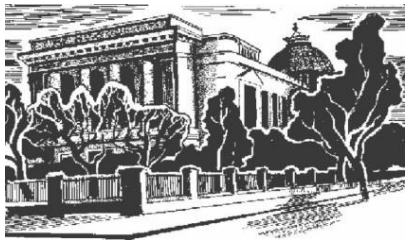

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Testing Nanodiamond as an Antibacterial and Antifungal Agent

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Introduction

The utilization of the optical – scattering and spectroscopic – properties of diamond nanoparticles (nanodiamonds, ND) for bio-imaging/monitoring and bio-sensing is developed and often combined with additional ND functionalities like drug delivery and other treatments currently.

A newly found property of NDs important for their multifunctional theranostic abilities is antimicrobial activity. The use of the antibacterial properties of nanostructures is currently considered an alternative to antibiotics.

In the presented work **the activity of ultradispersed nanodiamonds was tested against pathogenic microorganisms** – gram-negative and the gram-positive bacteria.

Additionally, **for the first time, systematic study of the ND antifungal activity against several fungi strains was carried out**.

Nanocarbons and nanocarbon-based hybrids have revealed significant antibacterial activity studied during last decade and has been demonstrated in

Ivanova V T et al 2012. Interaction of nanodiamonds materials with influenza viruses. J Physics: Conference Series 345, 012019
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Panáček D et al, 2021 Silver Covalently Bound to Cyanographene Overcomes Bacterial Resistance toSilver Nanoparticles and Antibiotics. Adv. Sci. 8, 2003090
Cumont A et al 2021. Properties, mechanism and applications of diamond as an antibacterial material, Functional Diamond, 1:1, 1-28

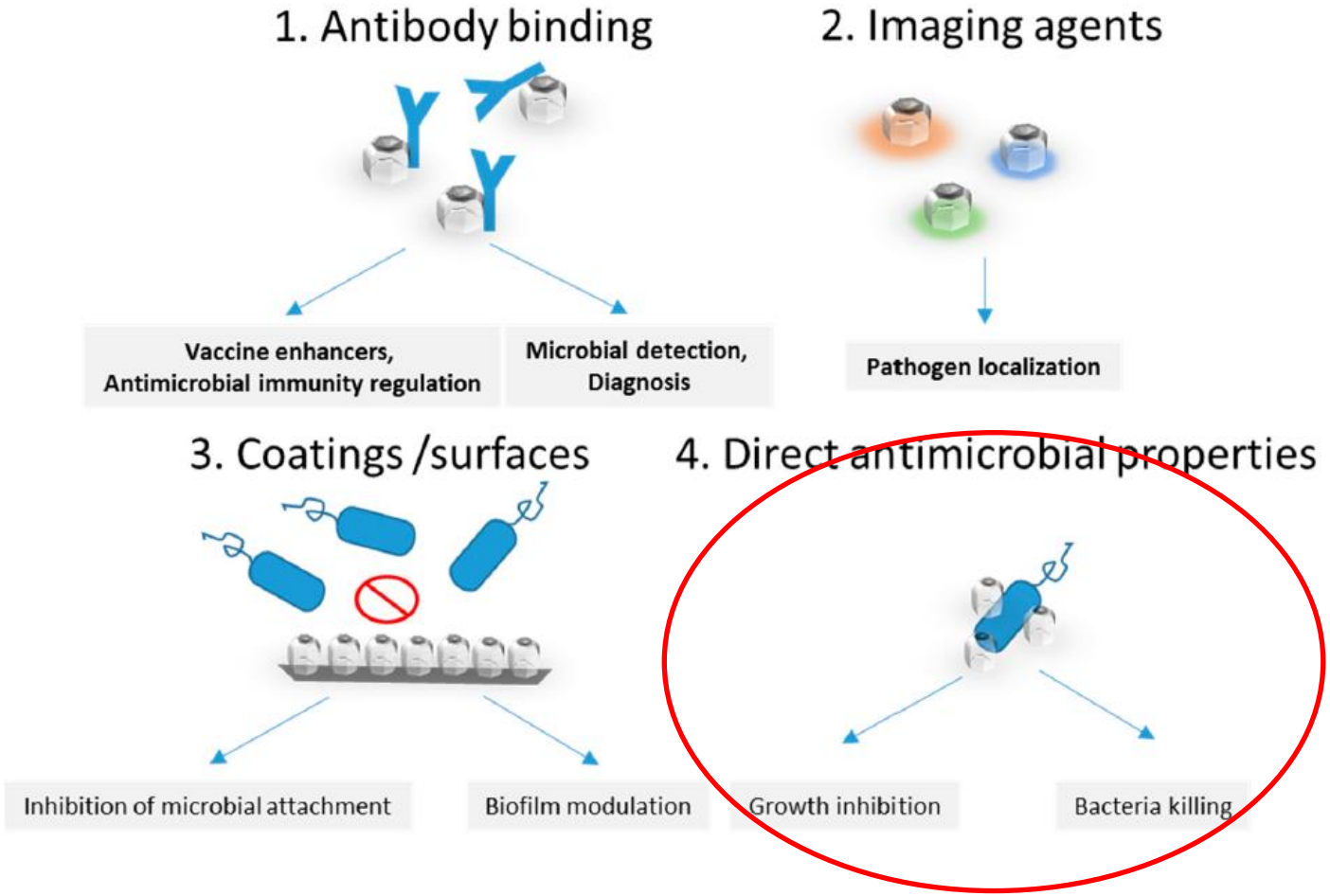
The effect depends on the physical-chemical properties, size, surface structure.

The possibility to modify properties of the film and the NP embedded allows the optimization of the antimicrobial effect.

NDs are currently considered as highly promising antibiotics alternative

[Cumont A, Pitt A R, Lambert P A, Oggioni M R and Ye H 2021 Properties, mechanism and applications of diamond as an antibacterial material Functional Diamond 1 1–28] .

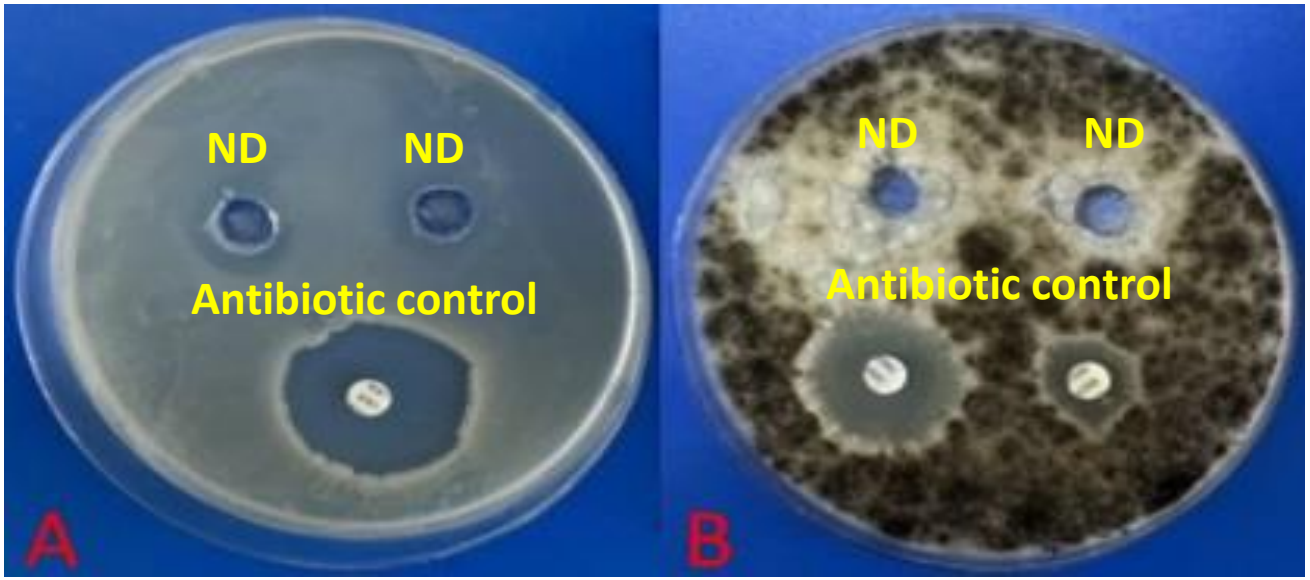
Proposed routes for infection, detection, and antimicrobial therapy, including – via ND particles, to be further investigated:



Torres Sangiao E et al. 2019 Materials 12 1639

We are focusing on direct antimicrobial properties

The antimicrobial activity of ultradispersed NDs at the concentration of 500 μg/mL was assessed by the agar diffusion method with the test strains of gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*; gram-positive bacteria *Bacillus subtilis*; *Staphylococcus aureus* and *Micrococcus luteus*. The antifungal activity was estimated for the fungi of the genus *Aspergillus* (*A. niger, A. fumigatus*) and the yeast *Candida albicans*. Amphotericin B, nystatin, and amoxiclav were used as a positive control.



Antimicrobial activity of ultradispersed NDs against bacteria and fungi detected by agar-duffision methods: A - *Staphylococcus aureus;* B - *Aspergillus niger*

European Committee on Antimicrobial Susceptibility Testing Antimicrobial susceptibility testing. EUCAST disk diffusion method. Available online at https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Disk_test_documents/2021_manuals/Manual_v_9.0_EUCAST_Disk_Test_2021.pdf (Accessed 22.07.2022)

Materials and Methods

Detonation nanodiamond (DND) powder (FRPC “Altai”, Russia) was suspended in deionized water and treated with ultrasound to decrease aggregation.

ND samples were tested in a concentration of 500 μg/mL. Inhibition zones were measured manually using digital caliper. Assays were performed three times in triplicate. The amphotericin B 40 μg («NII Pasteur», Russia,), nystatin 80 μg («NII Pasteur», Russia) and amoxiclav 10 μg («NII Pasteur», Russia) were used as a positive control. The antibacterial activity was assessed with the following test strains: gram-negative bacterium *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853; gram-positive bacterium *Bacillus subtilis* ATCC 6633; *Staphylococcus aureus* 209P and *Micrococcus luteus* NCTC 8340. The antifungal activity was assessed with the following test strains: fungi of the genus *Aspergillus* – *A. niger* INA 00760, *A. fumigatus* KPB F-37 and yeast *Candida albicans* ATCC 2091.

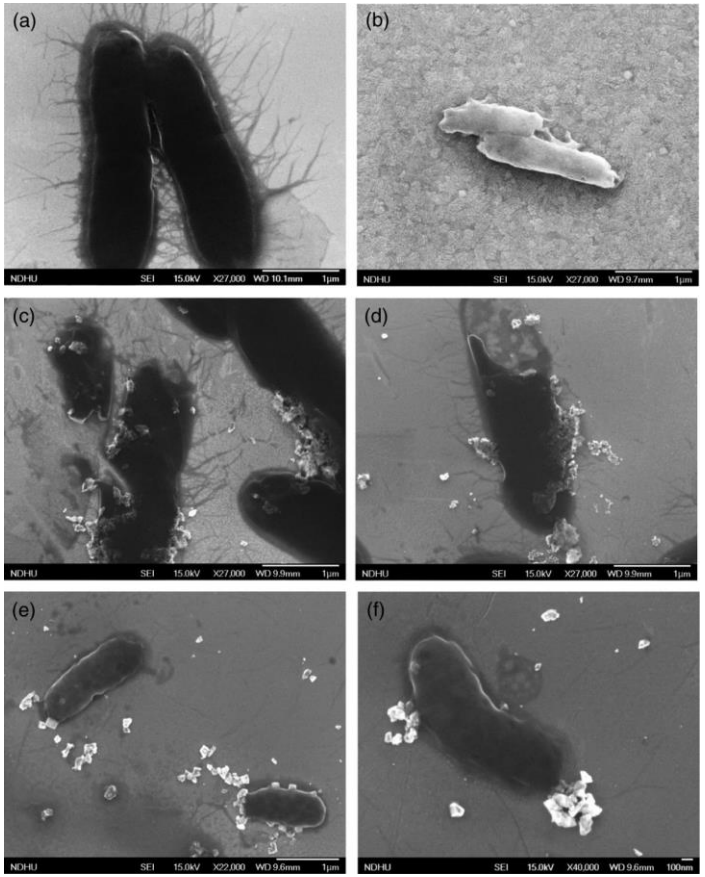
The test culture of *B. subtilis* ATCC 6633 was grown on the Gause 2 medium (g/L): 2.5 tryptone (or30 mL Hottinger broth), peptone - 5, sodium chloride - 5, glucose - 10. *S. aureus* 209P, *M. luteus* NCTC 8340, *P. aeruginosa* ATCC 27853 were grown on MPA medium and *E. coli* ATCC 25922 was grown on LB medium. Fungi *A. niger* INA 00760, *A. fumigatus* KPB F-37 and *C. albicans* ATCC 2091 were grown on PDA (potato dextrose agar). The cultures were preliminarily grown in the test tubes containing nutrient agar slant.

Afterwards the cells were suspended in saline to a turbidity of 0.5 according to the McFarland standard (1.5×10^8 CFU/mL) and were used within 15 min. One-day cultures of bacteria and 5-day cultures of fungi and yeasts were used. All test cultures were obtained from the culture collection of Gause Institute of New Antibiotics.

Results

Scanning electron microscopy images of (a) E. coli, E. coli incubated with (b) antibacterial agent lysozyme (50 μg⁄ml), (c) and (d) detonation ND. With crystallites of size 5-10 nm (100 μg⁄ml), and (e) and (f) 100 nm carboxilted NDs (100 μg⁄ml) after 4 h of interaction.

Detonation ND mechanically destroys the bacterial cell wall



The ND detrimental effects on microorganisms were previously observed to a more significant extent for the ND with higher content of sp^2 carbon on the surface typical for detonation ND (and similar structures)

Chatterjee et al, J Biomed Optics 20(5), 051014 (2015)

An activity of the ND against *E. coli*, *B. subtilis* and *S. aureus* was observed.
As for fungi, the ND effect was detected for all the test strains including yeast.

Interestingly, **ND can combine antibacterial and significant antifungal properties**.

Table. Antimicrobial activity of DND

Sample	Zone of inhibition, mm							
	B. subtilis ATCC 6633	*P. aeruginosa* ATCC 27853	*M. luteus* NCTC 8340	*S.aureus* 209	*E. coli* ATCC 25922	*A. niger* INA 00760	*C. albicans* ATCC 2091	*A. fumigatus* KPB F-37
DND	11	0	0	9	11	11	11	11
amoxiclav	18	11	24	25	11	nt	nt	nt
amphotericin B	nt	nt	nt	nt	nt	17	11	10

Nt – non tested

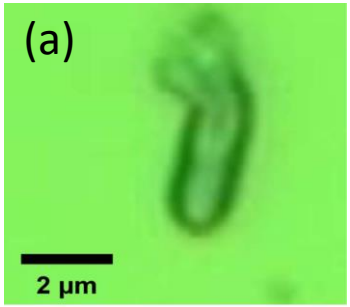
Discussion

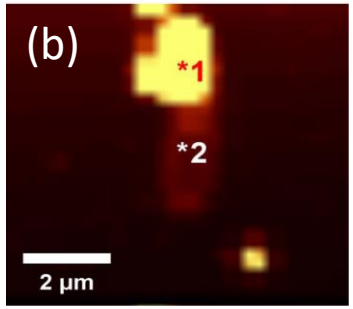
The antimicrobial action of nanoparticles is still poorly understood.

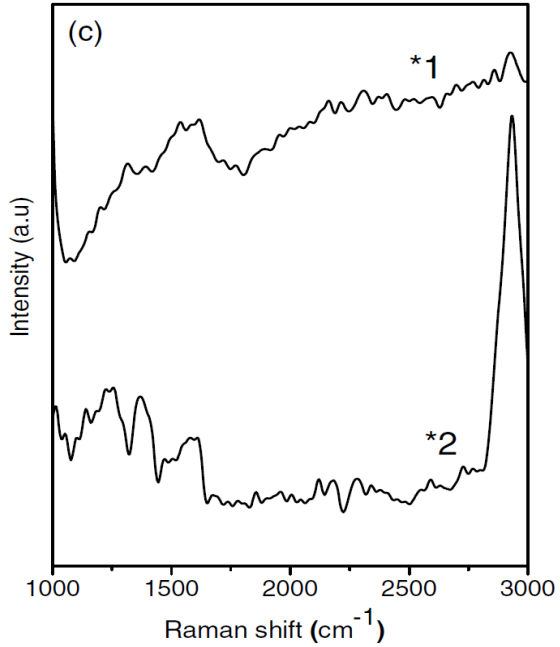
Using the methods utilizing the ND's optical-spectroscopic properties can be beneficial for the study of the ND interaction with the bacteria and fungi

Interaction of E. coli with DND (25 µg/ml) for 30 s in aqueous medium observed by

Raman spectroscopy and mapping







(a) Optical image of a single E. coli interacted with DND aggregate

(b) Raman mapping in the 1000 to 3000 cm^{-1} wavenumber region obtained with a 488 nm laser excitation and at 6 mW laser power at the 100× (NA 0.90) objective output.

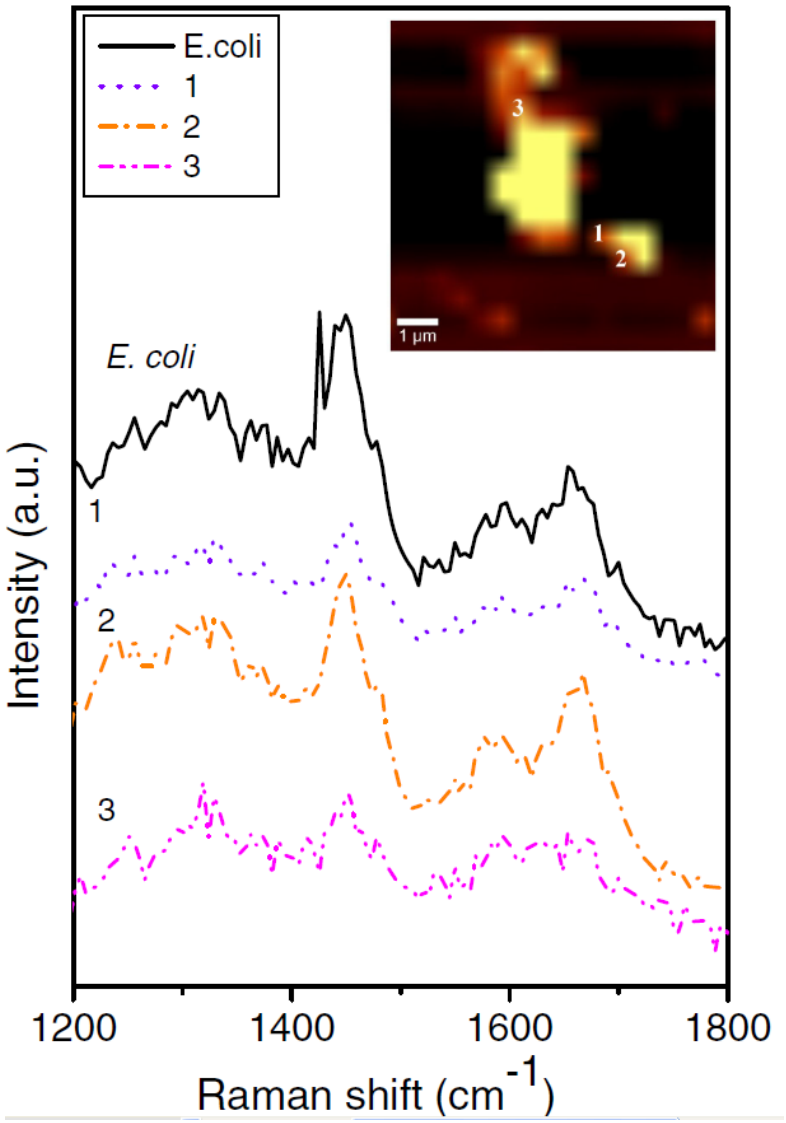
(c) Raman spectra from DND (revealing the lines in 1290-1370 cm^{-1} range from sp^2-sp^3 Carbon) attached at the bacterial cell membrane measured in point 1 marked in (b) and for E. coli corresponding to point 2 marked in (b) with pronounced Raman signal attributed to C-H bonds (2820 - 3030 cm^{-1})

Chatterjee et al, J Biomed Optics 20(5), 051014 (2015)

Raman spectra of a single untreated/undamaged E. coli (control) and E. coli treated with 5 nm DND (25 µg/ml) in a dry state on a silicon substrate measured at different points of the bacterial cell wall surface.

The inset shows the Raman mapping image of the single E. coli treated with DND; the distribution of intensity of the spectra in the range from 1000 to 3000 cm^{-1} was mapped.

Shown Raman spectra correspond to the points marked in the image. They are compared with the Raman spectra of untreated E. coli.



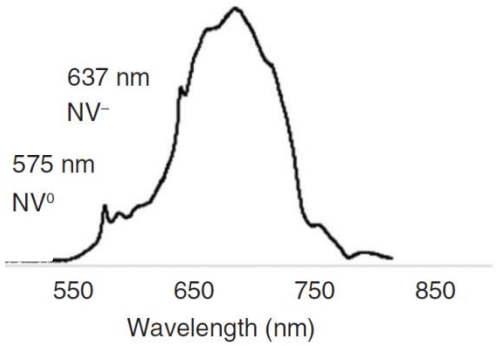
The spectra and mapping reveal the following:

- ND was adsorbed at the bacterial cell wall.
- The relative intensities of the peaks in the spectrum of the bacterium interacting with cNDs differs from the non-interacting bacteria.
- A shift of the Amide I (protein) peak from 1654 to 1667 cm^{-1} specifies that changes occur in the structure of the cell wall components in the presence of surface-adsorbed ND particles.

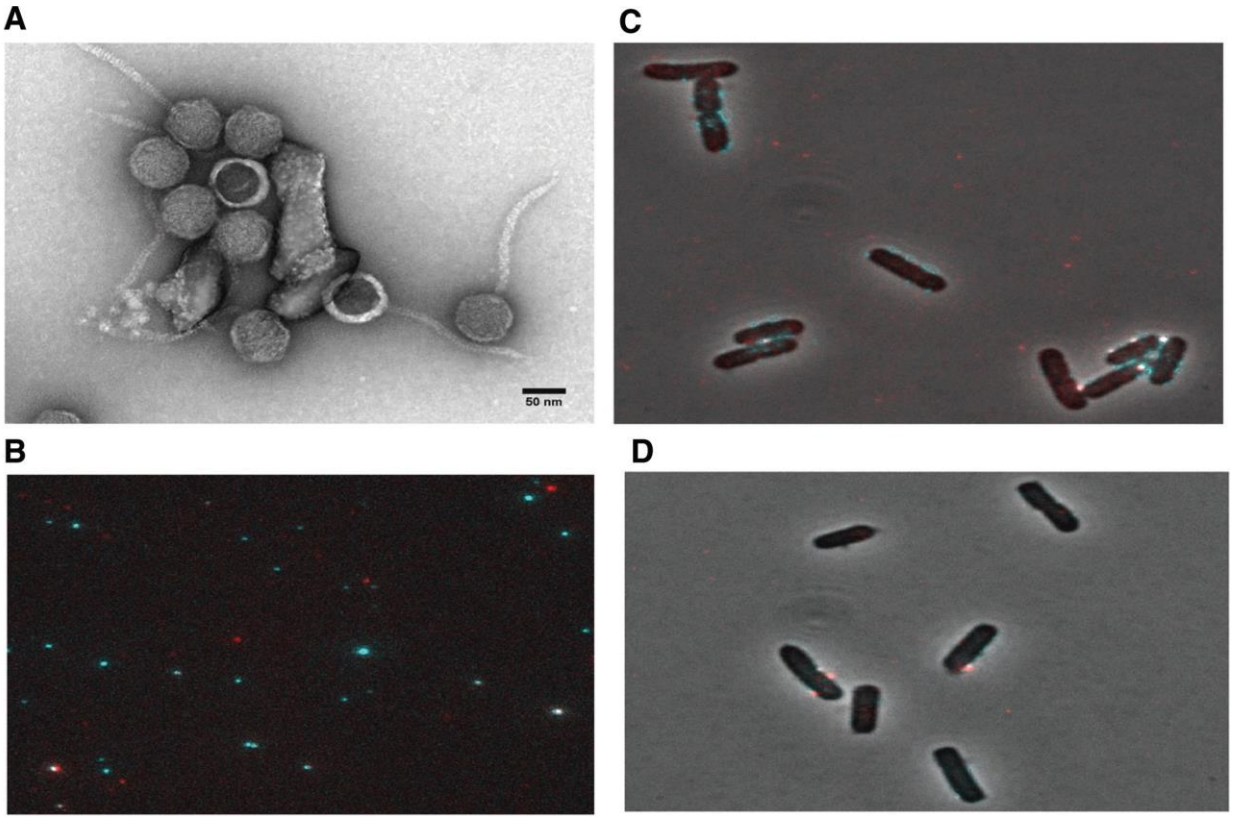
Chatterjee et al, J Biomed Optics 20(5), 051014 (2015)

Visualization of bacteria-bacteriophage interaction using detection of

FND fluorescence



Luminescence spectrum of fluorescent ND (FND) (excitation 514 nm)



Fluorescent nanodiamonds-bacteriophage conjugates allow host identification.

(A) High-magnification TEM images of biotinylated bacteria phages bound to streptavidin-functionalized FNDs.

(B) Optical image of biotinylated bacteria phages (blue spots) bound to streptavidin-functionalized FND (red spots).

(C and D) Optical images of biotinylated bacteria phages (blue spots) bound to streptavidin-functionalized FND (red spots) that have infected bacterial cells (*E. coli).*

Trinh JT,, et al. Biotechnol Bioeng 2018; 115, 1427–36.

Conclusion

Antimicrobial properties of detonation ND were assessed.

A prominent inhibition effect was observed for a number of bacteria and all the test strains of fungi. This makes DND perspective for the development of antibacterial agents and coatings.

DND optical-spectroscopic properties are convenient for spectroscopic studies of the interaction between pathogenic microorganisms and the nanodiamond or nanodiamond-based complexes.

Raman spectroscopy allows the suggestion that structural changes occur in the cell wall components in the presence of the surface-adsorbed ND particles.

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