

NANOMATERIALS FOR STERILIZATION AND PHOTOSTERILIZATION

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Introduction

In many fields of human activity, the environment free of harmful microbes, is required. To combat the pathogenic organisms is a main concern of health care or in the food production. The prevention of the spread of infections or food contamination may be ensured by proper disinfection or sterilization.

The procedure of destroying microorganisms is called sterilization. Heat, chemicals, irradiation, high pressure or filtration are commonly used to perform a sterilization. Removing all pathogenic agents from objects, refers often to the term: disinfection. Disinfectants are used to remove microbes from surfaces, medical appliances, linen etc., whereas antiseptics are antimicrobial substances that are applied to living tissue in order to protect against infection, sepsis, or putrefaction.

Many pathogenic species have the ability to survive during the passage from person to person. They may survive in the air or on the various objects. It was demonstrated that many microbes may survive on glass, fabrics, metals, plastics or even computers, etc.^{1, 2, 3, 4, 5}

Nanomaterials as antimicrobial agents

The application of nanotechnology and nanomaterials has increased rapidly in the last decade due to valuable and extraordinary properties of these materials. By controlling the phenomena that occur in nano-dimension, it is possible to create a new class of materials with tailored unique properties and functions. Various types of nanomaterials may be produced, e.g. be metals, ceramics, polymers, or composites. They may have various shapes and geometry: they can be one-, two- or three dimensional.

Many authorities worldwide believe that nanotechnology may have a big impact on the economic development and sustainable quality of life (see e.g. the welcome letter⁶ to EuroNanoForum 2009)

In the last years increased resistance of many pathogenic species to commonly used disinfectant and antibiotics, is observed. Therefore, many studies are conducted in order to find new types of safe and cost-effective antimicrobial active materials. Performed studies demonstrated that various nanomaterials exhibit excellent antimicrobial properties through diverse mechanisms (see Fig. 1).

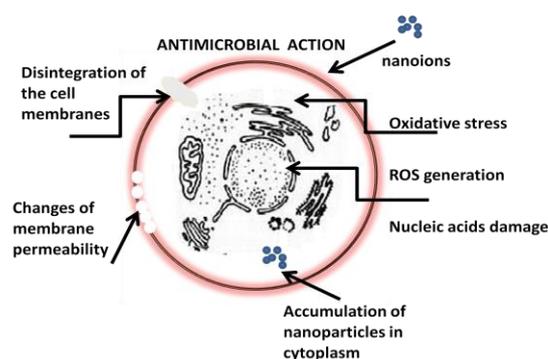


Fig. 1 Types of mechanism of cytotoxic interaction of nanomaterials.

Nanomaterials may interact with cellular wall and disintegrate it. They can accumulate inside the cell or may lead to ROS (reactive oxygen species) production, thus to destroy the cell integrity. The another cytotoxic mechanism is via DNA damage.

Recently, many papers were published, presenting the results of examination of antimicrobial activity of various nanoparticles. One of the most popular study objects are silver compounds and silver ions that have been recognized as active antimicrobial agents. The attempts are made to compare the activity of various nanomaterials. The examinations demonstrate that the activity depends on the microbial species, as well as on the type of ions⁷. The activation with light may increase the

antimicrobial activity of nanoparticles⁸.

Experimental

Materials

Silica nanospheres were prepared using modified Stöber synthesis [9]. The method bases on catalyzed hydrolysis of TEOS in water-ethanol mixture. Ethyl alcohol (95%) ammonia solution (25%), hydrofluoric acid (35%), all from Polish Chemicals and tetraethylortosiliane (TEOS from Aldrich) were successively added at room temperature. Sediment was washed in methyl alcohol, and dried in 70°C for 24 hours.

Impregnation process was performed basing on Tollen's method [10]. The spheres were dispersed in the distilled water. Further, silver ammonia complex $Ag(NH_3)_2^+$ [0.4 M] was added and the Tollen's-soaked silica spheres were formed. Glucose solution [0.4M] at room temperature, was used as the reducing agent. Sediment materials were washed in methyl alcohol and double distilled water. Following, the second reduction reaction was performed by adding silver nitrate [0.5 M], ammonia solution (25%) and solutions of glucose [0.4M]. After 24 hours in 70°C temperature silica spheres with silver nanoparticles were prepared.

The Ag-Au doped material was prepared by exploiting the cementation process. Solution of hydrate chloroauric acid, hydrate potassium carbonate, and distilled water were added to the materials after first reduction reaction. After one hour, cementation process was finished, and then solution of glucose [0.4M] was added as a reducing agent. After 24 hours in 70°C temperature, sediment materials were washed in methyl alcohol and double distilled water.

Total concentration of silver nanoparticles in sediment materials Ag-SiO₂ after I reduction reaction was 9.4 [mg/dm³], and after II reduction reaction was 35.6 [mg/dm³]. Total concentration Ag and Au after cementation process were 8.7 [mg/dm³] (for Ag) and 16.3 [mg/dm³] (for Au), respectively.

Method

Bacterial cultures of *Escherichia coli* were prepared on Mac Conkey's agar (Sis Biomed product). Three types of nanomaterials (I

reduction reaction, II reduction reaction and cementation process) in various proportions, with different amount of silver were used in this study (see Tab. 1). Nanomaterials were added to the bacteria cultures.

Tab. 1 Volumetric contents of nanomaterials

Sample	Bacteria suspension volume [ml]	Volume of sediment materials in distilled water [ml]
1.0 : 2	1.0	2
1.0 : 10	1.0	10
1.0 : 15	1.0	15
1.0 : 20	1.0	20
1.0 : 50	1.0	50
1.0 : 100	1.0	100
1.0 : 250	1.0	250

After 24h incubation in 37°C, the antimicrobial activity against *Escherichia coli* was evaluated by counting the number of colonies.

Results

The goal of the research was to examine the antibacterial properties of silica based materials (nanosilver and nanogold). Figure 2 shows some exemplary results.

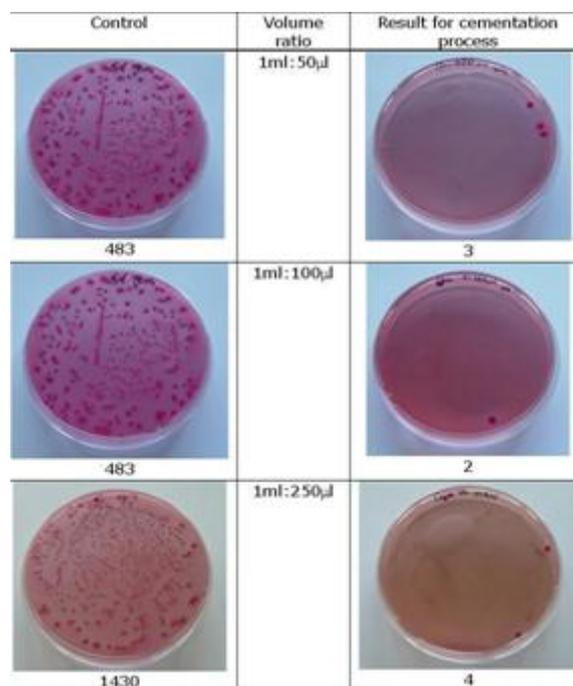


Fig. 2 Exemply results of antimicrobial activity of sediment materials prepared by cementation process: Ag-SiO₂-Au doped nanomaterial.

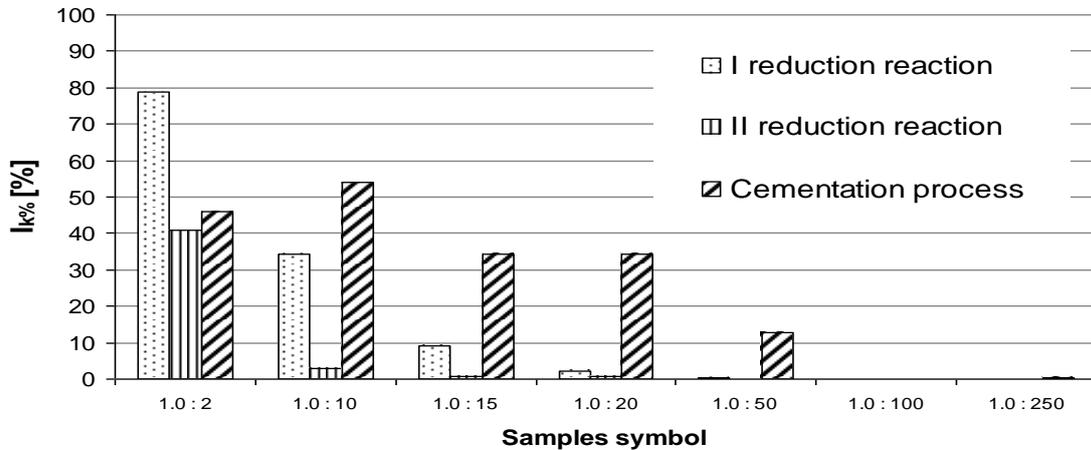


Fig. 3 Survivability of Escherichia coli bacteria treated three types of silica compound doped nanosilver and nanogold.

The antimicrobial activity $I_{k\%}$ was determined as

$$I_{k\%} = \frac{I_K}{C} \cdot 100\%$$
, where I_K is an average number of bacterial cultures (CFU – colony forming units) treated with nanomaterial, C is the average number of CFU in control group. The results are shown in 3 and Tab. 2.

Tab. 2 The rate of bacterial survivability.

Samples symbol	I reduction reaction	II reduction reaction	Cementation process
	$I_{k\%}$ [%]	$I_{k\%}$ [%]	$I_{k\%}$ [%]
1.0 : 2	78.81	40.89	46.16
1.0 : 10	34.26	2.91	54.18
1.0 : 15	9.01	0.71	34.43
1.0 : 20	2.19	0.58	34.39
1.0 : 50	0.43	0.00	12.84
1.0 : 100	0.00	0.00	0.17
1.0 : 250	0.03	0.03	0.21

All tested nanomaterials materials demonstrated an antibacterial activity. The most efficient material was that one produced on the way of II reduction reaction.

Analogical experiments were performed with supernatant materials. The antimicrobial activity was tested and compared with the activity in case of photosterilization. For irradiation TopGan laser emitting at 410 nm, peak optical power 200 mW, was used. The exposure time was 4 minutes. The nanomaterials in various concentrations were added to the bacterial suspension and then the

bacteria were seeded on agar plates and the samples were incubated. To 1 ml of bacteria suspension 0,25, 0,5 or 1 ml of supernatant was added. The CFU indices (colony forming units) were counted. Some of the results (for Ag-Au nanoparticles) are demonstrated on Fig. 4.

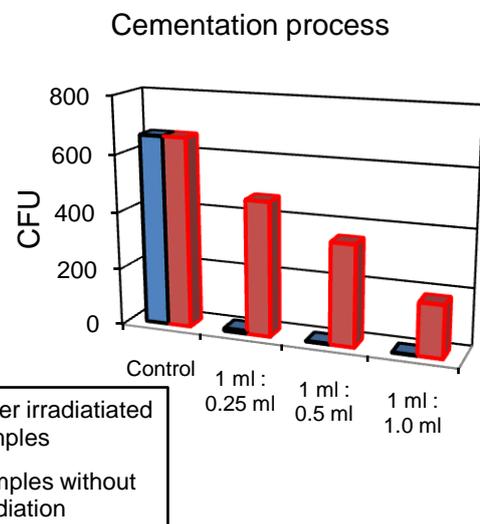


Fig. 4 The influence of laser irradiation on Ag-Au nanomaterials antibacterial activity. A decrease of CFU is seen after 4 minutes laser irradiation.

The rate of bacterial survivability after the laser irradiation depicts Tab. 3. One can see that there is a synergy in antimicrobial activity of nanomaterials and additional laser irradiation.

Tab. 2 Survivability rate in % of bacteria treated with nanomaterials and irradiated by 415nm laser light.

Sample	I red	II red	cemen-tation
1 ml : 0.25 ml	0,22	0,00	0,01
1 ml : 0.5 ml	0,12	0,00	0,00
1 ml : 1.0 ml	0,05	0,00	0,00

Conclusion

Recently, many reports were published from the studies on various materials with antimicrobial activity. The growing resistance of microbes against popular sterilization methods, caused the great interest in a new type of materials and new methods. Here, nanotechnology may offer promising solutions. Nanomaterials may be used as antimicrobial agents on solid bodies, as well as in water¹¹.

In our study, it was proved that silver and silver-gold doped silica nanospheres revealed antibacterial activity against *Escherichia coli*. For the higher nanoparticles concentration, the antimicrobial effectiveness was higher.

Sol-gel route is a very convenient method to produce various materials and it is frequently exploited for nanomaterials. Composite materials in form of nanopowders may be produced or they may be deposited on various substrates, e.g. fibers, metals etc. The photocatalytic properties of such materials may be used for photosterilization processes. Moreover, it is possible to apply not only UV but the light in visible range¹².

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References

1 Neely A. N., Maley M.P. Survival of Enterococci and Staphylococci on Hospital Fabrics and Plastic, J Clin

Microbiol. 2000 February; 38(2): 724–726

2 Kramer A., Schwebke I., Kampf G., How long do nosocomial pathogens persist on inanimate surfaces? A systematic review Published online 2006 August 16. doi: 10.1186/1471-2334-6-130.

3 Neely A. N., Orloff M. M., Survival of Some Medically Important Fungi on Hospital Fabrics and Plastics, J Clin Microbiol. 2001 September; 39(9): 3360–3361. doi: 10.1128/JCM.39.9.3360-3361.2001.

4 Neely A. N., Sittig D. F., Basic Microbiologic and Infection Control Information to Reduce the Potential Transmission of Pathogens to Patients via Computer Hardware, J Am Med Inform Assoc. 2002 Sep–Oct; 9(5): 500–508. doi: 10.1197/jamia.M1082.

5 Brady RRW, Kalima P., Damani NN, Wilson RG, Dunlop MG, Bacterial Contamination of Hospital Bed-Control Handsets in a Surgical Setting: A Potential Marker of Contamination of the Healthcare Environment, Ann R Coll Surg Engl. 2007 October; 89(7): 656–660. doi: 10.1308/003588407X209347.

6 www.euronanoforum2009.eu

7 Son, Won Keun; Youk, Ji Ho; Park, Won Ho, Antimicrobial cellulose acetate nanofibers containing silver nanoparticles, Carbohydrate Polymers Volume: 65, Issue: 4, September 13, 2006, pp. 430-434.

8 Takashi Sato and Masahito Taya, Copper-Aided Photosterilization of Microbial Cells on TiO₂ Film under Irradiation from a White Light Fluorescent Lamp Biochem. Eng. J. 30(2), 2006, p.199-204.

9 Stöber W., Fink A., Bohn E., Controlled growth of monodisperse silica spheres in the micron size range, J. Colloid and Interface Science 26, 1968, pp. 62-69.

10 Peterson M.S.M., Bouwman J., Chen A., Deutsch M., Inorganic metallodielectric materials fabricated using two single-step methods based on the Tollen's process, J. Colloid and Interface Science 306, 2007, pp. 41-49.

11 Li Qilin; Mahendra, Shaily; Lyon, Delina Y.; Brunet, Lena; Liga, Michael V.; Li, Dong; Alvarez, Pedro J.J., Antimicrobial nanomaterials for water disinfection and microbial control: Potential applications and implications, Water Research Volume: 42, Issue: 18, 2008, pp. 4591-4602.

12 Wua,P, Xie R, Imlay J, Ku Shang J, Visible-light-induced photocatalytic inactivation of bacteria by composite, photocatalysts of palladium oxide and nitrogen-doped titanium oxide, Applied Catalysis B: Environmental 88 (2009) 576–581.