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ORIGINAL ARTICLE

Fluorescence of intact human teeth enamel *in vivo*

Fluorescencja szkliwa nieuszkodzonych zębów *in vivo*

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Abstract

In this article the fluorescence of different anatomical areas of intact teeth of the upper and the lower jaw in vivo has been investigated. It was found that on average the enamel in cervical area has the highest fluorescence intensity and the enamel in the incisal edge area has the lowest. This fact is connected with the uneven homogeneity of enamel thickness, which depends on the anatomical area of a tooth and the influence of dentine-enamel junction and dentine layer on the overall fluorescence signal. To prove this hypothesis, enamel thickness in different anatomical areas of intact teeth has been measured using the methods of multilayer spiral computed tomography and scanning electron microscopy. The obtained results are very important for the investigation of mechanism of dental hard tissues fluorescence as well as for the prospective practical application of the method of laser-induced fluorescence to diagnostics of carious and non-carious lesions.

Streszczenie

W artykule przedstawiono badania in vivo różnych anatomicznych obszarów nieuszkodzonych zębów górnej i dolnej szczęki metodą fluorescencji. Stwierdzono, że średnio dla wszystkich zębów szkliwo w obszarze szyjki ma największą intensywność fluorescencji a szkliwo w obszarze krawędzi siecznej ma najniższą. Fakt ten jest związany z niejednorodną grubością szkliwa, która zależy od anatomicznego regionu zęba i wpływu połączenia szkliwno-cementowego oraz warstwy zębiny na całkowity sygnał fluorescencji. W celu udowodnienia tej hipotezy, zmierzono grubość szkliwa w różnych anatomicznie obszarach nieuszkodzonych zębów z zastosowaniem metody spiralnej tomografii komputerowej i mikroskopu skaningowego. Uzyskane wyniki są bardzo ważne w celu zbadania struktury twardych tkanek zęba a w przyszłości do diagnostyki ubytków pochodzenia próchnicowego i nie próchnicowego przy pomocy fluorescencji laserowej.

KEYWORDS:
laser-induced fluorescence, enamel, dentine-enamel junction

HASŁA INDEKSOWE:
fluorescencja laserowa, szkliwo, połączenie szkliwno-cementowe

Introduction

Today, the development of non-invasive diagnostic techniques based on low intensity laser radiation is one of the priority directions in laser medicine.[1] It is worth noting that laser-induced fluorescence method (LIF) is quite popular in dentistry due to its high sensitivity and non-invasive character.

The very promising LIF technique is indicated, for example, in the cases of dental caries, even at the early stages of the disease.[2,3] However, the investigation of hard tissues of intact teeth *in vitro* showed that the fluorescence signal of enamel greatly depends on the anatomical area of the probed tooth and may also depend on enamel thickness.[4] Besides, it is well-known that variation in enamel thickness may not only depend on the anatomical area of a given tooth, but also on its function.[5]

It seems obvious then that to make a diagnosis of various dental pathologies *in vivo* more accurately using LIF technique one should take into account an anatomical area of the tooth in which the pathology occurs. Thus, the present study is aimed at examining fluorescence of enamel in different anatomical areas of human teeth in the upper and the lower jaws *in vivo* and exploring the possibility of correlation between fluorescence signals and enamel thickness in these areas.

Materials and methods

Investigations have been carried out in a group of thirty female patients with intact dentition aged 20 to 30 years. In addition, sixty-five human teeth were removed for medical reasons. All the extracted teeth were intact, according to preliminary clinical and X-ray examinations.

Fluorescence spectra were recorded with a computer-controlled patented device, based on a fiber-optic spectrometer USB4000-VIS-NIR (Ocean Optics).[6] Laser diode, radiating at the wavelength of 405 nm, was used as an excitation source. Fluorescence spectra were recorded *in vivo* for different anatomical areas of teeth (cervical area, tooth equator area and incisal edge area).

To record enamel thickness at various anatomical areas of the teeth of different types, the method of multi-layer spiral computed tomography (MSCT) was used. The examination was performed using a scanning device Philips Brilliance ICT 64 with section thickness 0.55 mm in the axial plane. Enamel thickness *in vitro* was also recorded with scanning electron microscopy (SEM), using vacuum scanning electron microscope with JEOL energy dispersive spectrometer (Japan), 6380 LV. The images were taken using secondary electron and back-scattered electron modes.

This study was performed according to the Helsinki Declaration on proper treatments of human subjects.

Results

Fig. 1 represents the dependences of the integral fluorescence intensity of dental enamel on the type of teeth averaged over all of the patients: (a) precervical area, (b) equatorial area, (c) scalprum area. Solid colour corresponds to the upper jaw teeth; line art corresponds to the lower jaw teeth. Fluorescence spectra were recorded for the vestibular surface of teeth.

Fig. 1(a), (b), (c) shows that the values of the integral intensity of the enamel fluorescence in the equatorial area and in the scalprum area are considerably lower than for the precervical area for all of the groups of teeth both in the upper and lower jaw dentitions. It should be noted that on average the overall intensity of the enamel fluorescence for the lower dentition is higher than that for the upper dentition. That corresponds with the fact that the thickness of enamel in the upper dentition is on average greater than that in the lower dentition. As an example, Fig. 1(d) represents fluorescence spectra of enamel for the different anatomical areas of a tooth averaged for all of the patients: precervical area, equatorial area and scalprum area of the first mandibular premolar.

Table 1 shows the values of the enamel thickness averaged for all of the patients at different anatomical areas of different tooth types (incisors, canines, premolars and molars) of the upper and the lower jaws fixed with MSCT technique and measured from the vestibular surface of teeth.

As seen in Table 1, on average the scalprum area of each tooth has the largest thickness of

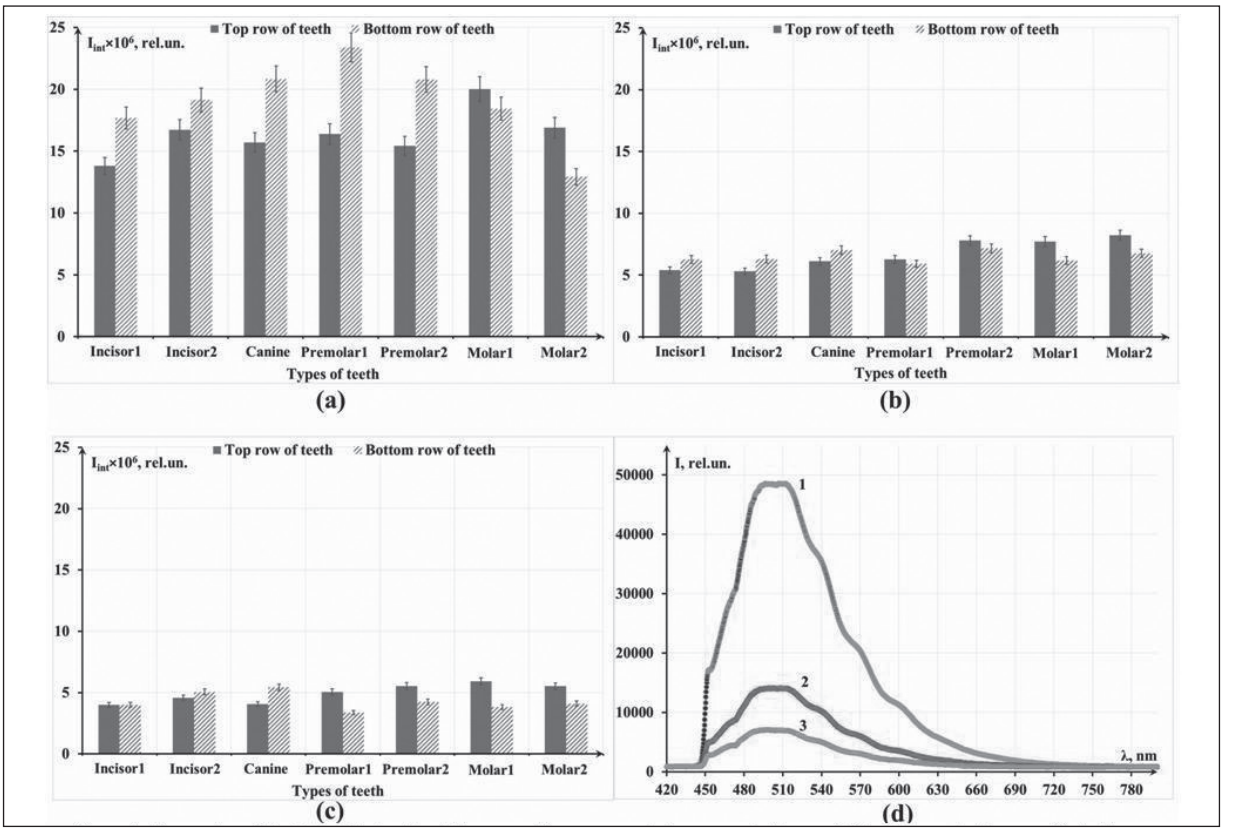

Fig. 1. *Mean value of the integral intensity of the enamel fluorescence in the precervical area - (a); in the equatorial area - (b); in the scalprum area - (c) (of the upper (solid colour fill) and lower rows of teeth (line-art fill) of the intact teeth in a dependence of their type. (d) - averaged fluorescence spectrum of enamel in the precervical area (1), in the equatorial area (2) and in scalprum area (3) of premolar in the lower jaw.*

enamel while precervical area has the lowest value of enamel thickness. For each group of teeth an increase in the mean thickness of enamel is typical practically for all of the anatomical areas along with the increase in the serial number of a tooth both in the upper and the lower jaw. From Table 1 it is also seen that the mean thickness of enamel in all the investigated anatomical areas of teeth in the upper jaw is slightly greater than for the teeth of the lower jaw.

The averaged values of the thickness of enamel for the upper and lower teeth measured with the use of Scanning Electron Microscopy (SEM) are presented in Table 2.

Comparison of the two applied techniques concerning the measurements of the enamel thickness at different anatomical areas in the lower and the upper jaws indicates a good correlation of the obtained results.

Discussion

there are a lot of studies on the fluorescence of hard dental tissues in the intact teeth.[2-4,7] For example, in the paper[4] where the extracted teeth were described, it was shown that the highest fluorescence signal is provided by enamel in the precervical area of a tooth, while the lowest signal is observed for the enamel in the scalprum area of a tooth. The results presented in our work mean that this tendency persists for all of the teeth of the upper as well as of the lower dentition, and not only *in vitro* but also *in vivo*.

Moreover, we tried to reveal the correlation between the signal of the enamel fluorescence and enamel thickness at different anatomical areas of teeth. This correlation is possible, because during investigations of fluorescence in dentine, enamel and dentine-enamel junction (DEJ), according to *Sarycheva* et al.,[4] it was noted that

Table 1. Thickness of enamel in precervical area, equatorial area and scalprum area. MSCT method was used

	Tooth area / Type of tooth	Precervical, thickness, mm	Tooth equator, thickness, mm	Scalprum, thickness, mm
Upper jaw	Incisor 1	0.27±0.01	0.84±0.05	0.97±0.06
	Incisor 2	0.32±0.01	0.92±0.06	1.04±0.06
	Canine	0.28±0.01	0.79±0.05	0.89±0.05
	Premolar 1	0.31±0.01	1.01±0.06	1.33±0.08
	Premolar 2	0.36±0.02	1.11±0.07	1.56±0.09
	Molar 1	0.38±0.02	1.25±0.08	1.76±0.11
	Molar 2	0.39±0.02	1.31±0.08	1.89±0.11
Lower jaw	Incisor 1	0.17±0.01	0.73±0.04	0.89±0.05
	Incisor 2	0.23±0.01	0.83±0.05	0.98±0.06
	Canine	0.19±0.01	0.78±0.05	0.84±0.05
	Premolar 1	0.21±0.01	0.98±0.06	1.41±0.08
	Premolar 2	0.23±0.01	1.02±0.06	1.58±0.09
	Molar 1	0.24±0.01	1.17±0.07	1.68±0.10
	Molar 2	0.30±0.02	1.21±0.07	1.72±0.10

the area adjacent to DEJ showed much greater luminescence than the enamel. In *Cloitre* et al.,[7] using multi-photon microscopy, it was shown that DEJ itself demonstrated very low fluorescence signal. The observed bright luminescence of the DEJ area is due to the parts of enamel and dentine adjacent to this boundary (DEJ), that have multi-level structure of the arc-like notches, with their convex sides faced dentine and their concave sides faced enamel. The width of this multi-level cavity-like structure of DEJ depends on the anatomical area of a tooth as well as on the type of a tooth.

On the other hand, the analysis of results obtained with MSCT and SEM techniques shows that the thickness of enamel in the precervical area for all the teeth both in the upper and the lower jaw dentition is smaller than in the equatorial or the scalprum areas. Therefore, one can assume that the lower the enamel thickness, the less the optical path for the exciting radiation directed at the most brightly luminescent area; hence, the intensity of fluorescence will be higher. In fact, the tendency for the fluorescence signal increase was observed for molars and premolars in the precervical area (see Fig. 1(a)) along with the decrease of enamel thickness (see Tables 1 and 2).

Fig. 1(a) also reveals that the total intensity of the precervical area signal for all of the mandibular teeth is higher than for the maxillary ones, and this correlates with the fact that the average thickness of teeth from the lower dentition is lower than for the upper teeth (see Tables 1 and 2).

However, the analysis of all the sets of data showed that the correlation between the fluorescence signal and the thickness of enamel for other types of teeth and in the other anatomical areas proved to be rather complicated and not so unambiguous.

Table 2. Thickness of enamel in precervical area, equatorial area and scalprum area. SEM method was used

	Tooth area / Type of tooth	Precervical, thickness, mm	Tooth equator, thickness, mm	Scalprum, thickness, mm
Upper jaw	Incisor 1	0.19±0.02	0.73±0.04	0.90±0.05
	Incisor 2	0.23±0.02	0.89±0.05	0.97±0.06
	Canine	0.20±0.02	0.76±0.05	0.80±0.05
	Premolar 1	0.22±0.02	0.98±0.06	1.20±0.07
	Premolar 2	0.27±0.03	1.05±0.06	1.30±0.08
	Molar 1	0.30±0.03	1.20±0.07	1.88±0.11
	Molar 2	0.34±0.03	1.27±0.08	1.93±0.12
Lower jaw	Incisor 1	0.15±0.02	0.67±0.04	0.80±0.05
	Incisor 2	0.20±0.02	0.80±0.05	0.91±0.05
	Canine	0.17±0.02	0.70±0.04	0.78±0.05
	Premolar 1	0.19±0.02	0.92±0.06	1.20±0.07
	Premolar 2	0.21±0.02	0.97±0.06	1.20±0.07
	Molar 1	0.24±0.02	1.11±0.07	1.70±0.10
	Molar 2	0.29±0.03	1.15±0.07	1.77±0.11

For example, for the incisors no unambiguous dependence of the fluorescence signal of enamel in the precervical area on its thickness was observed. As seen in Fig. 1(a), a tendency of increase of the fluorescence signal is observed for enamel in the precervical area for the incisors with an increase of the serial number of a tooth. However, the enamel thickness of the lateral incisor (2) in the precervical area is greater than that of the central incisor (1) (see Tables 1 and 2). At the same time, this experimental fact can be explained within the frameworks of the proposed concept. In fact, the lower the thickness of the multi-level notch-like structure of DEJ, the lower the registered fluorescence signal. The data obtained by *Scott* et al.[8] indirectly support the idea that DEJ thickness depends on the anatomical area of tooth. Therefore, there is possibly some critical value of the enamel thickness if the exceeding thickness of the notch-like area adjacent to DEJ does not depend on it for the given anatomical area of a tooth.

Also it was found that on average the intensity of enamel fluorescence near the equatorial area of the tooth is higher than near the scalprum edge, while the enamel thickness in the equatorial area is lower than in the scalprum area. Thus, the signal of fluorescence depends on the tooth type as well; this can be observed, for example, in the structure or chemical composition of dental enamel. However, this issue requires further investigations. We can only note here that, for example, the chemical structure of DEJ is not homogeneous and depends on the anatomical area of a tooth.[9]

Conclusions

Investigations of fluorescence in different anatomical areas of the intact teeth in the upper and the lower human jaws were performed *in vivo*

with the use of LIF. It was found that on average for all of the teeth enamel in the percervical area provides the highest fluorescence signal while enamel in the scalprum area yields the lowest fluorescence. In order to explain such spectral behavior we assumed a possible influence of the enamel thickness and multi-level region of DEJ. The obtained results mean that the correlation of the fluorescence signal with the enamel thickness seems rather ambiguous.

It was shown that for the precervical area fluorescence signal of enamel actually depended to a high extent on the thickness of enamel and on its smallest variations. However, the results obtained for the equatorial and scalprum areas mean that the fluorescence signal is determined not only by enamel thickness and the DEJ structure but also, quite probably, by inhomogeneity of the structure and chemical composition of dental enamel.

Obviously, the obtained results are very important for the understanding of mechanism of fluorescence of dental hard tissues, as well as for the development of LIF-based medical devices for the diagnosis of non-carious dental diseases.

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