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RESEARCH ARTICLE

MODIFICATION OF GLASS IONOMER FILLING MATERIAL BY METAL NANOPARTICLES AND THEIR COMPOUNDS: EFFECT ON ANTI-MICROBIAL ACTIVITY

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Background: Glass ionomer filling materials are widely used in stomatology, but do not have an antimicrobial effect that effectively prevents the development of secondary and recurrent dental caries. Researchers are attempting to modify these materials by introducing various biologically active additives into them.
The aim of the study was to evaluate the antimicrobial properties of a dental ionomer filling material modified withadditives based on high–energy nanoparticles of silver, copper, titanium and their compounds.
Material and methods: Colloidal aqueous and alcoholic solutions of metals and their oxides with stabilizers were obtained by the electroerosion method. Citric acid, cetylpyridinium chloride and Trilon B were used as stabilizers. The zeta potential and the distribution of particles of the dispersed phase in solutions were measured. Samples of dental glass ionomer cement "Cemion-Aqua" were impregnated with colloidal solutions of nanoparticles. The microbiological activity of glass ionomer fillings samples in relation to plaque was determined by disc diffusion and suspension methods.
Results: The results showed that modification of glass ionomer cement samples with silver hydrosols in citric acid solutions with concentrations of 0.04% and 0.0025% increases the zone of radial lysis of microbial plaque colonies around the cement samples by 1.5 and 2.5 times compared with the control. By the suspension method, it was determined that silver hydrosols in a solution of citric acid and without it reduce the formation of colonies of microorganisms to several units up to 72 hours of exposure compared with the control. And copper hydrosols in solutions of cetylpyridinium chloride prevent an increase in the number of colonies of microorganisms after 24 hours of exposure compared with the control. Silver hydrosol in a solution of citric acid with a concentration of 0.0025% and silver alcohol sol reduce the number of colonies of microorganisms to several units after 3 hours of exposure.

Keywords: glass ionomer dental material, nanoparticles of metals and their oxides, antimicrobial activity.

Introduction

Glass Ionomer (glass polyalkenate) cements are popular with dentists due to their positive properties. Among them: the presence of chemical adhesion to hard tooth tissues, good edge fit, a coefficient of thermal expansion comparable to tooth tissues, minimal shrinkage, the ability to release fluorine, satisfactory mechanical and aesthetic properties. It is precisely due to these properties that glass fibers are used as base and liner insulating gaskets (including sandwich equipment), permanent seals, and for fixing non-removable orthopedic structures. They are indispensable for minimally invasive filling of carious cavities (in ART technique)[1].

At the same time, in the latter case, when the carious cavity is not fully processed or is not treated with dental borons at all, the problem of preventing secondary and recurrent caries becomes urgent. Infected dentin remaining on the bottom or walls of the cavity may eventually cause the development of pulpitis or the appearance of a defect in glass ionomer restoration. Therefore, giving the filling material prolonged antimicrobial properties is an important task of science, which researchers are trying to solve on the basis of modern nanotechnology [2,5]

The aim of the study was to evaluate the antimicrobial properties of a dental ionomer filling material modified with additives based on high- energy nanoparticles of silver, copper, titanium and their compounds.

Material and methods. The dental two-component water-curing material "Cemion-Aqua", produced by JSC SEZ "VladMiVa" (Belgorod, Russia), was used as the glass ionomer (glass polyalkenate) filling material under study.

Chemical reagents used: Trilon B (99.20%), cetylpyridinium-N chloride 1-aqueous (99.46%), citric acid (99.80%), luxury ethyl alcohol (96.30%) and distilled water.

Modification of the glass ionomer material was carried out with high-energy nanoparticles of silver, copper, copper (I) oxide, copper (II) oxide andtitanium dioxide with an average size from 2 to 4 nm, obtained by a new, fairly simple and cheap method ofcooling low-temperature plasma in a liquid medium [4, 5]. To obtain a stable dispersed phase of metals and their compounds, cooling was carried out in various dispersion media: distilled water, ethyl alcohol, aqueous solutions of citric acid, cetylpyridinium chloride (CPC) and disodium-ethylenediamine tetra acetic acid (Trilone-B). In some cases, aqueous solutions of citric acid of various concentrations were used as a dispersion medium: 0.0025%, 0.005%, 0.02% and 0.04% and aqueous solutions of CPC of the following concentrations: 0.001%, 0.003%, 0.01% and 0.03%. The zeta potential and the distribution of dispersed phase particles in solutions were measured using the "Malvern Zetasizer Nano ZS analyzer" (GreatBritain). To measure the zetapotential, the "DTS1060" cuvette, which has a U-shaped shape with two holes, was washed with distilled water and filled with the test solution. A colloidal solution was poured into the cuvette to the edges of the holes, thenit was closed with plugs on both sides and placed in the cuvette compartment of the analyzer for furthermeasurements.

The same cuvette was used to study the distribution of aggregates of dispersed phase particles in colloidal solutions. The studied colloidal solution with a volume of 1-1.5 cm^3 was poured into a clean cuvette, covered with a lid and placed in a cuvette compartment.

Preparation of seal samples. Glass ionomer cement powder weighing 3 g was impregnated with hydro- or alcohol salt of metal nanoparticles or its oxide with a volume of 0.8-1 ml to a state of complete absorption without excess of the liquid phase. A crucible with impregnated cement was placed in a drying cabinet at a temperature of 388 ± 2^0 K, and drying was carried out for 30 minutes, during which the liquid phase evaporated. After cooling, the cement was kneaded to a visually observable homogeneous mass. The resulting paste– like mixture was placed in a special cylindrical titanium mold to simulate seals (height – 4 mm, diameter – 6 mm) and tightly tamped. The ends of the cylindrical shape were covered with new plates on both sides. The seal in the cylinder was kept for 24 hours at room temperature until it was completely cured under a weight of 1000 g. From the volume of the resulting cement impregnated with one type of colloidal solution, 2 samples of seals were obtained using such a mold. The finished samples were carefully removed from the mold and subsequently used in research.

Microbiological research. To study the bactericidal properties of the cement samples, an agar culture of plaque bacteria was used. It was received from 6 dental patients (aged 19 - 33 years, 3 men and 3 women) who applied to the clinic for rehabilitation and had a satisfactory hygienic condition of the oral cavity. Plaque removed from thesurface of the tooth enamel with a sterile cotton- gauze swab was suspended in Hottinger broth. The tube with the broth seeded with bacteria wasincubated for 24 hours at a temperature of 310^0K. After growing a broth culture of bacteria and determining their content in 1 ml of culture using the optical industry turbidity standard CCA 42-28-85-2014 (10 IU), a working suspension of bacteria of the required concentration was prepared on a sterile isotonic sodium chloride solution. The resulting suspension was used in further work.

During the disco-diffusion determination of the antibacterial activity of cement samples, the grown culture of plaque bacteria was washed off with an isotonic solution of sodium chloride, after which a bacterial suspension was prepared at a concentration of $(1.2\text{-}1.3)\times10^6$ CFU/ml. This suspension was applied to the surface of a dense nutrient medium (meat-baking agar) in Petri dishes. After drying the cups for 10-13 minutes, samples of fillings were placed on top of the nutrient medium with plaque bacteria culture. An indispensable element of the success of the experiment is the moistening of the samples before placing them on the surface of the nutrient medium with the sowing of bacteria. After 24 hours of exposure at a temperature of 310^0K, the presence and size of zones for inhibiting the growth of colonies of microorganisms in the radial direction were visually determined around the seals.

An "iCAP 6300 Radial View" spectrometer manufactured by "Thermo Fisher Scientific Inc." (USA) was used to analyze the mass concentration of metal nanoparticles and their oxides in aqueous and alcoholic solutions.

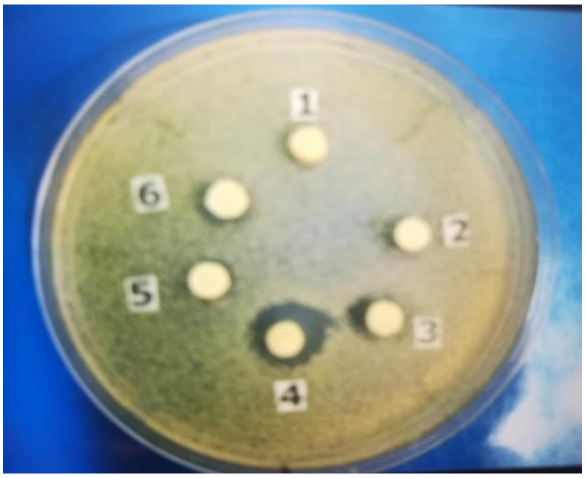
Aqueous solutions of Ag, Cu and Ti were subjected to intensive mixing for 3-4 minutes immediately before measurements on the device. Standard samples with known concentrations of Ag, Cu and Ti ions in solution were used to calibrate the device. HNO_3 solution (1:20) was used as a background solution. Certain spectral analytical lines were used in the measurements: Ag – 328.07 nm, Cu – 324.75 nm and Ti is 323.45 nm. Measurements on the spectrometer were carried out no earlier than 20 minutes after the plasma was switched on, and the drift of the calibration characteristics was taken into account every 15 minutes.

A transmission electron microscope "LEO 912 AB OMEGA" (Germany) was used to study the size and structure of particles of the dispersed phase of colloidal solutions of metals and their oxides. This transmission microscope is equipped with an energy filter and a Keller system. Modes that set the accelerating voltage: 60, 80, 100, 120 kV. The illumination area of the microscope is 1-75 microns, and the aperture reaches up to 5 millimeters. The metal hydrosol was applied to a special copper mesh with a diameter of 3.05 mm, which was coated with a thin polymer film of polyvinyl formal.

Results

Microbiological studies have shown that samples of dental fillings made of cement "Cemion-Aqua" modified with colloidal solutions of silver hydrosol and citric acid with different concentrations exhibit bactericidal properties against plaque microorganisms.

The most significant zones of inhibition of plaque colony growth were observed in samples No.



3, 4 and 6 (Fig. 1). In samples No. 2 and 5, the sizes of bacterial colony growth inhibition zones are less pronounced and amount to about 1 mm.

Figure 1: Suppressive activity against plaque culture of nanoparticle–modified samples of glass ionomer fillings:
1 - silver hydrosol in 0.005% citric acid solution,
2 – silver hydrosol in 0.02% citric acid solution,
3 – silver hydrosol in 0.04% citric acid solution,
4 – silver hydrosol in 0.0025% citric acid solution,
5 – silver hydrosol,
6 – control.

It seemed important to establish possible correlations between bactericidal activity, qualitative and quantitative indicators of the dispersed systems used. The identification of such patterns is an important fact from the point of view of practical evaluation of the obtained hydrosols, organozoles of metals and their compounds. Such correlations in the future will make it possible to predict the biocidal properties of the obtained dispersed systems without conducting microbiological studies. Such indicators related to bactericidal activity, along with the particle size of the dispersed phase, may be the zeta potential and the size of particle aggregates in the dispersed phase. Table 1 shows these two parameters of the initial hydrosols used to modify glass ionomer cement.

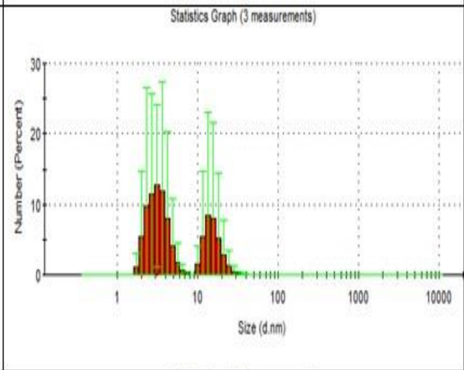
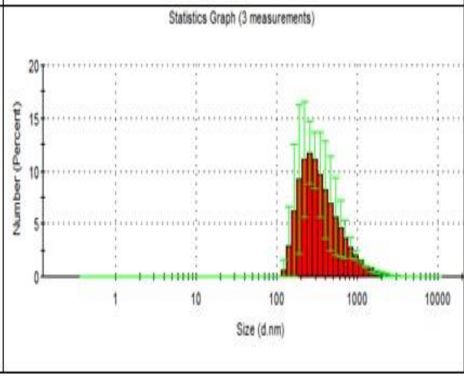
Table 1: Suppressive activity of samplesof glass ionomer fillings modified with silver hydrosols in relation to plaque cultures

Samples	Radial culture growth suppression zone, mm	The concentration of citric acid in silver hydrosols, %	Zeta potential, mV	Distribution of particle aggregates, nm
1	–	0,005	17	2 – 38
2	1	0,02	15	122 – 2669
3	2-3	0,04	9	142 – 1990
4	3-5	0,0025	– 4	1 – 16
5	1	0	– 12	5 – 255
6	2	control	–	–

From the Table No. 1 it can be seen that the highestvalues of the zones of inhibition of the growth ofplaque cultures were shown by samples of fillingstreated with silver hydrosols in 0.04% and 0.0025%solutions of citric acid. The distribution of particleaggregates in the colloidal solution, which wastreated with sample No. 4, does not exceed 20 nm. Itcan be assumed that the small size of particleaggregates in colloidal solutions improves bactericidal properties, but this is not observed in sample No. 1. And in the colloidal solution with which sample No. 3 was treated, the distribution of particle aggregates is more than 100 nm. It ispossible that the improvement in the bactericidalproperties of the seal samples in this experiment isdue to several factors at once: the concentration of citric acid, the distribution of particle aggregates andtheir zeta potential. The combination of the optimalvalues of three factors increased the bactericidalactivity of samples Table No. 3 and 4.

When studying the distribution of aggregates of particles of the dispersed phase by the method of dynamic light scattering, the minimum values were shown by samples No. 1 and No. 4 with dimensions of 2 - 38 nm and 1 – 16 nm, respectively. The size distribution curves of the dispersed particles of colloidal solutions turned out to be both unimodal in samples No. 2 and 3, and polymorphic in samples No. 1, 4 and 5 (Table 2).

Table 2: Distribution of aggregates of silver particles in an aqueous solution of citric acid

Samples	Distribution of particle aggregates, nm	Percentage of particles in interval
1		5% ≤ 2 – 4 нм ≤ 10% 5% ≤ 12 – 18 нм ≤ 10% 10% ≤ 3 – 4 нм ≤ 15%
2		5% ≤ 164 – 531 нм ≤ 10% 10% ≤ 220 – 295 нм ≤ 15%

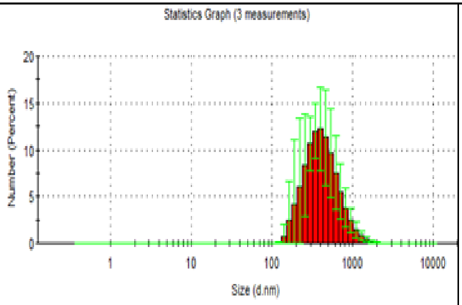
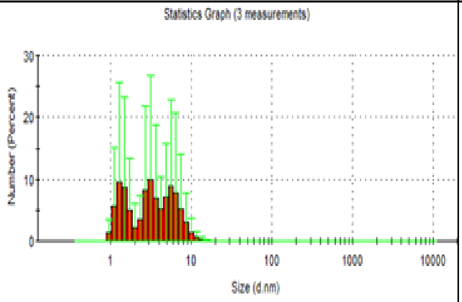
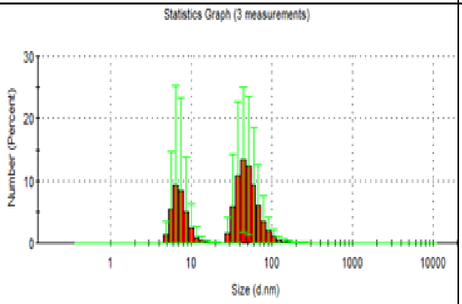
3		5% ≤ 220 – 712 нм ≤ 10% 10% ≤ 295 – 459 нм ≤ 15%
4		5% ≤ 1 – 2 нм ≤ 10% 5% ≤ 3 – 8 нм ≤ 10%
5		5% ≤ 6 – 9 нм ≤ 10% 5% ≤ 33 – 68 нм ≤ 10% 10% ≤ 38 – 51 нм ≤ 15%

Table 3 shows the results of studies of the bactericidal properties of colloidal solutions determined by the suspension method. All samples, except for the control, showed a decrease in the number of colonies of microorganisms after 48 and 72 hours of exposure. Colloidal solutions of silver hydrosols in citric acid and without it have shown an effective effect on plaque microorganisms up to 72 hours of exposure.

Figure 2 shows samples of dental fillings after 24 of exposure to a nutrient medium with plaque culture.

The sizes of the radial zones of suppression of the growth of colonies of microorganisms (except for control sample No. 1) areapproximately equal and vary from 1 to 3 mm.

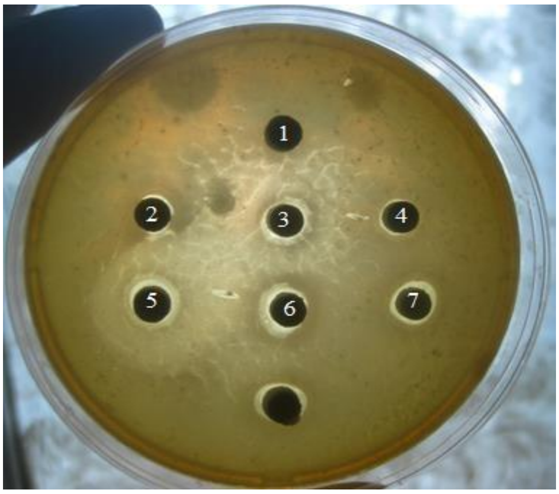


Figure 2: Antimicrobial activity of samples of glass ionomer fillings modified with hydrosols of nanoparticles and their oxides with various stabilizing additives in relation to plaque culture:
1 – control,
2 – copper hydrosol in 0.001% cetylpyridinium chloride solution,
3 – copper hydrosol in 0.003% cetylpyridinium chloride solution,
4 – copper hydrosol in 0.01% cetylpyridinium chloride solution,
5 – copper hydrosol in 0.03% cetylpyridinium chloride solution,
6 – silver hydrosol in 0.0025% citric acid solution,
7 – titanium dioxide hydrosol in a solution of trilon B,
8 – silver alcohol

Table 3: Antimicrobial activity of colloidal solutions determined by the suspension method (initial concentration of microorganisms – 10^6 CFU/ml)

Samples	Concentration of citric acid in silver hydrosols, %	Number of colonies of microorganisms depending on exposure time (CFU/ml)				
		0	5 min	24 hours	48 ч hours	72 hours
1	0,005	10^6	10^5	s. col.*	s. col.*	s. col.*
2	0,02	10^6	10^5	s. col.*	s. col.*	s. col.*
3	0,04	10^6	$5 \cdot 10^4$	11	s. col.*	s. col.*
4	0,0025	10^6	10^6	10^6	s. col.*	s. col.*
5	0	10^6	10^6	10^5	s. col.*	s. col.*
6	control	10^6	10^6	18	20-25	150

*- single colonies of microorganisms

Table 4 shows the results of determining the antimicrobial activity of dental fillings by the suspension method. The number of colonies in cultures of microorganisms with copper hydrosols at different concentrations of CPC after 3 hours of exposition is 10^5, and in the control sample it is an order of magnitude higher. After 24 hours of exposure, colonies of microorganisms were completely absent from samples No. 2 and 3, and in the control, it returned to its original value. It can be seen from the table that an increase in the concentration of CPC in all colloidal copper solutions increases their bactericidal properties. Only in the tested samples No. 4 and 5, the number of colonies of microorganisms was 10^2 and 10, respectively, after 24 hours of exposure.

Table 4: Antimicrobial activity of dental seal samples determined by suspension method (initial concentration of microorganisms – 10^6 CFU/ml)

Samples	Concentration of cetylpyridinium chloride in copper hydrosols, %	Number of colonies of microorganisms by exposure time (CFU/ml)	
		3 hours	24 hours
1	control	10^6	10^6
2	0,001	10^5	0
3	0,003	10^5	0
4	0,01	10^5	10^2
5	0,03	10^5	10

A similar experiment was conducted with other colloidal solutions, the results of which are shown in Table 5. Single colonies of microorganisms were observed in samples No. 6 and 8 after 3 hours of exposure.

Table 5: Antimicrobial activity of dental seal samples determined by suspension method (initial concentration of microorganisms – 10^6 CFU/ml)

Samples	Dispersed phase	Dispersion medium	Number of colonies of micro-organisms after 3 hours of exposure (CFU/ml)
1	control	–	10^6
6	Ag	Ag aqueous solution of citric acid (0.0025%)	single colonies
7	TiO_2	TiO_2 aqueous solution of trilon B	5×10^5
8	Ag	ethyl alcohol	single colonies

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The distribution of particle aggregates in copper hydrosols in CPC solutions lies approximately in the same interval, and their zeta potential is greater than 20 mV in modulus, that is, the particles of the dispersed phase are stable (Table 6). If we compare these values with the values from Table 4, we can conclude that the bactericidal properties were influenced by the concentration of CPC, since the distribution of particle aggregates in copper hydrosols is approximately the same. Consequently, aqueous colloidal solutions of copper with different concentrations of CPC increase the antimicrobial activity of modified dental fillings.

Table 6: Zeta potential and particle aggregate size distribution of colloidal solutions

Samples	Discrete phase	Dispersion medium	Concentration of the stabilizing substance, %	Zeta potential, mV	Distribution of aggregates of particles, nm
2	Cu	aqueous solution of CPC	0,001	31	51 – 459
3	Cu	aqueous solution of CPC	0,003	39	21 – 295
4	Cu	aqueous solution of CPC	0,01	50	44 – 396
5	Cu	aqueous solution of CPC	0,03	65	44 – 295
6	Ag	aqueous solution of citric acid	0,0025	– 13	9 – 59
7	TiO_2	aqueous solution of Trilon B	–	– 29	51 – 531
8	Ag	ethyl alcohol	–	– 14	44 – 458

Thus, as a result of the conducted studies, it was revealed that the modification of samples of glass ionomer seals with hydrosols of high-energy metal nanoparticles and their oxides significantly increases the antimicrobial activity of the sealing material. Unlike the known methods[6,7], a fairly simple and low-cost method for obtaining hydro- and alcohol salts of metal nanoparticles, proposed by us, can be easily applicable in the practice of production and use of such ionomeric materials. This, in turn, can contribute to improving the effectiveness of dental caries treatment.

Discussion

It is known that any filling material after curing in the carious cavity shrinks. In addition, over time, chips appear on the border between the honey seal and the cavity wall[1]. These phenomena can lead to the development of secondary caries along the edges of the restoration. That is why it is important that the filling material has prolonged antimicrobial properties[2]. In this study, we modified the glass ionomer cement "Cemion-Aqua" with colloidal solutions of silver hydrosol and citric acid, as well as the addition of CPC in different concentrations. It was possible to identify a correlation between the qualitative and quantitative indicators of the dispersed systems used and the bactericidal activity of the filling material. Thus, the highest antimicrobial activity was shown by samples of fillings treated with silver hydrosols in 0.04% and 0.0025% citric acid solutions. We believe that this phenomenon is due to the influence of citric acid on the size and distribution of particle aggregates, as well as on their zeta potential[4]. In addition, the presence of CPC in copper solutions not only stabilizes the colloidal system, but also increases their bactericidal properties. We consider this approach to the modification of colloidal nanosystems of metals added to a glass ionomer composite to give it antimicrobial properties to be justified and most effective[5]. In addition, from a practical point of view, this does not pose great difficulties.

Conclusions

1. Modification of glass ionomer cement samples with silver hydrosols in citric acid solutions with concentrations of 0.04% and 0.0025% increases the zone of radial formation of microbial plaque colonies by 1.5 and 2.5 times compared with the control.
2. By the suspension method, it was determined that silver hydrosols in and without citric acid solution reduce the formation of colonies of microorganisms to several units up to 72 hours of exposure compared with the control. And copper hydrosols in solutions of cetylpyridinium chloride prevent an increase in the number of colonies of microorganisms after 24 hours of exposure, which is not observed in the control.
3. Silver hydrosol in a solution of citric acid with a concentration of 0.0025% and silver alcohol sol reduce the number of colonies of microorganisms to several units after 3 hours of exposure.

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Ethics approval and consent to participate

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Consent for publication Not applicable.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. None of the authors have any relevant financial relationship(s) with a commercial interest

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