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The evaluation of 20 MHz ultrasonography, computed tomography scans as compared to direct microscopy for periodontal system assessment

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Abstract

The aim of the study was to identify by ultrasonography (DermaScan C, Cortex Technology1) the reference points necessary to monitor the horizontal bone resorbtion and to assess the accuracy of the measurements by comparing with Cone Beam Computed Tomography images, having direct microscopic section measurements as a gold standard. **Material and methods:** We performed the examinations on the lingual side of the alveolar bone of 4 pig mandibles. We tried to identify the enamel-cementum junction, the root, the periodontal space at the appearance of the tooth from the alveolar bone, the edge of cortical bone at its coronary limit (in order to assess the horizontal bone resorption). We measured the distance between the enamel-cementum junction and the coronal edge of cortical bone. **Results:** There was a strong positive correlation between the alveolar bone level measured with the 3 techniques. Correlation parametric R2 tests were statistically significant for DermaScan measurements compared to microscopy ones ($p<0.0001$). Linear regression showed statistically significant correlations for alveolar bone level measurements made by CBCT compared to microscopy ($p<0.05$). **Conclusions:** The cementum-enamel junction may be identified on ultrasonic images with high accuracy according to the tooth anatomical convexities, therefore this method can be used for monitoring the periodontium.

Keywords: periodontium, ultrasonography, cone beam computed tomography, microscopy

Rezumat

Scopul a fost identificarea reperelor anatomice necesare in monitorizarea atrofiei osoase orizontale, cu ajutorul ultrasonografiei si stabilirea acuratetei masuratorilor efectuate in acest sens pe imaginile de ultrasonografie cu DermaScan C, CORTEX TECHNOLOGY1 fata de cele efectuate pe imagini de Cone Bean Computed Tomography avand ca si gold standard masuratori pe imagini de microscopie directa. **Material si metoda:** Am examinat 4 mandibule de porc la nivelul suprafetei linguale a procesului alveolar. Am incercat sa identificam jonctiunea smalt-cement, radacina dintelui, spatiul parodontal la emergenta procesului alveolar, portiunea coronara a corticalei osoase (in scopul evaluarii resorbtiei osoase orizontale). Am masurat distanta dintre jonctiunea smalt-cement si limita coronara a corticalei osoase. **Rezultate:** A existat o corelatie statistic semnificativa intre masuratorile facute prin cele 3 tehnici. Testele parametrice de corelatie tip R2 au furnizat rezultate statistic semnificative pentru masuratorile effectuate cu DermaScan, comparativ cu cele efectuare pe imagini de microscopie directa ($p<0.0001$). Regresia liniara a aratat o corelatie statistic semnificativa ($p<0.05$) pentru masuratorile nivelului osului alveolar, efectuate pe CBCT comparativ cu cele de microscopie. **Concluzii:** Am putut identifica jonctiunea smalt-cement pe imaginile de ultrasonografie, pe baza convexitatilor anatomice ale dintelui. Acuratetea masuratorilor fiind foarte ridicata, aceasta metoda ar putea fi utilizata in monitorizarea afectiunilor parodontale.

Cuvinte cheie: parodontiu, ultrasonografie, cone beam computed tomography, microscopie

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The elements of the dento-alveolar system have undergone significant changes through time and continuous modifications. These modifications are more important especially if there is an associated pathology, such as periodontal disease, of infectious etiology, generated by occlusal traumas or excessive forces applied during orthodontic treatments [1,2]. In these situations a cost ef-

fective early diagnostic test is required in order to be able to prevent and monitor the disease evolution.

Nowadays in order to diagnose periodontal diseases, clinical signs and symptoms are assessed and different tests are performed during clinical examination (such as periodontal pockets depth recording, teeth mobility evaluation) and paraclinical examination (such as radiological examination, blood tests, microbiological analysis) [3]. The clinical examinations are time-consuming, are user-dependent and not very reliable (inter- and intra-user reliability is rather low) [4]. Since no other reliable method has been found for assessing the periodontium, clinical examinations continue to be used on daily basis in the dental practice. Yet there is hope that sometime soon other examination methods will be found. These should overcome the shortcomings of currently used methods and bring much higher precision and accuracy to periodontal examinations [5].

One of the less expensive and most used imaging techniques used is the X-ray (retroalveolar, bitewing, panoramic X-rays, ortopantomography). The radiological examination is not very expensive and offers important information. The exposure to the radiation of the patient and the medical staff, and the lack of information about bone resobtion from the lingual and buccal surfaces of the teeth, are the most important disadvantages of the method [3]. Computed tomography (CT) and Magnetic Resonance Imaging (MRI) studies would easily overcome these disadvantages, but they are costly and the amount of radiation is much higher for CT than for the above mentioned X-rays, therefore not routinely used. Identifying the anatomical elements of the dento-periodontal system is rather easy. According to previous studies, very accurate measurements of the periodontal space are better on 2D X-rays than on Cone Beam Computed Tomography (CBCT) images [6].

After the onset of periodontal disease, the dento-alveolar system suffers modifications as well as also in the horizontal bone resorption. The best way for monitoring the disease progression in the cases of horizontal atrophy, is the position of the bone level in the sagital plane for any type of examination, compared to a reference point which does not change, such as the enamel-cementum junction [7-9]. For a close follow-up of periodontal disease, more accurate imaging techniques that would facilitate the measuring process of those parameters are required. These measurements have to be made quite often, so a non invasive cost effective imaging method is desired. It should not have side effects on the patients health, neither cause irradiation, nor increase and accelerate tissue destruction. Such a method would provide additional clinical information in order to make a timely diagnosis and would also improve the follow-up of the disease progression.

Ultrasonography (US) could be an alternative, because it is a noninvasive method, with no radiation exposure and relatively low costs. Currently ultrasonography is used mostly for soft tissues and is considered to be operator-dependent.

Although previous studies [10-12] concluded that the anatomy of dento-periodontal system is too complex for ultrasonographic examinations, our previous research [13] suggested that this minimal invasive diagnostic method could be used in the future as a routine investigation in the evaluation and monitoring of periodontal disease.

The aim of this study was to identify by ultrasonography the reference points necessary to monitor the horizontal bone resorbtion. Also to establish the accuracy of the measurements made between the reference points comparing the ultrasonography with Cone Beam Computed Tomography (CBCT) images having direct microscopic section measurements as a gold standard.

Material and methods

We performed the examinations on the lingual side of alveolar bone of 4 pig mandibles, on areas corresponding to 20 teeth. We glued gutta-percha-landmarks on the lingual surface of lateral teeth. The gutta-percha landmarks were bonded with a flowable composite (fig 1, fig 2). All 4 mandibles were examined using three methods of examination: the first method was US with DermaScan C, CORTEX TECHNOLOGY 1, with a 20 MHz, 2D transducer. The second method was radiological method CBCT with a New Tom 3G machine and the third one was direct microscopy with 4x magnification on a Olympus BX51 microscope equipped with Olympus cell B software. The pig mandibles were examined, without prior refrigeration, not later than 3 hours after the sacrifice of the animal. We used mandibles belonging to animals from farms organized according to EU legislation, not from experimental animals, therefore the Medical Ethics Commission of our university considered no further agreement was necessary.

The area of interest was between the dental crown near the bonded gutta-percha-landmarks, enamelo-cementum junction, till the external cortical bone 2-3 mm below its coronary limit. The elements we tried to identify were: the enamel-cementum junction, the root wall down to the emergence of the tooth from the alveolar process, the periodontal space at the appearance of the tooth from the alveola, the edge of cortical bone at its coronary limit (in order to assess the horizontal bone re-

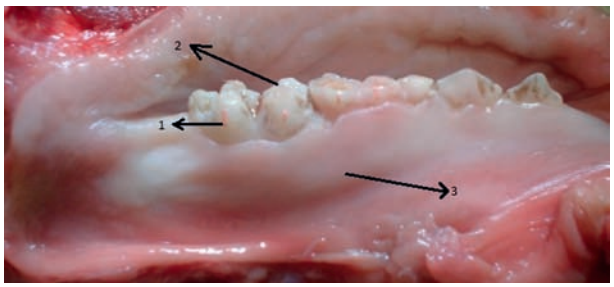


Fig 1. Pig mandible with glued gutta-percha-landmarks: 1-glued gutta-percha-landmarks, 2- posterior tooth, 3-alveolar process.

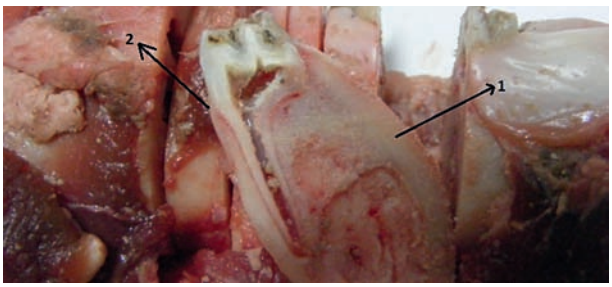


Fig 2. Pig mandible, sagittal sections: 1-tooth in sagittal section, 2-periodontal system- interest area.

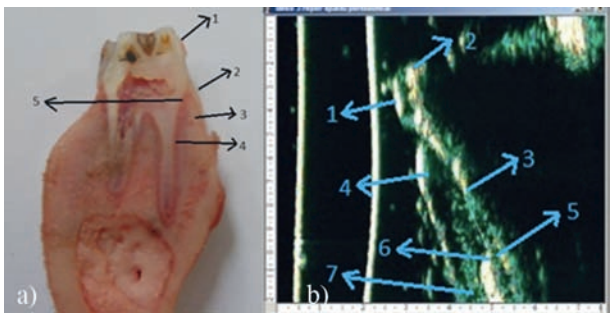


Fig 3. a) Sagittal section through a tooth:1-glued gutta-percha-landmark, 2-gingival margin, 3-cortical bone, 4-periodontal space, 5-tooth wall covered by cementum. b) DermaScan sagittal section through a tooth, visualized elements: 1-glued gutta-percha-landmark, 2- crown enamel, 3-enamel-cement junction, 4- gingival margin, 5-tooth root, periodontal space, 6-cortical bone (coronal edge), 7-fix mucoasa

sorption), the fixed mucosa from the alveolar process and the free gingival margin (fig 3a).

The distance we measured between the reference points was between the enamel-cementum junction and the coronal edge of cortical bone. The software of each device: DermaScan C Cortex Technology, CBCT New Tom 3G and Olympus cell B software of the Olympus BX51 microscope enabled us to measure the distance between those two anatomical reference points we were looking for in order to evaluate the horizontal atrophy.

Using the three methods of investigation, we performed the measurements for each place marked with gutapercha landmarks. For each place we took 3 images, one for each imaging method used. First on the DermaScan image we defined the enamel-cementum junction (ECJ) according to the convexities of the crown and of the root wall, due to teeth morphology . On the CBCT image for the same section, the ECJ was easily identified due to different radioopacity between the enamel and the other tissues. Also on direct microscopy the ECJ was easily indentifiable. We intended to verify if the reference point we assumed to be the ECJ on DermaScan images was indeed that, therefore we compared the measurements made by all the 3 methods. Through this comparison, we also verified the accuracy of the measurements. The microscopy was used as a gold standard and the measurements performed with the other two were compared with the data obtained using this method.

The first imaging method was the ultrasonography. The images were obtained by positioning the transducer in a longitudinal plane in the lateral area of mandible alveolar bone. On the sonograhic image, we were able to do the measurements at a micrometric level.

The second method used in our study was the CBCT scan. All the mandibles were scanned with Cone Beam Computed Tomograph (CBCT), whose software enabled us to do measurements within 0.2 mm intervals.

The third method we used was microscopy. The mandibles were sectioned in sagital plane. Our sections were realized through the bonded gutta-percha-landmarks, positioned on the oral surfaces of the teeth. The measurements were accomplished with direct visualization of the tooth without any previous histological preparation. This was done because the histological preparation dissolves the tooth enamel during the decalcification process. As one of our reference points was the enamel-cement junction, the damage of the tooth enamel would make the measurements impossible.

The statistical analysis was performed using Epi Info v6 software. The descriptive statistics were used for representing the graphical distribution of the data in order to verify if they follow the normal law and therefore to decide if liniar regression analysis is appropriate or if correlation tests for reduced data set should be used.

Results

We identified anatomical reference points on ultrasonography images of 20 MHz. Using the software of

the DermaScan C, Cortex Technology1 we performed the necessary measurements to assess the position of the bone in the sagitally plane, between the coronal edge of the cortical bone and the enamel-cementum junction. The sections obtained on the ultrasonography images were considered for measurements only if this gutta-percha landmark was clearly visible in order to be able to look for radiological and microscopy images exactly in the same zone (fig 3b).

The gutta-percha-landmarks necessary were identified also on each CBCT image. This confirmed that the measurements were made in the same area both by the ultrasonographical as well as by the radiological method. The first reference point, the enamel-cementum junction, was easily identified based on the differences of radiopacity between the enamel and dentine or enamel and cementum. The second reference point sought was the cortical bone, whose coronal edge was able to be identified (fig 4).

The sections for microscopy were made at the gutta-percha landmarks bonded initially. These landmarks were partially or completely destroyed during cutting, but, as can be seen on images no 2 and 3, the pig mandible was cut exactly around these landmarks. On the microscopy images we succeeded in easily identifying, both the coronal edge of the cortical bone and the enamel-cementum junction (fig 5).

The gold standard was direct microscopy. Comparative measurements performed between the sections obtained through the 3 methods described above, showed important similarities regarding the shape and the distances between the pre-set landmarks.

These similarities are clearly visible in the images shown above obtained by ultrasonography DermaScan and by radiology CBCT (fig 6). Four different sections are presented. The teeth shown in these images belong to the same mandible and the examinations began with the molars, towards the anterior area till the premolars, at the previously bonded gutta-percha landmarks. Only the first two sections belong to a permanent tooth; the adjacent ones belong to temporary teeth. The permanent tooth bud can be seen on the radiological images. The distance between the ECJ and the coronal edge of the cortical bone is smaller for posterior teeth compared to anterior ones. This was confirmed also by the measurements done with the software of the 3 devices we used. The 4th section, was made in the interradicular zone of a temporary molar (as seen on the radiological image). Both on the radiological and the ultrasonography image, the root of the tooth cannot be seen, just the crown and the root.

In order to prove the accuracy of the measurements, we took as a gold standard the direct microscopy measurements and we compared those made with DermaScan C and CBCT softwares.

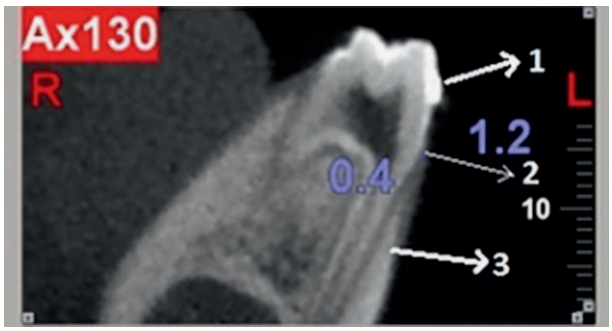


Fig 4. CBCT sagittal section through a tooth, visualized elements: 1-glued gutta-percha-landmark, 2-enamel-cement junction, 3- external cortical bone under fix mucoasa

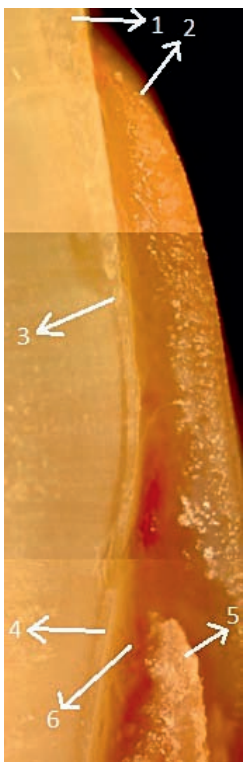


Fig 5. 4X microscopy brought extremely precise informations regarding the obtained sections: 1 – tooth crown, 2 – gingival margin, 3 – enamel-cement junction, 4 – root wall covered by cementum, 5 – cortical bone, 6 – periodontal space

Although the measurements were done only on 20 teeth, generating a rather small data set (table I), the obtained values had a normal distribution, which allowed us to perform a linear regression analysis.

There was a strong positive correlation between the alveolar bone level measured with the 3 techniques as shown in fig 7 and fig 8. The regression analysis showed as statistically significant for DermaScan measurements compared to microscopy ones ($p<0.0001$). Linear regression showed statistically significant correlations also for

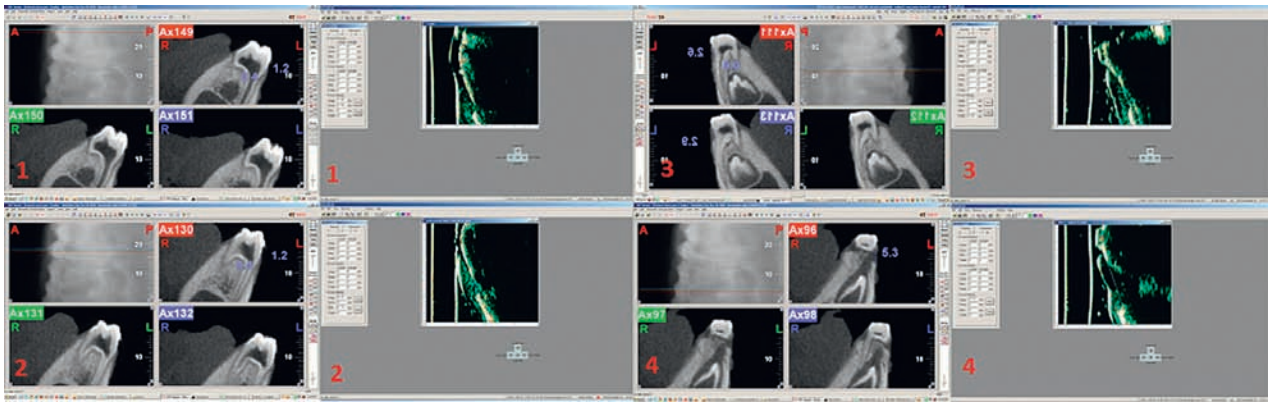


Fig 6. CT scan images compared with DermaScan images on the same sections

Table I. Measurements of bone level on the longitudinal section by Microscopy (mb), DermaScan (dscb) and CBCT (cbct)

	Mb(µm)	cbct(µm)	dscb(µm)
Mandible 1	2378,75	1200	1036
	1887,21	1200	1716
	4505,66	2900	2397
	5879,91	5300	5516
	7437,63	6400	5741
	5294,63	4900	5012
Mandible 2	3726,17	3100	3474
	3350,58	4300	4033
	4952,88	7400	7766
	9103,13	7200	7366
Mandible 3	4198,56	3000	3158
		4100	4339
	5236,41	5100	5167
		7800	7202
	4949,19	6700	7371
Mandible 4	1925,77	1500	1193
	2529,24	2200	1354
	4802,04	3800	3231
	5855,92	5600	4890
	5711,33	5200	5525

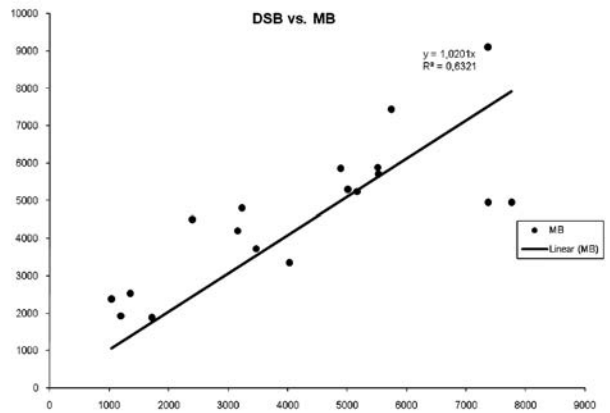


Fig 7. Correlation between measured values of the alveolar bone by Microscopy (MB) and DermaScan (DSB)

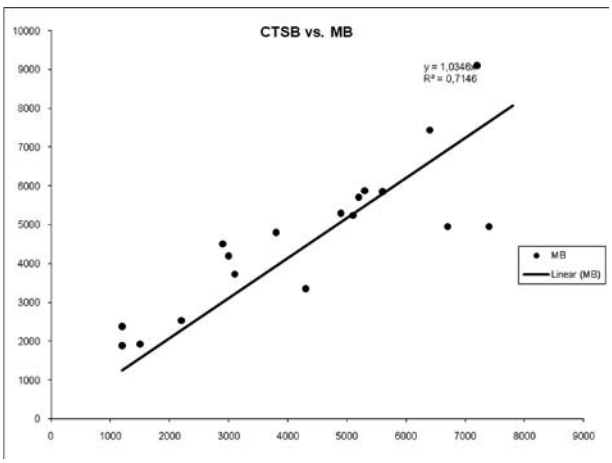


Fig 8. Correlations between alveolar bone values measured by computer tomography (CTSB) and microscopy (MB)

alveolar bone level measurements made by CBCT compared to microscopy ($p<0.001$).

The correlation coefficient R2 value show that more than 50% of the values were highly related which proves the accuracy of the measurements.

Discussions

Considering that the anatomical reference points were identified, the main aim of the study was achieved.

The measurements done could be extremely useful for prediction, evaluation and follow-up of periodontal disease. Peridodontits is also characterized by horizontal atrophies of the alveolar processes, an atrophy that could be monitored with a very high precision and without unwanted side-effects for patients' general health. Compared to the measurements of the areas from the coronary limit of the alveolar processes performed on radiological images, the ultrasonographic data will bring equal information. The ultrasound method has as its main advantage the absence of the unwanted side-effects of radiation, which is the case during radiological examination or computertomography performed for that area. The measurements could be repeated at small time intervals, obtaining reproducible data without negative effects on the patients health. The measurements will be more accurate than during the radiological examinations. On ultrasonographic images the measurements could be done with a precision of micrometers unlike with Computer Tomography, where the precision is within a tenth of millimeters. The resolution is higher for ultrasonography. In the same respect, the information given by the ultrasonographic images is much fuller for buccal and lingual sides of the teeth than the information given by retroalveolar X-rays, where the cortical bone is projected over the root and it cannot be clearly seen, due to the superimposition. In our study we compared the quantity of information obtained with the retroalveolar X-rays. The retroalveolar radiography is the radiological examination mostly used in dentistry because this form of examination has the smallest irradiation dose during the examination, much smaller compared to other types of radiological investigations. The retroalveolar X-ray done by parallel technique are very frequently used for evaluation and follow-up of the periodontal disease.

A limit of the ultrasound method appears when visualizing the elements from the interdental septum [13]. Due to increased frequency of ultrasounds, the penetrability is low in the interdental area and the examinations cannot be done in the same manner. If a miniaturized transducer could have been made available, it could have compensated for these drawbacDue to the strong positive correlations ($p<0.001$) and the high values of r2 (which could be interpreted as the percentage of common/shared variance) it can be concluded that the cementum-enamel junction may be identified with high accuracy according to the tooth anatomical convexities. The high resolution of the images demonstrates these convexities with a very good precision, therefore enabling the user to easily identify the enamel-cementum junction. This is a very important step because previous studies [14,15] positioned the alveolar bone level compared to landmarks drilled into the enamel, which may certainly not be done on real clinical cases, because it is out of the question from an ethical point of view to drill cavities into healthy enamel just in order to get reference points for further measurements.

This method will have a high applicability and utility in the follow-up of bone resorptions due to forces applied during orthodontic treatments, giving the possibility of follow-up and monitoring the effects of these forces on bone remodeling in the coronary area of alveolar processes. Measurements can be done at each activation of the orthodontic appliances, having the possibility of recording modifications of ten, hundreds and thousands parts of a millimeter, instead of just doing an evaluation at the beginning and at the end of the treatment. The virtual 3D models could be compared by sections in the transversal or sagital plane in order to see the evolution of vertical and horizontal bone resorptions. Constructing 3D models based on the information obtained by high-frequency ultrasonography will be very useful both for diagnostics as well as for follow-up of periodontal diseases of infectious etiology and in the follow-up of unwanted or negative effects in orthodontic treatments as well as in oclusology. In oclusology this method will provide very important data relating to the atrophies that have appeared due to premature contacts and occlusal interferences.

Therefore, we consider that this method of generating 3D models, which will be reproducible and comparable, does not have any side effects on the health of patients and medical staff, providing precise data for an exact follow-up of the bone level of alveolar processes in different periodontal disease stages.

Conclusions

The data from this study showed that ultrasound examination may be a reliable method to assess the periodontal system because the measurements made by ultrasound have a high correlation with, microscopy, which is the gold standard. The cementum-enamel junction may be identified on ultrasonic images with high accuracy according to the tooth anatomical convexities, therefore

this method could be used in the future for monitoring the periodontium if the size of the transducer will permit this.

Conflict of interest: none

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