Colloidal metal oxide nanoparticle systems: the new promising way to prevent antibiotic resistance during treatment of local infectious processes

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Abstract. New bactericidal containing nanoparticles colloids for application in dentistry, maxillofacial surgery, urology, obstetrics, gynaecology, ENT, proctology have been developed. The various water colloidal nanodispersive systems of metals and oxides have been obtained by means of electric impulse – condensation (electroerosion) method. These systems are based on pure elements and alloys of argentum (Ag), titanium dioxide (TiO₂), iron oxide (Fe₂O₃), tantalum oxide (TaO), vanadium oxide (VO₂), cobalt oxide (CoO), tantalum dioxide TaO₂, zinc oxide (ZnO), copper oxide (CuO) and mixed suspensions of titanium, aluminium and molybdenum oxides. The research has been made on culture of dentobacterial plaque and mixed culture issued from gingival spaces. The composition of culture was identified with S.aureus, S.epidermidis and nonfermentable kinds of E.coli. The observation period lasted more than nineteen days. All solutions showed highly prolonged bactericidal activity in dilutions from the whole solution 1-20 mg/L. The bactericidal activity of powder specimen of silica containing Ag and Fe₂O₃ nanoparticles used as dental filling material and disintegrates of composite materials (produced by “StomaDent” CJSC) have been studied. Tested materials have long (up to 19 days and more) bactericidal activity.

1. Introduction

The principally new drugs with high antibacterial activity, which would significantly reduce the scale of using antibiotics in medical practice and thereby reduce further spread of antibiotic resistance are in the spotlight now. A lot of different antibiotics which make parts of materials for topical treatment of odontogenic infections are used in dentistry and oral surgery. However the activity of antibacterial components in these medicines is short-term. In addition an attempt to add resistant antibacterial properties to cements, restorative materials and adhesives has so far failed. And after all it has long been observed that the use of light-cure composite materials, even those of the latest generation, in practice does not guarantee further protection of dental hard tissues from appearance of secondary caries. Therefore, current research is related to the future use of colloidal solutions of metal nanoparticles as an antibacterial component of dental materials.
The high pH of oral fluid, a great variety of biochemical processes, a wide range of natural and pathogenic oral microorganisms, peculiarities of structure and chemical composition of tooth tissues, etc., determine the following requirements for filling materials and adhesive systems:

1. A wide range of bactericidal action.
2. Prolonged bactericidal effect.
3. Absence of local and systemic toxic effects.
4. Adhesion to dental hard tissues and prosthetic surfaces.
5. Insolubility in oral fluid.
6. Preservation of physicochemical properties when interacting with other dental materials.

The possibility of using nanomaterials in medicine has been studied recently by many researchers. The bactericidal activity of silver nanoparticles on various individual strains of microorganisms is studied well enough. From a practical point of view it is necessary to analyze the antimicrobial properties of aqueous dispersions of silver and other metals directly with plaque microflora.

2. Materials and methods

Water dispersions of Ag, ZnO, FeO, TaO₂, TiO₂, CuO, VO₂ nanoparticles were produced by means of so-called electro-erosion method. was filled into the arc Glass chamber was filled with distilled water and electric impulses of capacity of 6.5 kW were fed to the metal electrodes made of Ag, Zn, Fe, Ta, Ti, Cu, VO₂ accordingly to type of nanoparticle systems. As a result of evaporation of electrodes the formation of colloidal solution of metals in water occurs. This method allows to obtain the stable dispersions of oxide nanoparticles. The nanoparticle systems are stable due to formation of hydrate multilayer shells on the surface of nanoparticles.

Information about dislocation microstructure of nanoparticles was obtained with using of transmission electronic microscopy (TEM) using a microscope JEM 2100 (Jeol, Japan). The suspensions were dropped on TEM copper grids with amorphous carbon substrate. All the measurements were conducted under voltage of 200 kV; cathode LaB6 was used as a source of electrons. The microbiological samples were dehydrated prior TEM experiments by means of treatment with acetone.

The culture of coliform bacterium E.Coli was used to evaluate antibacterial activity. Solid nutritional medium (agar) was made on the base of Hottinger broth (protein base used for nutritional media preparation for microorganisms. PanEco, Moscow.). Combination of the medium components provides the necessary nutritional elements for the growth of cultures in the form of certain colonies on the solid medium surface. Hottinger agar was melted on a boiling water bath, after that it was cooled to 45…50 °C and was dispensed into sterile 100 mm Petrie dishes by 25 ml with the resulting layer of 4.0 ± 0.5 mm. After setting the Hottinger agar was dried at 37 ± 1 °C for 45-60 min. Two days prior to researches the test culture was reinoculated from the storage medium onto slant nutritional agar in test tubes. The next day the obtained agar culture was used to produce suspension of the test strain employing turbidity standard.

To control dilution 0.1 ml (100 mkl) of suspension from 6th and 7th dilutions of culture was plated to the dishes with nutritional agar by method of direct surface inoculation. Three such inoculations were made out of each culture. After inoculation test tubes with culture were immediately placed in a refrigerator. Dishes with cultures were incubated in a thermostat at 37 ± 1 °C within 18…24 hours. On the day of the research the average number of colonies grown in the three dishes was calculated for each series of inoculations. All laboratory glassware was treated with 3-% water solution of phenol or 2% chloramine solution for disinfection.

Disk diffusion test was used to assess antibacterial properties of the studied dispersions. It consists of measuring inhibition zone around the paper carrier of of antibacterial preparation. The inhibition zone forms as a result of the preparation diffusion from the carrier into the media. Within certain limits the inhibition zone is reversely proportional to the minimum suppressing concentration (MSC). The study results in attributing the microorganism to one of the sensitivity categories (sensitive, intermediate or resistant). For the research small batches of disks (wafers) were made of filtering
Laboratory glassware was washed twice in 2% HNO₃ solution and rinsed 7 times with distilled water. All the surfaces including inner surfaces of air hoods and tables were treated with disinfecting compositions of sodium bicarbonate. Application of the discs was implemented with the help of sterile tweezers. The distance from the disc to the edge of the dish and between disks was 15…20 mm.

3. Results and discussion

There are two ways to obtain hydrosols of metals and their oxides: the chemical method of producing nanocolloids based on reduction of cations and physical method based on evaporation of metal in different liquid media. The first method has a various significant shortcomings. Aqueous dispersion of metal and oxide nanoparticles obtained by equilibrium chemical reduction have relatively low surface energy level, these colloids have some toxicity due to the presence of ions that makes its applications to living organisms undesirable [1-4].

Therefore the most promising methods of production of such nanosystems of metals and oxides for biological applications are physical. One of these methods is electrical erosion of metal electrodes by a voltaic arc in liquid media. A method is based on electrical erosion of electrodes under the influence of spark arc discharge occurring between them. The resulting low-temperature plasma in the voltaic arc is cooled by liquid (water or another liquid) which condenses nanoparticles at ambient temperature. The result of huge temperature difference is condensation of electrode material in liquid with formation of polydisperse colloidal system which consisted of nanosized metal or metal oxides in amorphous or crystalline phase homogeneously dispersed in liquid. Thus obtained colloidal solutions usually are stable to precipitation during several months or several years, The concentrations of nanoparticle depends of production conditions and has a range of 0.1 - 30 mg/l.

Analysis of transmission electron microscopy photos of as obtained various nanosystems on the base of Ag, ZnO, FeO, TaO₂, TiO₂, CuO, VO₂ (Figures 1-4) indicates that colloidal systems vary in different distribution of particles and the ratio of crystalline and amorphous phases, , what allows to suggest a high potential surface energy of particles in these systems. The potential energy will be transferred to the dispersion medium for a long time as a result of the processes of crystallization of the amorphous phase, coagulation and coalescence of the particles, recrystallization of the crystalline phase and redox processes associated with changes in the chemical composition of the dispersed phase.

Figure 1. TEM-images of silver nanoparticles obtained.
Figure 2. TEM-images of copper oxide nanoparticles (I) and zinc oxide (left), iron oxide (II) (right).

Figure 3. TEM-images of tantalum dioxide nanoparticles (left) and titanium dioxide (right).

Figure 4. TEM-images of copper oxide nanoparticles (I) (left) and vanadium dioxide (right).

Antibacterial properties of silver, titanium, copper, cobalt, nickel and zirconium nanoparticles are caused by the presence of double electric layer around the nanoparticles that has high reaction activity and interact with adsorption centers of cell membrane peptidoglycans [2, 3]. Breaking the integrity of
microbial wall and cytoplasmic membrane, metal nanoparticles penetrate into the cell and react as a catalyst in oxidation processes with the release of free radicals and destruction of cell structure [1, 4-8]. For example, such high antibacterial activity of Ag-ZnO, Ag-CuO composite nanoparticles composite against microorganisms P.gingivalis; F.nucleatum and P.intermedia was shown [9].

In our study the bactericidal properties of colloidal solutions of metals and metal oxides have been analyzed by means of disk diffusion method. We obtained the clearly defined bactericidal effect for solution concentrations from 1 to $10 \times 10^{-7}$ mg/l. For evaluating the antimicrobial activity a mixed culture of dental deposit biofilm was used. The calico tests – objects previously sterilized and impregnated with test solutions – were placed in Petri dishes with the seeding of indicator bacteria, on the surface of FT – agar based on Hottinger’s broth. The incubation of Petri dishes with the seeding of indicator culture and test samples was carried out at a temperature of 370 °C for 24 hours.

The investigated aqueous colloidal nanoparticles solutions of silver, iron oxide (II), nickel oxide (II), titanium dioxide, tantalum dioxide, mixed solution of titanium dioxide and molybdenum dioxide with corresponding concentrations of 12.2 mg/l, 12.4 mg/l, 8.2 mg/l, 10.3 mg/l, 7.5 mg/l, 6.8 mg/l were multiply diluted to $10, 10^2, 10^3, 10^4, 10^5$ and $10^6$ times. During incubating no bacterial medium was found under the test objects for all solutions and all dilutions.

The results of bactericidal activity researches of calico tests impregnated with colloidal solution of silver and iron oxide (II) nanoparticles against dental deposit on the second day are presented by Figures 5 and 6. Figure 7 presents the results of research after nine days of incubations, one can see that silver and iron oxide nanoparticles have prolonged bactericidal effects.

![Figure 5](image)

**Figure 5.** Bactericidal activity of calico tests impregnated with colloidal solution of silver against dental deposit on the second day.
Figure 6. Bactericidal activity of calico tests impregnated with colloidal solution of iron oxide (II) against dental deposit on the second day.

Figure 7. Bactericidal activity of calico tests impregnated with colloidal solutions of silver and iron oxide (II) against dental deposit on the ninth day.

It was established that the maximum values of antibacterial activity against dental bacteria were shown by following water solutions of nanoparticles:
- silver with the concentration from 1 to $10^{-5}$ mg/l;
- iron oxide (II) with the concentration from 1 to $10^{-6}$ mg/l;
- nickel oxide (II) with the concentration from 10 to $10^{-5}$ mg/l;
- titanium dioxide with the concentration from 10 to $10^{-6}$ mg/l.

The results of microscopic researches of model bacterial system (Bacillus cereus) with colloidal solution of titanium dioxide nanoparticles have shown the destruction of bacterial cell wall and spores membrane with the release of organelles into the environment (Figures 8 and 9).
Therefore the long-term bactericidal effect of metal nanoparticles against pathogenic germs of dental deposit proven by our and earlier bacteriological studies [8-13] makes possible the introduction of these nanoparticles in the composition of dental restorative filling materials.

As a result of our research we can stipulate that obtained by means of electroerosion method metal and metal oxide nanoparticles could be potencially new alternative to traditional antibiotics in the treatment of local dental infectious processes. Such nanoparticles colloids have a prolonged bactericidal effect and wide range of antibacterial activity. Therefore it is possible to use these colloidal solutions as an antibacterial component of dental composite filling materials, adhesive systems and pickling gels. It would allow to improve the results of therapeutic treatment and help to prevent dental caries.

4. Acknowledgments
The project was supported by the Ministry of Science and Education of Russian Federation (project RFMEFI57814X0080).

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