The repeatability and reproducibility of gingival thickness measurement with an ultrasonic device

Powtarzalność i odtwarzalność pomiaru grubości dziąsła biometrem ultrasonograficznym

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Abstract

Background. Successful periodontal and implant surgery as well as orthodontic treatment often depends on gingival and mucosal thickness. So far there has been no generally accepted protocol of measuring the thickness of gingiva by non-invasive methods.

Objectives. The aim of the study was to evaluate the repeatability and reproducibility (%R&R) of the 20 MHz A-Scan ultrasonic device in measuring gingival thickness (GT) in the mucogingival complex.

Material and methods. A 2-stage study utilizing non-invasive ultrasonic methods was conducted. In the 1st stage, 3 operators got calibrated by measuring previously established GT in porcine cadaver jaws. In the 2nd stage, 1 periodontally healthy subject was recruited in the study. Three operators performed the measurements of GT in maxillary left central and lateral incisors and canines, using the 20 MHz A-Scan ultrasonic device with a probe of 1.7 mm in diameter. The thickness was measured in 4 standardized points located in the free gingiva (FGT), supracrestal gingiva (SGT), crestal gingiva (CGT) and the mucosa (MGT).

Results. The analysis of variance (ANOVA) method was used to quantify %R&R. The repeatability and reproducibility of the measurements was 8.4%. Interobserver reproducibility varied from 0.8% to 13.4%. The average intraobserver coefficient of variation (CV) was 6.6% (1.9–13.6%). The median of the reproducibility of all measurements was 8.1 %. Nevertheless, the median of CV was variable to the observer, i.e. 5.4%, 6.5%, 6.4%.

Conclusions. The obtained results in %R&R prove the good recognition of methodology as well as the usefulness of the device. Non-invasive ultrasonic biometer GT measurements are crucial in periodontology as well as in other fields of dentistry.

Key words: gingiva, periodontium, anatomy and histology, ultrasonography, reproducibility of results

Słowa kluczowe: dziąsło, przyzębie, anatomia i histologia, ultrasonografia, odtwarzalność wyników

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Introduction

The first report on the utility of ultrasound in dental diagnostic process appeared in 1963.¹ The authors used a 15 MHz converter to visualize intradental structures. However, the results were not satisfying. Since that time, several research studies on using ultrasound in imaging hard and soft tissues of the oral cavity have been published.² Researchers have worked on fractures and cracked teeth,³ caries detection,⁴ periapical lesions,⁵ alveolar ridge and maxillofacial bones structures,⁶ temporomandibular joints,⁷ and on the measurements of soft tissue of the oral cavity, in particular of the gingiva and the masseter muscle.^{8–12} Furthermore, it is possible to use ultrasounds to examine pathological structures, such as neoplastic lesions of the mucosa,¹³ but also to detect the implant-abutment connection or to assess the margin of the alveolar ridge and the clinical attachment level, which can be used to evaluate the probing depth of the gingival sulcus and biologic width.¹⁴

Nowadays, there is an increasing interest in evaluating the quality of the gingival margin. This is widely used in orthodontics, periodontology, implantology, and prosthodontics.^{15–18} Knowing the amount of the keratinized attached gingiva, the orthodontist can assess the risk of gingival recession and future problems in the treatment process, especially during proclination and labial bodily movements.¹⁶ Thus, the orthodontic treatment is safer and gingival recession can be avoided. In the field of periodontology, especially periodontal surgery, the assessment of the quality of the gingiva is helpful in planning the technique and making a prognosis of the surgery.^{15–17} In implantology, the thickness of tissue plays a key role in the long-term stability of the dental implant.¹⁸ Accumulating evidence indicates that the atrophy of the buccal bone is lower when the attached gingiva is thicker.^{19–21} Gingival thickness determines the method of preparation of the prosthetic crowns. Dental esthetics is one of the most common reasons for improving dental procedures. Nowadays, dental restoration or filling as well as teeth whitening no longer satisfy the needs and expectations of the patients and dentists. Pink-white esthetics has become a challenging task and the balance between restorations and the gingiva seems to become a standard of the optimal dental treatment, consistent with the patient's expectations.

The quality of soft tissue around the teeth and dental implants plays an important role in the diagnostic process as well as in the treatment of gingival problems.²² The quantity of the gingiva can be measured with 2 parameters: the width of keratinized tissue and the thickness of soft tissue at different levels of the dentogingival unit.²³ The 1st one is relatively easy to assess by periodontal probing, but the 2nd parameter can be particularly difficult to measure for inexperienced clinicians.^{8,10,17}

Measurements of soft dental tissue can be done with invasive methods, e.g., with an injection needle or an endodontic file with a silicone stop (bone sounding method)

or computed tomography (CT). Until now, the gold standard procedure have been the invasive methods.¹⁰ However, using an ultrasonic biometer is a non-invasive method that has been reported to be the most efficient procedure.^{10,17,24–26} The procedure is based on the pressure-free application of the head of an ultrasonic probe to the surface of tissue at a right angle. The signal bounces off the tooth or the alveolar bone and comes back to the head of the probe. The time it takes for the signal to return to the device is taken into account to determine the distance, which then can be used to calculate the actual gingival thickness (GT). The main advantage of the presented method is its safety for the patient and the doctor.²⁶ It reduces the concerns many patients have about excess radiation exposure and the need for local anesthetics. Measurements are very precise (the accuracy to the 2nd decimal place) and immediately available. Moreover, the cost of a single examination is relatively low. Nevertheless, correct measurements require an experienced examiner.

An ophthalmic ultrasonic biometer has been used for the examinations of GT, but the diameter of the periodontal probe was too wide for dental purposes, since the size of the transducer probe head was larger than the examined area.¹⁰ The other challenges of using the discussed method include the curved surface of bone and roots. The shape causes a nonparallel reflection of the signal, which is not received by the transducer.¹⁷ Müller et al. reported the difficulty in interpreting the results as well as high measurement inaccuracy.⁹ The repeatability of the measurements of 1 clinician and between all the examiners is a critical parameter.

The present study was focused on the ultrasonic biometry of soft tissue around the tooth, and was aimed at validating whether the method is precise and repeatable to be used in a dental office by 1 examiner, and whether the measurements can be reproduced among 3 operators. The ultrasonic device used to obtain all the measurements was PIROP® (Echo-Son S.A. Puławy, Poland) (Fig. 1) and has been described in previous articles.^{10,27}

Material and methods

Project plan

The study was designed to validate the ultrasonic method of GT measurements in dentistry. The 1st stage of the project was to perform the preclinical training and calibration of the researchers. In the 2nd stage of the project, a periodontally healthy volunteer was examined in the clinical environment. The results were taken for statistical analysis. The study was conducted in accordance with the Declaration of Helsinki of 1975 as revised in 2000, and the study protocol was approved by the ethical committee of Wrocław Medical University (No. KB-126/2018). The authors declare the lack of the conflict of interests and the manufacturer Echo-Son S.A. declares the lack of third-party involvement in our research.

Fig. 1. PIROP® (Echo-Son S.A., Puławy, Poland)
Specifications: probe frequency – 20 MHz; velocity range – 1400–3000 m/s;
measurement range – 0.25–6 mm; axial resolution – 0.01 mm (10 µm);
4 predefined maps of periodontal areas.

Determining the variability of the measurement process

A diagnostic tool, which guides us in making any clinical decisions, should reflect the real conditions as accurately as possible. Hence, the trial was conducted according to Measurement System Analysis (MSA), which is the first critical step in qualifying the measurement method by quantifying its precision, repeatability, reproducibility, and accuracy.²⁷ Measurement System Analysis is an experimental, mathematical method of determining the variability of the measurement process. To verify usefulness of the measurement process, the Repeatability and Reproducibility (R&R) test is carried out. Repeatability means obtaining identical measurement results by 1 examiner using the same device. Reproducibility means obtaining identical measurement results by a few operators using the same device. The R&R result is expressed as a percentage (%R&R) assuming a 90% confidence interval (CI) with lower and upper borders. In the R&R test, 10 measurements of 3 trials are carried out by 3 examiners. The MSA standards of acceptance of a measurement system are shown in Table 1.

Table 1. Measurement System Analysis (MSA) standards

Combined R&R value [%]	Level of acceptance of the system
$R\&R \leq 10$	acceptable
$30 \leq R\&R < 10$	conditionally acceptable
$R\&R > 30$	not acceptable

R&R – repeatability and reproducibility.

Calibration and training

The study was conducted at the Department of Periodontology in Specialist Outpatient Clinic in Gorlice, Poland. To reduce the potential bias related to the human factor (caused by an examiner's performance), the preclinical calibration and training of the 3 examiners were provided. For both the training and clinical trial, PIROP was used. A fresh porcine jaw was prepared for practicing purpose. In the preclinical calibration, the porcine jaw was placed on the laboratory scale, which was subsequently reset to zero to enable the pressure control. One point was marked on keratinized tissue on the palatal side of the porcine jaw. The probe was applied to the point with minimum pressure, lower than 25 g, which was controlled by sight in the scale screen as shown in Fig. 2. The perpendicular position of the probe enabled the return of the echo. The result was calculated on the basis of the time it took for the transducer to receive the signal and the velocity of the pulse, which was then displayed digitally on the screen of the PIROP biometer. To move to the next phase of the calibration, it was necessary to repeat the measurement 10 times with the appropriate pressure maintained. Each examiner performed 10 measurements consisting of 10 signals each. The researcher was blind to the values of

Fig. 2. Placement of the probe with the pressure of 9 g

the pressure exerted by the ultrasound probe on tissues, presented on the screen of the scale; they were controlled by other person. Calibration was finished after obtaining 10 measurements of GT at the pressure <25 g. One researcher applied the bone sounding method in the designated point with an endodontic file, which is considered to be a gold standard for measuring the thickness of soft tissue (Fig. 3,4). The direct measurement with a calliper (calibrated to the 2nd decimal place) was a control reference. All 3 examiners positively completed the training.

Clinical examination

One periodontally healthy volunteer was recruited. Three teeth in the left upper jaw were selected for the examination: a central incisor, a lateral incisor and a canine tooth. First, the probing depth, the clinical attachment level and the width of keratinized gingiva were estimated in each dentogingival unit. According to the previous study, showing a statistically significant difference between the thickness in the supracrestal and crestal gingiva,²⁸ 3 points were marked on the labial surface of the gingiva and 1 point on the alveolar movable mucosa (Fig. 5). The 1st point – free gingival thick-

Fig. 3. Bone sounding method with an endodontic file

Fig. 4. Measuring the distance with a digital calliper

ness (FGT) – was localized on the free margin of the gingiva. The head of the probe was positioned with its lower border at the clinical attachment level, more coronally (Fig. 6). The 2nd point – supracrestal gingival thickness (SGT) – allowed us to measure the thickness of the supracrestal gingiva attached to the tooth surface. The upper border of the probe was placed at the clinical attachment level, more apically (Fig. 7). The 3rd point – crestal gingival thickness (CGT) – was localized more apically on the keratinized gingiva and represented the thickness of the gingiva attached to the alveolar ridge. The head of the probe was placed with its upper border at the mucogingival junction, more coronally (Fig. 8). The 4th point called mucosa thickness (MGT) was placed on the lining mucosa. The head of the probe was positioned with its lower border more apically of the mucogingival junction (Fig. 9). At the FGT point, the head of the probe was in partial contact with the free gingiva, but as signals are produced by the whole surface of the probe, the examination enabled thickness measurement of the free gingiva. All trials were carried out without local anesthesia. The clinical examination was entirely performed in 12 points (4 points for each tooth), with 5 attempts each (automatic 10-fold measurement of every attempt, 50 values in total), by 3 examiners (Fig. 6). The chlorhexidine 0.2% bioadhesive gel (Elugel® – 40 mL gel tube; Pierre Fabre Oral Care, Boulogne-Billancourt, Paris, France) was used to allow conduction of the ultrasonic impulses.

Statistical analysis

Data analysis was performed using statistical software STATISTICA v. 10.0 (StatSoft Polska Sp. z o.o., Kraków, Poland). The statistical units in the analysis were: patient, dentogingival units, single points, and operators. The analysis of variance (ANOVA) method was used to quantify the repeatability and reproducibility percentage (%R&R). The mean, median, standard deviation (SD), and coefficient of variation (CV) were calculated. A p-value ≤0.05 was considered statistically significant.

Fig. 5. Four new points marked on the surface of soft tissue of the lateral incisor

Fig. 6. Examination of the free gingival thickness (FGT point) with the ultrasonic probe

Fig. 7. Examination of the supracrestal gingival thickness (SGT point) with the ultrasonic probe

Results

In the 1st stage, the master sample was chosen and the preclinical part was conducted. There was no statistical difference ($p = 0.732$) between ultrasonic measurements made by 3 operators with the PIROP device, bone sounding technique and the real value as a control, which is presented in Table 2.

The 2nd stage was a clinical trial performed on 1 generally and periodontally healthy volunteer. Three teeth were used in the examination. Four measurement points were marked on soft tissue for every chosen tooth. Three examiners took measurements 5 times at 1 point. The data corresponding to the different points and operators is presented in Table 3.

The average intraobserver CV was 6.6% (from 1.9% at the SGT point in researcher 1 to 13.6% at the SGT point in researcher 2). Nevertheless, the median of CV

was variable to the observer, i.e., 5.4%, 6.5% and 6.4%. Interobserver reproducibility varied from 0.8% to 13.4%. The median of the reproducibility of all measurements was 8.1%.

Considering the results related to the dentogingival units, the combined R&R for tooth 21 was 7.43%, for tooth 22 – 12.48% and for tooth 23 – 4.67%. All the results were acceptable or conditionally acceptable, but still very close to 10%. The combined R&R ratio for all measurements carried out by 3 researchers was 8.4% (CV) (Table 4). Following the results obtained with PIROP in this trial, this device can be placed in the first group of acceptance according to the MSA standards.

Fig. 8. Examination of the crestal gingival thickness (CGT point) with the ultrasonic probe

Fig. 9. Examination of the mucosa thickness (MGT point) with the ultrasonic probe

Table 2. Average values of preclinical gingival thickness (GT) measurements carried out by 3 operators

GT value [mm]	USG 1	USG 2	USG 3	BS	RV	p-value
mean	3.39	3.36	3.38			
SD	0.14	0.13	0.14	3.44	3.40	0.732

USG 1 – operator 1; USG 2 – operator 2; USG 3 – operator 3; BS – bone sounding; RV – real value (measured directly by the caliper); SD – standard deviation.

Table 3. Intraoperator coefficient of variance (CV) repeatability and interoperator reproducibility for individual gingival thickness (GT) measurements

Tooth No.	GT measurement point	Researcher 1			Researcher 2			Researcher 3			Reproducibility [%]
		mean of GT [mm]	SD	CV [%]	mean of GT [mm]	SD	CV [%]	mean of GT [mm]	SD	CV [%]	
21	FGT	0.86	0.08	9.4	0.88	0.10	10.9	0.88	0.06	7.4	1.4
	SGT	1.59	0.17	10.5	1.75	0.24	13.6	1.68	0.20	11.8	8.0
	CGT	0.69	0.04	5.1	0.89	0.08	9.6	0.79	0.04	5.3	10.1
	MGT	0.82	0.03	3.1	0.79	0.04	4.7	0.81	0.05	6.7	1.5
22	FGT	0.61	0.03	5.3	0.79	0.04	5.5	0.84	0.05	6.0	11.9
	SGT	1.37	0.03	1.9	1.13	0.04	3.5	1.39	0.03	2.1	13.4
	CGT	0.88	0.05	5.5	0.84	0.06	7.6	0.87	0.06	7.4	1.8
	MGT	0.57	0.06	9.8	0.54	0.03	5.6	0.70	0.07	9.2	8.4
23	FGT	0.76	0.02	2.8	0.71	0.05	6.5	0.74	0.04	6.0	2.3
	SGT	1.70	0.07	4.2	1.62	0.11	6.6	1.52	0.13	8.4	9.3
	CGT	0.61	0.05	7.6	0.77	0.05	6.9	0.75	0.04	5.6	8.3
	MGT	0.99	0.06	6.1	1.01	0.06	5.8	1.00	0.03	3.5	0.8
Median	–	–	–	5.4	–	–	6.5	–	–	6.4	8.1

FGT – free gingival thickness; SGT – supracrestal gingival thickness; CGT – crestal gingival thickness; MGT – mucosa thickness; SD – standard deviation; CV – coefficient of variation; 21 – central incisor; 22 – lateral incisor; 23 – canine.

Table 4. The combined repeatability and reproducibility (R&R) of the ultrasonic measurement at the central incisor (21), lateral incisor (22) and canine (23)

Tooth No.	Estimated	90% lower CI	90% upper CI	Combined R&R [%]
21	0.120	0.115	0.221	7.43
22	0.110	0.097	0.298	12.48
23	0.092	0.085	0.103	4.67
Total	0.107	0.105	0.141	8.39

CI – confidence interval.

The summary plot in Fig. 10 shows 3 boxes successively for each researcher. In each box, there are 12 columns corresponding to the points of measurement (from the left: 1 – FGT tooth 21; 2 – SGT tooth 21; 3 – CGT tooth 21; 4 – MGT tooth 21; 5 – FGT tooth 22; 6 – SGT tooth 22; 7 – CGT tooth 22; 8 – MGT tooth 22; 9 – FGT tooth 23; 10 – SGT tooth 23; 11 – CGT tooth 23; 12 – MGT tooth 23). In each column, 5 values of the deviation from average are marked with green points. Considering the results in relation to the single points, the deviation from the average value of the measurement at the SGT point in the lateral incisor is visibly higher than in the other points. The shape of the root of the upper lateral incisor is biconcave and narrow, which may cause a nonparallel reflection of the signal at the surface. This may indicate that the method used requires experienced examiners, who are able to assess the value provided by the device.

Discussion

Gingival thickness measurement with an ultrasound has been recognized as a reliable, reproducible and non-invasive method.^{8,9,17,26} The authors compared results obtained by ultrasonic, invasive^{8–10} and CT methods.¹⁷ Slak et al. used a phantom made from materials with similar

Fig. 10. Deviations from average for 3 operators, 5 times at each measurement point
 Staring from the left: 1 – operator 1; 2 – operator 2; 3 – operator 3.

properties of ultrasound wave transmission to bone and gingival tissue.²⁶ Ten places were marked on a polyurethane surface resembling the gingiva; on each of these points, the thickness was measured with an ultrasound method and an invasive method using an endodontic K-file, with individual measurements taken directly. An optical microscope was used to calculate the thickness of the polyurethane based on the direct method. In experimental conditions using an ultrasound and transgingival probing method, 10 measurements of the GT of the swine maxilla were taken at each of 4 GT1 points (midway through the keratinized gingiva) and 4 GT2 points (2 mm apically from the mucogingival junction). Measurements of the thickness of polyurethane by means of 3 methods produced results that were very similar to one another. However, the greatest inaccuracies when compared to direct measurements occurred with the invasive method. Most of the GT values measured on the swine mandible using the invasive method were higher than the values recorded with ultrasonography. The greatest inaccuracies recorded with the ultrasound method concerned measurements of the mobile mucosa in one of the GT2 points, and amounted to 10.3%. The data obtained in clinical conditions are confirmed by the occurrence of smaller measuring deviations noted with ultrasound biometrics compared with the invasive method.

Eghbali et al. measured the palatal masticatory mucosa of 4 human cadavers using an ultrasonic device with a measurement frequency of 5 MHz, with a transducer probe of 4 mm in diameter.¹⁷ To assess the validity of the ultrasonic device, one of the researchers performed GT measurements of 100 sites marked on the mucosa surface with copper wires. The other researcher performed CT and compared the results obtained by ultrasonic and imaging methods. A strong correlation between both methods used were observed; however, the ultrasonically measured GT was significantly lower than GT measured with micro-CT. To evaluate the reproducibility of 1 researcher, the ultrasonic GT measurement of the 50 sites was performed twice. There was no statistically significant difference between the former and the latter GT results. Moreover, a strong positive correlation was observed between the 2 sets of results.

Müller et al. assessed the degree of repeatability of the ultrasonic method of measuring GT using a 5 MHz device in 33 volunteers at all dentogingival units on the tooth/occasion and subject/patient level.²⁹ Patients with gingivitis were examined 3 times in 2 weeks with respect to each tooth on the clinical attachment level. The highest repeatability level (95%) was obtained in the anterior teeth and premolars.

In the studies conducted by Bednarz et al., 30 patients with healthy periodontal tissue were tested.¹⁰ Measurements of periodontal soft tissue were taken in each patient at 2 measuring points GT1 and GT2, around each of 10 teeth in the maxilla and 10 teeth in the mandible, covering pre-

molars, canines and incisors. The average GT achieved with the invasive method was significantly greater than the values recorded with an ultrasound, amounting to 0.828 mm and 0.784 mm, respectively. Similarly, the average GT values noted at points GT1 and GT2 were higher with the invasive method. The biggest differences in measurements at these points between the invasive and ultrasound methods were observed in the lowest values <0.5 mm.

Müller et al. obtained higher values of ultrasonic GT measurements than those achieved with the invasive method,²⁹ whereas Eghbali et al. obtained values lower than in the case of micro-CT.¹⁷ In our research, verification of the stability, repeatability and reproducibility of GT measurement using an ultrasonic device was carried out in accordance with the guidelines of MSA.²⁷ Appropriate conditions for carrying out the R&R test should be provided. The measurement process should be precisely described and intelligible, investigators should be well-trained and the device should be calibrated. The frequency of measurements has to be determined. The environmental conditions must be comparable for all the trials. Our study meets all the mentioned criteria. According to the authors, the most important element of the study is the calibration by the researchers of the pressure with which the head of the probe is applied; it should not exceed 25 g. Firstly, self-control training on the porcine jaw placed on the laboratory scale was performed. Ten results that met the above-mentioned criterion allowed the researcher to proceed to the next phase of the study, in which the pressure was controlled by other researcher. Ten correct measurements (with a pressure lower than 25 g) in a row were considered as a positive completion of the calibration.

The median of the reproducibility of all measurements was 8.1%. Nevertheless, the median of CV was variable to the observer, i.e., 5.4%, 6.5% and 6.4%. The overall combined R&R parameter for all the values indicates that ultrasonic measurements of GT are repeatable within 1 examiner and reproducible between 3 of the examiners.

Müller and Könönen underlined that the GT measurement is assessed mostly with the variables on the tooth level, not the patient level.³⁰ In the previous experiments, the diameter of the probe used was about 4 mm.^{17,29} This size did not allow the researcher to place the probe exactly where planned. The present experiment visualized the differences between the values for the supracrestal and crestal tissue. It can be achieved using a probe with the head diameter of 1.7 mm, which makes it possible to measure GT in a few points with greater accuracy. Differences within R&R for individual dentogingival units may occur because of the curvature of the roots. It is particularly visible in FGT and SGT of the lateral incisor, whose root is very thin and in a biconcave shape. This may cause problems with placing the probe perpendicularly to the root surface as the signal goes back straight to the probe. The first 2 parameters of free gingiva and supracrestal gingiva,

in which the signal is bouncing from the root surface, show the highest R&R value. This proves that knowledge of anatomy and experience of the examiner are the key factors in the proper conduct of the examination, which is consistent with the conclusions of others.^{10,17,28–30} To avoid the mentioned bias in the future, flat plastic may be placed into the sulcus to create a flat reflection surface and ensure a perfect measurement of the FGT point.

Non-invasive ultrasonic biometer GT measurements are crucial in periodontology as well as in other fields of dentistry. Knowing the value of GT is necessary to predict the functional and esthetical outcomes as well as to minimize possible complications.

It is widely known that the quality and quantity of soft tissue around the teeth and implants matters. In the era of body worship and increasing esthetic expectations, it is not enough to restore the function of the stomatognathic unit. To improve the functional as well as the esthetic results of the treatment, a precise diagnostic tool to assess soft tissue is needed. Moreover, knowledge of the anatomy of each dentogingival unit can make the treatment safer for a patient and more predictable for a clinician. This trial showed that the ultrasonic method of measuring GT can be used as a basis for a diagnosis and clinical decisions.

Conclusions

The obtained results in repeatability and reproducibility prove the good recognition of methodology as well as the usefulness of the device. Within the limits of the study, the 20 MHz A-Scan ultrasonic device may be used to measure GT in the oral cavity. The study is an important basis for a large clinical study with more participants.

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