Laser-Induced Fluorescence and X-Ray Spectral Analysis of Carious Process in Hard Dental Tissues

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 149, No. 3, pp. 350-354, March, 2010
Original article submitted January 18, 2010

Morphological and spectral X-ray analysis of carious and noncarious extracted teeth showed the patterns of dentin ossification in caries of different degree. Parietal ectopic ossification of the canal and cavity lumens in stages III and IV dental caries is regarded as a specific structural marker of pathological regeneration. The X-ray spectral analysis showed that the progress of carious process is paralleled by loss of mineral components. Laser-induced fluorescent study of tissues in extracted teeth showed 4 spectral bands corresponding to mineral and protein components of the tooth. The progress of carious process was associated with reduction of the fluorescence intensities of the spectral bands characteristic of dental collagen and mineral components.

Key Words: *carious process; hard dental tissues; morphology; fluorescence; x-ray spectral analysis*

According to WHO data, dental caries can be regarded as the most prevalent disease. It often leads to loss of teeth, thus causing temporary disability of young and adult people [4]. Caries develops in the overwhelming majority of humans; its incidence in adult age is 91%. Numerous studies showed that the development and progress of caries can manifest by systemic locomotor diseases, cardiac infections, and other visceral diseases [1]. The most important diagnostic problem is detection of the demarcation zone between the intact and involved tissues, determining the volume of dental tissue resection and treatment strategy [5]. At present these problems are solved by traditional methods used for the diagnosis of caries (electro-odontodiagnosis, dental roentgenography, *etc.*). However, these methods are imperfect and their potentialities are limited.

On the other hand, the development of optical diagnostic methods based on the analysis of spectral characteristics of laser-induced fluorescence (LIF) for cardiovascular tissues is in progress. These methods can be applied to analysis of the mineral constituent of calcified heart valves and large vessels [2,3,7]. Moreover, the DIAGNODENT™ device for the fluorescent diagnosis has been created and clinical trials of its pilot specimens are now in progress, which will more precisely define the diagnostic value of the device for various forms of dental involvement at λ=655 nm [6,8]. The objective need in the development of a new method for accurate diagnosis of dental diseases prompted us to undertake this study.

We carried out a comparative morphological and X-ray spectral analysis of intact and carious teeth with evaluation of the LIF spectra in order to evaluate the diagnostic significance of these approaches.

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0007-4888/10/1493-0373

MATERIALS AND METHODS

A total of 229 teeth from patients (128 men and 101 women aged 19-75 years) were studied *in vitro*. The material was collected at specialized dental department of medical center in Novosibirsk in 2005-2007 and classified by patients' age, gender, tooth type, caries degree, and complications.

The material was divided into 5 groups by patients' age: 1) patients aged 19-30 years; 2) 31-40; 3) 41-50; 4) 51-60, and 5) 50-75 years. Dystopic third molars were removed in 74% cases, premolars in 23%, and canines in 3% cases. By caries stages, the material was distributed as follows: stage I in 21% cases, stage II in 47%, stages III and IV 16% each. Noncarious lesions were detected in 39% of all extracted teeth; of these 66% were third molars, 7% dystopic premolars, and 3% retained canines. The teeth were extracted because of progressive periodontitis in 24% cases; 52% noncarious teeth were extracted in men and 48% in women.

Native tooth specimens were studied by LIF. The KrF eximer laser (λ=248 nm) served as the radiation source exciting fluorescence. The length of laser pulse was 5 nsec, energy 5-10 mJ. The data were recorded on a spectrograph with holographic diffraction lattice. The PZC camera with brightness amplifier served as the radiation receiver. A tissue specimen was placed on the microscopic stage from slightly fluorescent material (stainless steel). The fluorescence was collected with a spherical reflector (with the spectrograph input slit in the focus), that is, the spectrum averaged by the specimen surface was measured. As the brightness amplifier photocathode was not standardized by spectral sensitivity, the actual shape of the spectrum on the curves is distorted, but the percent shifts can be traced on them with high precision.

Macroscopic analysis of the specimens and histological studies (hematoxylin and eosin staining and staining by van Gieson method) were then carried out. Preparations for scanning microscopy and subsequent X-ray spectral analysis were made. The X-ray spectral microprobe chemical analysis of the studied dental substances was carried out using the Si (Li) energy detector (OXFORD). Due to the use of X-ray spectral microprobe chemical analysis, quantitative chemical analysis could be carried out on microvolumes (from a section area) and the distribution of elements could be obtained from the scanning area. Quantitative chemical analysis by references was carried out using INCA Energy300 software.

Statistical analysis of quantitative data was carried out using Student's test.

RESULTS

Virtually all destructive manifestations of surface caries were observed in middle caries, but they were more disseminated. On the other hand, later stages of caries were characterized by specific features intrinsic to such common pathological processes as atrophy and pathological regeneration. The destruction zones were often separated from intact dentin by ossification sites, which was associated with dentin tubule degeneration. One of the most interesting features was "blocking" of dentin tubules in intact dentin far beyond the focal destruction (Fig. 1, *a*). A stable sign of stages III and IV caries was secondary dentin, characterized by abnormal distribution of dentin tubules and uneven mineralization. We detected a special morphological symptom of the late stages of caries: parietal ectopic ossification of the dental canal and cavity lumen (Fig. 1, *b-d*).

X-Ray spectral analysis of the material showed changes in the mineral composition of hard tooth tissues (content of Ca, P, Mg and Ca/P proportion), depending on caries involvement. The chemical composition of human dental enamel and dentin apatites is liable to change under the effects of numerous factors, such as biogeochemical conditions of the place,

TABLE 1. X-Ray Spectral Analysis of the Levels of Ca, P, Mg and Ca/P in Normal and Pathological Enamel and Dentin at Different Stages of Carious Process ($M \pm m$)

Mineral composition	Stages I, II		Stages III, IV	
	intact teeth	carious teeth	intact teeth	carious teeth
Ca	21.10±0.27	20.30±0.39	20.20±0.34	18.70±0.42*
P	14.00±0.44	14.00±0.25	13.80±0.24	13.50±0.17
Ca/P	1.58±0.60	1.47±0.30	1.48±0.22	1.43±0.18
Mg	0.90±0.17	0.75±0.04*	1.20±0.09	0.97±0.05*

Note. *p<0.05 (ANOVA) compared to intact teeth.

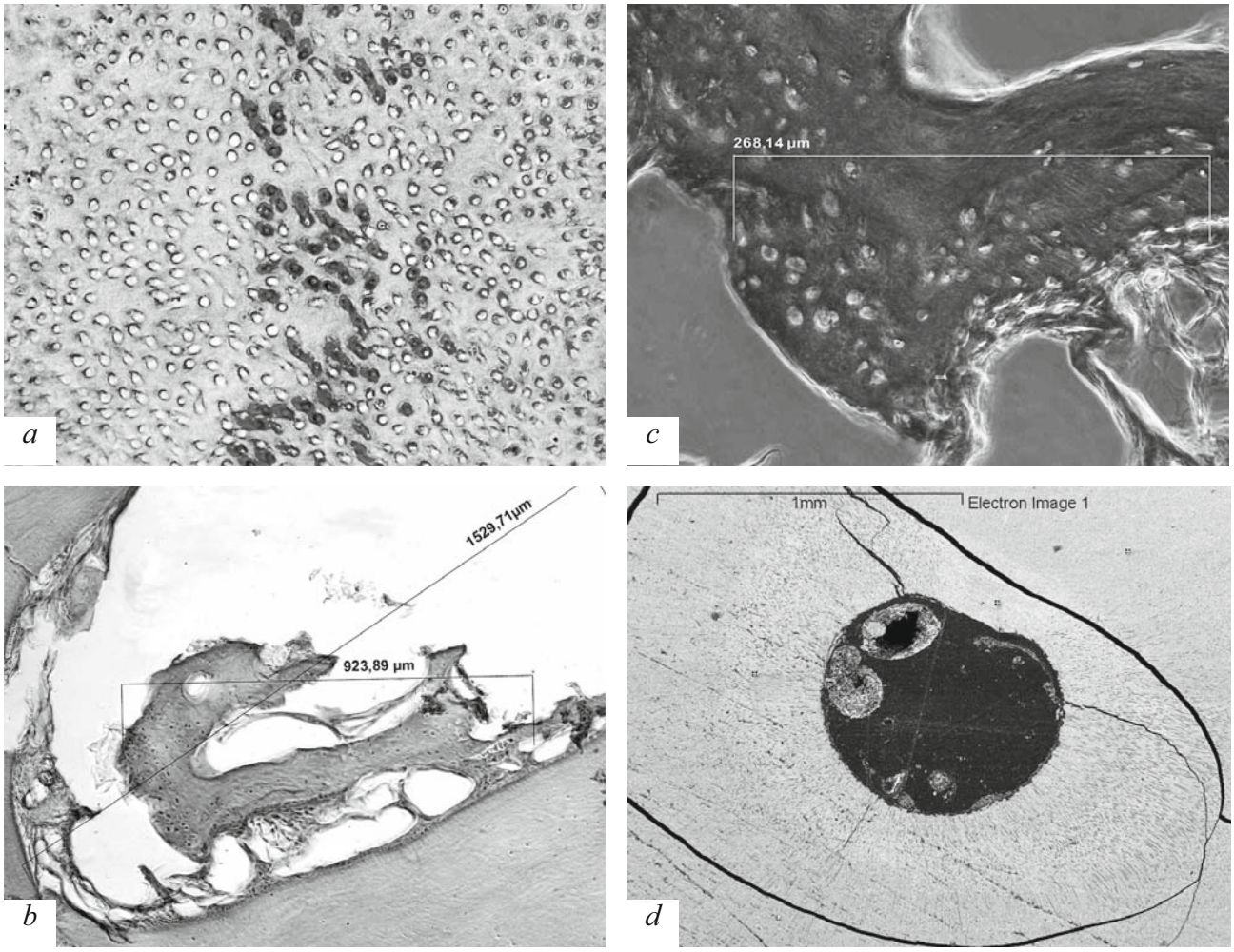

Fig. 1. Structural changes in dentin in caries. *a*) middle caries, "blocking" of dentin tubules by eosinophilic mass, ×914; *b*) deep caries, parietal ossification in dental cavity, ×114; *c*) deep caries, ossification in dental cavity, ×360; *d*) dental section, deep caries, parietal ossification in dental cavity (*1*: secondary dentin; *2*, *3*: intact dentin). *a*) hematoxylin and eosin staining; *b*, *c*) van Gieson staining, phase contrast; *d*) scanning electron microscopy.

ecological and occupational factors, patients' age, concomitant oral diseases, *etc.* The difference between the studied apatite and the hydroxyapatite with the ideal formula $Ca_{10}(PO_4)_6(OH)_2$ is usually evaluated by the Ca/P proportion. The greater calcium deficiency, the lesser is this proportion in comparison with the 1.67 value, characteristic of the ideal composition. This proportion can reach 1.3 with the progress of caries. That is why we used the Ca/P coefficient as the index of caries resistance of hard tooth tissue.

The levels of Ca, P, Mg and of the Ca/P proportion in intact and carious enamel differed negligibly at stages I and II of caries. A trend to a reduction of the mineral component levels is seen (Table 1). At stages III and IV of carious process the levels of mineral components of dental tissues clearly reduce in the pathological compared to intact dentin.

Study of LIF spectra of intact and carious teeth has shown at least four fluorescent components in dental tissue (Fig. 2). One of them, responsible for spectral band at 450 nm (Fig. 2, *b*, *c*), most likely belongs to the mineral constituent hydroxyapatite [2]. The second spectral band with maximum at 390 nm (Fig. 2, *a*) is present in connective tissues (collagen and elastin). Two more fluorophores are responsible for the spectral band of 330-370 nm, with presumable maximums at about 340 and 360 nm (Fig. 2, *c*). These LIF spectra are characteristic of tryptophan-containing proteins. Laser-induced spectrum of the tooth represents a sum of spectra of numerous components. The percent contribution of each component depends on individual characteristics of the patient and severity of pathological processes. For more convenient comparison, the studied spectra were standardized for the intensity of λ=340 nm.

The complex multicomponent LIF impedes the comparison of various spectra, but despite the difficulties, we can trace a certain relationship between LIF

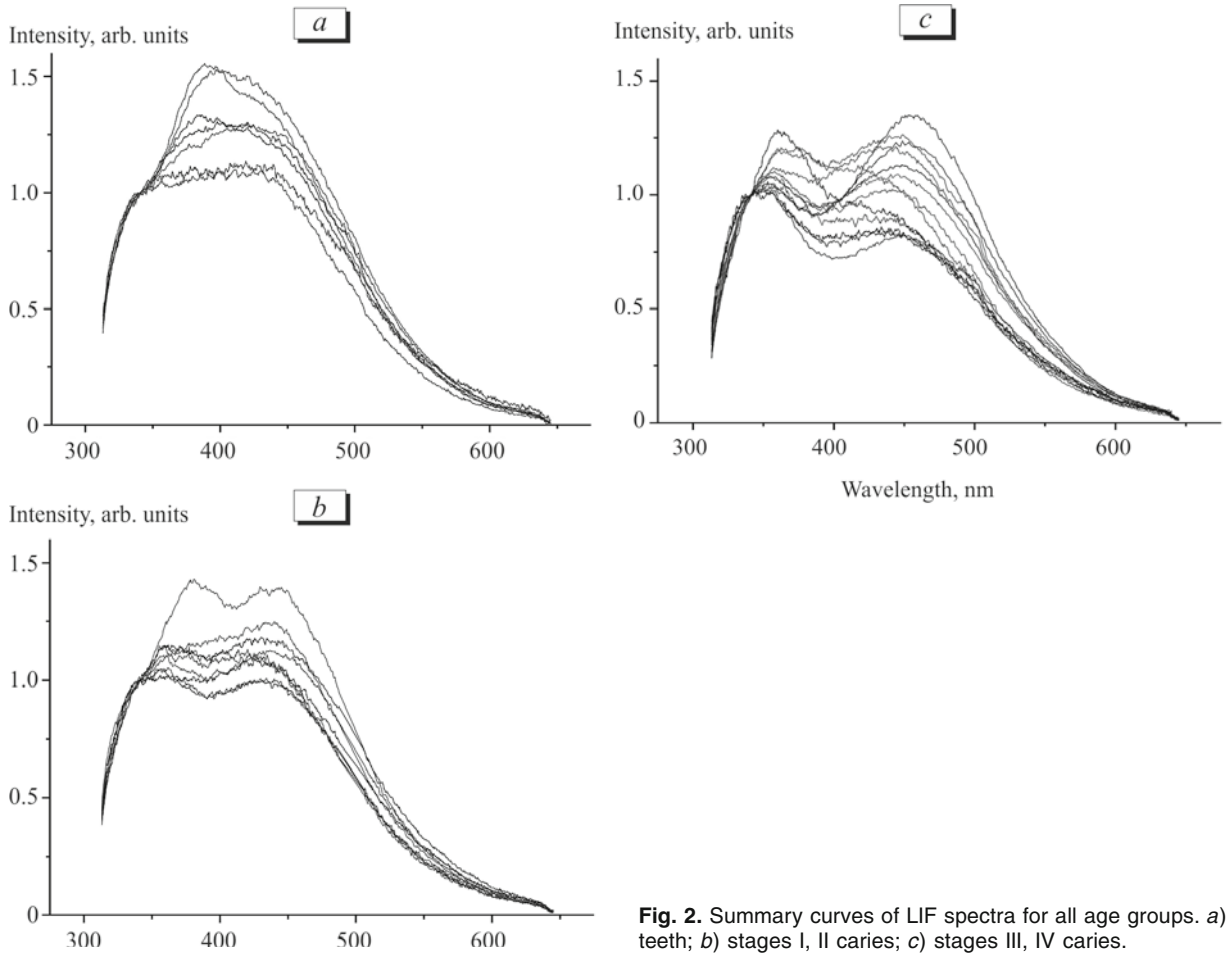

Fig. 2. Summary curves of LIF spectra for all age groups. *a*) intact teeth; *b*) stages I, II caries; *c*) stages III, IV caries.

spectra patterns and carious process stages in comparison with the intact tooth spectra. An intact tooth LIF spectrum is characterized by the formation of a wide spectral band in the entire measured range. The contribution of all fluorescent components in this process is rather uniform. Some spectra exhibited a clear-cut "collagen" maximum (λ=390 nm; Fig. 2, *a*). All spectra were similar at 330-370 and 410-600 nm. In other words, intact teeth are characterized by a certain proportion of the protein and mineral constituents, while the differences caused by individual features are determined by the contribution of the collagen constituent.

A "gap" at 380-400 nm forms in stages I and II caries because of a lower intensity of the collagen spectral band fluorescence (Fig. 2, *b*). The intensities of the protein (330-370 nm) and mineral (410-600 nm) spectral bands were close to those of intact teeth. However, the fluorescence at 330-370 nm was not uniform: the maximum was differently shifted to the long-wave spectrum in different specimens, which fact indicated changes in the protein composition in the destruction zone. At stages III and IV caries, the "gap" at 380-400 nm clearly increased: collagen spectral band in fact completely disappeared (Fig. 2, *c*). The fluorescence patterns at 330-370 nm also varied. In some specimens the spectral band with the maximum at 450 nm notably reduced, this indicating tissue demineralization.

Hence, studies of hard dental tissues by the LIF method can be used for evaluating the structural changes in dentin at different stages of caries. This opens new prospects for the diagnosis of carious involvement.

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