Úpravy povrchů biomateriálů

Surface Modification of Biomaterials

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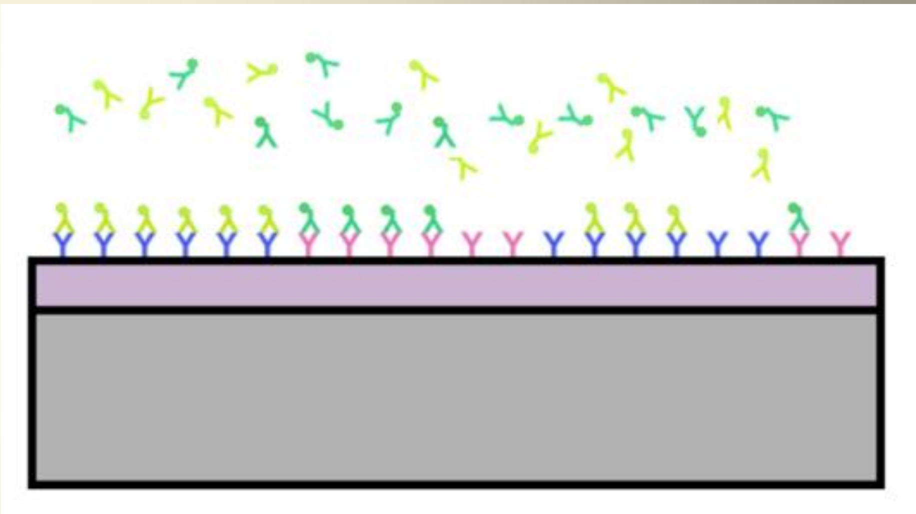
Thanks for Katarzyna…

Surface functionalization

Surface functionalization introduces chemical functional groups to a surface. This way, materials with functional groups on their surfaces can be designed from substrates with standard bulk material properties. Prominent examples can be found in biomaterial research.

Protein Patterning

The function of surface modification is to change the physical and chemical properties of surfaces to improve the functionality of the original material. Protein surface modification of various types biomaterials is performed to ultimately increase biocompatibility of the material and interact as bioactive material for specific applications. In various biomedical applications of developing implantable medical devices (such a spacemakers and stents), surface properties/interactions of proteins with a specific material must be evaluated with regards to biocompatibility as it plays a major role in determining a biological response. For instance, surface hydrophobicity or hydrophilicity of a material can be altered.



Surface modification can be done through various methods, which can be classified through three main groups: **physical** (physical adsorption, Langmuir blodgett film), **chemical** (oxidation by strong acids, ozone treatment, chemisorption, and flame treatment) and **radiation** (glow discharge, corona discharge, photo activation (UV), laser, ion beam, plasma immersion ion implantation, electron beam lithography, and γ-irradiation).

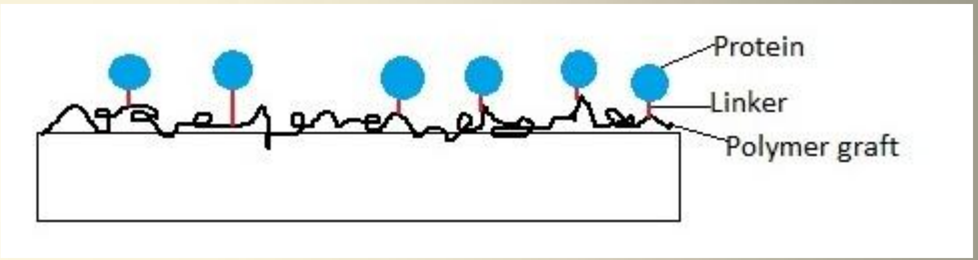
Engineering biocompatability between the physiological environment and the surface material allows new medical products, materials and surgical procedures with additional biofunctionality.

Physical modification

- Physical immobilization is simply coating a material with a biomimetic material without changing the structure of either. Various biomimetic materials with cell adhesive proteins (such as collagen or laminin) have been used in vitro to direct new tissue formation and cell growth. Cell adhesion and proliferation occurs much better on protein-coated surfaces. However, since the proteins are generally isolated, it is more likely to elicit an immune response. Generally, chemistry qualities should be taken into consideration.

Chemical modification

Alkali hydrolysis, covalent immobilization, and the wet chemical method are only three of the many ways to chemically modify a surface. The surface is prepped with surface activation, where several functionalities are placed on the polymer to react better with the proteins.



Covalent binding of protein with polymer graft

Plasma treatment

Plasma techniques are especially useful because they can deposit ultra thin (a few nm), adherent, conformal coatings.

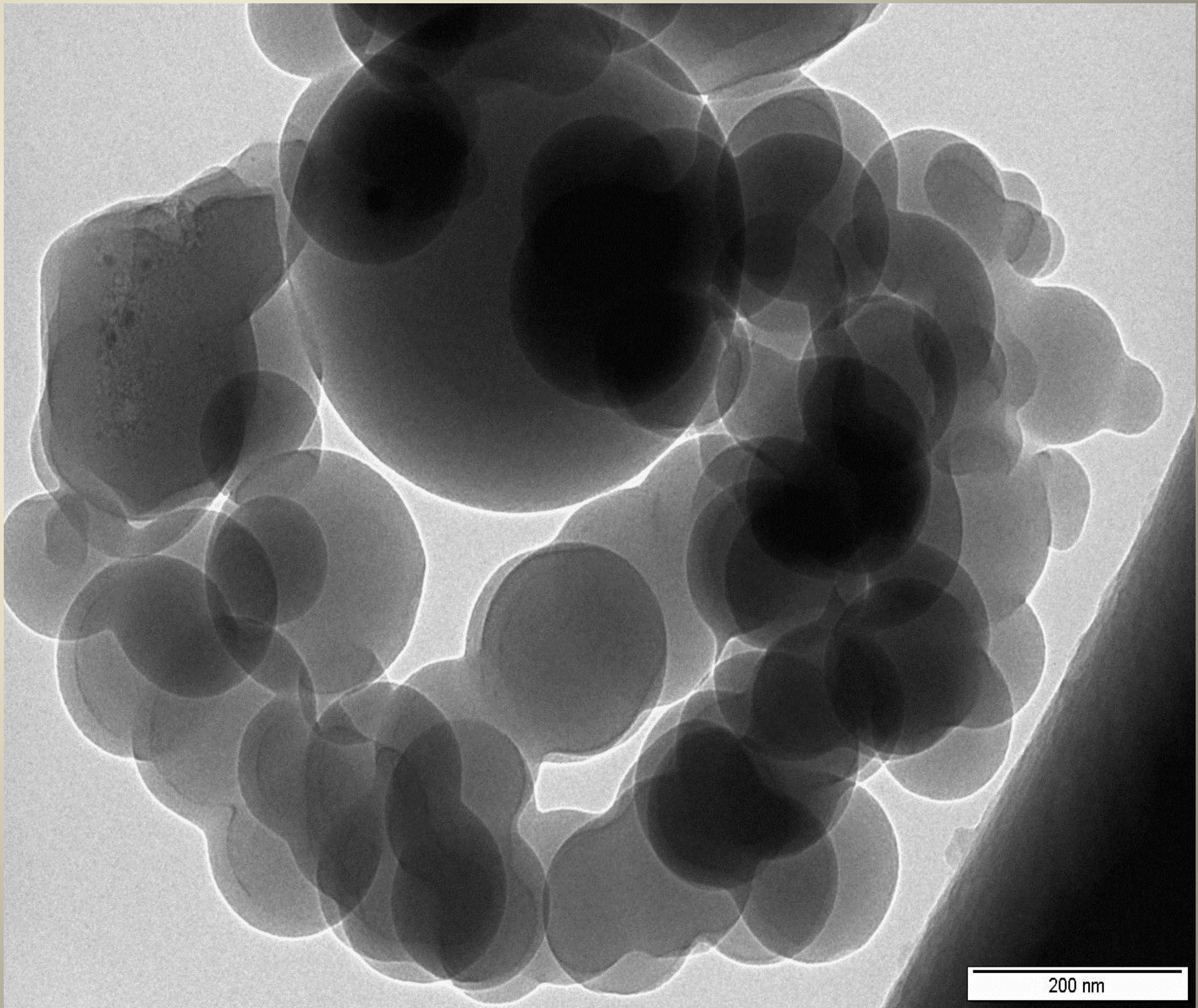
Experiments

examples

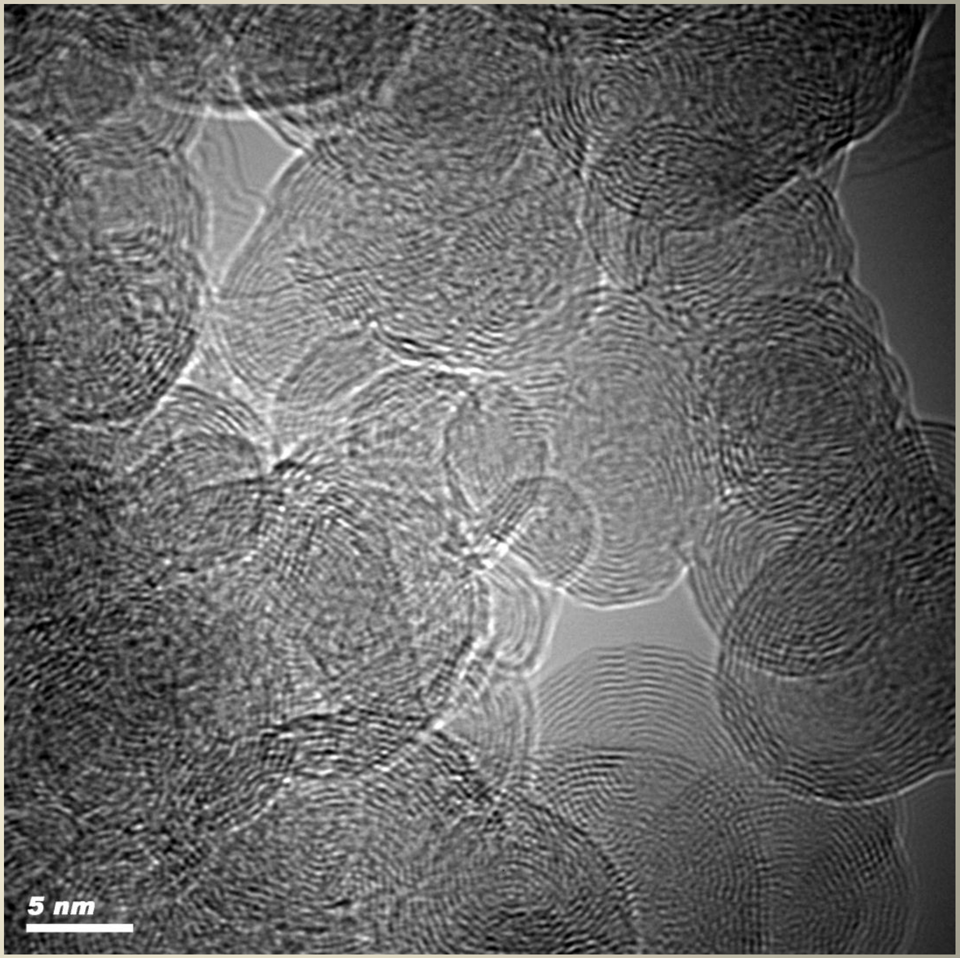
NANODIAMOND PARTICLES – RF/PACVD METHOD – HR TEM

K.Mitura ,

HR TEM examinations of nanodiamond particles for biomedical applications,
Journal of Achievements in Materials and Manufacturing Engeenering,
37, (2009), 317-322.



NANO-DIAMOND Particle HR TEM – detonation method



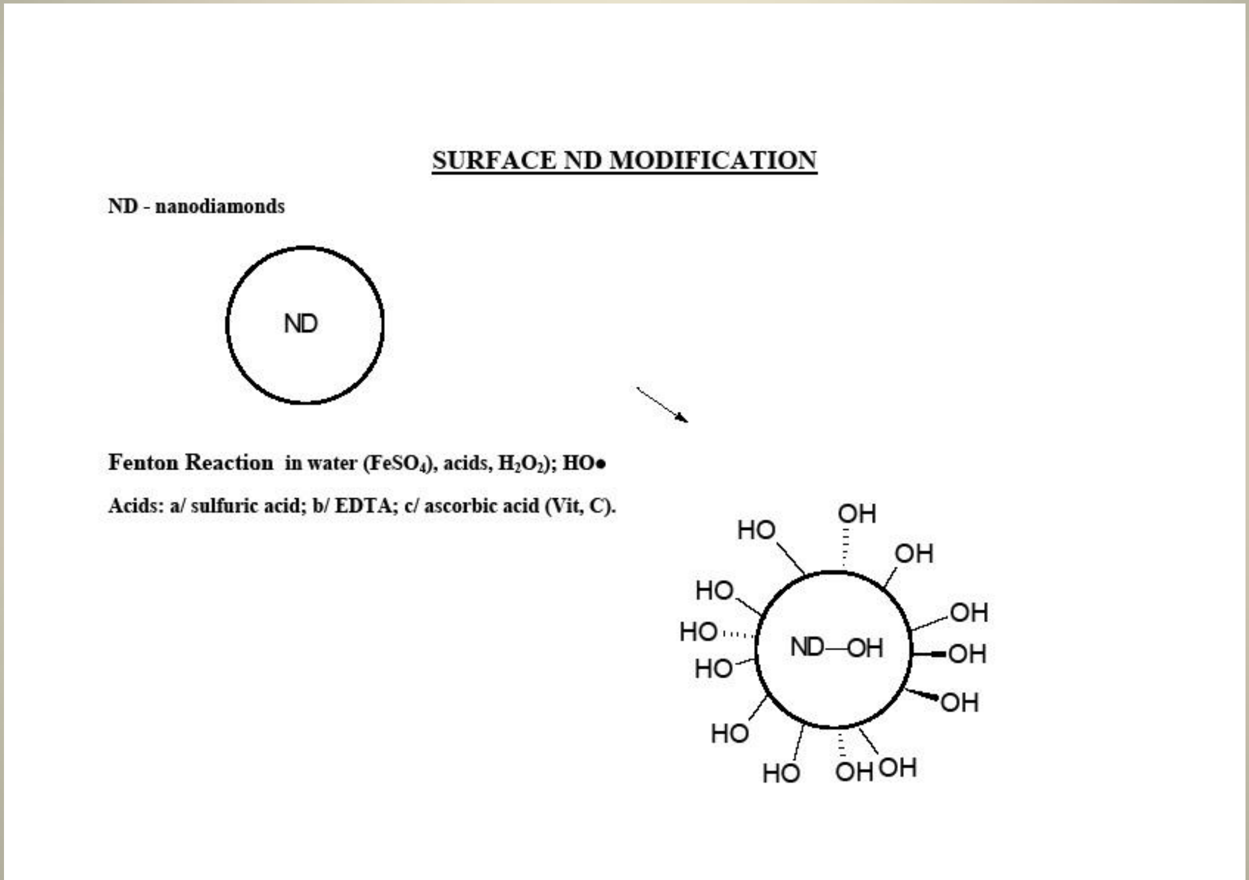
K.Mitura ,

HR TEM examinations of nanodiamond particles for biomedical applications,

Journal of Achievements in Materials and Manufacturing Engeenering,

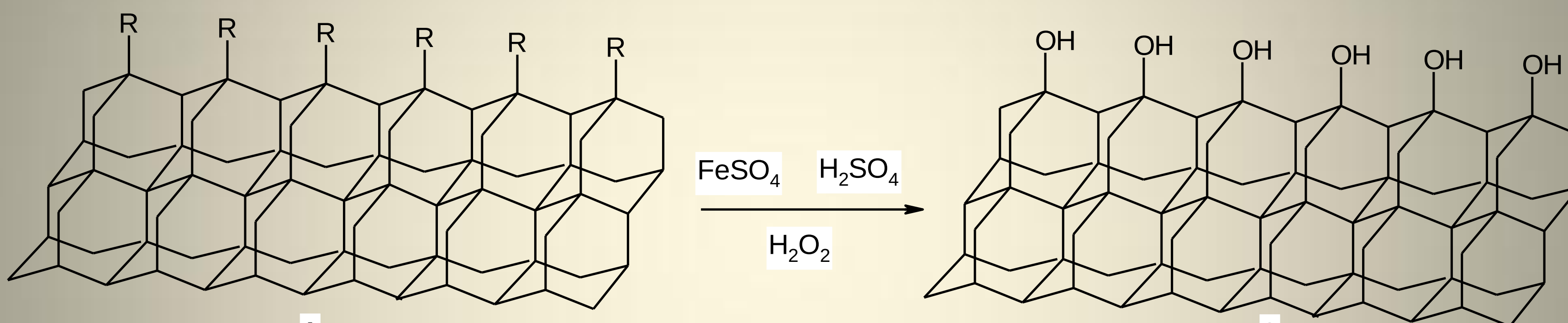
37, (2009), 317-322.

Surface modification of nanodiamond



Solarska-Ściuk, K., Gajewska, A., Skolimowski, J., Mitura, K., Bartosz, G.,: *Stimulation of production of reactive oxygen and nitrogen species in endothelial cells by unmodified and Fenton-modified ultradisperse detonation diamond.* **Biotechnology & Applied Biochemistry,** 60, (2013), 259-265.

FENTON's REACTION



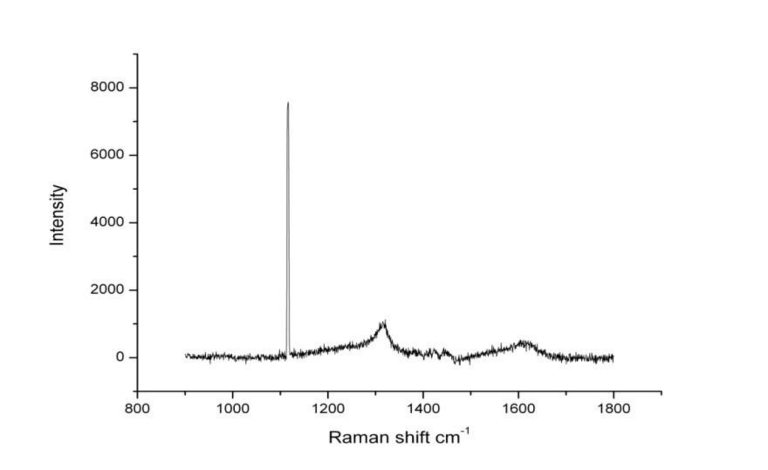
1 6

$$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + {}^{-}OH + {}^{\cdot}OH$$

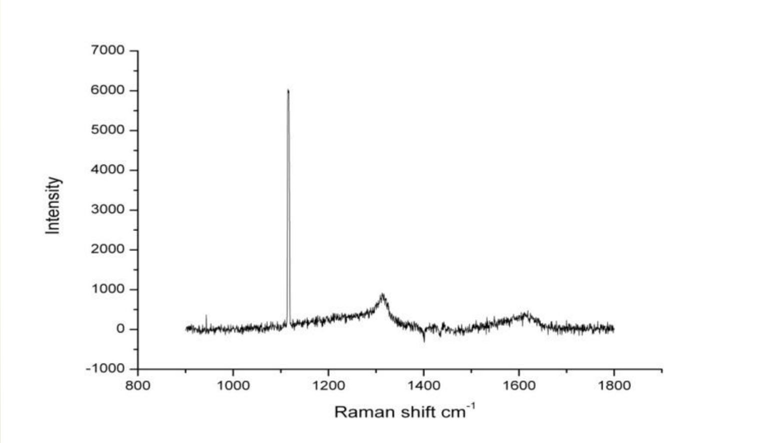
$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + H^{+} + HO_2^{\cdot}$$

Raman Spectroscopy of detonation nanodiamond

Before modification

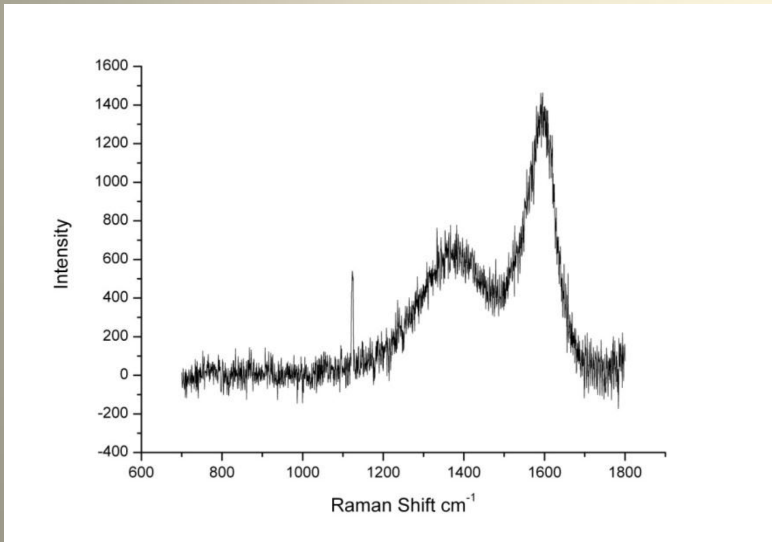


After modification

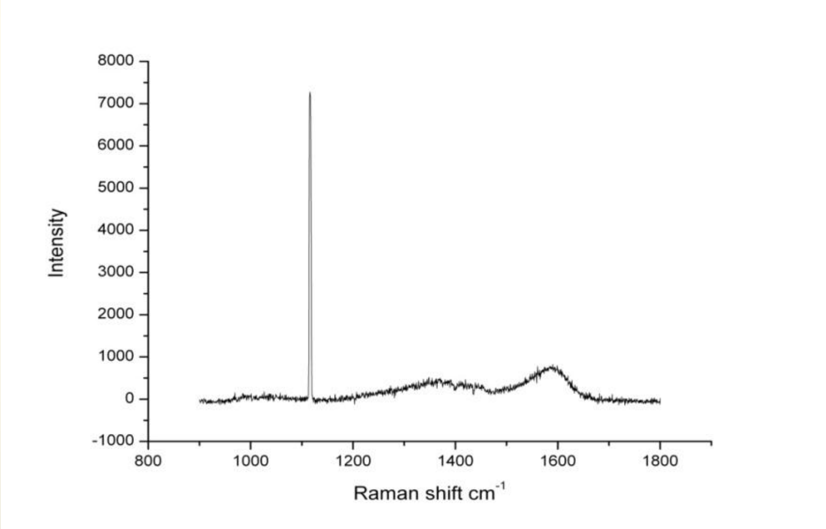


Raman Spectroscopy of RF PACVD nanodiamond

before modification

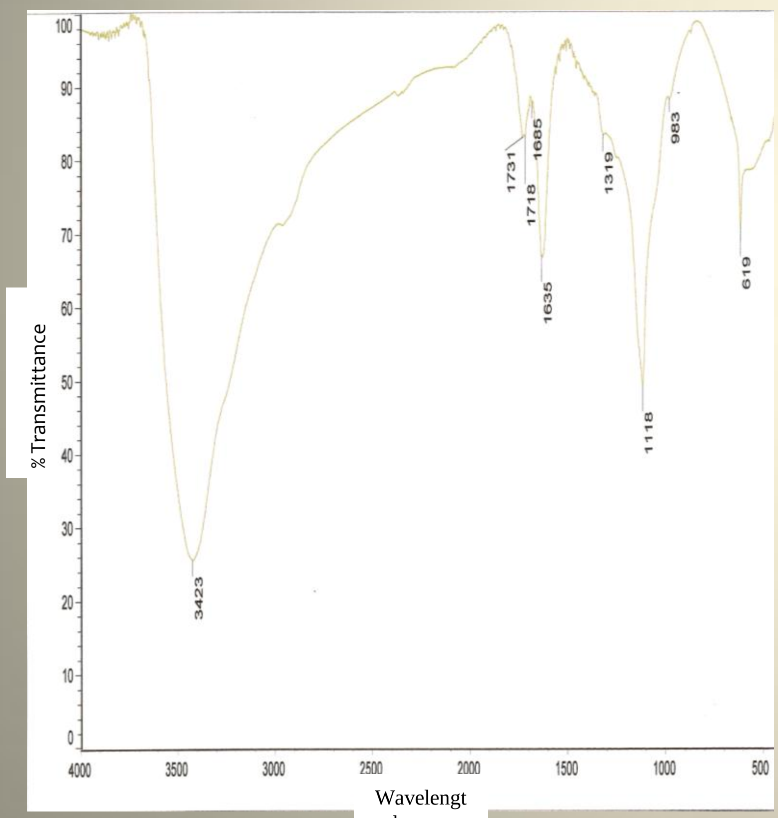


after modification

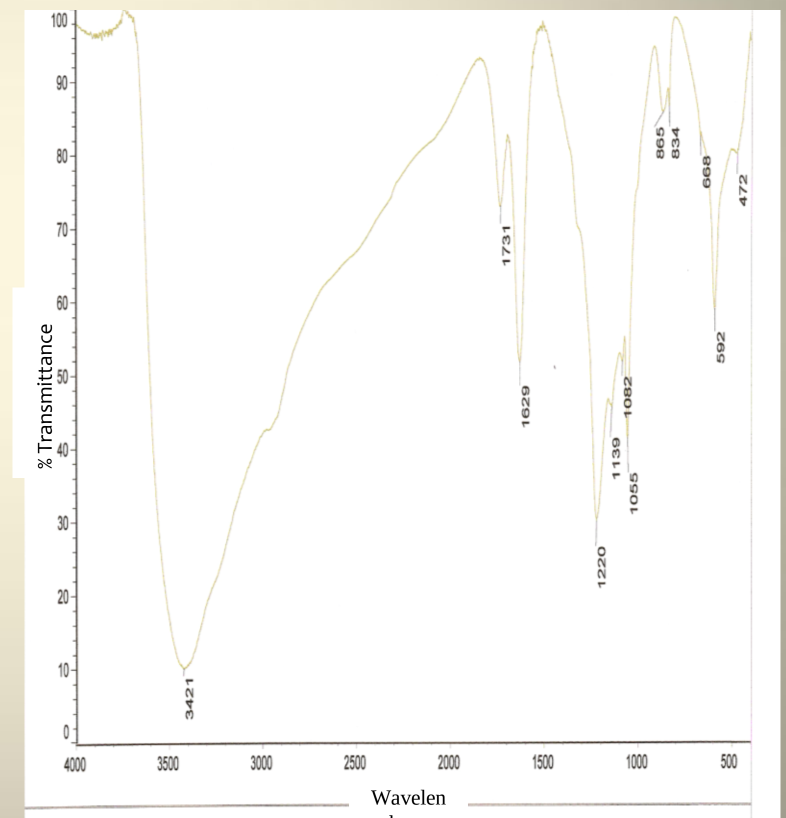


FT-IR spectroscopy of detonation nanodiamond

before modification

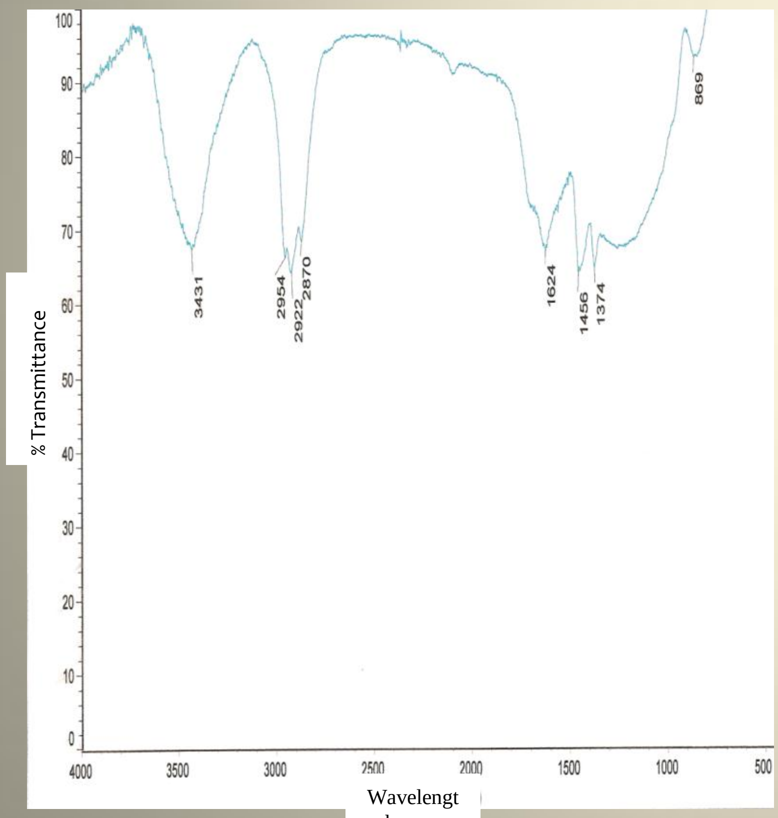


after modification

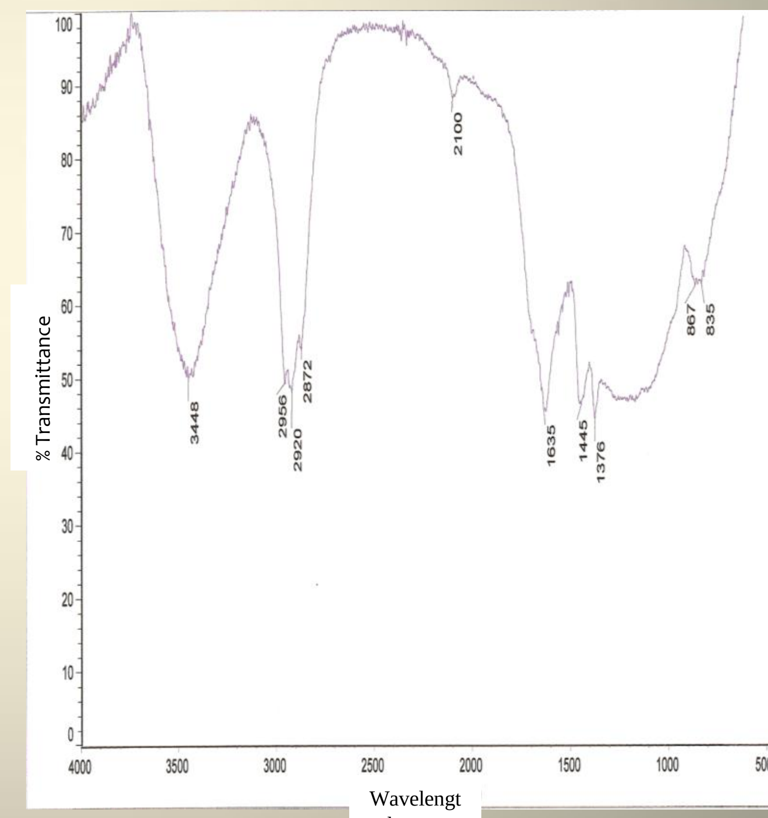


FT-IR spectroscopy of RF/PACVD nanodiamond

before modification



after modification



Raman spectra

Raman spectra for the UDD shows peaks corresponding to interest us diamond bonded at 1318 cm^{-1} and peak at 1600 groups OH (hydroxyl) at 1600 cm^{-1}

After fenton treatment it was observed that the carbon perecentage of pristine DNs was reduced.We interpret this decrease as a reflection of the introduction of oxygen functionalities

Raman spectra for the RF PACVD shows clear (distinct) peak at 1555 cm^{-1} coresponding to amorphous sp^2 bonded and peak 1600 cm^{-1} come from OH groups.

After fenton treatment it was observed that experienced a decrease and shift of the peak which may indicate a reduction sp^2 bonds and OH groups .

Ferrari and Robertson also argue that the peaks near 1150 cm^{-1} and near 1450cm^{-1}should not be assigned to nanocrystalline diamond or other sp3 – bonded phases. They think that these peaks are assigned to transpolyacetylene segments at grain boundaries and surface , so sp2-bonded configurations

A.C. Ferrari, J. Robertson, Phys. Rev. B 63 (121405), (2001), doi:10.1103/PhysRevB.63121405. (2006), 15, 296-299.

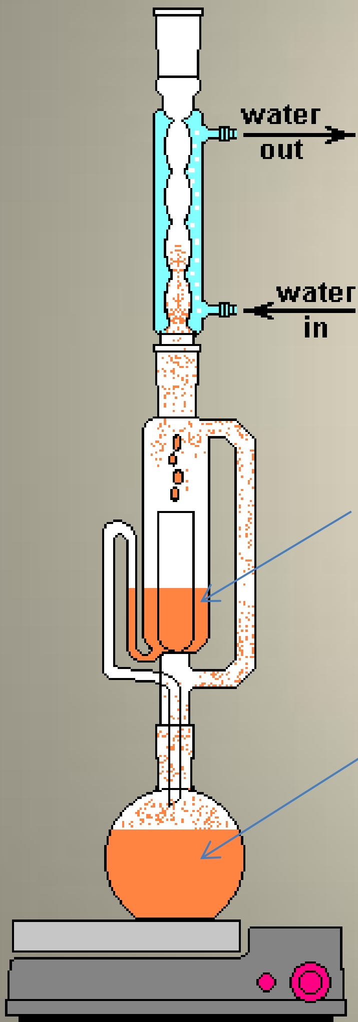
FT-IR

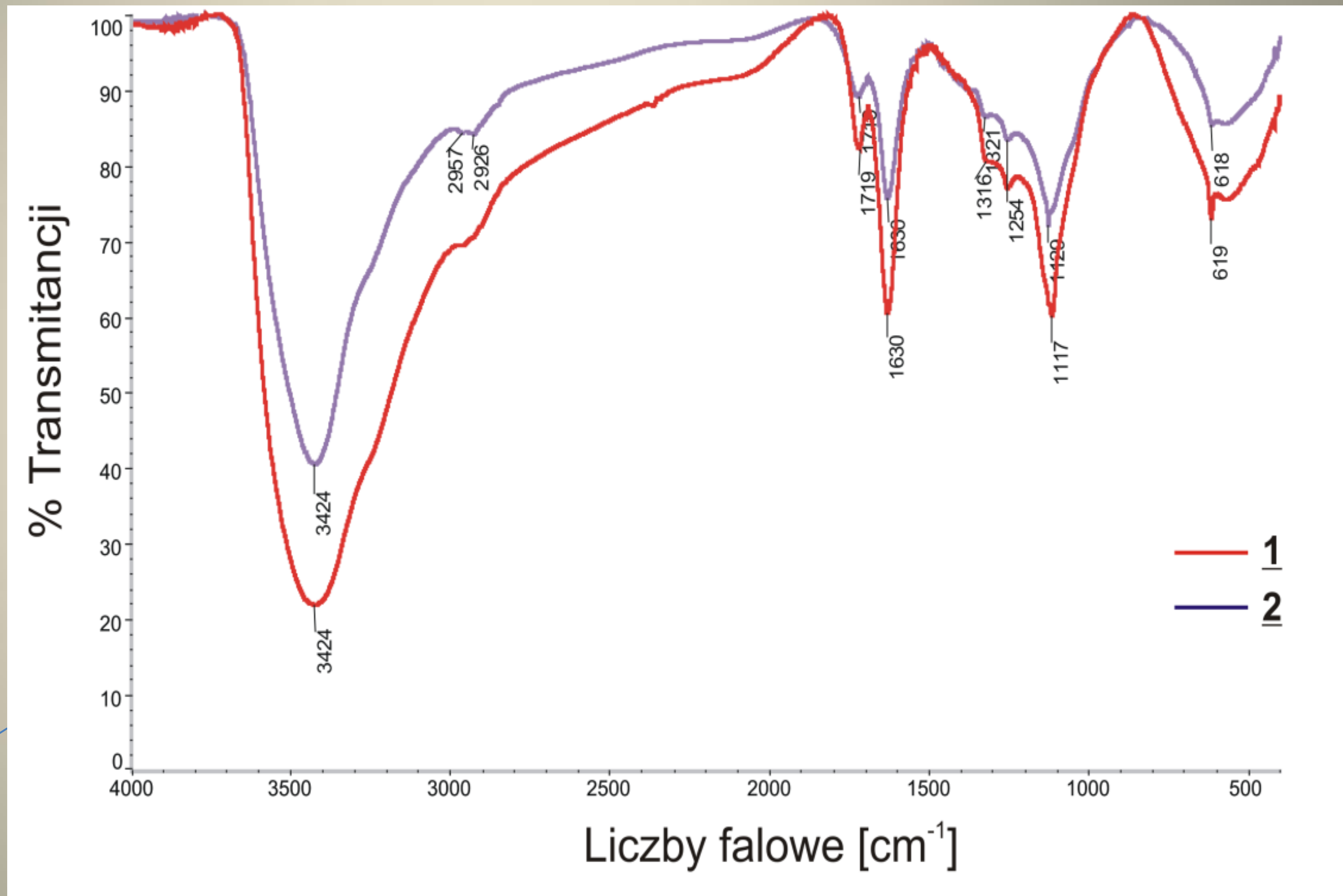
This slide shows FTIR spectra of pristine detonation nanodiamond and spectra after fenton treatment. The intensity of the band (broad) about 3423 cm^{-1} corresponding to hydroxyl groups grows and concomitant it was observed apperance of the carbon- oxygen (C-O) single bond at about 1124-1205 cm^{-1}. It is evidence of surface xydroxylation by fenton.

This slide shows FTIR spectra of the RF PACVD NDs sample before and after fenton treatment . It was observed apperance band about 2870-2954 cm^{-1} corresponing to appearing of methylene CARBON-HYDROGEN (C-H) groups.

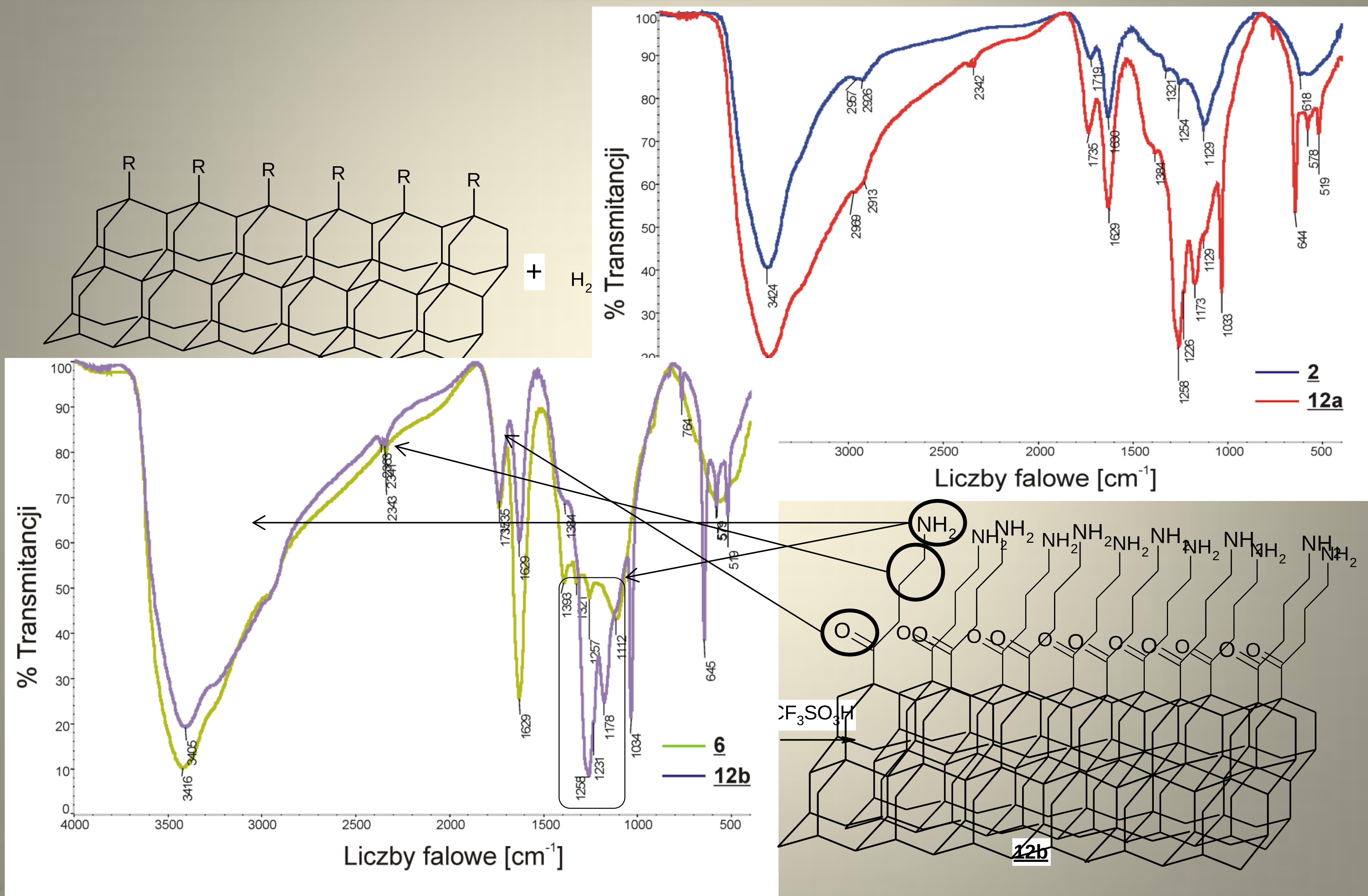
No such bands in the FTIR spectrum of the detonation powder. Since the intensity of spectras of RF PACVD powder before and after the reaction remains unaltered we state that this reaction occured much more poorly.

Purification of nanodiamond surface in ethanol

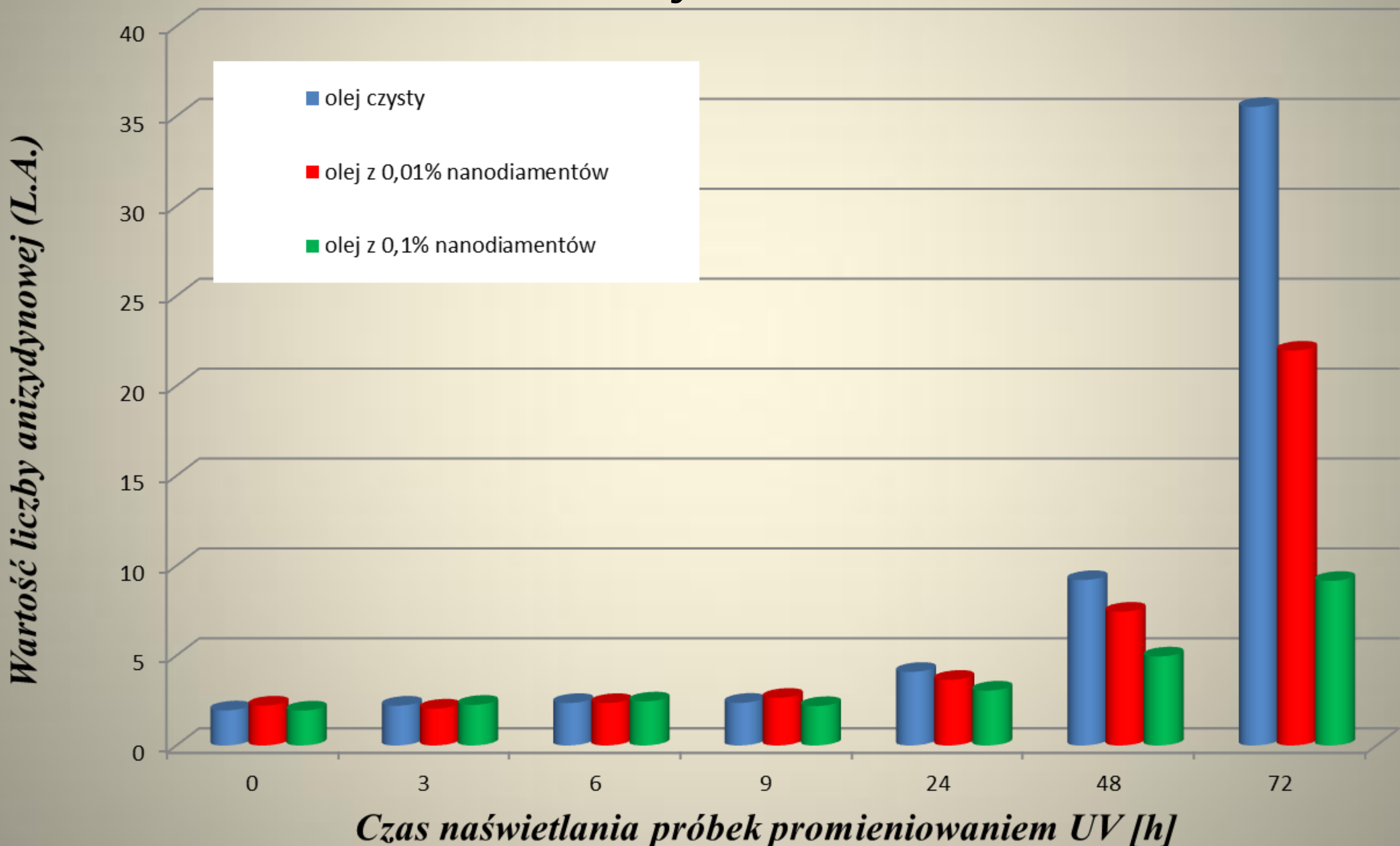




Modification of nanodiamond surface by carbon connections



Antioxidant properties of nanodiamond particles in soybean oil



Anisidine value (LA) for soybean oil UV-irradiated

Research on bacterial strains:

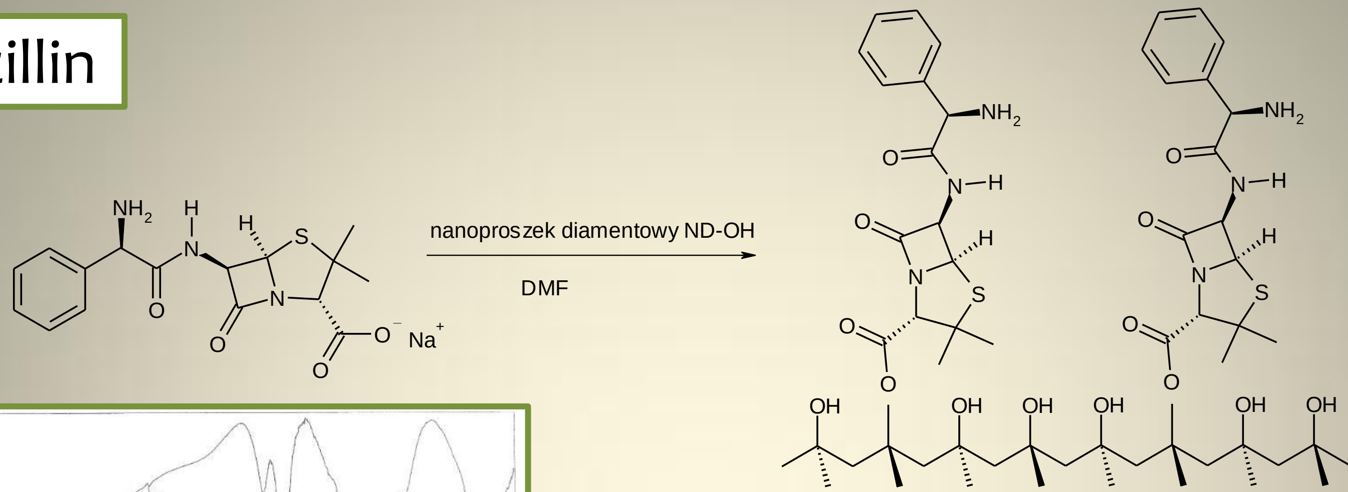
1 *Pseudomonas aeruginosa* ATCC 9027

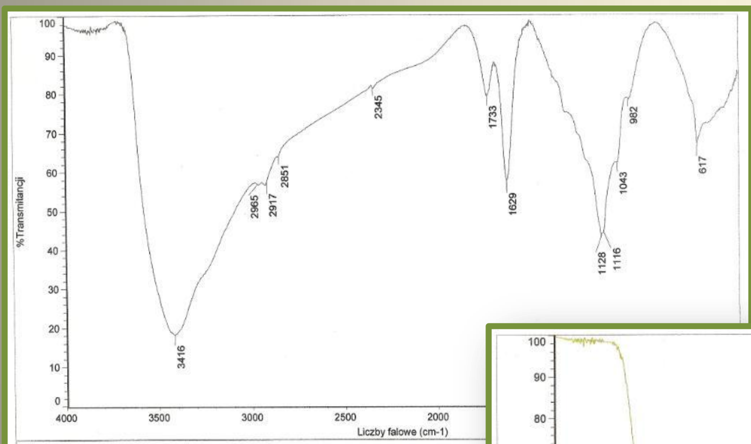
2 *Staphylococcus ureus* ATCC 6538

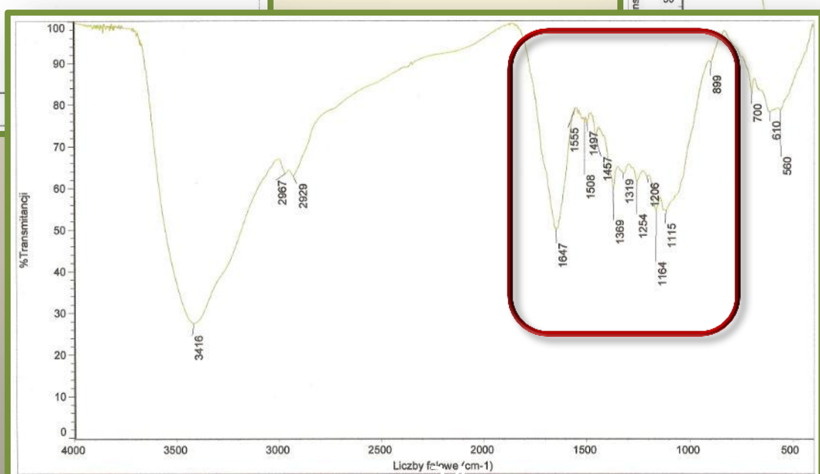
3 *Escherichia coli* ATCC 8739

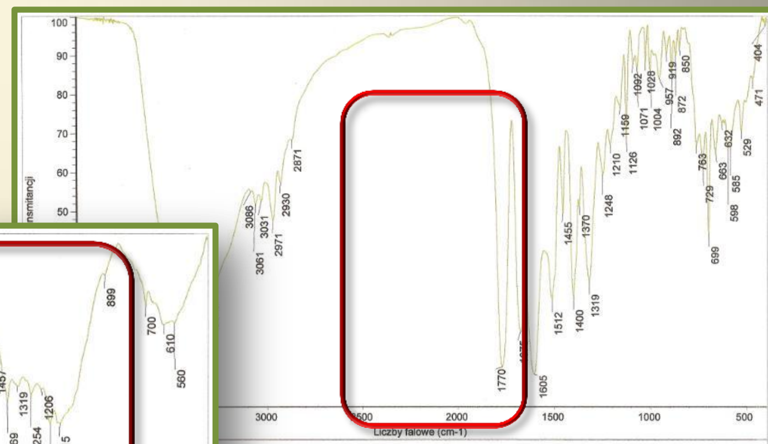
Synthesis of ampicillinum on the surfece of nanodiamond particles

ampicillin







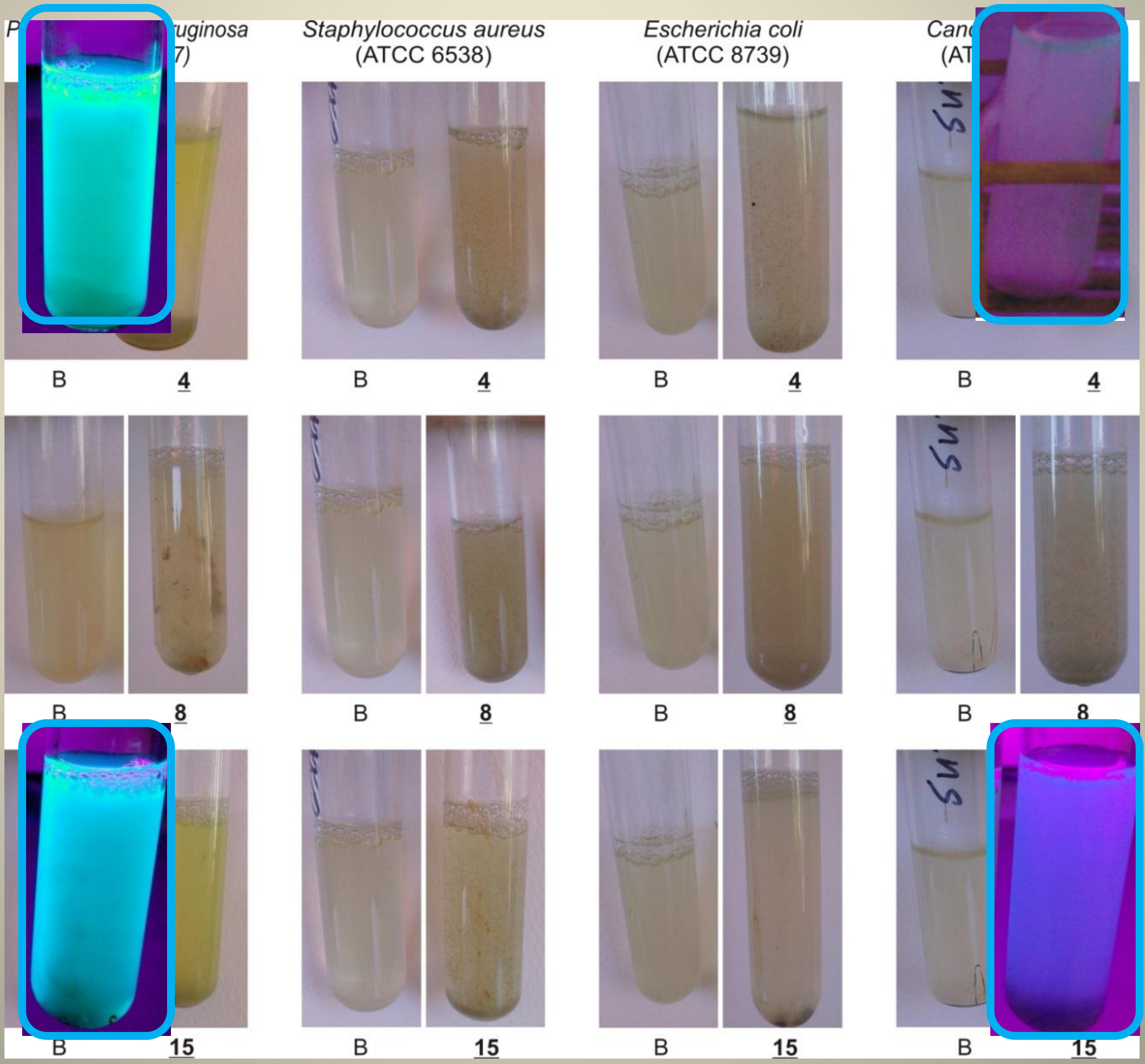


The FTIR spectrum of the modified diamond nanopowder Fenton's reaction

Fluorescence of bacteria and fungi in presence of nanodiamond particles

Kinga Adach „Chemical modification of nanodiamond particles manufactured by detonation method",
PhD Thesis, Lodz University of Technology, 2013.

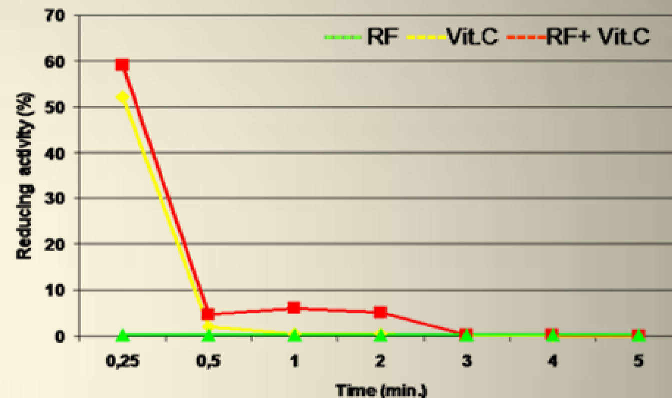
K.Mitura, I.Gisterek, P.Ceynowa, A.Wachowicz, G.Pich, R.Woś, K.Adach: *Badania mikrobiologiczne bioaktywnych proszków nanodiamentowych modyfikowanych chemicznie*, in: ed. Lucyna Leniowska, Zbigniew Nawrat ; **Postępy inżynierii biomedycznej**, Wyd. Uniwersytetu Rzeszowskiego, (2013), 157-167. ISBN 978-83-63151-02-7.



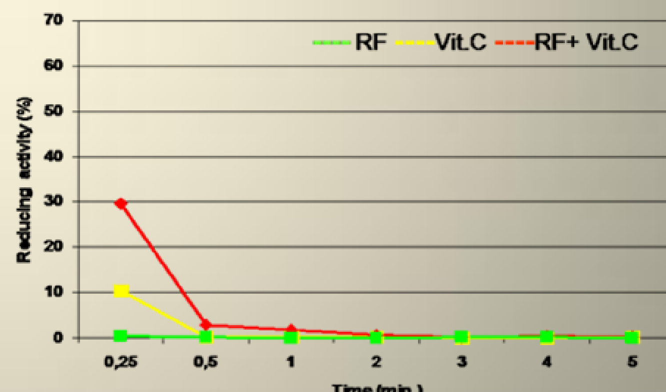
Why carbon powder RF PACVD

Reduction of vitamin C by a DPPH a)without boiling b)after boiling

In the experiment (in vitro)
assessed the effect of powder
carbon, obtained by RF PACVD
the reducing power of ascorbic acid (vitamin C).
In the experiment was used as an oxidizing agent
DPPH radical.
DPPH Test showed that the reactions catalyzed carbon
with ascorbic acid.
After 15 seconds incubation with DPPH radical,
coal increases the activity of vit. C.
High temperatures reduced the activity of reducing
Vitamin C from 50% to 10%, and the carbon powder in
the presence of only 30%.



a)



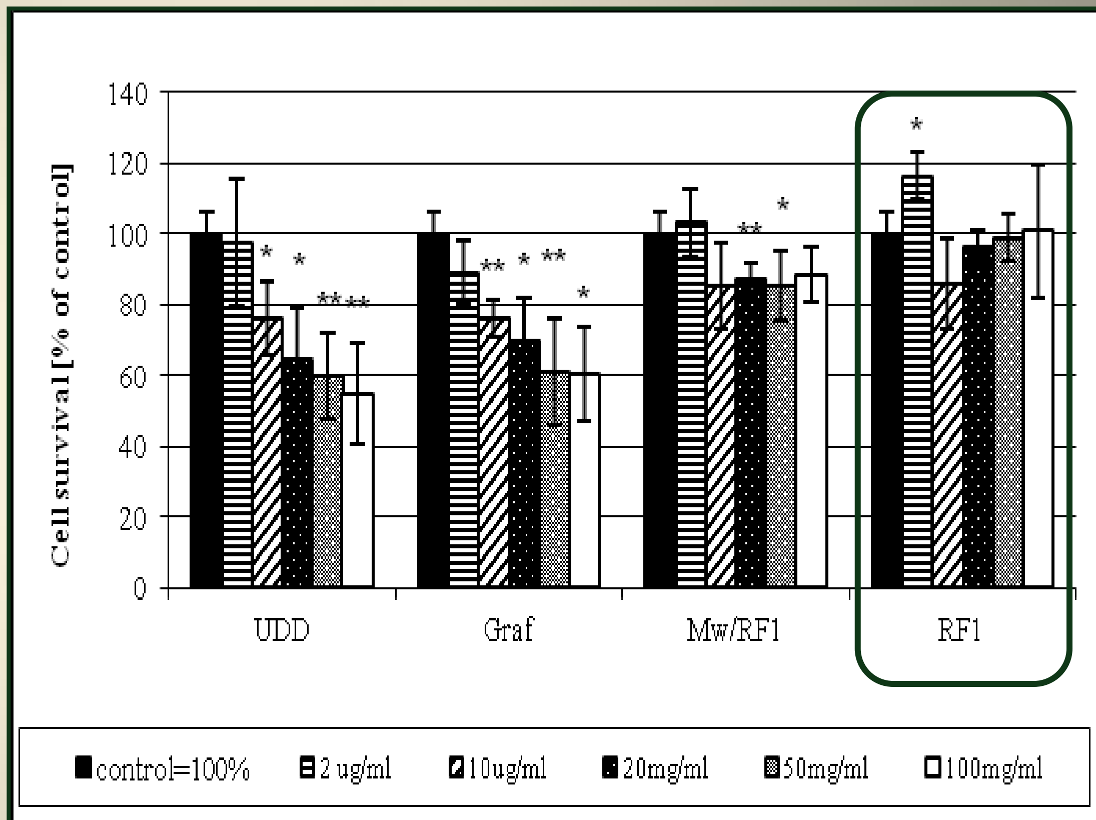
b)

- TOMASZ NIEMIEC, MACIEJ SZMIDT, ANNA KRUK-ROSZKOWSKA, EWA SAWOSZ-CHWALIBÓG , KATARZYNA MITURA: Carbon synthetized by RF PACVD method enhances the activity of antioxidants, Annals of Warsaw University of Life Sciences-SGGW. Animal Science, vol. 52 (2013).
- K. Mitura, M. Szmidt, J. Sikorska, E. Sawosz, A. Kruk, M. Grodzik, T. Niemiec, Carbon Synthetized by Chemical Vapor Deposition Method Enhances the Activity of Antioxidants, Abstract Book, 28 June - 2 July, 2010, Zakopane, Poland

Why carbon powder RF PACVD

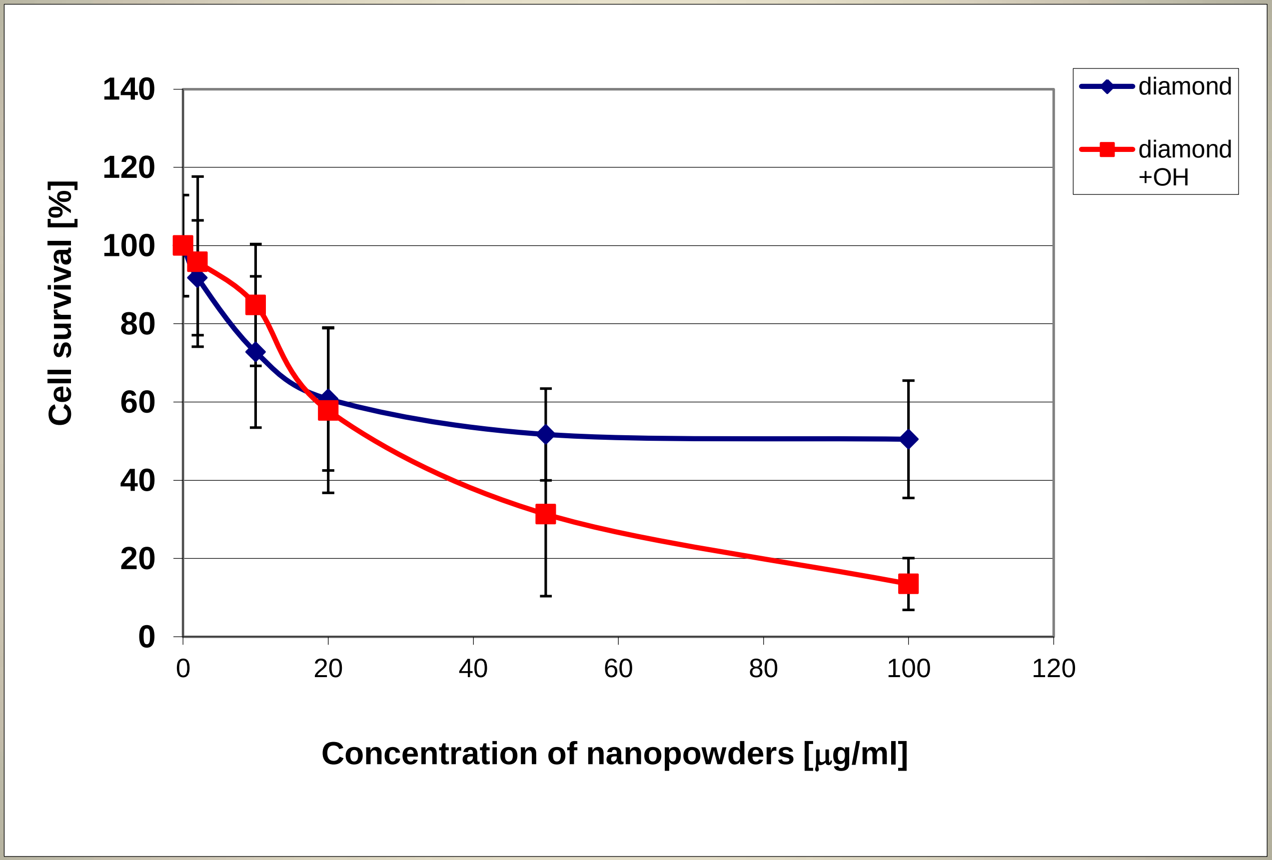
Spectrophotometry with MTT test

The results of the study of carbon powder RF PACVD on HUVEC in comparison with other powders



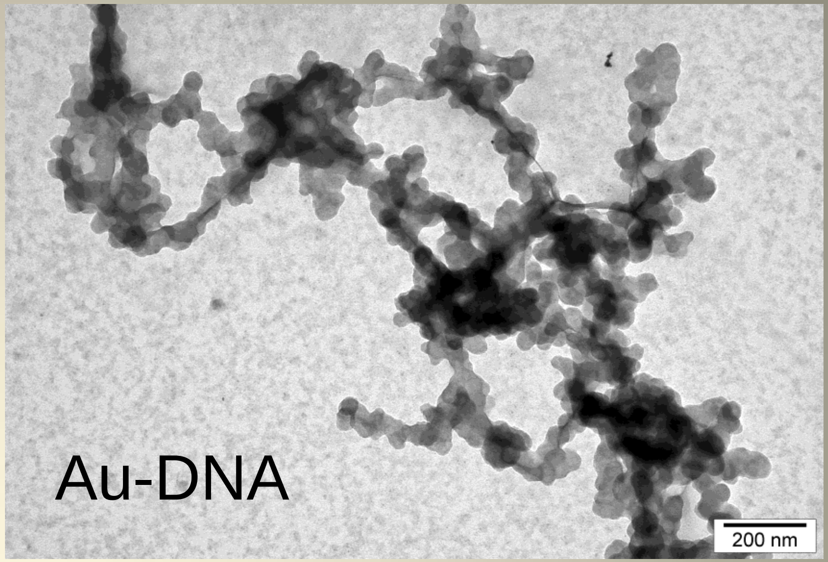
K. Solarska, A. Gajewska, W. Kaczorowski, G. Bartosz, K. Mitura, Effect of nanodiamond powders on the viability and production of reactive oxygen and nitrogen species by human endothelial cells, Diamond & Related Materials 21 (2012) 107–113

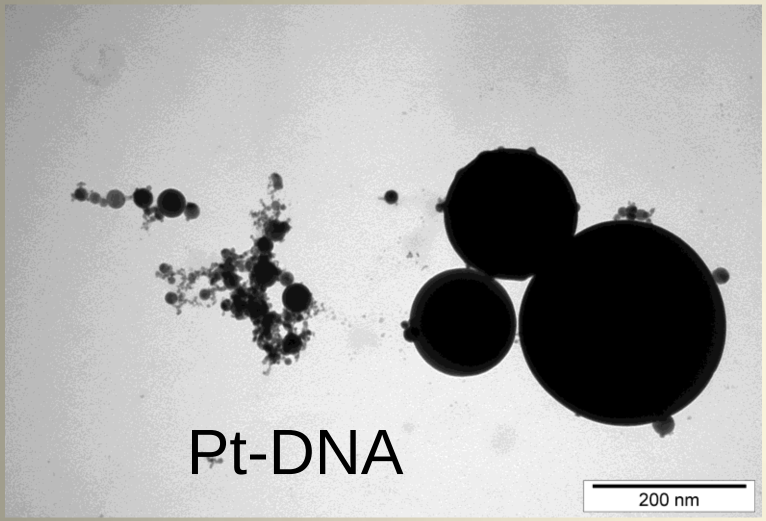
The influence of detonation nanodiamond on viability HUVEC-ST cells

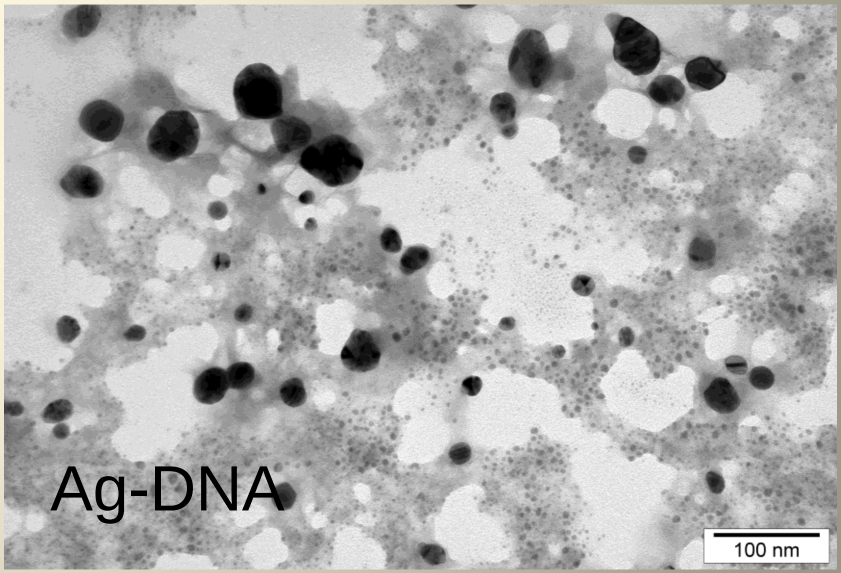


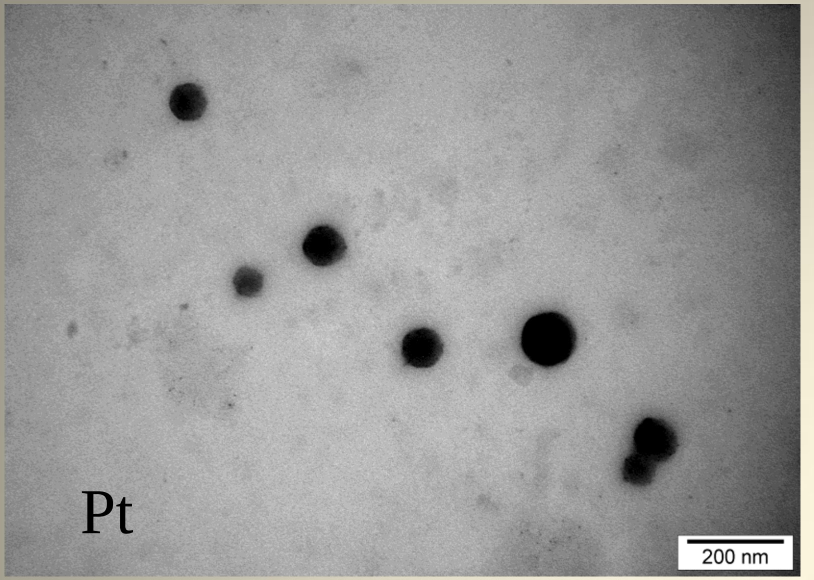
K. Solarska, A. Gajewska, W. Kaczorowski, G. Bartosz, K. Mitura: *Effect of nanodiamond powders on the viability and production of reactive oxygen*. **Diamond & Related Materials**, vol. 21, (2012), 9037-9046.

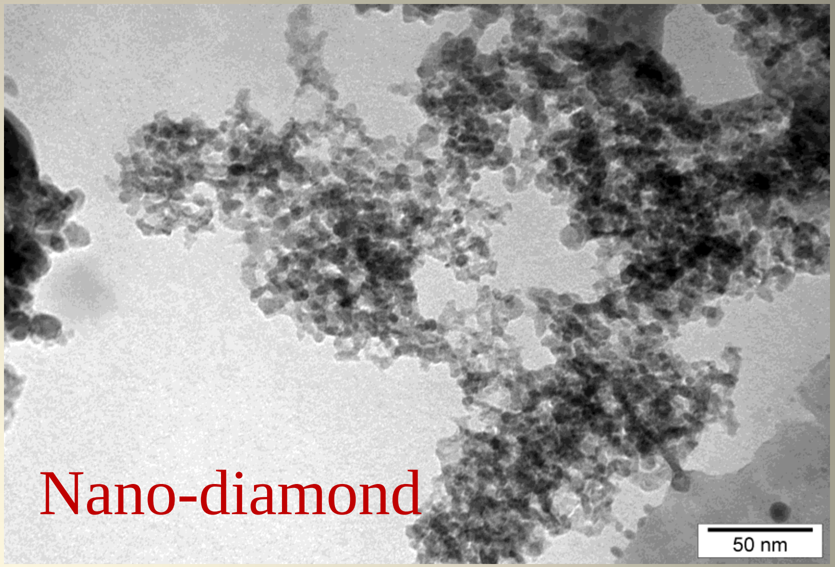
DNA
and metal nanoparticles

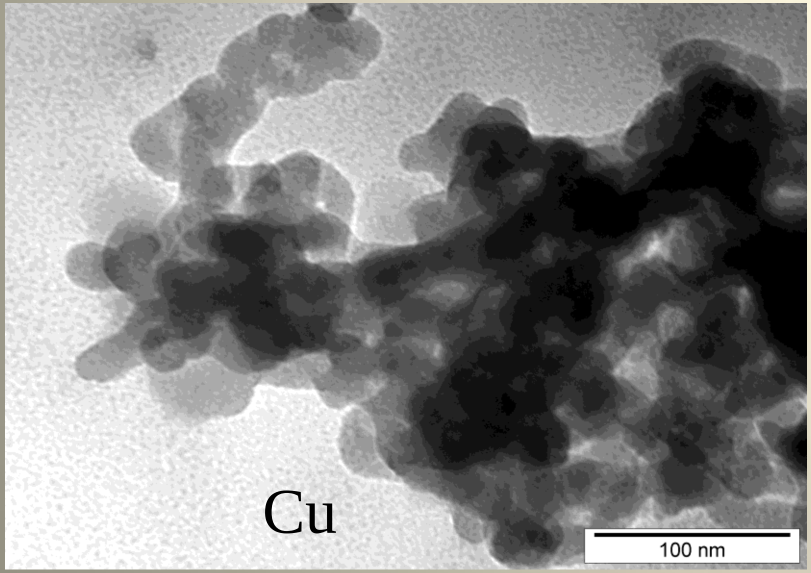


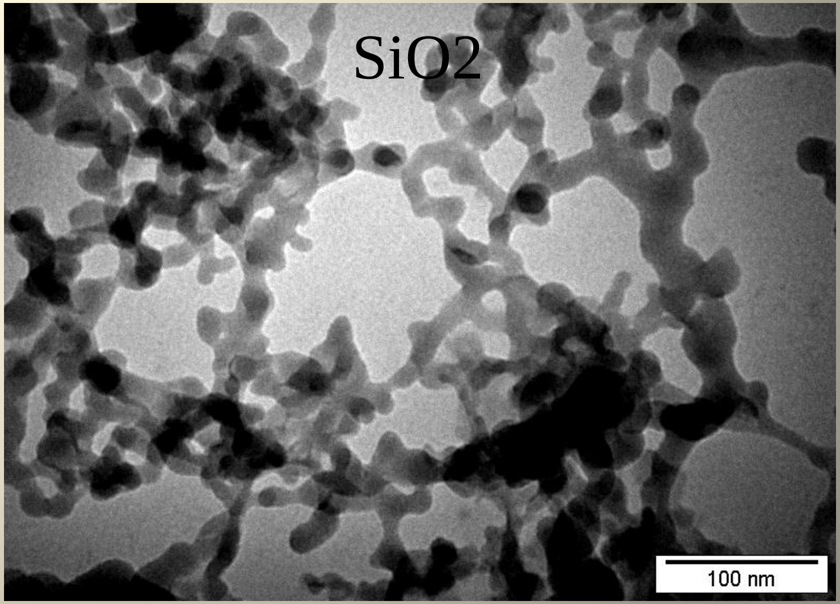





Pt
200 nm

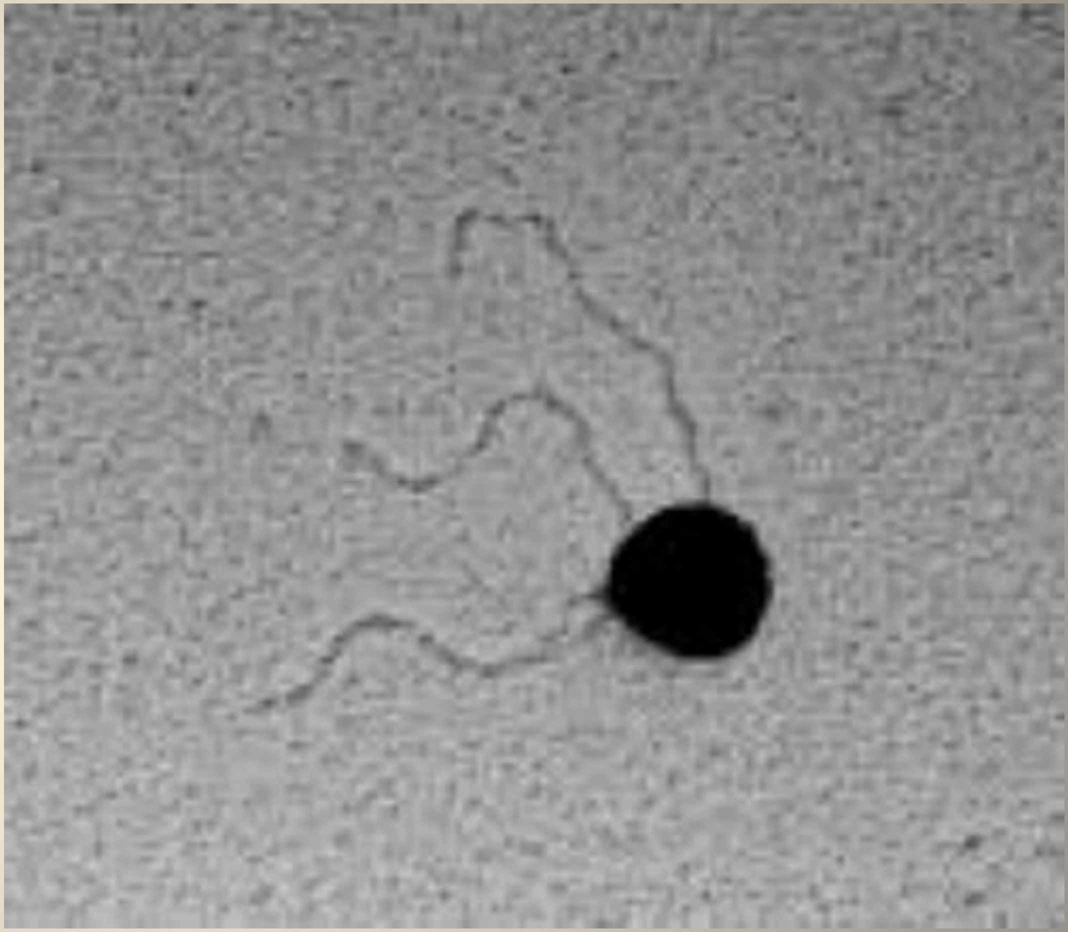

Nano-diamond
50 nm


Cu
100 nm


SiO2
100 nm

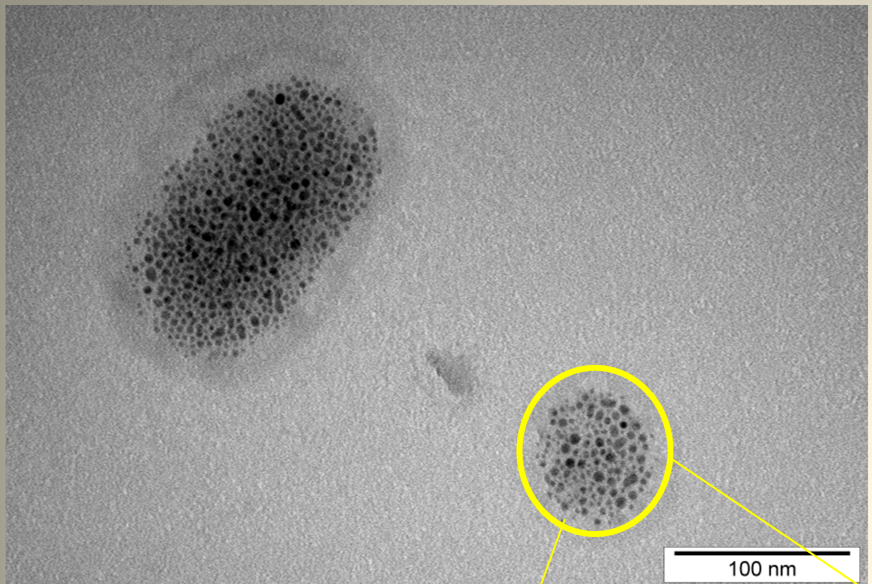
Nanocarrier of DNA

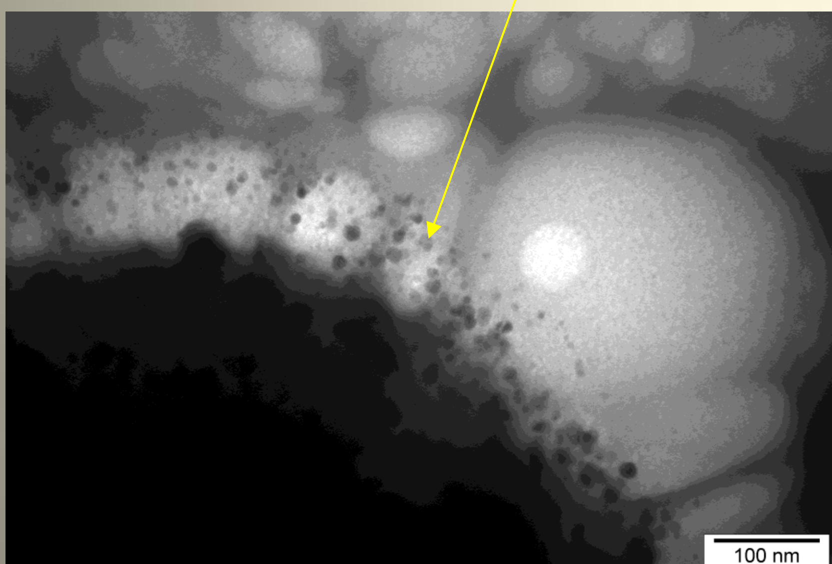
Gold nanoparticle with DNA

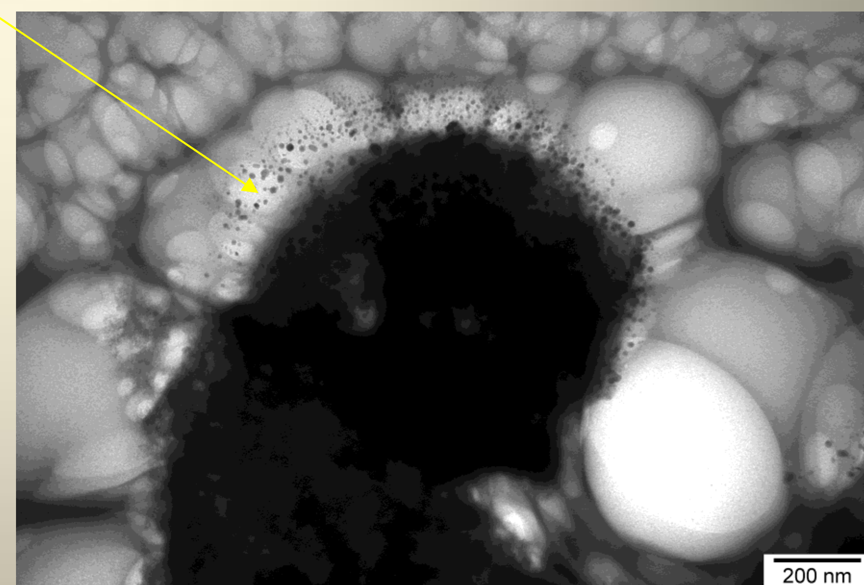


J. Griffith, 2009. Univ. of North Carolina

Varicella-Zoster Virus in presence of nanodiamond particles

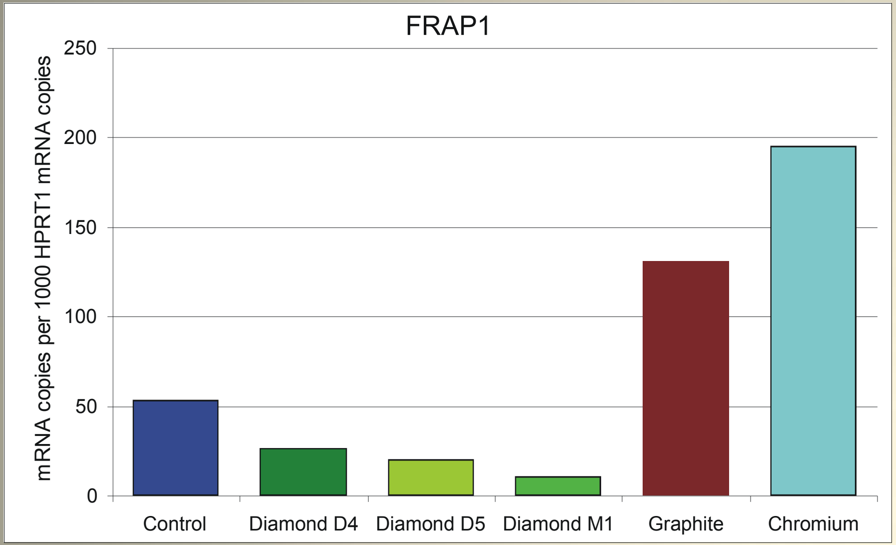


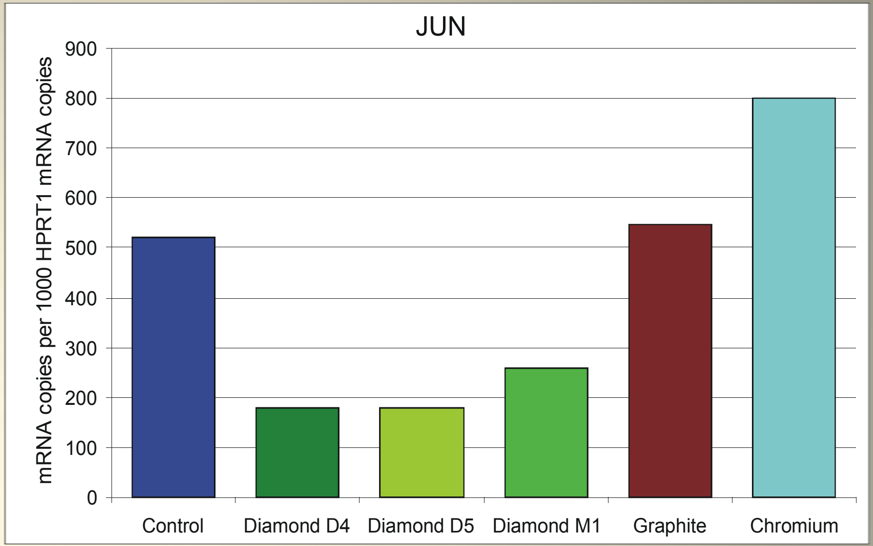




Discovery of diamond bioactivity

Human gene expression



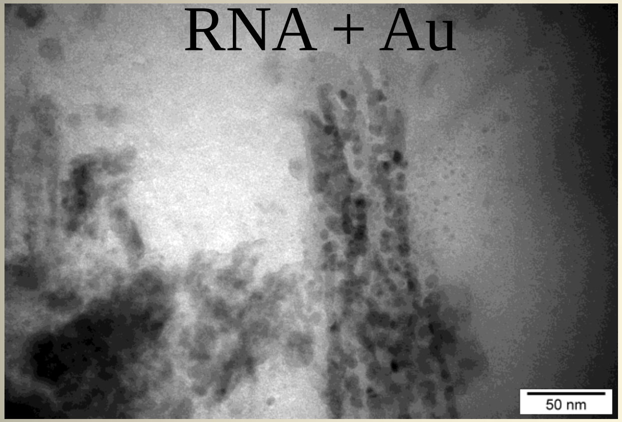


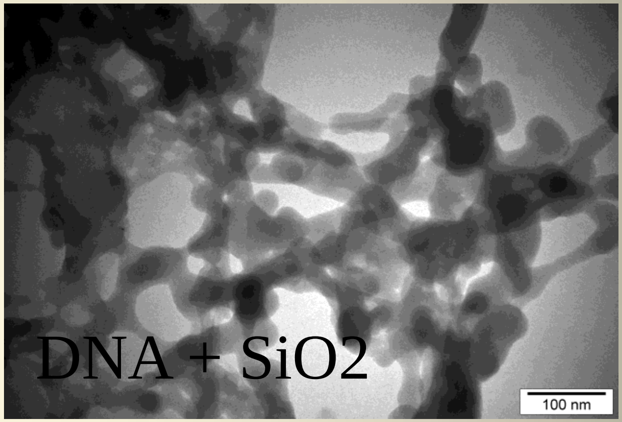
Gene name	Encoded protein	PCR product length	Main inducing stimulus
JUN	Jun activation domain binding protein	118	Cellular stress
FRAP1	FKBP-rapamycin associated protein 1	141	Genotoxic stress

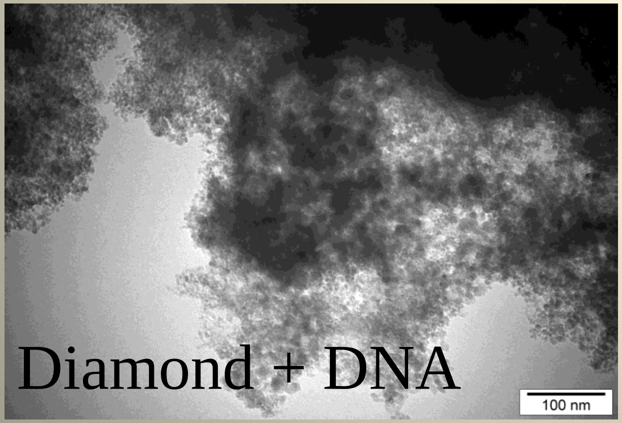
JUN – is responsible for oxidative stress
FRAP1 – is responsible for proliferation of cancer cells

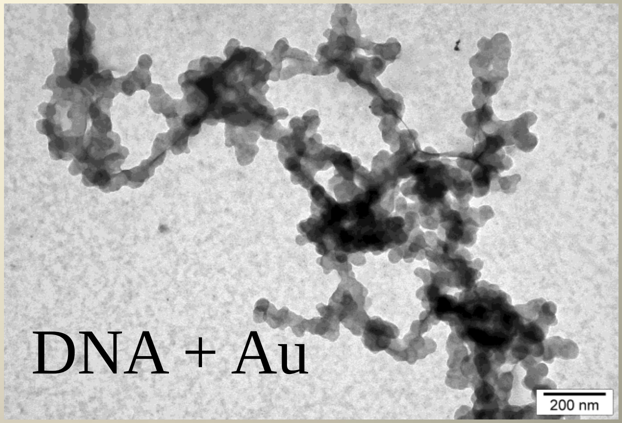
- K.Mitura, Ł.Pułaski, K.Kania, S.Mitura, G.Bartosz: „*Biocompatibility assay of nanocarbon preparations based on transcriptomic stress biomarkers”,* **Int. Conf. 4-th Nanodiamond and Related Materials jointly with 6-th Diamond and Related Films, Zakopane**, **Abstract Book, (**2005**),** 165.
- Katarzyna Bakowicz–Mitura, Grzegorz Bartosz, Stanislaw Mitura: *”Influence of diamond powder particles on human gene expresion”,* **Surface and Coatings Technology**, 201, (2007) 6131-6135.

DNA and RNA and nanopowders



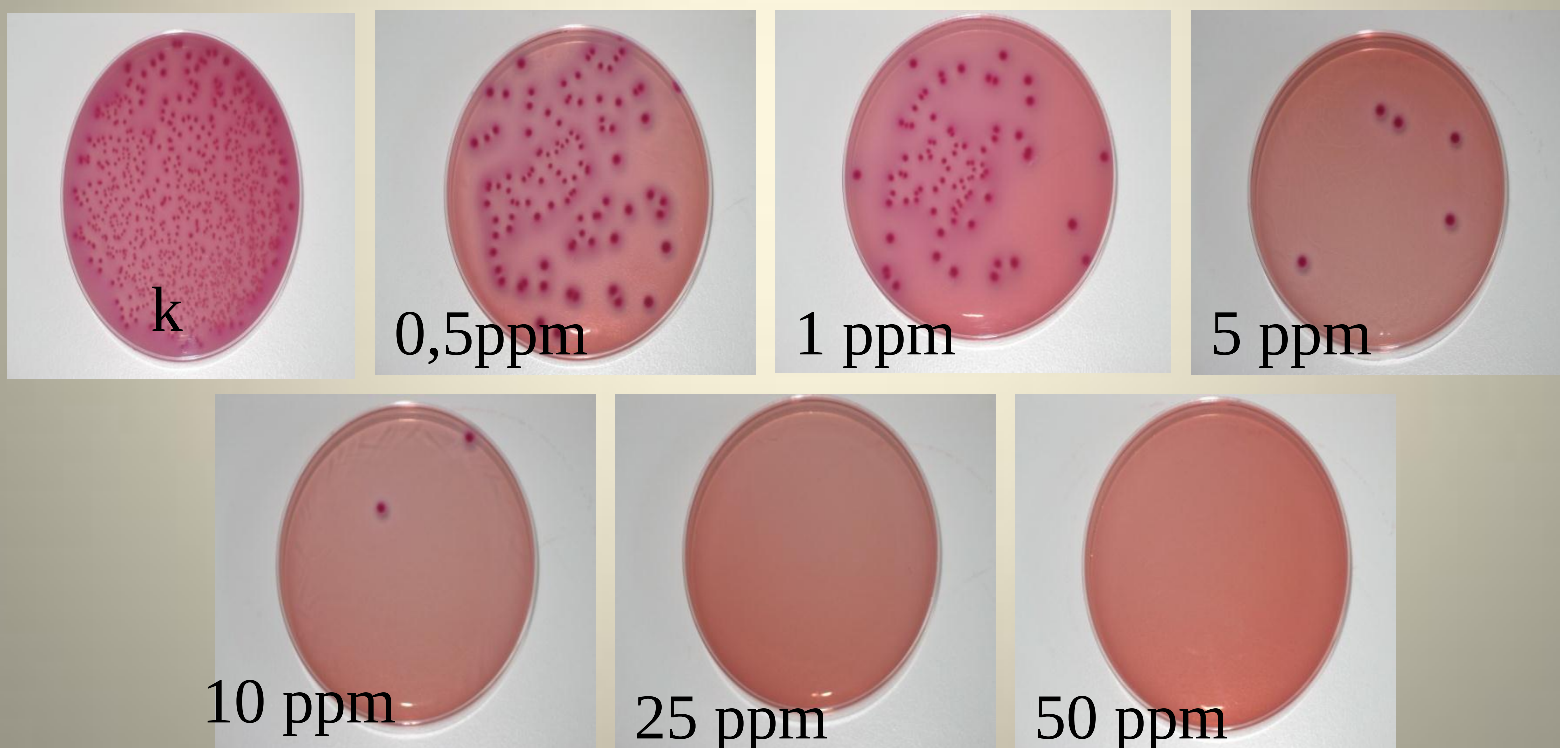




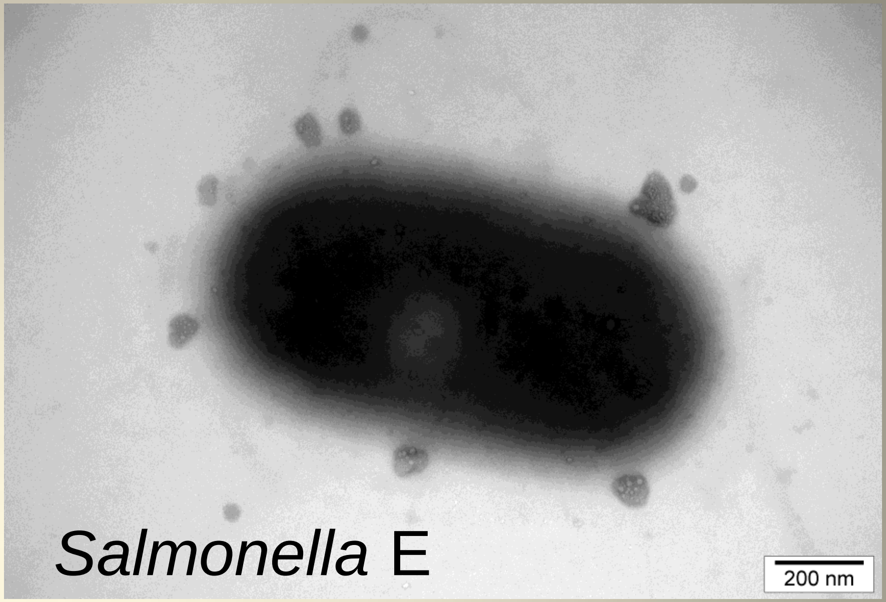


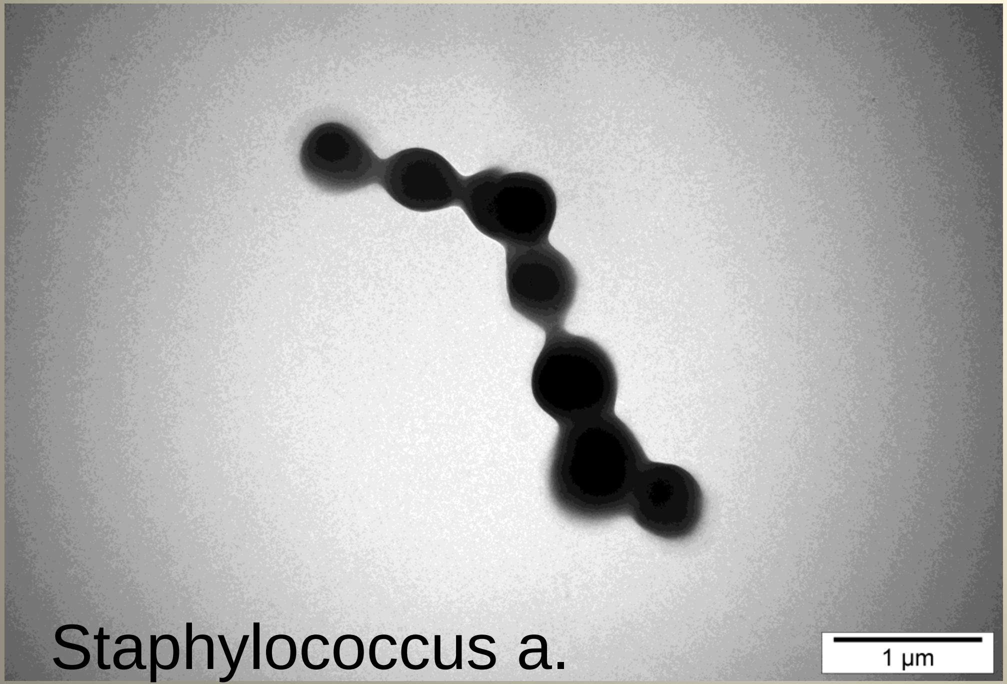
Bacteria and nanoparticles

Antibacterial properties of
Ag + Salmonella E and Escherichia colioli



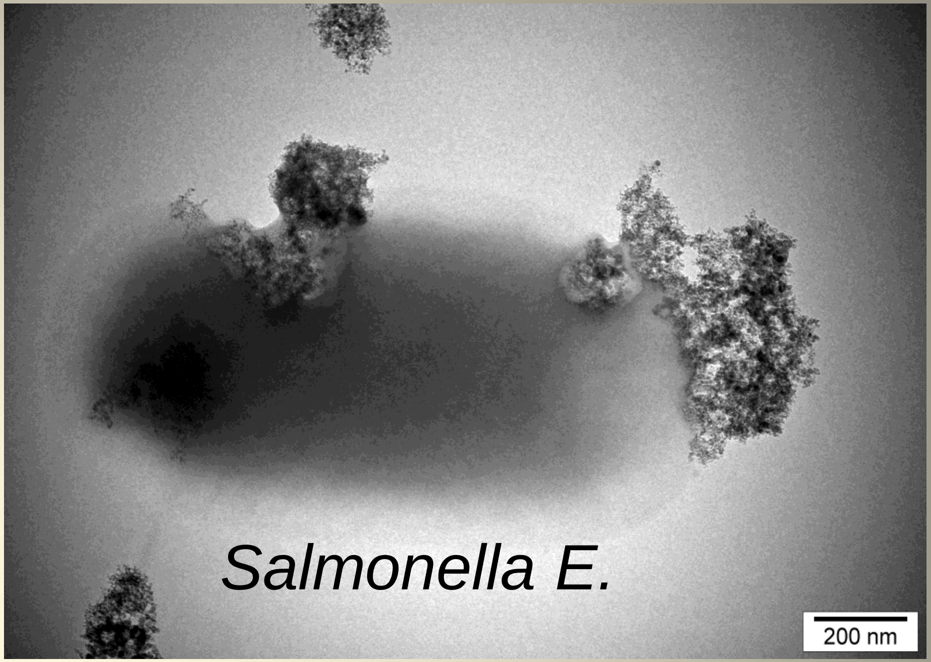
Microorganisms

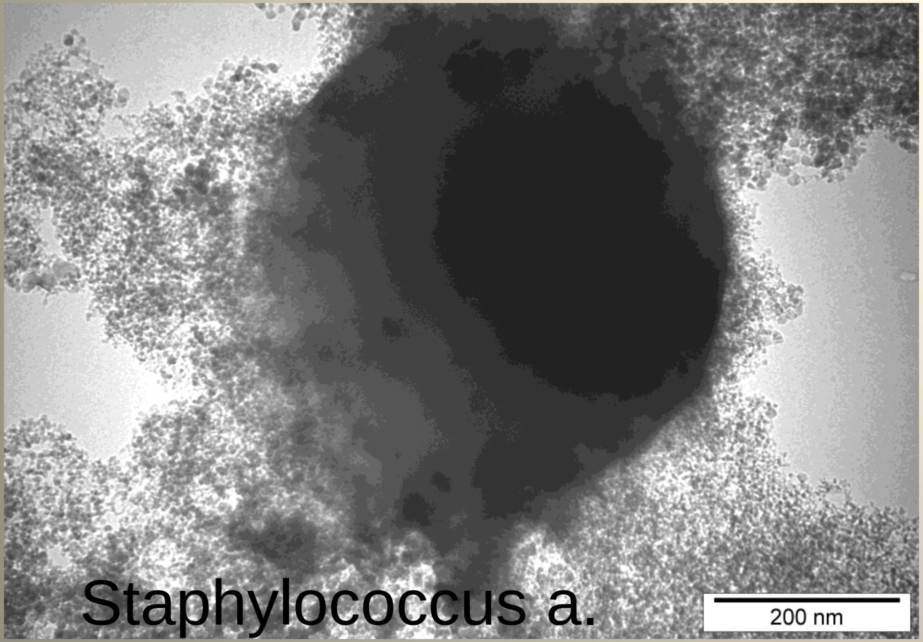


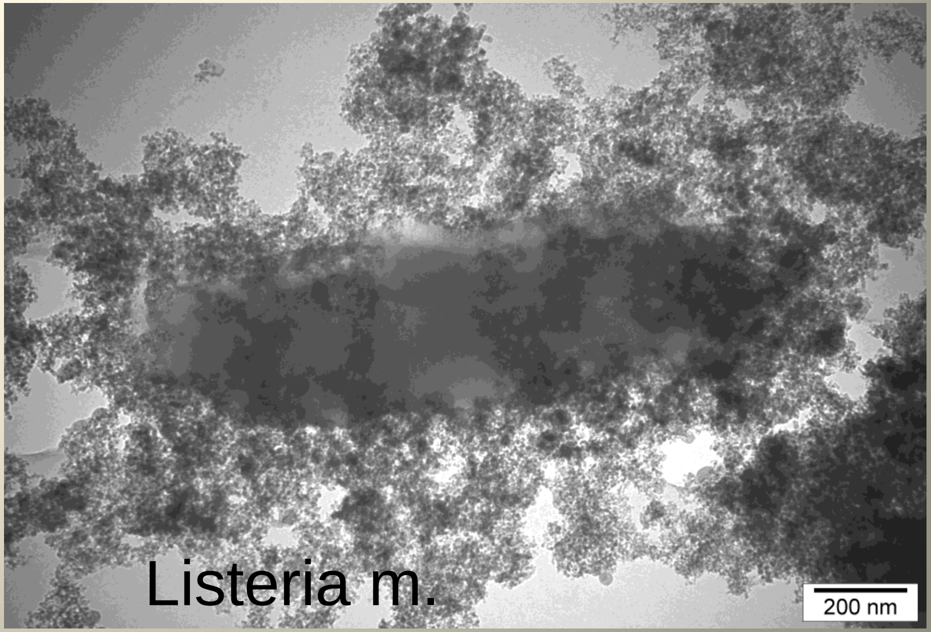


Nano-Diamond

Sawosz E., Chwalibog A., Mitura K, Mitura S., Szeliga J., Niemiec T., Rupiewicz M., Grodzik M, Sokołowska A.: *Visualisation of morphological interaction of diamond and silver nanoparticles with Salmonella enteritidis and Listeria monocytogenes*. **(**project 357/ERA-NET/2008**), Journal of Nanoscience and Nanotechnology, 11, (2011), 1-7.**

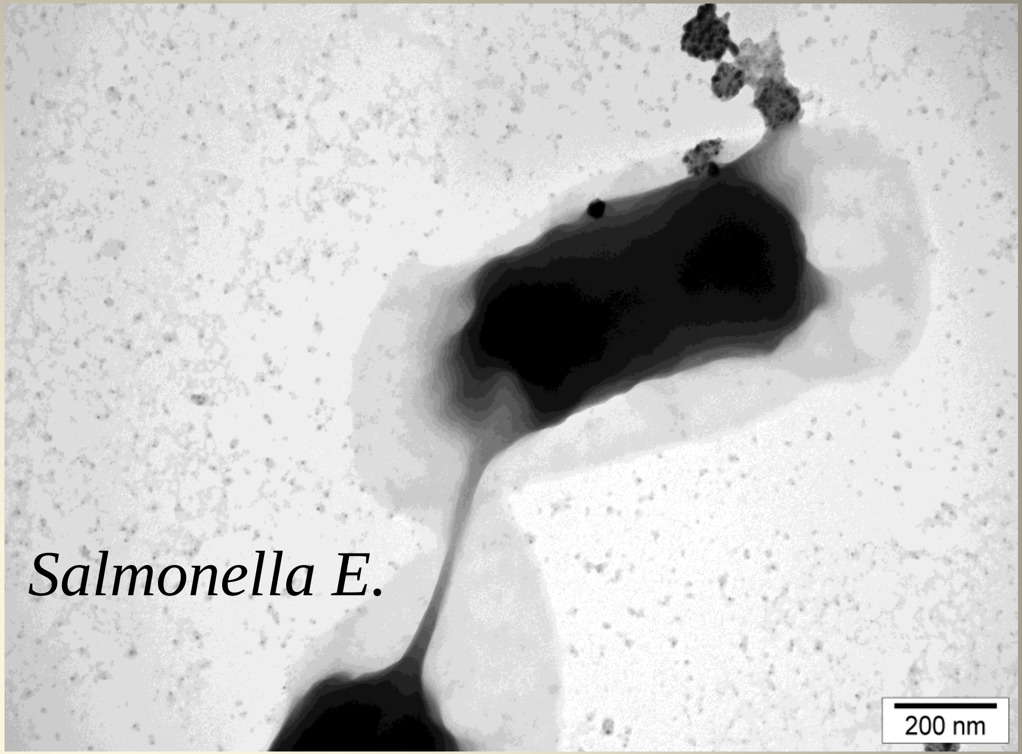


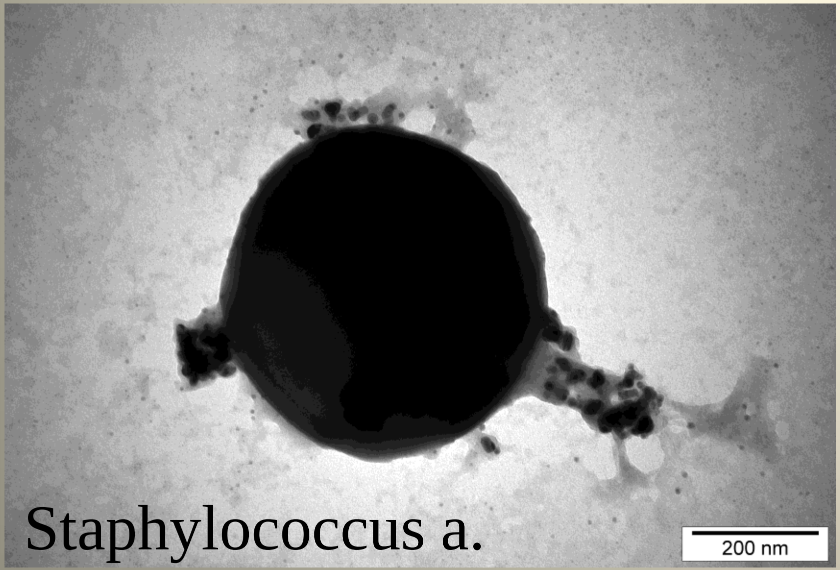


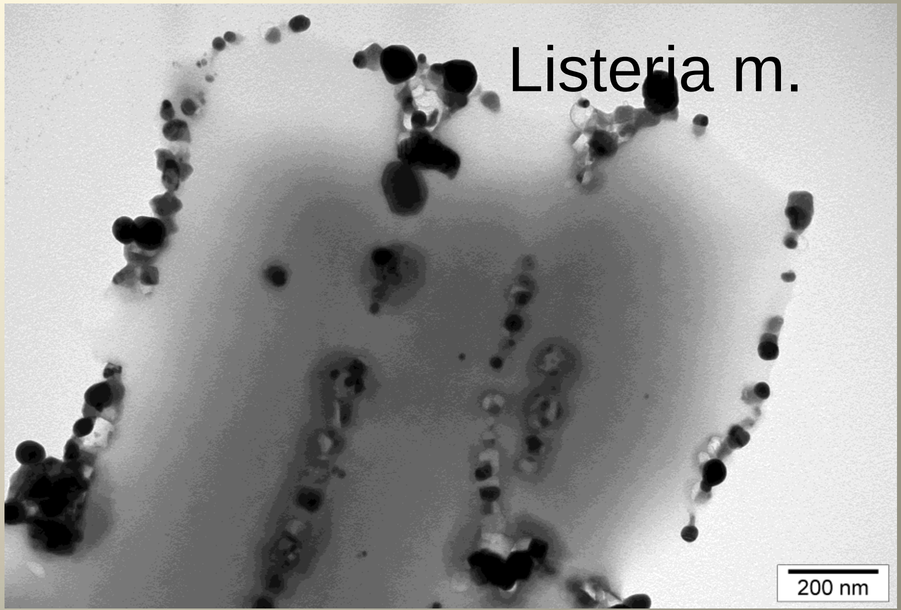


Ag

Sawosz E, Chwalibog A, Mitura K, Mitura S, Szeliga J, Niemiec T, Rupiewicz M, Grodzik M, Sokołowska A.: *Visualisation of morphological interaction of diamond and silver nanoparticles with Salmonella enteritidis and Listeria monocytogenes*. **(**project 357/ERA-NET/2008**), Journal of Nanoscience and Nanotechnology, 11, (2011), 1-7.**







Au

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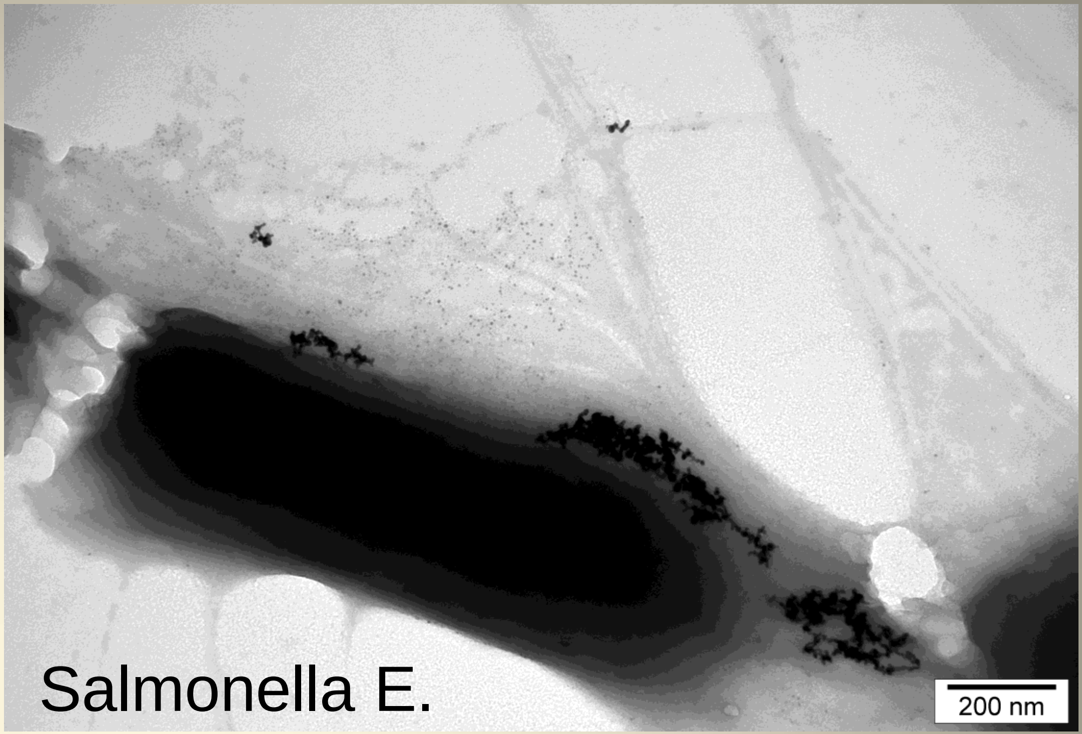
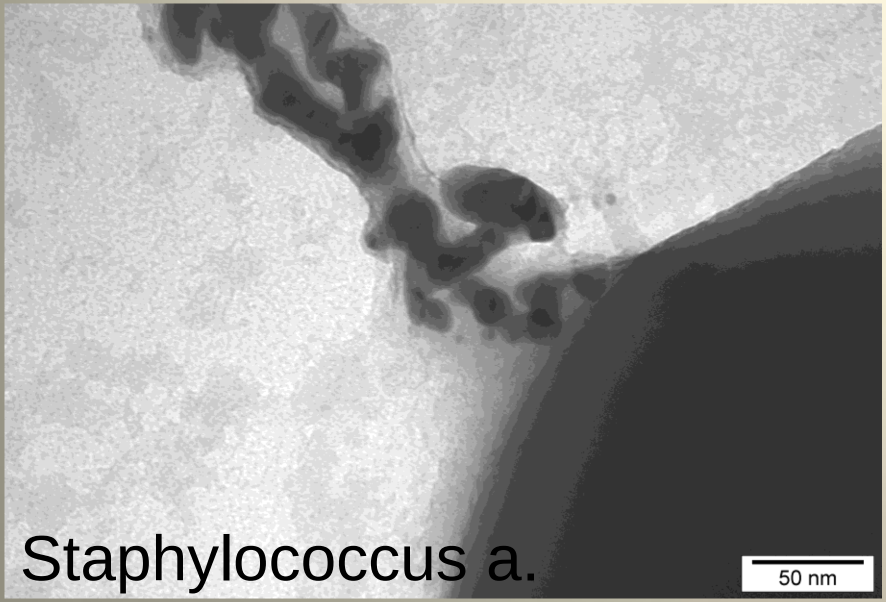
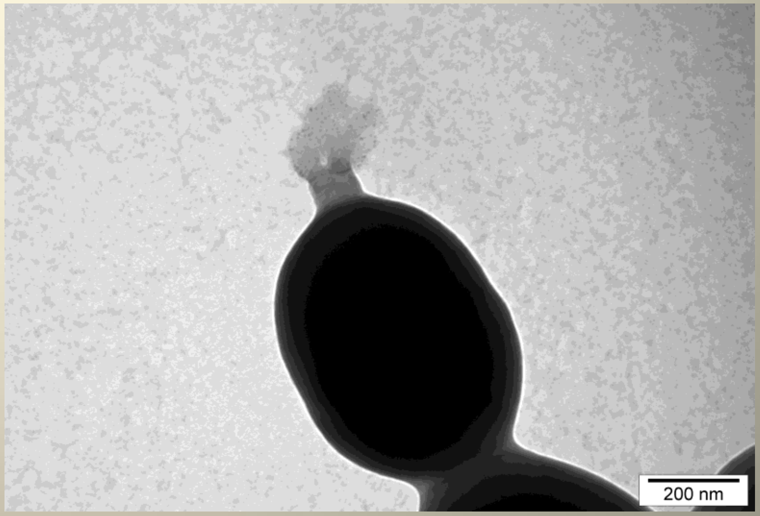
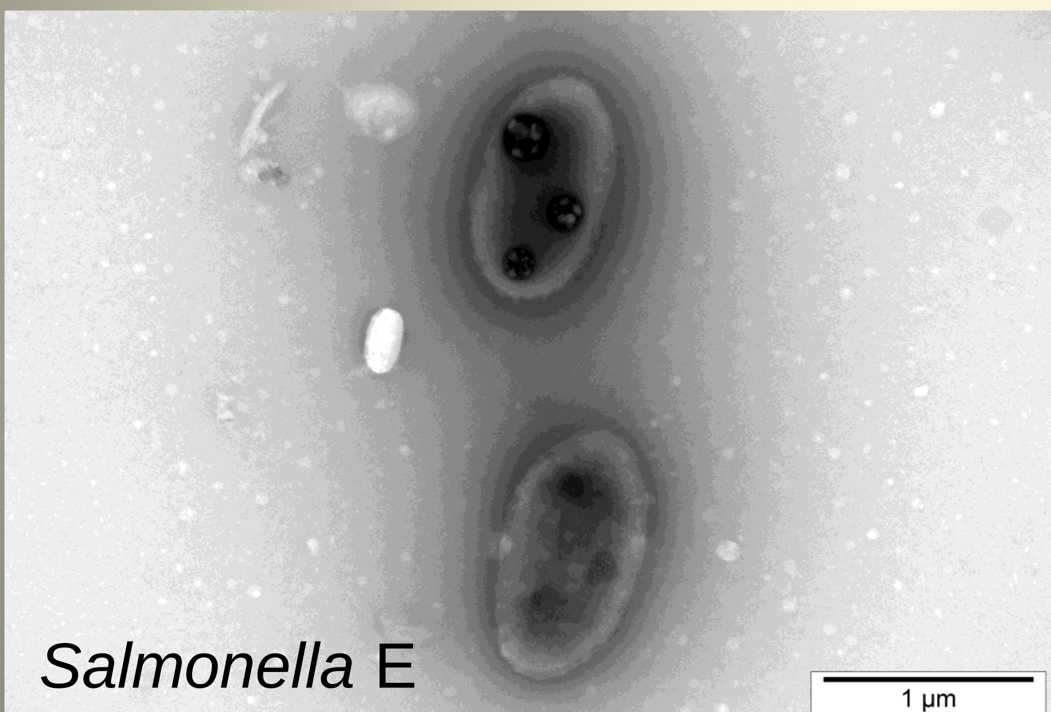




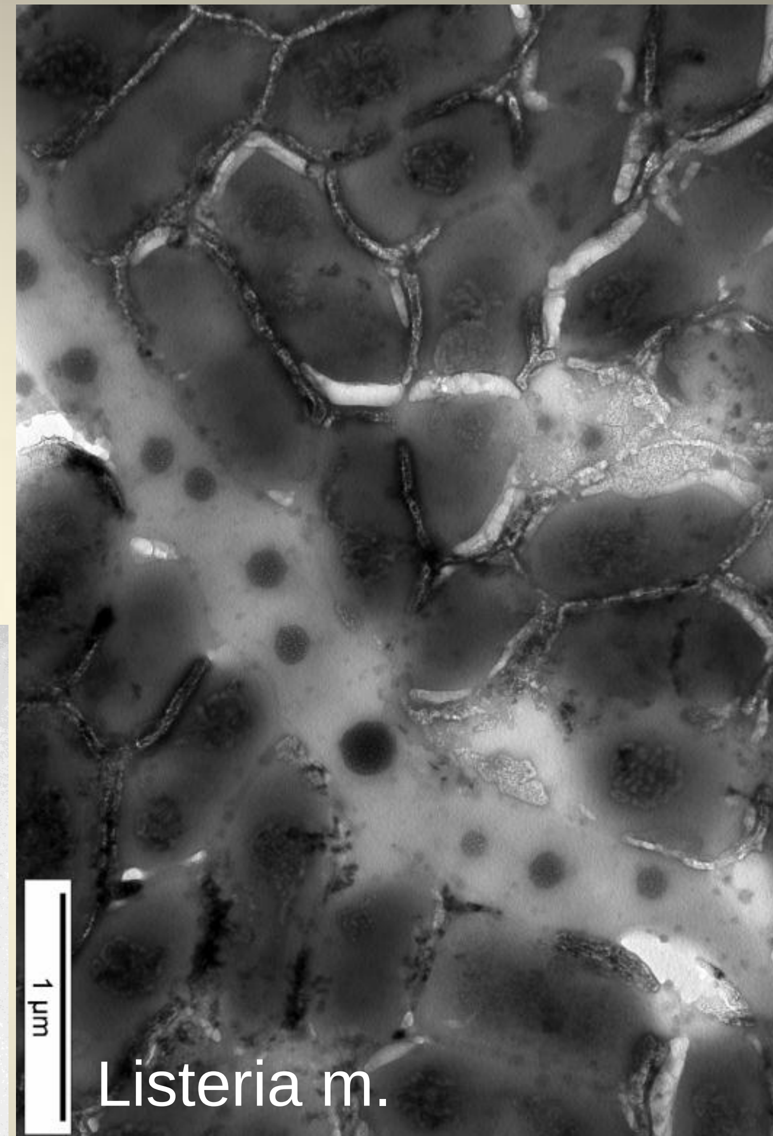


Pt

Sawosz E., Chwalibog A., Mitura K, Mitura S., Szeliga J., Niemiec T., Rupiewicz M., Grodzik M, Sokołowska A.: *Visualisation of morphological interaction of diamond and silver nanoparticles with Salmonella enteritidis and Listeria monocytogenes*. (project 357/ERA-NET/2008**), Journal of Nanoscience and Nanotechnology, 11, (2011), 1-7.**



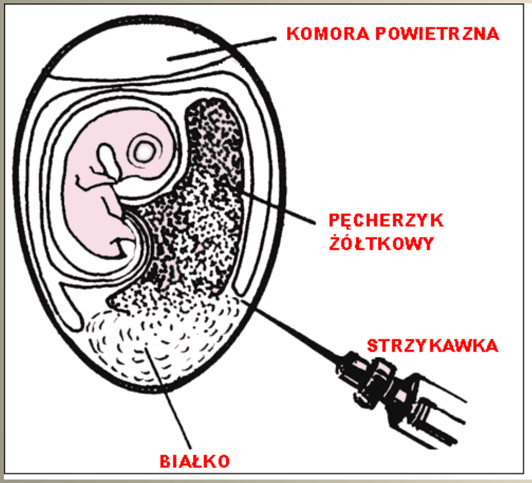
Salmonella E

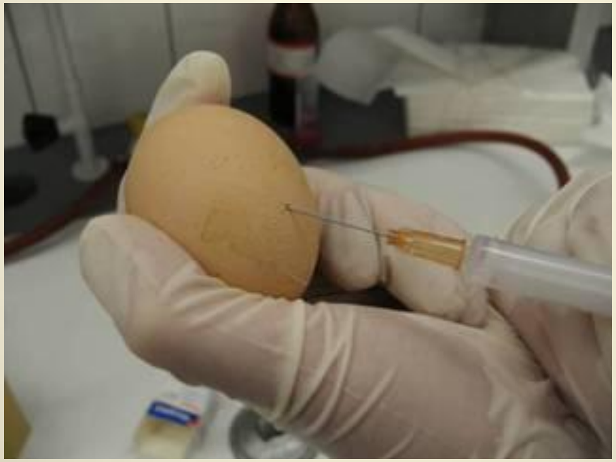


Listeria m.

In ovo examinations with nanodiamond particles

(model tests on chicken embryo)

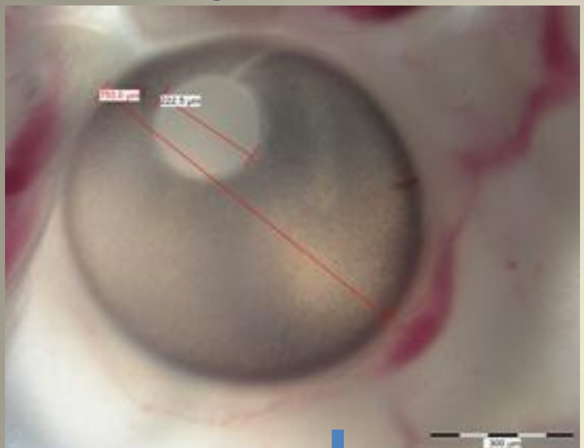




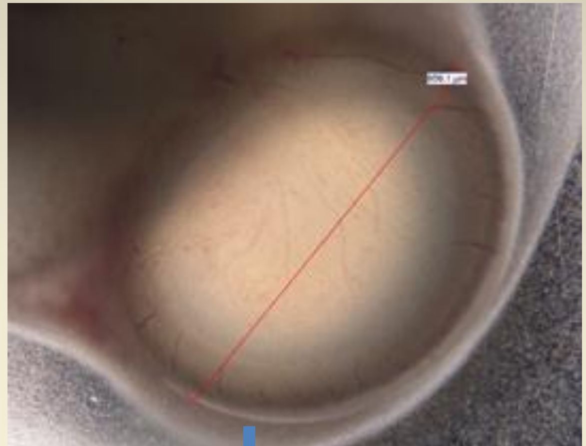
K. Mitura, S. Mitura, E. Sawosz, T. Niemiec, J. Sikorska, M. Grodzik, A. Chwalibog, M. Szmidt: Nanoparticles of diamond do not stimulate angiogenesis in chicken embryo model, NS243, in: Proc. **4th International Conference on Surfaces, Coatings and Nanostructured Materials, NANOSMAT 2009**, 19-22 Oct. Rome, p. 103.

6 day of chicken embryogenesis

eye

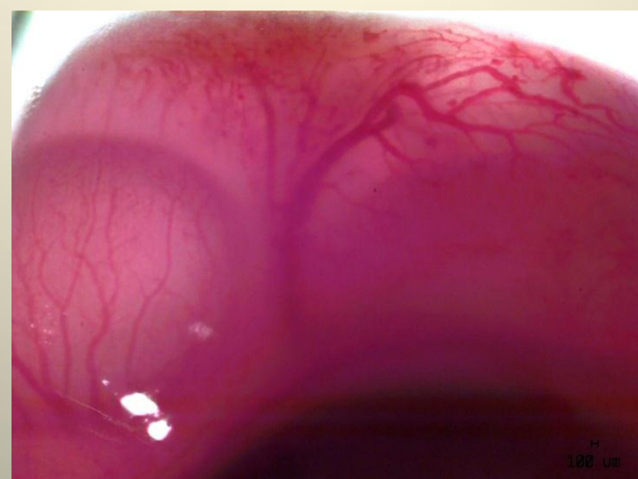


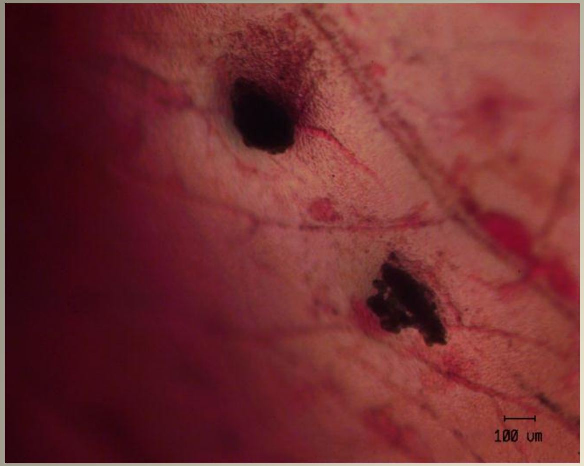
brain



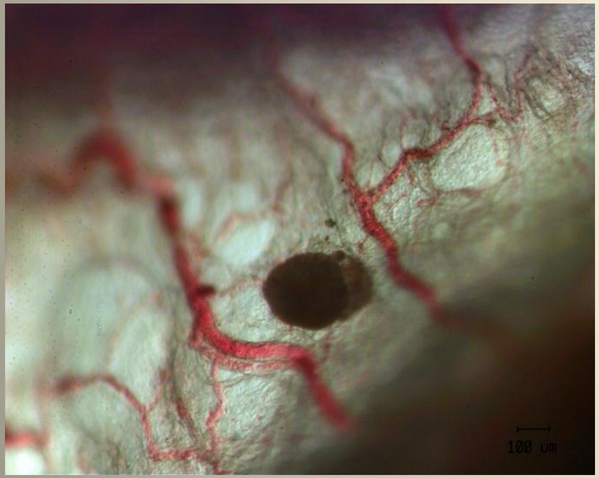
heart

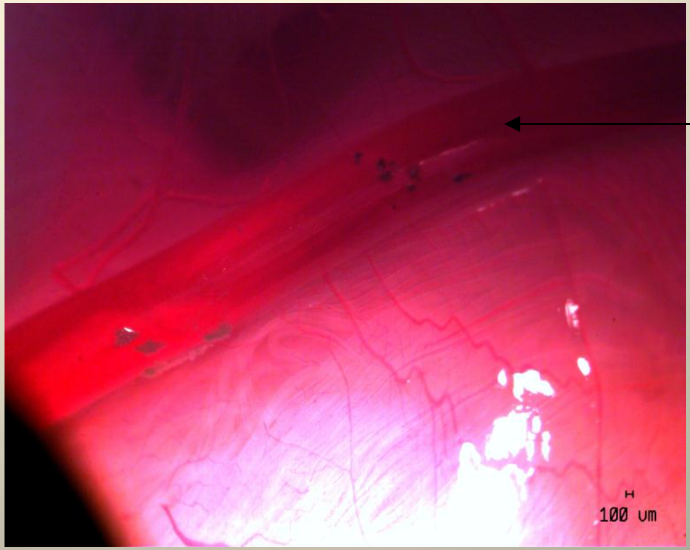
12 day of chicken embryogenesis





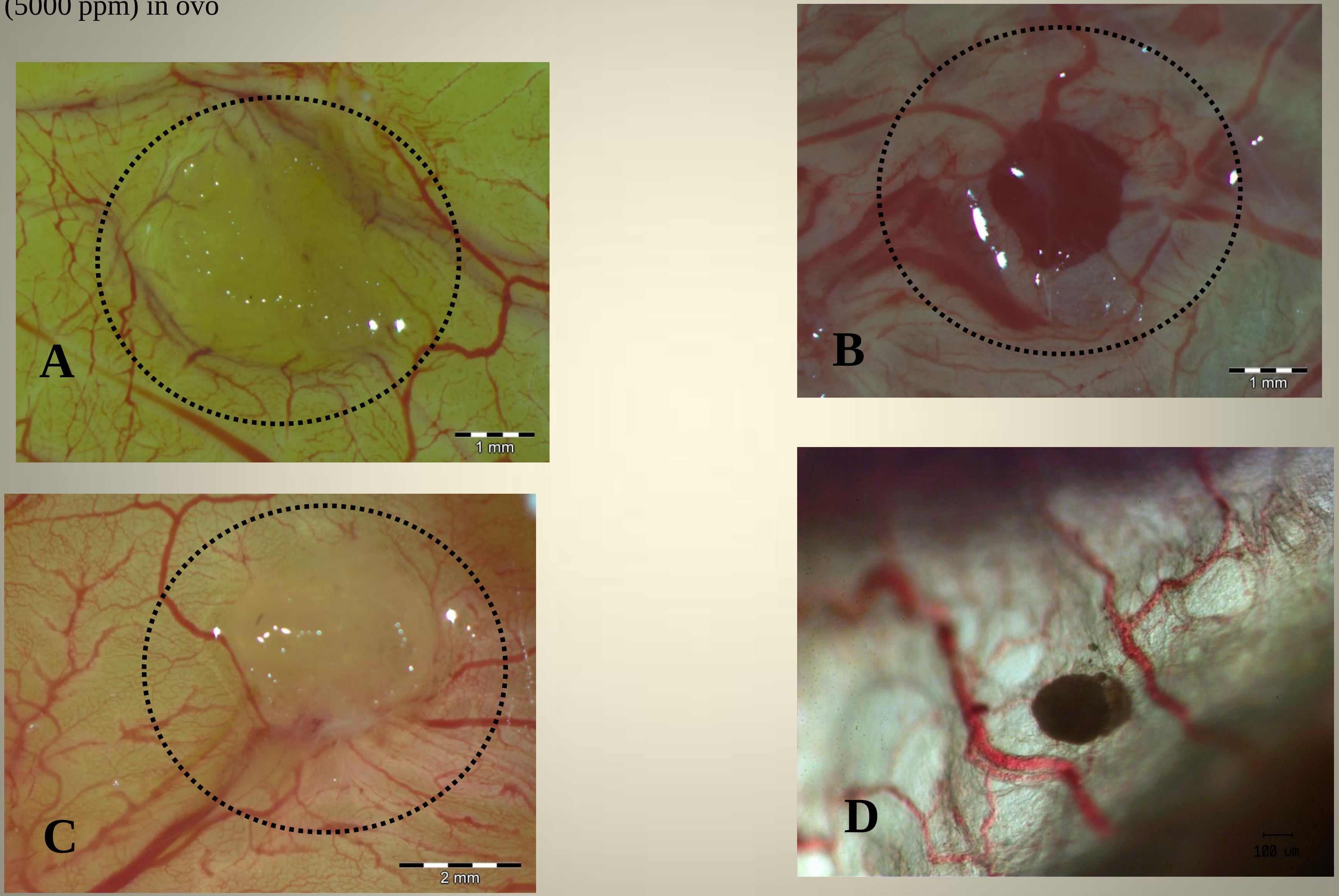
E.Sawosz, T.Niemiec, K.Mitura: Chicken embryon 12 day – vessel system nanodiamond particle introduced to embryon in the second day (300ul-50 ppm C), **NanoSMat, Rome, 2009.**



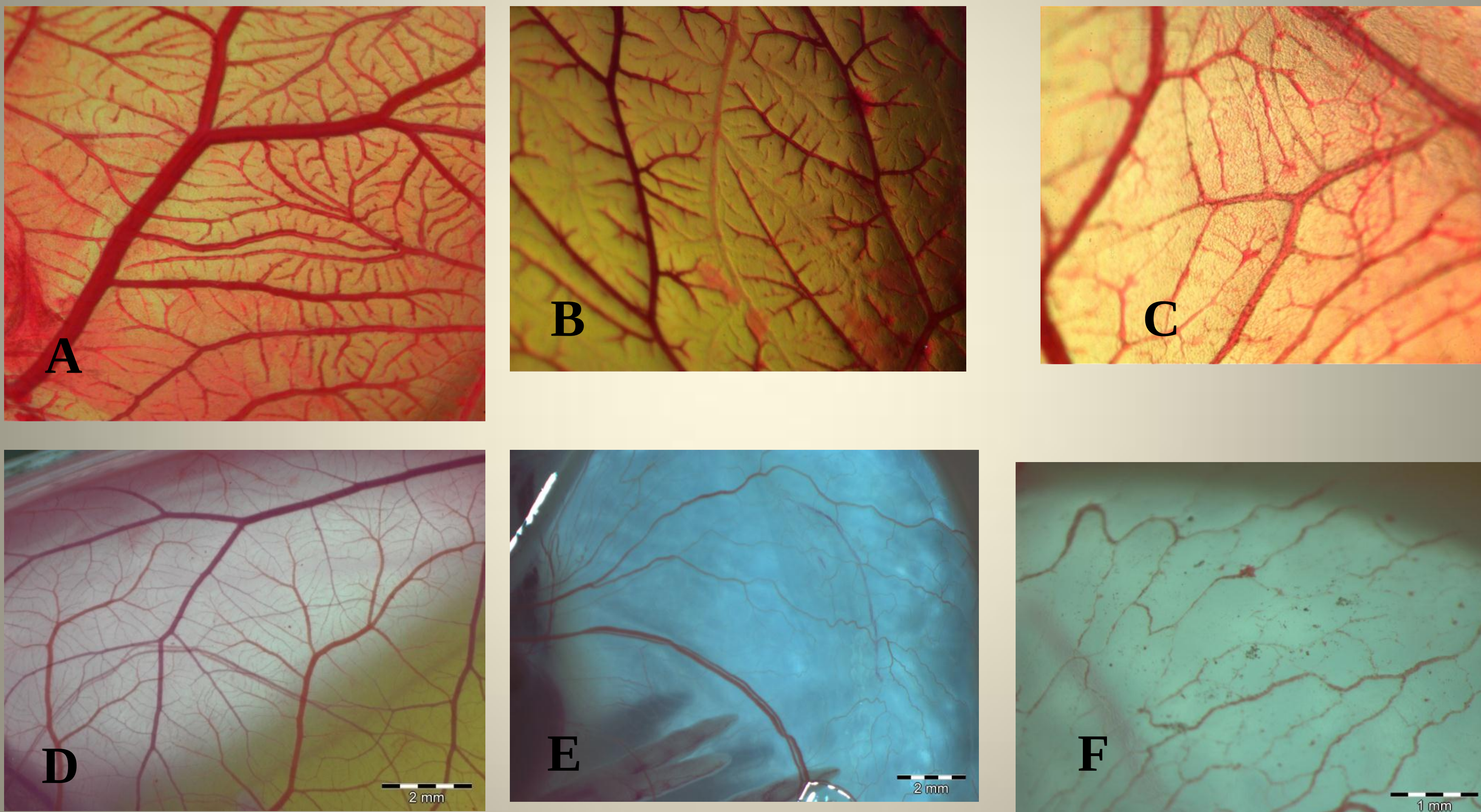


vessel

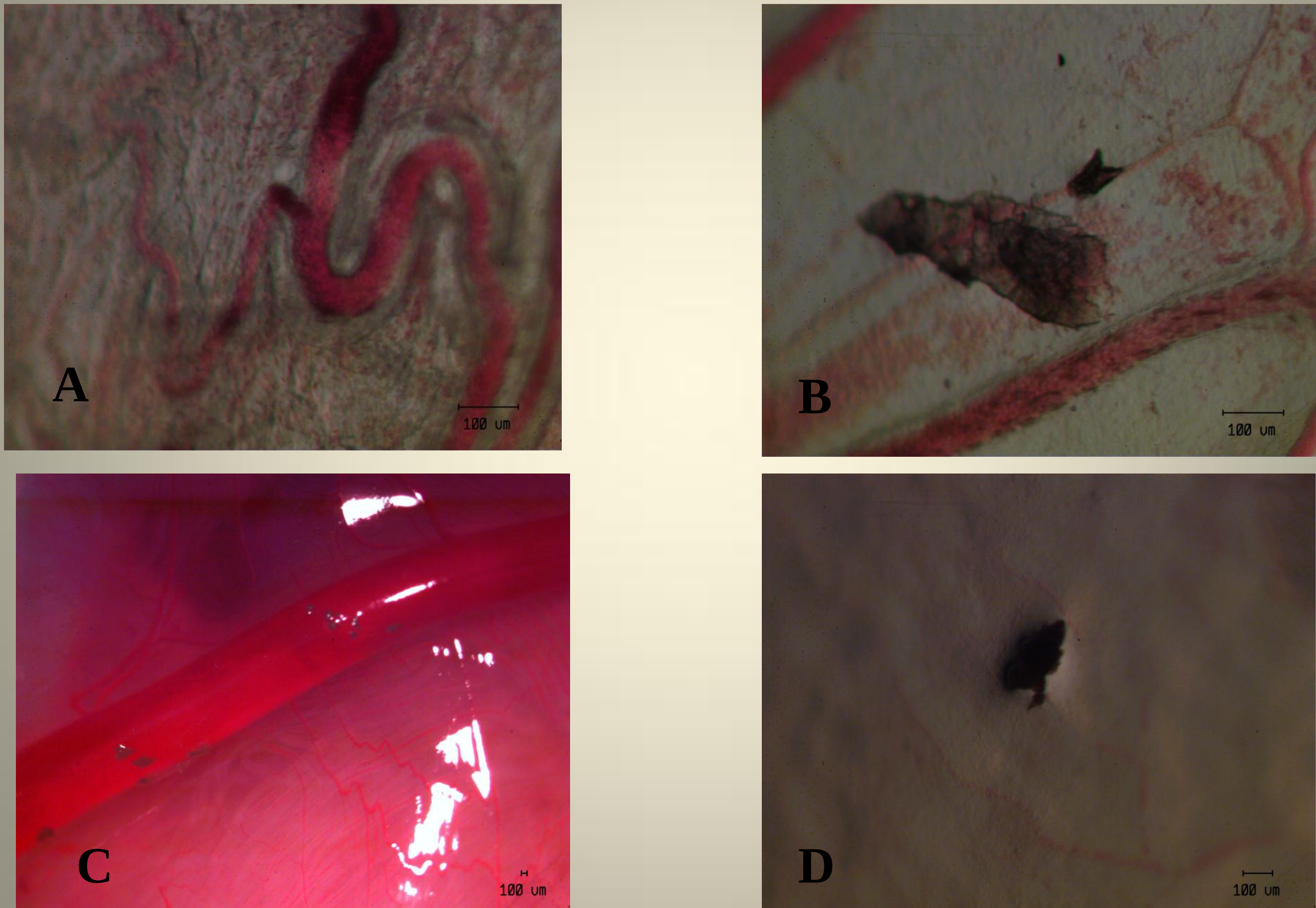
CAM of 12-day old chicken embryos implanted with a sponge loaded with; (A) PBS (control), (B) VEGF (proangiogenic control), (C) diamond nanoparticle suspended in water at a level of 50 ppm and (D) with aglomerate of nanodiamond placed onto CAM after injection of diamond nanoparticles (5000 ppm) in ovo



14-day old chicken embryo’s chorioallantoic membrane (CAM) after treatment with; (A,D) control (PBS) and diamond nanoparticles suspended in PBS at a level: (B,E) 500 ppm, (C,F) 5000 ppm.



Distribution of diamond nanoparticles and their aglomeration in chicken embryo's CAM after injection of diamond nanoparticles hydrosol (5000 ppm) in ovo; (A) diamond nanoparticles flow inside blood vessels, (B) diamond nanoparticles flow inside blood vessels and aglomerates placed outside the vessels, (C) diamond nanoparticles aglomerates located arounded vessels to force them, (D) diamond nanoparticles force membrane



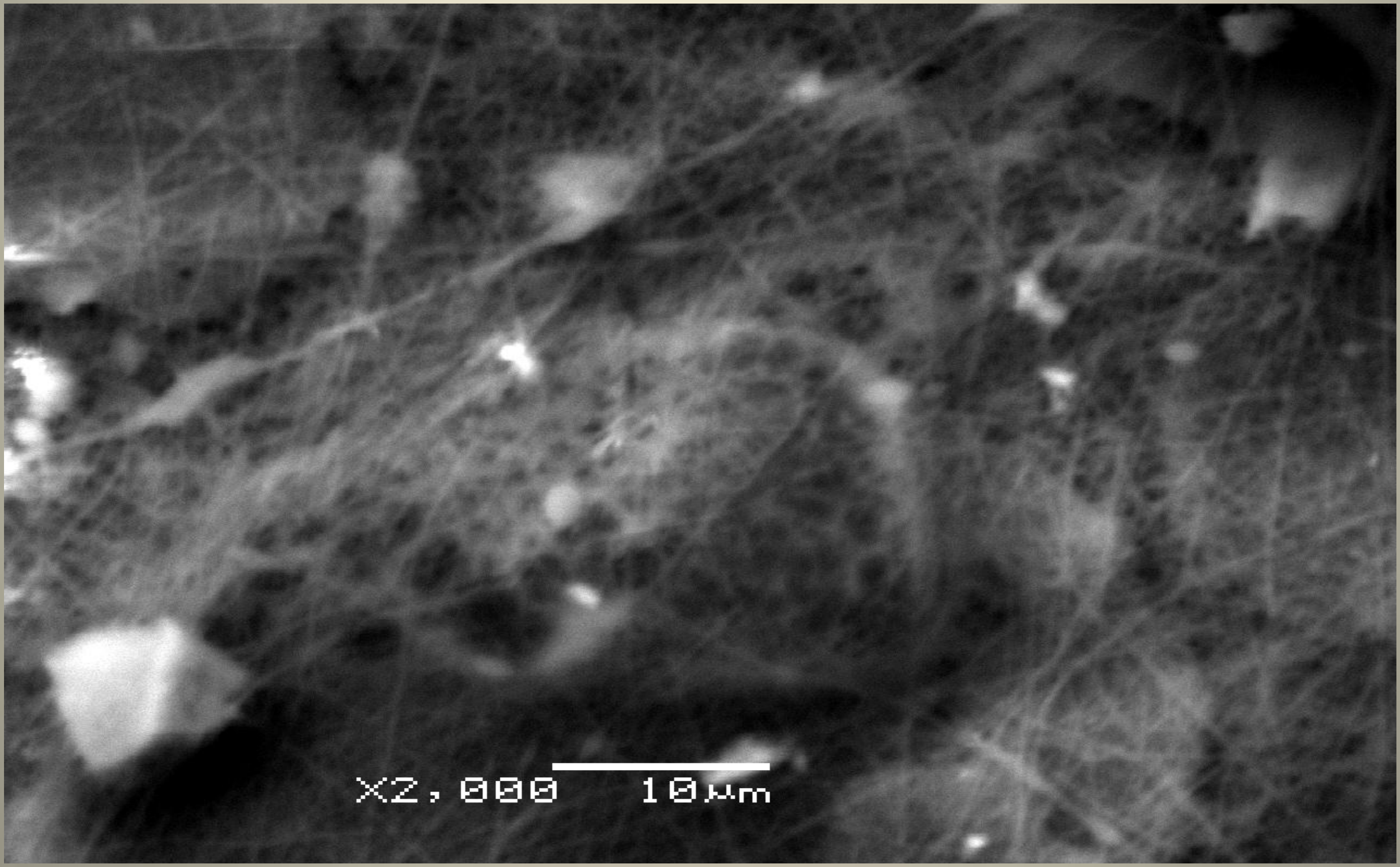
Summing up the results of research, it shows that diamond nanopowder affects the antioxidant activity of superoxide dismutase (SOD), which, in addition to the role of sweeping free radicals, is an anti-inflammatory factor. This results from its action at the molecular level, involving slowing down of immunological and inflammatory process, driven by a Tumour Necrosis Factor (TNFα). Oxidative stress induces an inflammatory process, stimulating the production of pro-inflammatory interleukins. Diamond nanopowder weakens the process of phagocytosis of neutrophils. It is likely that mechanism of an interaction between diamond nanoparticles and cellular membrane has a receptor nature and is associated with the presence of a large amount of sp3 free bonds on the extended surface of diamond nanopowder, to which antibodies may join by Fc regions (FcR). Diamond nanopowder, produced by detonation method, changes the oxidation-reduction potential of erythrocyte membrane cell and it acts as an immunosuppressant on innate immune cells.

Last experiments

- Modification of nanospider wires by incorporation of diamond nanoparticles

Incorporation of nanodimond particles in polymer nanofibres (SEM)

Julie Soukupova, PhD Thesis, Technical University of Liberec, in preparation.



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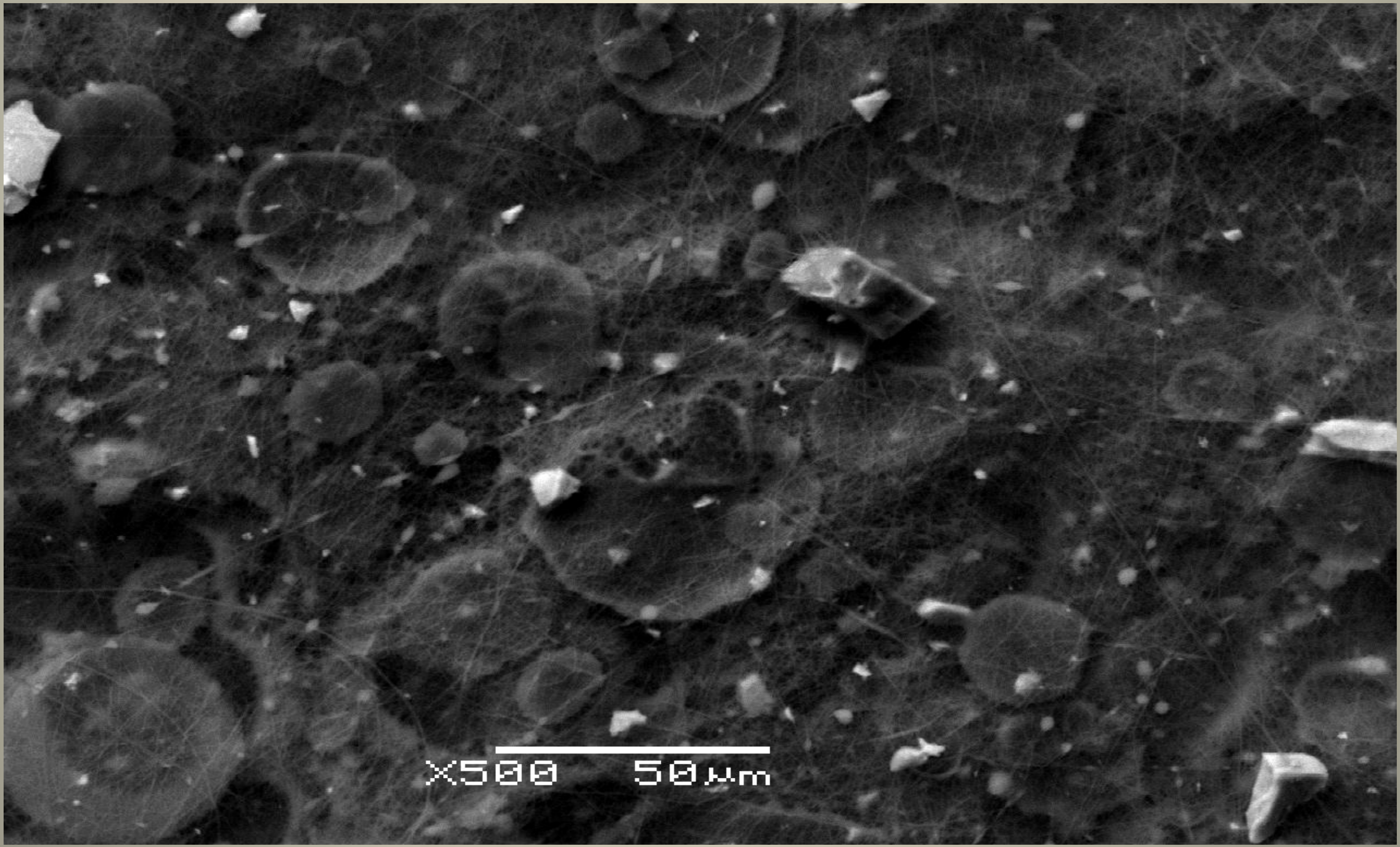

Conclusions

- Biomaterials with functional groups on their surfaces (**surface functionalization)** can be designed from substrates with standard bulk material properties.
- Surface functionalization alter surface properties to enhance performance in biological environment while retaining bulk properties of device.