Monitoring of copper nanoparticle penetration into dentin of human tooth in vitro

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ABSTRACT

Study of the penetration depth of synthesized copper nanoparticles into cut samples of human dentin was conducted. The scanning electron microscopy was used to determine the elemental composition of fresh transverse cleavage of the dentin cut for determination of the copper nanoparticles penetration with an effective antiseptic effect. The morphology of the cut surface of the dentin of a human tooth was studied and the lower limit of the diffusion boundary was determined. It was found that copper nanoparticles penetrate into the dentin cut to a depth of ~ 1.8 μm with the diffusion coefficient of 1.8×10^{-11} cm^2/s. Despite the rather small size of the synthesized copper nanoparticles (20-80 nm), a rather small penetration depth can be explained by the high aggregation ability of copper nanoparticles, as well as the ability of a micellar solution of sodium dodecyl sulfate, in which nanoparticles were stabilized, to form conglomerates in micelles of much larger sizes.

Keywords: biological tissues, biomedical optical visualization, stomatology, nanotechnology, copper nanoparticles, dentin, diffusion, antimicrobial properties

1. INTRODUCTION

Modern science shows great interest in the development of nanotechnologies that use nanoparticles of various metals. The nanoparticles of metals and materials modified by them have unique specific properties [1], that are widely used as catalysts [2, 3], sensory systems, optical devices, drugs with high biological activity, used in ecology, medicine and agriculture. There are great prospects for the usage of metal nanoparticles in biology and medicine [4-6]. Applications of nanoparticles in conjunction with optical methods were demonstrated for diagnosis and treatment of various (including oncological) diseases, as well as for immunochemical research [7, 8]. The leading role in this field of application of metal nanoparticles belongs to such metals as gold and silver. For example, silver nanoparticles are used to produce a variety of materials with bactericidal properties [9] and gold nanoparticles - to increase efficacy and reduce undesirable side effects in radiotherapy of tumors [10-13]. The positive properties of gold nanoparticles are used for treatment of defects in the jawbone in an animal experiment [14] and periodontal disease [15], and as additives to dental implants [16]. Copper nanoparticles have pronounced antibacterial activity, for example, greater than iron nanoparticles relative to *Staphylococcus aureus* [17]. The study of the antimicrobial properties of copper nanoparticles with the usage of soy extract showed the antibacterial efficacy of nanoparticles towards *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus faecalis* [18]. Copper nanoparticles synthesized by the green method using the extract of ginger also show antimicrobial efficacy against various bacterial and fungal pathogens [19].

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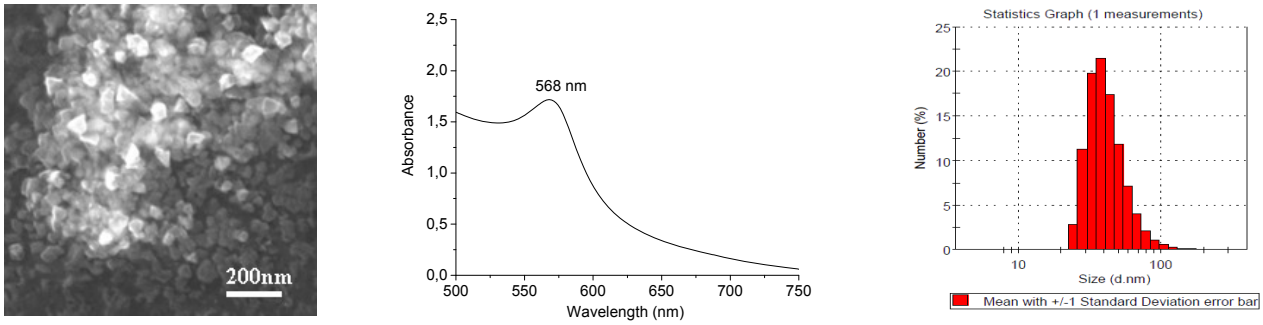
With the insertion of nanoparticles in dentine tubules, the development of methods for reducing hypersensitivity of the tooth is associated. Embedded nanoparticles can exert bactericidal effect, enhance photodynamic effect, perform cosmetic functions, promote whitening. In this regard, the urgent task is the development of optical methods for simple and non-invasive control of the delivery of nanoparticles to the tooth tissue. Studies were carried out with the usage of TiO_2 and ZnO nanoparticles, light activated to generate free radicals, that are prospective to be used for bleaching and eliminating hypersensitivity of teeth [19-22], as well as early diagnostics of carious lesions at the cretaceous stain stage, using optical diagnostic methods [23]. Bacteria are one of the main factors of the emergence of primary [24] and permanent [25-27] endodontic infections. The presence of bacteria in the dentinal tubules is associated with infection in the root canals [28]. The presence of bacteria is dangerous not only in volume, but also on the surface of the tooth, where bacteria play a key role in the formation of microbial biofilms. The directions used to prevent the formation of biofilms include the physicochemical modification of the surface of the biomaterial to create antiadhesive surfaces, the incorporation of antimicrobial agents into medical polymer devices, various mechanical cleansing and treatments, and the release of antibiotics, photodynamic antimicrobial technologies. Inactivation of *Enterococcus faecalis* biofilms by light has been proven in studies *in vitro* [29]. Portable red LED for excitation of exogenous toluidine blue photosensitizer and blue LED for excitation of endogenous porphyrins showed good efficacy of photodynamic inactivation of the three main pathogenic bacteria of periodontitis - *Prevotella melaninogenica*, *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* and the prospects for use in clinical settings for photodynamic therapy [30]. The selective effect of the laser on pathogenic microflora due to the difference in the absorption of photon energy by pathogens and host tissues can also be used in dentistry, and the depth of the bactericidal effect is determined by the parameters of the laser [31]. Polyurethane foams with a coating of copper nanoparticles can be used as an excellent antibacterial filter for water against *E.coli* [32].The study of the interaction of nanoparticles of different nature with biological tissues, in particular, with human dentin, is an urgent problem. Composites with nanoparticles can be used as effective antiseptics (for antiseptic treatment of root canals with tooth pulp), as an additive to implants, antiseptic rinsers, toothpastes, filling materials, etc.

2. METHODOLOGY

The preparation of copper nanoparticles was carried out in the system: an aqueous solution of copper (II) chloride, a complexing agent and a pH regulator: an ammonia-reducing hydrazine hydrate in a micellar solution of a sodium dodecyl sulfate surfactant, described in detail in the Ref. [33]. The size of the copper nanoparticles obtained in the solution of dodecyl sulfate is smaller (average size 20-80 nm) than in the solution of cetylpyridinium chloride (average size 40-180 nm) [34]. Confirmation of the presence of copper nanoparticles in the resulting suspension was carried out using a spectrophotometric method on a SHIMADZU UV-2550 spectrophotometer. The absorption spectra were recorded in the wavelength range from 190 to 900 nm. The appearance of a maximum absorption of the solution with copper nanoparticles corresponds to surface plasmon resonance, in the wavelength range λ = 570-590 nm (Fig. 1b). Later on, the morphology of the surface of copper nanoparticles (Fig. 1a), samples of cuts of a human tooth and the elemental composition were studied on the SEM of the Tescan Mira II LMU in the mode of secondary electron detection (at an accelerating voltage of 30 kV). For this, the samples were fixed on a special carbon substrate (carbon scotch) and gold was deposited on their surface.



a b c

Figure 1. Electron micrograph obtained by copper nanoparticles (a); the absorption spectrum of the solution with products of copper nanoparticle synthesis (b); the histogram of copper nanoparticle size distribution (c).

The size of the synthesized copper nanoparticles was determined on Zetasizer nano ZS Malvern, the size distribution of the obtained copper nanoparticles is shown in Fig. 1 (c).
Samples of sections of a human tooth were prepared as follows. The teeth were removed in the dental clinic for medical reasons. The removed teeth were stored in physiological solution at 4 °C in a dark place. A significant part of the human tooth is dentin, which is covered with enamel in the crown part, and with cement in the root part. The dentin determines the size, shape of the tooth and protects the dental nerve from damage. The dentinal layer and enamel have an uneven surface and are firmly adhered to each other due to the dentin-enamel compound (Fig. 2a). Wet dental samples were cut with a diamond disc into sections about 1 mm thick. One part of the dental samples was cut along the growth axis, and another part - across. On the longitudinal section (Fig. 2a) the dentinal tubules run in parallel to the cut, having a different shape, angle of bend and diameter. In the transverse section (Fig. 2b), the dentinal tubules are all cut and displaced at different angles to the surface.

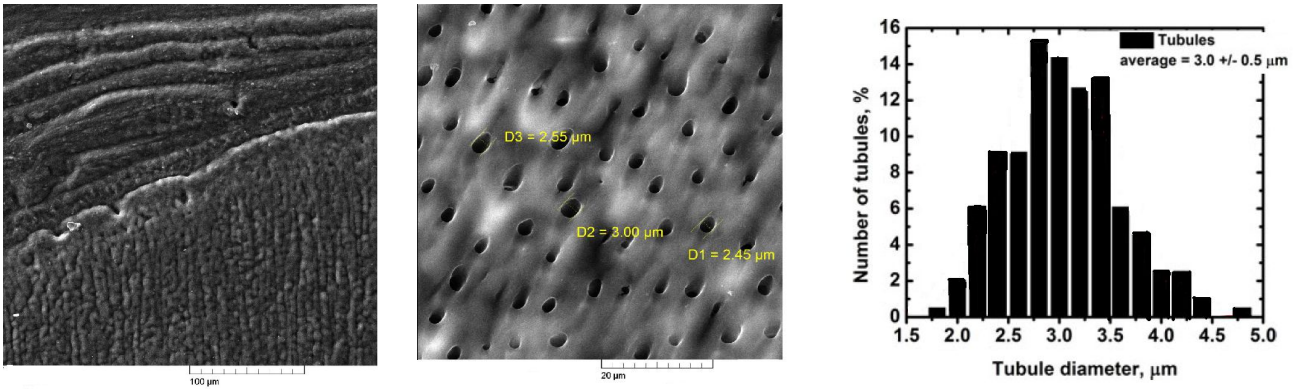


Figure 2. SEM images of sections of a human tooth: longitudinal (border enamel and dentin), magnification 1 kx (a); transverse section of dentin, magnification 5 kx (b); the histogram of the distribution of the dentinal tubule diameters (c).

Dentinal tubules are S-shaped in the region of the crown of the tooth; in the root region they pass rectilinearly, expanding fanwise upward to the outer surface. There is a different number and density of dentinal tubules in different parts of the transverse section of dentin. The diameter and volume of the dentinal tubules depends on the age of the teeth. The distribution of the sizes of the tubules, located mainly perpendicular to the transverse cut of the human tooth, are shown in Fig. 2 c. [23].

It can be seen that the average size of dentin tubules is 2.5 - 3.5 µm. The resulting sections of the human tooth were etched in 35% orthophosphoric acid for 1 min, then, using a brush and 95% ethanol, the surface was cleaned from the sawing products and other external contaminants; further, the acid was removed with a stream of distilled water for 30 s, the sample was placed in an ultrasonic bath for 10 min and wiped with a lint-free cloth dampened with alcohol; the samples were dried in air under normal conditions during the day. Prepared in this way, samples of tooth cuts were placed in a cuvette with 5 ml of a solution containing synthesized copper nanoparticles. Then the cuvettes with the samples were exposed to ultrasound (ultrasonic bath Techsonic UD100 SH-45 L, 35 kHz, 240 W) for 30 min. It was then removed from the solution, washed with a stream of distilled water and dried under ordinary conditions. Since only dentin has dentinal tubules, in which particles can penetrate due to capillary effects, a fresh cleavage was made across the sample from the transverse dried cut. Then, using a special fastener, the sample was fixed with fresh cleavage upward, and the elemental composition of this part of the sample was examined with a scanning electron microscope. In total, 4 samples of the transverse section of a human tooth were examined.

3. RESULTS

The synthesized copper nanoparticles, according to the studies carried out, have an average size of 20-80 nm. Nanoparticles have the form of polyhedra with different shapes approximating to spherical, cubic and irregular polygons. However, these particles tend to form conglomerates of much larger sizes, which can be observed on the surface of a human tooth sample without being rinsed with distilled water after the experiment (Fig. 3a), despite the application of ultrasonic action aimed at reducing of agglomeration.

a b

Figure 3. SEM image of the surface of the cut of a human tooth after 30 min of being in solution with copper nanoparticles without subsequent washing with water: the sample surface, magnification 500 x (a); end face of the sample, magnification 10 kx (b).

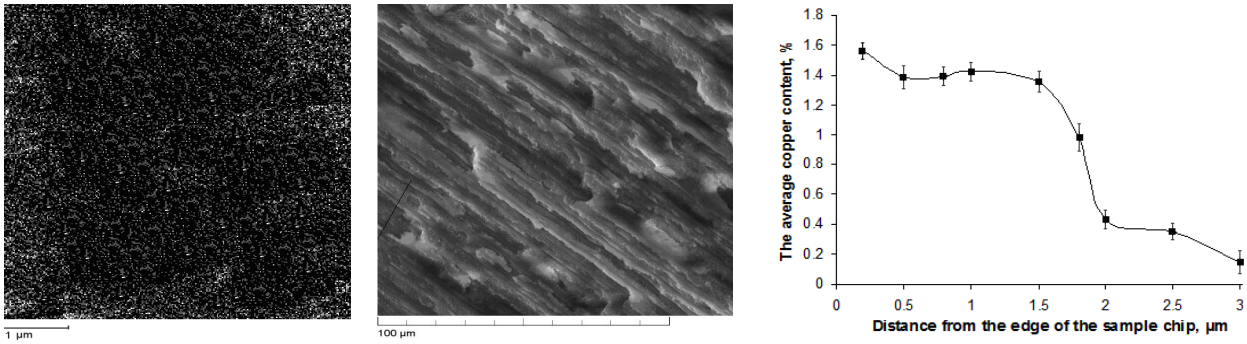
After the experiment described above, the human tooth samples were removed from the cuvette and dried under normal conditions. Since dentinal tubules have a non-smooth but very uneven curved scaly surface, the study of the morphology of the surface of the dentinal tubules does not give them the appearance of copper nanoparticles in visualizing the morphology of the surface (Fig. 3 b). To prove the penetration of copper nanoparticles into the cut of dentin, a fresh cleavage of the sample (denudation of the width of the cut) was made, on which elemental analysis was made on a scanning electron microscope (Table 1).

Table 1. The average microelement composition (for 4 samples) of dentin sections made on a scanning electron microscope

Distance from the edge of the sample chip, μm	C	O	Na	Mg	Al	P	Ca	Fe	Cu
0.2	23.28 ±3.15	41.09 ±3.56	-	0.68 ±0.06	2.74 ±0.58	9.50 ±1.02	16.53 ±1.78	4.26 ±0.23	1.56 ±0.15
0.5	17.04 ±2.50	40.84 ±3.25	0.91 ±0.05	1.26 ±0.09	-	15.53 ±0.36	19.62 ±2.12	3.42 ±0.19	1.38 ±0.17
0.8	13.22 ±1.38	45.24 ±4.12	-	0.58 ±0.08	2.83 ±0.45	16.12 ±0.25	18.36 ±1.35	2.14 ±0.14	1.39 ±0.14
1	18.13 ±1.05	41.43 ±3.67	-	0.36±0.05	1.96 ±0.18	18.45 ±0.31	16.12 ±1.26	1.57 ±0.18	1.42 ±0.15
1.5	17.51 ±1.65	41.47 ±3.56	0.45 ±0.05	0.26 ±0.06	-	10.35 ±0.25	23.15 ±2.13	5.46 ±0.89	1.35 ±0.14
1.8	26.36 ±3.45	38.12 ±3.75	0.16 ±0.03	1.54 ±0.28	-	11.11 ±0.78	19.54 ±1.85	2.19 ±0.16	0.98 ±0.13
2	8.62 ±0.56	45.71 ±4.16	-	0.61 ±0.05	1.63 ±0.16	12.72 ±0.75	25.26 ±2.67	5.02 ±0.56	0.43 ±0.12
2.5	23.22 ±3.15	43.32 ±3.86	-	0.23 ±0.04	-	8.47 ±0.45	20.85 ±2.16	3.15 ±0.42	0.35 ±0.10
3	20.56 ±2.45	44.05 ±4.58	-	0.19 ±0.04	1.19 ±0.01	10.56 ±0.63	20.36 ±2.23	2.71 ±0.12	0.15 ±0.11

After staying of the sample for 30 min in 5 ml of a solution containing copper nanoparticles with ultrasonic action, it was brought out that copper was found at a distance of 1.8 μm from the edge of the cleavage of the sample (Fig. 4 b). At a width of 1.8 μm, the average copper content was found to be 1.4% by mass (Fig. 4 a).

It is possible to estimate the lower limit of diffusion, the coefficient D of copper nanoparticles in the tooth tissue, taking into account the penetration depth d and the time t: $D \sim = d^2/t$. The depth of penetration into the dentin was 1.8 μm after 30 min of sonication. Thus, for copper nanoparticles, the lower diffusion limit in dentin was found to be 1.8×10^{-11} cm^2/s. Four samples were examined for the penetration of copper nanoparticles, all data are matched with each other (Fig. 4 c).



a b c

Figure 4. Electron microphotographs of fresh chipping of human dentin after being in solution containing copper nanoparticles for 60 min: elemental analysis of copper distribution, magnification 100 kx (a); morphology of the surface, magnification 1 kx (b); dependence of the average copper content on distance from the edge of the sample chip (c).

4. CONCLUSIONS

Penetration of copper nanoparticles into a cut of human dentin was investigated using elemental analysis obtained with the help of a scanning electron microscope. It was found that copper nanoparticles penetrate the dentin section to a depth of ~ 1.8 µm, with the diffusion coefficient of 1.8×10^{-11}. Despite the rather small size of the synthesized copper nanoparticles (20-80 nm), the depth of penetration is relatively small. This can be explained by the large aggregation ability of copper nanoparticles. Also, the micellar solution of sodium dodecyl sulfate, in which nanoparticles were synthesized, promotes the formation of aggregate of considerable sizes in micelles and has a lower penetrating ability (relative to water). This retarding property of the micellar solution can potentially be used for internal treatment of canals during dental treatment, when added to filling materials, implants, etc., in order to use the antimicrobial properties of copper nanoparticles and to prevent them from entering the bloodstream, since opinions about the safety of using nanoparticles when ingested is not unambiguous and requires additional research. The issue of the depth of penetration of copper nanoparticles over a much longer time is relevant and requires additional research.

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