P-2: Efficiency of the Combined Action of Ultrasound and Nanoparticles of Different Nature on Bacteria

Alexander Gopin, Svetlana Mazina, Alexander Nikolaev*

Faculty of Chemistry of Lomonosov Moscow State University
MSU, Faculty of Chemistry, Russia,119991,Moscow 1, GSP-1, 1-3 Leninskiye Gory, nic@radio.chem.msu.ru

Authors consider the effect of combined action of ultrasound and nanoparticles of different nature on the viability of the bacteria. It is shown that the ultrasonic treatment reduces the viability of the bacteria. The introduction of nanoparticles during ultrasound exposure in some cases leads to enhancement of the effect of up to 8 times.

In recent years we are developing a method of sonodynamic cancer therapy using nanoparticles as sonosensitizers (Nikolaev et al., 2009). The methodology of the combined action of ultrasound and nanoparticles can be applied in other areas, particularly in the antimicrobial treatment of various objects (Mazina et al., 2009). In this paper we study the influence of the combined action of ultrasound and nanoparticles of different nature on the viability of bacteria.

Enterococus spp were selected as the model object. They are adapted to survival in the human body at mesophilic temperatures and neutral values of pH, are characterized by high proliferative activity, are easily cultivated on nutrient media. Silver nanoparticles stabilized by citrate ions or miramistin and gold nanoparticles, and octasodium salt of cobalt octacarboxyphthalocyanine (theraphthal) were used as sonosensitizers. Theraphthal proved to be a sonosensitizer in the treatment of cancer (Nikolaev et al., 2009).

To estimate the effectiveness of the combined action of ultrasound and sonosensitizers 2 ml of the suspension of bacterial cells and 2 ml of nanoparticles suspension or 2 ml of theraphthal solution were introduced into the thermostated vessel with a ultrasonic transparent bottom and were exposed to ultrasonic treatment (0,88 MHz, 1 W/cm^2) for 10 minutes at 38 °C. Working concentration of gold and silver nanoparticles was 10^{-5} or 10^{-6} g/ml, and 10^{-5} mol/l for theraphthal solution. Then, 1 ml of the suspension was transferred to a sterile Petri dish. Bacterial cultures were grown on a PCA Standard Methods agar medium. The result of seeding was estimated after 24 hours by counting the number of colony-forming units.

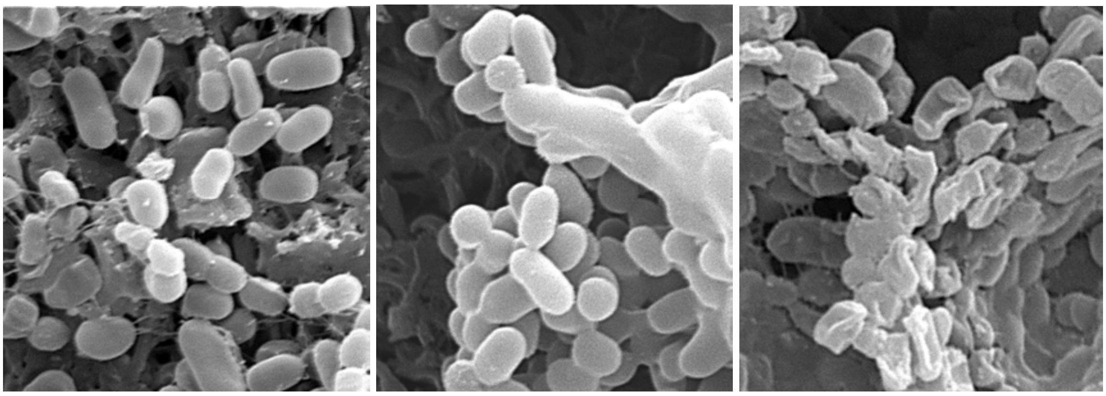

Figure 1: Electron micrographs of Enterococus spp. Cells: untreated cells (left), after ultrasonic treatment (center) and after combined action of ultrasound and theraphthal (right).

Figure 1 shows scanning electron micrographs of bacteria after the combined action of ultrasound and theraphthal. It is evident that as a result of ultrasonic treatment some of the bacteria exposed to destruction apparently accompanied by the leakage of cytoplasm. However, most of the cells keeps normal form. Bacteria pretreated with theraphthal show, a change in the shape and the destruction of the membranes that cover almost all the treated cells. A similar pattern is visible on the transmission electron micrographs of sections of cells (Fig. 2). It is evident that combined action of theraphthal and ultrasound leads to the destruction of cell membranes and organelles. In our opinion, theraphthal reacts with calcium ions contained in the cells and forms a solid phase of nanoparticles of calcium salts. This phase provides sonosensitizing action, stimulating the destruction of the cells under the action of ultrasound.

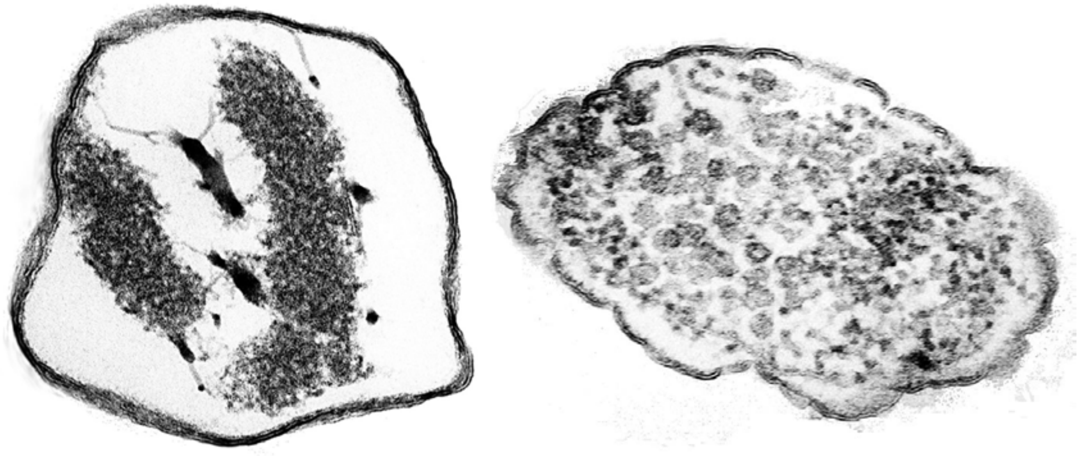

Figure 2: Ttransmission electron micrographs of sections of Enterococus spp. cells: untreated (left), after the combined action of ultrasound and theraphthal (right).

Table 1 presents data on the effectiveness of the combined action of ultrasound and sonosensitizers. Percentage of viable cells (*E*) was calculated on the basis of experimental data on the number of colony-forming units on seeding. The estimated percentage of viable cells (*C*) after combined action of the sonosensitizer and the ultrasound was calculated as the product of the percentages of viable cells after independent action of the sonosensitizer and ultrasound ($C=E_{SS}\cdot E_{US}$). Enhancement of the effect (*G*) was estimated by the value (100-*E*)/(100-*C*).

Table 1

The effectiveness of the combined action of ultrasound and sonosensitizers on the Enterococus spp. bacteria (E – experimentally determined percentage of viable cells, C – the estimated percentage of viable cells ($C=E_{SS}\cdot E_{US}$), G – enhancement of the effect).

Sample	*E*, %	*C*, %	*G*
Ultrasound	90		
Silver nanoparticles, stabilized by citrate, 10^{-6} g/ml	90		
Silver nanoparticles, stabilized by citrate, 10^{-6} g/ml + Ultrasound	82	81	0.95
Silver nanoparticles, stabilized by miramistin, 10^{-6} g/ml	85		
Silver nanoparticles, stabilized by miramistin, 10^{-6} g/ml + Ultrasound	75	77	1.05
Gold nanoparticles, 10^{-5} g/ml	100		
Gold nanoparticles, 10^{-6} g/ml	100		
Gold nanoparticles, 10^{-5} g/ml + Ultrasound	20	90	8.00
Gold nanoparticles, 10^{-6} g/ml + Ultrasound	93	90	0.70
Theraphthal 10^{-5} mol/l	98		
Theraphthal 10^{-5} mol/l + Ultrasound	77	88	1,92

From an examination of the results follows: (i) the combined action of ultrasound and sonosensitizer (theraphthal, silver and gold nanoparticles) in some cases leads to the superadditive antibacterial activity, the most striking example is the combined action of ultrasound and gold nanoparticles (10^{-5} g/ml) – enhancement of the effect in this case is 8; (ii) the antibacterial activity of silver and gold nanoparticles is determined by their concentration and the nature of the stabilizer.

References

Mazina S.E., Gopin A.V., and Nikolaev A.L. Assessing the Contamination of Water Flow in Soldatskaya Cave (Crimea), Water Resources, 2009, 36, 692–698.

Nikolaev A.L., Gopin A.V., Bozhevolnov V.E., Treshchalina E.M., Andronova N.V., and Melikhov I.V. Use of Solid-Phase Inhomogeneities to Increase the Efficiency of Ultrasonic Therapy of Oncological Diseases, Acoustical Physics, 2009, 55, 575–583.