Antibacterial nanocomposite coatings

Gold Against Bacteria

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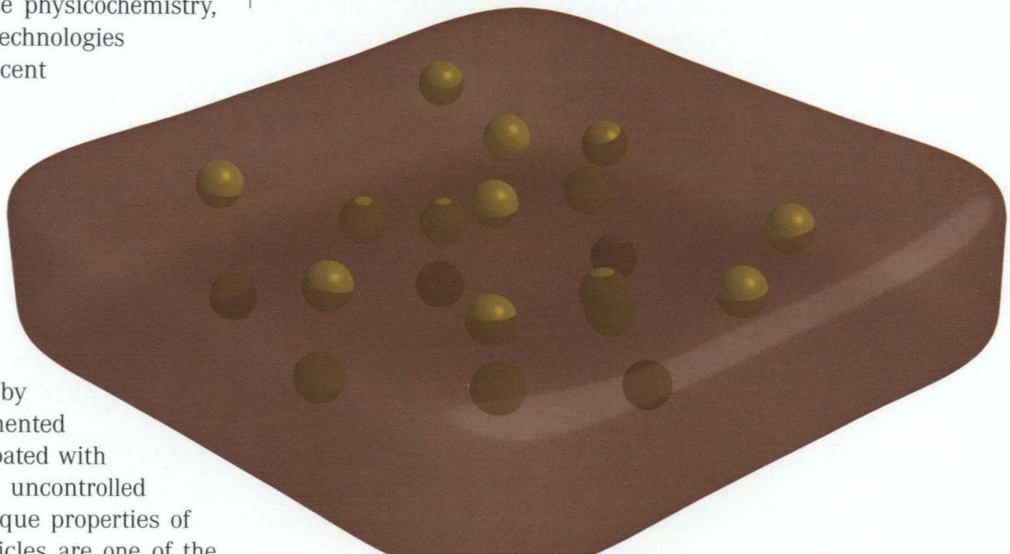
According to the most recent report from the European Centre for Disease Prevention and Control, one in eighteen patients in European hospitals has experienced at least one illness as a result of infection while receiving treatment. That's a total of 3.2 million of people per year. The pathogens are usually bacteria picked up from surgical tools and hospital equipment. At the PAS Institute of Physical Chemistry, we have devised a method of functionalizing surfaces with an antibacterial coating which is harmless to human cells

The progressing miniaturization of electronic devices, the dynamic development of surface physicochemistry, and the intensified research into technologies using quantum phenomena in recent decades have meant that nanoparticles are becoming increasingly important. They are usually spherical particles with at least one dimension not exceeding 100 nanometers, comprising from a few to tens of thousands of atoms. Their properties and potential applications are significantly different from those exhibited by the same substances in a less fragmented form. Nanoparticles are generally coated with a stabilizing layer, which prevents uncontrolled aggregation and the loss of the unique properties of nanoscale materials. Gold nanoparticles are one of the most durable metallic nanoparticles. Because of their unique properties, closely related to their size and shape, gold nanoparticles have many applications, for example in materials chemistry, catalysis, medicine, and biology.

Research conducted by our team at the PAS Institute of Physical Chemistry has led to the development of a nanocomposite material with powerful antibacterial properties which remains completely harmless to human cells. The key to creating this material was the immobilization of gold nanoparticles in a hierarchical oxoborane structure.

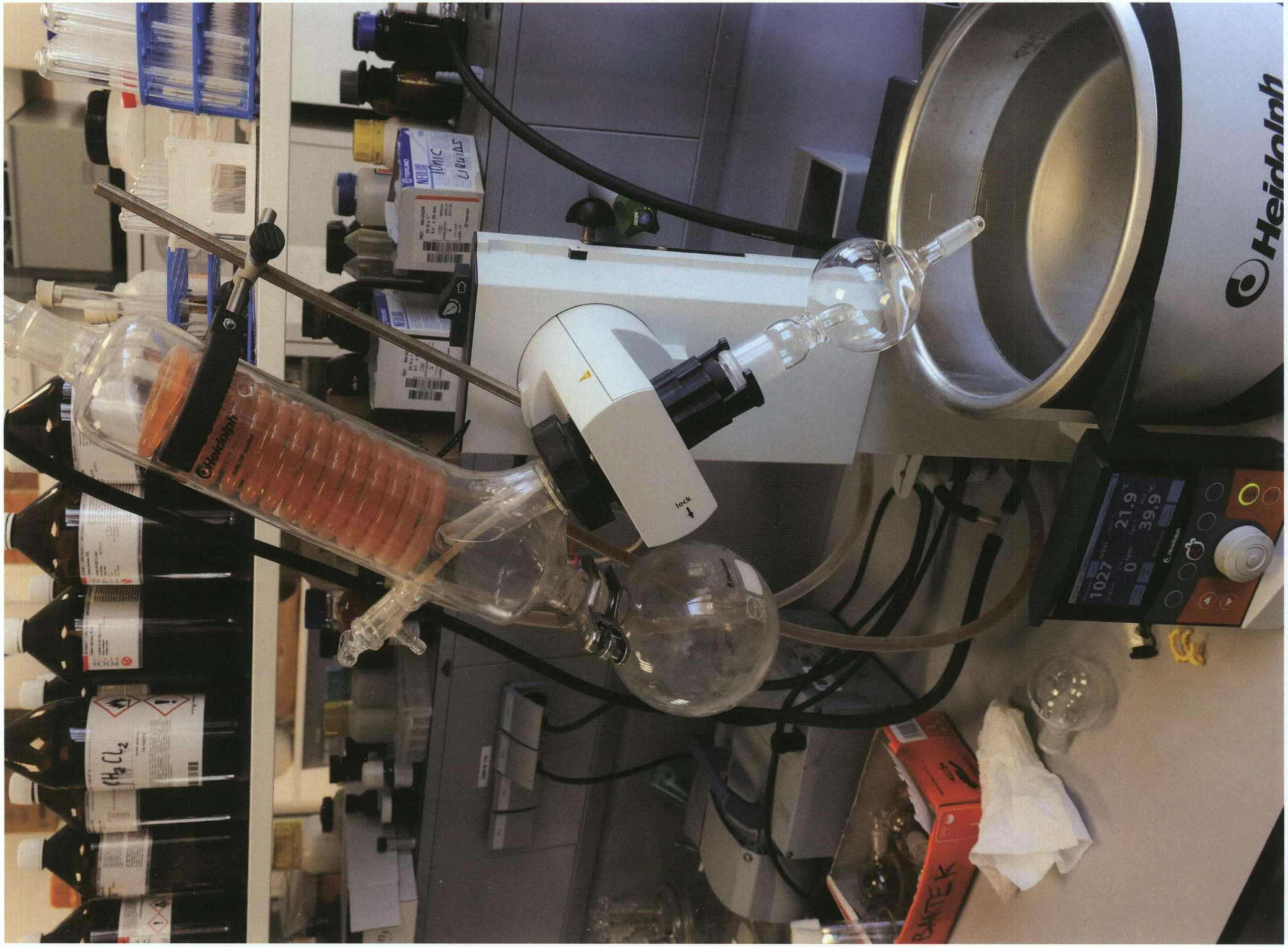
Naked gold

We used gold particles with a diameter of approx. 4 nanometers, obtained by reducing chloroauric ions with sodium borohydride. In the literature, these types of nanoparticles coated with inorganic compounds are frequently described as "naked," since they do not include an organic stabilizing ligand. The absence of an organic coating means that the surface of gold nanoparticles is accessible to bacteria. The resulting compounds of boron and oxygen (oxoboranes) stabilize the surface of the nanoparticles, and also play a part in forming the nanocomposite itself. The post-reaction solution contains various types of anions and polyanions, comprising basic units BO_3 and BO_4, whose number depends on the solution's pH. In solutions with a higher concentration of boric acid, we find an equilibrium between the non-ionized form of $B(OH)_3$ acid and polynuclear complexes $B_3O_3(OH)^{4-}$, $B_4O_5(OH)_4^{2-}$, $B_3O_3(OH)_5^{2-}$, $B_5O_6(OH)^{4-}$ and $B(OH)^{4-}$.



Aggregates composed of nanoparticles surrounded by oxoboranes aggregate to form nanocomposite bricks. Gold nanoparticles protrude from the matrix; they are accessible to microorganisms, which are destroyed as a result

Jakub Ostałowski



An evaporator, a piece of equipment that vaporizes a liquid in a controlled way, which was used at one of the stages of the project

The addition of H^+ or OH^- ions to the solution leads to condensation and the formation of polyoxoanions. The difference between condensation in an acidic or base environment concerns the morphology of the polyanions that arise during the process. In acidic environments, we observe a tendency to form flat networks which then form layers, while in base environments condensation leads to the formation of amorphous networks.

To synthesize our nanostructures, we applied condensation induced by H^+ ions. Following the acidification of the solution with hydrochloric acid, a process of condensation of oxoboranes starts around the gold nanoparticles (surrounded by boron compounds). The polyoxoborane networks around the nanoparticles have a planar structure.

The hydrogen bonds result in the formation of an almost hexagonal structure similar to graphene (as illustrated on the next page). The distance between the layers is 3.18 Å. The oxoborane condensation process involves the delocalization and reduction of the charge, resulting in increased hydrophobic properties of the structures. The aggregates of nanoparticles and the polyoxoborane sheath combine further due to hydrophobic interactions, and condense to form structures with a higher order of organization. The "bricks," formed of polyoxoboranes, are shaped like flattened blocks with a length of a few tens of nanometers and a thickness of around 10 nanometers. The blocks - acting as a matrix or scaffold - immobilize gold nanoparticles (as illustrated above). They are composed of boron, oxygen and gold, earning the composite material the name "BOA." The process of oxoborane condensation is accompanied by their chemical (covalent) bonding to the material surface, which is functionalized; the process is facilitated by hydroxyl groups. As a result, the surface is durably coated with the BOA material. It's worth adding that the ability of oxoborane ions to form strong complexes with OH groups, for example in cellulose or polyalcohols, is well known. However, this property has not been explored thus far in the modification of hydrophilic materials with gold nanoparticles.

The powerful antibacterial properties of our nanocomposite have been demonstrated for both Gram-negative and Gram-positive bacterial strains. In our tests, we used *Escherichia coli* and *Staphylococcus epidermidis*, respectively. The *E. coli* strain was mutated to give it resistance against two powerful antibiotics: chloramphenicol and kanamycin. The microorganisms are potentially dangerous, since certain *E. coli* strains cause serious infections such as pneumonia and peritonitis, which may lead to sepsis. They can also causes urinary tract infections and diarrhea. Additionally, toxins synthesized by *E. coli* cause damage to internal organs. The other pathogen, *S. epidermidis*, is a staphylococcus which can cause very serious opportunistic infections. This makes it especially dangerous for patients hospitalized following surgical procedures, since it can cause infections at the surgery site. The pathogen is also a potential threat for patients with burns, catheters, and implants.

In order to establish bactericidal properties of nanocomposite coatings, we conducted studies in which the bacterial strains were incubated in the presence of fragments of cotton wool modified with the BOA nanocomposite. For both pathogens, after 24 hours of incubation we observed a significant decrease in the number of bacteria, reaching two or three orders of magnitude of the original population. This demonstrates that our nanostructural coatings have excellent antibacterial properties.

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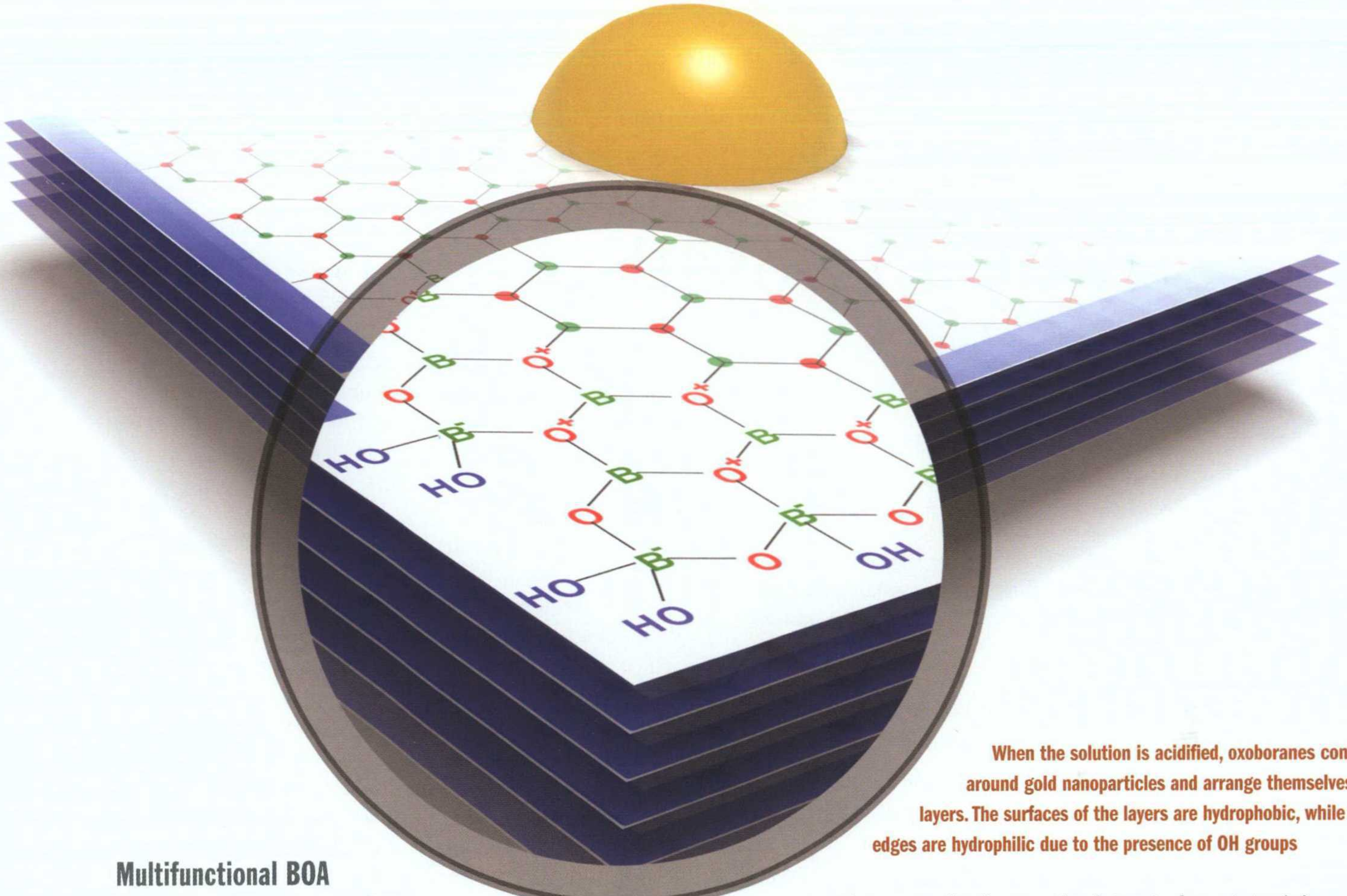
Cotton wool soaked in solution remaining from the nanocomposite synthesis has bactericidal properties



When the solution is acidified, oxoboranes condense around gold nanoparticles and arrange themselves in layers. The surfaces of the layers are hydrophobic, while their edges are hydrophilic due to the presence of OH groups

Multifunctional BOA

We were also interested in the antibacterial mechanism of action of BOA structures. During our studies we determined that the nanocomposite does not release any factors that are toxic to bacteria. It also turned out that no antibacterial factors were found in the solution remaining after the composite's synthesis. This means that the microorganisms are destroyed as a result of coming into direct contact with the nanostructure. We also established that it is the presence of gold in the nanoparticles that gives them antibacterial properties. We verified this by studying the bactericidal properties of surfaces coated with a layer of polyoxoboranes without gold nanoparticles. We do not yet know how direct contact between the pathogens and the gold nanoparticles trapped in the polyoxoborane matrix leads to their death, although there can be no doubt that the mechanism of destroying microorganisms exhibited by the BOA nanocomposite has great potential economic and environmental benefits. Used as a bactericidal coating, the material is highly durable, and it doesn't release any toxic substances into the environment.

One of the most important properties of antibacterial coatings is their effect on human cells. In collaboration with researchers from the Jagiellonian University's Collegium Medicum and Małopolska Centre of Biotechnology, we investigated the cytotoxicity of BOA nanostructures on four cell lines: glioma LN18, liver HepG2, pancreas BTC-6, and adipose tissue SVF. We used a quantitative method to study cytotoxicity, measuring the activity of lactate dehydrogenase (LDH). LDH is a cytosolic enzyme, released into the environment as a result of mechanical damage to the plasma membrane and cell death. Enzyme activity demonstrates the presence of a cytotoxic factor. Our research shows that cotton wool coated with the BOA nanocomposite is not cytotoxic for any of the cell lines we studied. Additionally, microscopic observations show that cells continued to develop on strands of modified cotton wool for up to four weeks after inoculation.

The BOA nanocomposite has vast potential. Its applications include making antiseptic materials, in particular coatings for various types of surfaces, including polymers (Petri dishes, implants, cell growth scaffolds), glass (cuvettes, glass fiber), metals (medical equipment, implants), and cotton (bandages, sutures). ■

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Further reading:

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