simulated Mastication by Loading

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Abstract

Purpose: The purpose of this study was to determine the effect of number of chewing strokes on change in blood flow in denture-supporting maxillary mucosa.

Methods: Subjects consisted of 11 healthy dentate men. Mastication was simulated by intermittent loading (10 N, 1/0.75 Hz) on a lateral area of the hard palate using a 2-cm² test plate. Loading duration was set at 1, 4, 8 or 12 minutes (80, 320, 640 or 960 chewing strokes). A non-contact laser Doppler blood flow meter was used to determine change in blood flow and time taken for recovery to 110% of the pre-loading value. Mean blood flow at pre-loading and at each duration of intermittent loading were compared using a repeated measures ANOVA (α =0.05) and the Dunnett test. Recovery times for each loading duration were compared using a one-way ANOVA (α =0.05) and the Bonferroni test.

Results: Three subjects showed no increase in mean blood flow with loading. Eight subjects consistently showed an increase in mean blood flow during intermittent loading relative to at pre-loading. Duration of loading yielded no significant difference in mean blood flow. Significant differences were observed in recovery time between at after 8 min loading and at after the other 3 loading (1 min, 4 min, and 12 min loading) durations.

Conclusion: Number of simulated chewing strokes showed no influence on mean blood flow during intermittent loading in denture-supporting mucosa. It did, however, affect
recovery time taken for blood flow to return to its pre-loading level.

**Key WORDS:** denture-supporting mucosa, mastication, palatal mucosa, intermittent loading duration, laser Doppler blood flow meter

**INTRODUCTION**

Denture-supporting mucosa is subjected to occlusal and masticatory stress transmitted through the denture base. A number of methods are employed to avoid overloading the mucosa. However, these methods are not always sufficient to avoid overloading in the denture-supporting mucosa. This may result in persistent pain due to transmission of excessive forces during clenching, forceful mastication, or unsuitably prolonged mastication.

To elucidate the effects of clenching, many studies have investigated the effects of continuous loading on underlying mucosa in denture wearers (1,3,4). Clenching was reported to decrease blood flow in underlying mucosa, resulting in circulatory disorder (1). In a study on the effects of simulated mastication, 15 sec repeated loading by tapping induced an increase in blood flow in underlying mucosa (5).

However, very few studies have investigated the effects of mastication on denture-supporting mucosa. Furthermore, the appropriate duration of mastication that has no effect on blood flow in underlying mucosa in denture wearers remains to be clarified.
In dentate subjects, individual differences in number of chewing strokes prior to the final swallow was affected by multiple factors, including personality, with some subjects executing 9-times the number of mastication strokes than others (6). An increase in number of chewing strokes was observed with aging (7-9), possibly due to the concomitant decrease in muscle mass and maximal occlusal force that occurs with age (10-13). In addition, many studies reported a 50-80% decrease in mastication efficiency in denture wearers compared with dentate subjects (14-18). Denture wearers compensate for this decrease in masticatory performance by increasing the number of chewing strokes, as well as the duration of chewing (15, 19, 20). As a result, the mucosa may be subjected to excessive loading. An increase in number of chewing strokes may have the same effect on the supporting mucosa as clenching. Therefore, it is necessary to determine whether increase in number of chewing strokes affects mucosal blood flow.

Our hypothesis was that blood flow in denture-supporting maxillary mucosa was dependent on number of chewing strokes, and that a large number of chewing strokes decreased blood flow, resulting in circulatory disorder. The purpose of this study was to determine the effect of number of chewing strokes on blood flow in denture-supporting mucosa.
MATERIAL AND METHODS

Participants consisted of 11 healthy dentate men ranging in age between 20 and 31 years (mean age, 26 years ± 3 (SD)). There were no visible palatal mucosal abnormalities. Informed consent was obtained from all subjects. Inclusion of women was ruled out to avoid the potential effect of sex as a variable in differences in pain threshold (23). All tests were performed in accordance with the requirements of the Revised Helsinki Declaration (Edinburgh). The study protocol was approved by the Ethics Committee of Tokyo Dental College (#110).

A customized acrylic resin palatal test plate was constructed for each subject. A 2-cm² circular area in the left lateral aspect of the hard palate was designated as the pressure application site (Fig. 1). Nakajima et al. divided the hard palate into 3 regions according to stress relaxation characteristics: the palatal rugae region, the median palatal area and the lateral palatal area (22). From the standpoint of denture retention, Pendleton et al. categorized the edentulous maxillary edentulous ridges into 4 regions, comprising the residual ridge (the primary stress-bearing area), the median portion (the relief area), the lateral slope portion (the secondary stress-bearing area) and the buccal and labial surfaces and posterior portion (the border seal area) (24). With this in mind, the test plate used in this study was placed in the lateral palatal area, as shown in Fig. 1 & 2. The lateral palatal area was chosen for two reasons: firstly, because it is considered the secondary stress-bearing
area for occlusal pressure, and secondly, because it is relatively easier to position a test plate here securely in dentate subjects.

The retainer was designed as a maxillary splint, and was retained by the premolar and molar teeth on either side of the jaw. The retainer covered the entire area of the hard palate between the premolar and molar teeth on each side (Fig 2). The 2 cm² area to be loaded and used as a reference site for measuring blood flow was identified by first extending a transverse line between two points on the distal lingual grooves of the left and right maxillary first molars (Fig 1). Another line was then extended at an angle of 90° to the transverse line from the left distal lingual groove. The median line of the hard palate served as another reference line for demarcation of the lateral palatal mucosa targeted for loading and measurement of blood flow. The 2 cm² circle was accordingly identified as the most central area of the lateral palatal mucosa, with the center of the circle located on the midpoint of the transverse line. The circular area had a radius of 8 mm. Figure 2 shows the position of the test plate and loading and blood flow measurement site. The pressure transducer used for manual application of pressure is also shown.

Mucosal blood flow was measured underneath the center of the test plate. Pressure was applied manually with degree of pressure registered by the pressure transducer, enabling the operator to control level of pressure. The transducer was placed under the test plate (Kyowa
Electronic Instruments/PS-5KC, Tokyo, Japan) (Fig. 2). Pressure was applied perpendicularly to the palatal mucosa of measurement region of blood flow. A non-contact laser Doppler blood flow meter (Advance/ALF21, Tokyo, Japan) was used to measure blood flow (21). A measuring probe was attached firmly to a stent fitted to the test plate. A pilot study confirmed that the probe remained in place during intermittent loading. Blood flow and applied pressure were recorded on a computer analysis system (AD Instruments/PowerLab 16sp, Tokyo, Japan). The data were analyzed using the Chart for Windows software v5.1.1 (AD Instruments, Sydney, Australia).

Loading and recording were performed with the subject in the supine position. Pre-loading blood flow was recorded with the subject at rest, at which time blood flow was considered to be at equilibrium.

To simulate mastication, intermittent loading of 10 N (5 N/cm²) was applied to the test plate at a frequency of 1/0.75 Hz. Loading was applied for 4 durations: 1, 4, 8 or 12 minutes (80, 320, 640 or 960 chewing strokes). Only one measurement was performed per day per subject, with duration selected at random. When the subject felt pain due to the applied load, measurement was terminated and the data discarded so as to avoid the effects of pain on blood flow.

Analysis was performed based on the following 2 parameters: 1) mean blood flow during intermittent loading, and 2) recovery time (time taken to reach 110% blood flow at
Mean blood flow during intermittent loading in each subject was calculated by averaging blood flow for each one-minute interval. A repeated measures one-way analysis of variance (ANOVA) was performed on mean blood flow for each one-minute interval. Mean blood flow prior to loading and that during intermittent loading were compared using the repeated measures one-way ANOVA and the Dunnett test as an ad hoc test. A one-way ANOVA and the Bonferroni-test were used as ad hoc tests to analyze the recovery time for each of the intermittent loading durations. A value of $\alpha=0.05$ was considered significant.

The SPSS 11.0J software for Windows was used for the statistical analysis (SPSS Inc, Illinois, U.S.A.).

Results

Two types of reaction pattern in blood flow were observed during intermittent loading. The subjects were divided into 2 groups on the basis of observed pattern of change in blood flow in response to intermittent loading. Subjects showing no increase were categorized as the “No-increase Group”, and those showing an increase as the “Increase Group”. Mean blood flow during intermittent loading in 3 subjects (No-increase Group) was $94 \pm 25\%$ of that at pre-loading. In the other 8 (Increase Group) it was $122 \pm 35\%$ of that at pre-loading. In the
No-increase group, mean blood flow during loading reached a minimum value of $91 \pm 25\%$ of that at pre-loading at the third minute of intermittent loading (Figure 3). However, mean blood flow during repeated loading reverted to $95 \pm 25\%$ of that at pre-loading after the fourth minute of intermittent loading. Before the 12th minute of loading, mean blood flow recovered to $97 \pm 23\%$ of that at pre-loading. Of the 3 subjects who showed no increase in blood flow during intermittent loading, one showed a mean blood flow value higher than that at pre-loading for the first 30 seconds of intermittent loading. However, blood flow decreased in the following 30 seconds. Mean blood flow then reverted to its pre-loading value at 30 seconds before the 12th minute of loading. Blood flow in the other 2 subjects showed an immediate decrease after initiation of intermittent loading, recovering to pre-loading levels in 30 seconds before the 12th minute of intermittent loading.

Mean blood flow during intermittent loading in comparison with at pre-loading in the Increase Group is shown in Fig. 4 as a function of time. No significant differences were observed in mean blood flow between any duration of intermittent loading. On the other hand, a statistically significant difference was observed in mean blood flow between at pre-loading and all loading durations. Blood flow was higher than at pre-loading for all intermittent loading durations.

Figure 5 compares recovery times between intermittent loading durations.
Significant differences were observed in recovery times between 1 minute loading and 8 minutes loading, 4 minutes loading and 8 minutes loading, and 12 minutes loading and 8 minutes loading. A loading duration of 8 minutes showed the greatest recovery time.

**Discussion**

The average force employed in food mastication in denture wearers was between 2.9 and 17.6 N (25). Ono et al. applied pressure stimulation to the palatal mucosa and determined the pain threshold in men to be between 5.8 N/cm² and 6.8 N/cm² (23). Therefore, in this study, load was set to 10 N (5 N/cm²) to ensure that the force was within the range of the masticatory force of average denture wearers and below the threshold of pain. Frequency of intermittent loading (1/0.75 Hz) was set in reference to the study of Ueda et al (6).

Blood flow rapidly increased after initiation of mechanical stimulation, and then decreased to lower than pre-stimulation levels before finally returning to the original level (23). Two patterns of change in blood flow were reported: reactive hyperemia or no reactive hyperemia after initiation of mechanical stimulation (26). In this study, the 3 subjects in the No-increase group showed no constant increase in blood flow with loading. These subjects might then be classified as showing no reactive hyperemia. No increase at all in blood flow
was observed in two of them. This may have been due to an extremely low level of reactive hyperemia, possibly due to compression of blood vessels.

Flexibility of blood vessels reduces with aging (27). A decrease in thickness of oral mucosa was observed with age (28, 29). Recovery in mucosa underlying a denture after removal of pressure takes longer with age (30). This suggests that recovery time in older persons may take much longer than that observed in the younger age group investigated in this study.

Blood flow showed a decrease with 40 seconds continuous loading of 3.92 N to the palatal area (2). Continuous loading of the palatal mucosa for 10 seconds resulted in a decrease in blood flow (31). Blood flow rates decreased to less than 15% of those at preloading with 20 seconds continuous loading at 9.8 N on an experimental denture base placed on a 2-cm² area of supporting mucosa corresponding to the mandibular lower first molar region (1). A decrease in blood flow rates was reported with 10 seconds continuous loading of palatal mucosa at various magnitudes (3). The results of these studies indicate that continuous loading causes a consistent decrease in blood flow and ischemia in the underlying mucosa. Circulatory disorders have been suggested to induce metabolites in underlying mucosa which accumulate and stimulate receptors, giving rise to pain (4). This implies that continuous loading leads to excessive burdening of the mucosa.
In the present study, reaction to simulated mastication by intermittent loading differed from that to simulated clenching by continuous loading in other studies. In the Increase Group in this study, mean blood flow during intermittent loading increased to 122% of the pre-loading value. Even in the No-increase Group, mean blood flow during intermittent loading reached a minimum 91% of the preloading value. On the other hand, blood flow during continuous loading decreased to 15% of that at pre-loading (1). Increases in blood flow during intermittent loading may be caused by reactive hyperemia (23, 32). Since each loading is very brief, blood vessels are immediately reopened after each temporary blockage. After each loading, reactive hyperemia is initiated by the brief blockage of blood flow. Kato et al. (5) studied the effects of intermittent loading on blood flow in subjects using test appliances during a mucosal tapping exercise for 15 seconds. They reported that blood flow showed an increase during repeated loading relative to blood flow before loading. The present study showed the same finding. However, other studies on the effects of intermittent loading dealing with short loading times of approximately 15 seconds to 1 minute did not find this (7, 23). The major difference between these earlier studies and this study is that we used extended periods of simulated mastication to mimic the kind of stresses occurring in denture-supporting mucosa during intake of a full meal.

The results of the present study showed no significant differences in blood flow
between the first and last minute of 12 minutes intermittent loading. This indicates that 12 minutes intermittent loading did not influence blood flow. This suggests that intermittent loading, as in mastication, over such a time period does not adversely burden the supporting mucosa.

Although no significant differences were observed in blood flow under the various loading conditions, significant differences in recovery times were observed. An intermittent loading duration of 8 minutes showed the longest recovery time. Variation in recovery time with loading duration is probably caused by reactive hyperemia. However, the underlying mechanism remains to be clarified. Reactive hyperemia is caused by the direct action of the expansion of blood vessels by a chemical mediator which is generated during blood vessel contraction (33). It is also caused by indirect action, where a chemical mediator activates the arachidonate cascade, facilitating the production of prostaglandins, causing the expansion of blood vessels (33-35). Differences in the responsiveness of direct and indirect action may be the reason for the observed differences in recovery time seen here.

Mechanical stimulation of the gingiva increased its hemoglobin oxygen saturation, suggesting an improved blood flow (36). The increase in blood flow seen in the present study, therefore, may have resulted from mechanical stimulation of the mucosa.

The hypothesis of the current study was that blood flow was dependent on the
number of chewing strokes. It was also hypothesized that a greater number of chewing strokes affected mucosal blood flow similarly to clenching. However, the results of the present study did not validate this. In fact, blood flow showed no correlation with number of chewing strokes, and the effects of larger numbers of chewing strokes on the mucosa were different to those reported for clenching. The present results further suggest that mastication does not readily elicit pain, as improvement of blood flow prevented the accumulation of metabolites.

In the present study, mucosal simulation by intermittent loading, as would occur during chewing, caused no blood circulatory disorder in the mucosa. This result differed from that obtained with simulated clenching by continuous loading. Even in cases where blood flow initially decreased to below pre-loading blood flow values, it returned to pre-loading levels during subsequent interruption of loading. This suggests that, as mastication does not result in an excessive burden on the mucosa, prolonged mastication should be recommended not only to dentate patients, but also to denture wearers. Further study is needed to determine how blood flow is affected at other mucosal sites and whether those effects vary with age.
**Conclusion**

Number of simulated chewing strokes showed no effect on blood flow pattern in denture-supporting maxillary mucosa during loading. On the other hand, duration of intermittent loading affected time taken for blood flow to return to pre-loading levels. Recovery time increased with increase in duration of intermittent loading until 8 min. This peaked at an intermittent loading duration of 8 min.

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Figure 2

- Retainer
- Pressure transducer
- Test plate
- Probe of laser Doppler blood flow meter
- Loading force
Figure 3

Mean blood flow during loading (n=3)

Repeated loading time [min]
Figure 4

Mean blood flow during loading

Repeated loading time

(min)

(n=8)
Figure Legends

Figure 1: Area of loading and blood flow measurement (M).

Figure 2: Position of test plate, pressure application and blood flow measurement sites.

Figure 3: Mean blood flow values in “No-increase” group (n = 3) before loading and at 1-min intervals during intermittent loading (T-bar: S.D.).

Figure 4: Mean blood flow values in “Increase Group” (n = 8) prior to loading and at one-minute intervals during intermittent loading ( * :p<0.05, T-bar: S.D.)

Figure 5: Average recovery times for each intermittent loading duration. (*: p<0.05, T-bar: S.D.)