Pokroky v biomedicínském inženýrství
Chytré nanotechnologie pro biomedicínské aplikace

Důmyslné formy hmoty otvírající široký prostor převratnému vývoji vědy a novým technologiím

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Contemporary science reached the level which makes possible to peep into very tiny pieces of matter to observe natural processes taking place inside.

On top of that the today’s technology allows interfere with these internal processes and working upon them; already achieved the levels that makes possible to copy the processes of formation tiny structures and to imitate these structures in “nanosize” scale, or to find the ways how to prepare them.

As these structures exhibit unique characteristics, unknown in the macro-world, it can be said that from the point of view of utilization of these structures for practical applications, doors are becoming wide open to undreamt-of development of science and technology.

As all these processes take place on nanometers scale, this area has received the name nanotechnology.
Nanotechnology is an inter-disciplinary branch of science

Problems of nanostructures belong to an inter-disciplinary field of research, where chemistry, physics, biology and mathematics, and perhaps some other branches of science as well, overlap in creating possibility to describe, study and employ these directions. Nanophysics, nanochemistry, nanomedicine, nanobiology, and particle nanostructures are categories of current nanoscience.
very small nano-material objects have unusually physical and chemical properties for example

- Black semiconductor like $\text{Cd}_3\text{As}_2$ is red (can be also yellow), exhibits strong luminescence, and is soluble.
- Carbon C is red and soluble.
- Iron Fe is soluble and yellow.
- Silicon Si is yellowish, exhibits strong luminescence, soluble in organic solvents.

Nanoceramics last temperatures up to 3000°C (Space shuttle) and so on.

Kelvar Liberec
Carbone-fiber composites 14x lighter than steel, 10 stronger than steel

**Luminescence of nanoparticles**

$\text{Cd}_3\text{As}_2$ in aqueous solutions in UV light Increasing particles size from left to right
Powder TiO<sub>2</sub> (big particles)

Nanoparticles TiO<sub>2</sub>
Energy and wavelength of free electron

Maxwell-Boltzmann \[ E = \frac{3}{2} kT \] \[ E_{300K} \doteq 0.04 \text{ eV} \]

where \( E \) is the kinetic energy of the carrier, \( T \) is temperature in K, and \( k = 1.38 \times 10^{-23} \text{ JK}^{-1} \).

DeBroglie - duality of particles

\[ \lambda = \frac{h}{\sqrt{2mE}} \Rightarrow \lambda = \frac{h}{\sqrt{3mkT}} \]

where \( \lambda \) is the wavelength of the carrier with mass \( m \) and \( h = 6.63 \times 10^{-34} \text{ Js} \).
In case of thermal electron \( m = 9.1 \times 10^{-31} \text{ kg} \), at 300 K

\[ \lambda \doteq 62 \text{ Å} \]
Metal nanoparticles in statu nascendi.
(From embryonic stage of Ag nanoparticles to the metal nanoclusters)
Surface localized plasmon resonance (LPPR):

**Absorption spectrum of SNPs sample 7**

Silvers

**Absorption spectrum of GNP sample 5**

Gold

Size of the bulk, particles and clusters
Process of dissolving of silver metal nanoclusters
SEM characterization of Ag nanostructures. Nanoparticle size ~18nm

\[
18.5 \text{ nm} = n_{\text{atoms}} = \frac{6.023 \cdot 10^{16}}{3.102 \cdot 10^{11}} = 194,165 \text{ Ag atoms}
\]
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10^8 bacteria cells/ml

Time 0 min
Cultivation at 37°C for 1 hour
End of cultivation

Dilution

2x10

Time 0 min
Uncountable amount of cells colonies
End of cultivation

n x 10^6 - 10^8 bacteria cells/ml
End of cultivation
Standard bacteria solution
Dilated $10^{-6} - 10^{-7}$

End of cultivation
Blank samples

End of cultivation
Standard bacteria solution + Ag solution
Dilated $10^{0} - 10^{1} - 10^{2}$
Effectivity = \frac{\text{CFU/ml in control sample} - \text{CFU/ml in experimental sample}}{\text{CFU/ml in control sample}} \times 100% \%

CFU – Colony forming unit; its mean one bacterie forming one colon of bacteries

CFU in control sample: \(1,36 \cdot 10^9 / \text{ml}\)
CFU in experimental sample: \(4,5 \cdot 10^3 / \text{ml}\)

Effectivity = \frac{1,36 \cdot 10^9 - 4,5 \cdot 10^3}{1,36 \cdot 10^9} \times 100% = 99,9996 \%
Ag particle sizes 4–12 nm

No Ag nano

\[ E. \text{coli} \text{ bacterial survival on cotton alone in the dark,} \]

Ag nano

\[ E. \text{coli} \text{ bacterial survival on Ag–cotton samples in the dark,} \]
Time dependence of interaction between staphylococcus aureus and silver nanoparticles

Efficiency of silver nanoparticles,
a/ bacteria : silver = $10^7$ bac./ mL : $10^{11}$ particles/mL
b/ bacteria : silver = $10^7$ bac./ mL : $10^9$ particles/mL

$\text{Ag}_n + \text{R} - \text{SH} + \text{O}_2 \rightarrow \text{Ag}_{n-1} + \text{R} - \text{SAg} + \text{H}_2\text{O}$
**Antrax**

Anthrax is an infection caused by the bacterium *Bacillus anthracis*. It's frequently used as filling in to biological weapons. Is using in practice broil fit for transmission airy on the way.

*Bacillus anthracis*, its **grampozitiv** stick form of bacterie, which created endospory.

The 2001 anthrax attacks, also known as Amerithrax one week after the September 11 attacks. Letters containing anthrax spores were mailed to several news media offices and two Democratic U.S. Senators, killing five people and infecting 17 others. Dr. Bruce Edwards Ivins was a scientist who worked at the government's biodefense labs at Fort Detrick in Frederick, Maryland.
Centrum of biological protection, Těchonín, Antrax

Využití v medicíně a biologii
Příklad: el.mag.ventil pro nekrotizaci tumorů, vychytávaní HIV virů in vitro, dopravníky a nosiče léků v biolog.systémech
Requirements on Nanoparticles in Medicine

- **SMALL SIZE**
  - Longer blood half-life
  - Wider biodistribution
  - Surface for coupling of biomolecules

- **MONODISPERSY**
  - Uniform properties
  - Constant drug content
  - Equal probability of capture for each magnetic microsphere

- No tweezer
Chemical synthesis

- coprecipitation of \((\text{NH}_4)_2\text{Fe(SO}_4\text{)}_2\cdot6\text{H}_2\text{O}\) (or \(\text{FeCl}_2\cdot4\text{H}_2\text{O}\)) and \(\text{Fe(ClO}_4\text{)}_3\cdot9\text{H}_2\text{O}\) (or \(\text{FeCl}_3\cdot6\text{H}_2\text{O}\)) salts by \(\text{NH}_4\text{OH}\)
- synthesized under \(\text{N}_2\) atmosphere and vigorous stirring
- nanocrystals of \(\text{Fe}_3\text{O}_4\) formed after heating
Magnetic PGMA Microspheres

GMA → \( \gamma \text{-Fe}_2\text{O}_3 \) → Sipomer

Ring-opening reaction

H\(_2\)O → OH

NH\(_3\) → NH\(_2\)

Carboxylic Acid

RCOOH → COOR
Beating pathogens \textit{in Vitro}

**Chemical Structures:**
- $\gamma$-Fe$_2$O$_3$
- $\text{H}_2\text{N}$-$\text{NH}_2$
- $\text{NH}_2$

**Diagram:**
- Monoclonal Antibody
- Functionalized Magnetic Nanoparticles

**Equation:**
- $\text{H}_2\text{N}$-$\text{NH}_2$-$\text{NH}_2$-$\text{NH}_2$-$\gamma$-Fe$_2$O$_3$ + MONOCLONAL ANTIBODY
- HOOC-$\text{H}_2\text{N}$-$\text{NH}_2$-$\text{NH}_2$-$\text{NH}_2$-$\gamma$-Fe$_2$O$_3$
Beating pathogens *in vitro* □ innovative, efficient and simple

- **Pathogens**
  - Antibody with charged end
  - Negatively charged magnetic nanoparticle

- **Separation**
  - Attracting the nanoparticles by external magnetic field
  - Separation of the pathogens from the solution

- **Antibodies**
  - Antibodies bind to pathogens
  - Functionalyzed magnetic nanoparticles
  - Specifically bound pathogens
Results of practical applications

TUMOR NECROTIZING

Necrotizing tumour

Magnetic particles seal the capillary
Area of hydrodynamic dispersion sphere for RFA with magnetic nanoparticles

THERMAL ABLATION
RFA agar-phantom

Temperature increase in agar phantom

different size of nanoparticles
Patient at hyperthermia application
New approach
Biological Application of Laser-generated Nanoparticles

Stephan Barcikowski
For biomedical applications, purity matters.
Purity of "Gold Colloid" (Material Safety Data Sheet)

All unconjugated gold colloids contain approximately 0.01% HAuCl₄ suspended in 0.01% tannic acid with 0.04% trisodium citrate, 0.26 mM potassium carbonate, and 0.02% sodium azide as a preservative.

\[ \text{NaN}_3 \]
Ligand-free Nanoparticles from Laser Ablation in Liquids
When it begun ...

Communications

Laser Ablation of Films and Suspended Particles in a Solvent: Formation of Cluster and Colloid Solutions

Anton Fojtik and Arnim Henglein
Hahn-Meitner-Institut Berlin, Abteilung Photochemie, 1000 Berlin 39

Clusters / Colloids / Photochemistry

A strong 694 nm Ruby laser beam was used to ablate films of gold, nickel and carbon in a solvent (water, 2-propanol, cyclohexane). Colloidal solutions of these materials were obtained. The mean size of the colloidal gold particles depends on the laser intensity. Small graphite particles (several microns) suspended in toluene were also exposed to the laser flash. Ablation of these particles in the plasma generated by the laser leads to an orange solution which contains carbon-60, carbon-70 and other carbon clusters which have not yet been identified.

A: Experimental arrangement for ablation of films in liquid by a strong laser beam
V: glass vessel, F: film on glass support
G, L: lens, B: laser beam, O: optical cuvette

A. post-irradiation, fragmentation, melting
B. coalescence and reaction of activated species (ions, atoms, cluster)
C. ablation
D. cavitation
E. lens
Absorption spectra of the colloidal gold solutions obtained at different laser intensities. The golg film was 500A thick and illuminated in 2-propanol without stabilizer. Laser intensity: a: 2.3 J/cm², b: 7.0 J/cm², c: 27 J/cm². The figure also contain the absorption spectrum of a nickel sol obtained by ablation of nickel film (on glass) in aqueous 10⁻³M sodium polyacrylate.
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[C 60]

[C 60]

[C 90]
Formation of Nanometer-Size Silicon Particles in a Laser Induced Plasma in SiH₄

Absorption spectra of centrifuged and filtered silicon particles. Right: thiol-stabilizer present in the cyclohexane-propanol solvent mixture. The spectra of solution were taken towards blanks containing the solvent mixture (plus stabilizer). Insert: luminescence spectra of the etched particles in solution; excitation wavelength: 360nm.
Could lasers be put to a good use?
Attempts were made, but without any particular breakthrough...
At that time, we aimed to new type of nanostructures
and we really had not expected
that usage of lasers could bring us something revolutionary.
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Biomedical Application
... to grow rapidly within connected disciplines

Surface Charge of laser-generated nanoparticles

Noble metal nanoparticle surface is partially oxidized

\[ \zeta = -28 \text{ mV} \pm 17 \text{ mV} \]

adsorption of anions

negative zeta potential (pH-dependent)

Rehbock; Barcikowski et al.; *PCCP* 2013, 15 (9), 3057-3067.
Pfeiffer, Barcikowski, Parak et al., *J. R. Soc. Interface* 2014, 11, 20130931
Single (!) Laser Pulse Ablation of Metal in Water

500 W, MHz, ps Laser Ablation at High-Speed (500 m/s)

R. Streubel, S. Barcikowski, B. Gökce, Optics Letters 41 (2016), 1486
Nanoparticle Targeting & Binding
Bioconjugation of Nanoparticles
Ligand Exchange

\[ \text{O}^\text{−} \rightarrow \text{S} \]

\[ \text{Na}^\text{+} \]

obtained surface coverage of ssO \([\text{pmol cm}^{-2}]\) for particles with mean diameter \([\text{nm}]\) of Au, Au\(^{3+}\), in situ BSA conjugation (multi layer formation) calculated saturation conjugation by ligand exchange. Non specific binding (no thiol).
Conjugation Efficiency

Chemical reactants block the surface

Laser-generated NP  → 4 x higher surface coverage
Morbus Alzheimer
Morbus Alzheimer

Alzheimer’s brain

Neurofibrillary tangles

Amyloid plaques

APP (amyloid precursor protein)

β

γ

Biochemical Society Transactions (2005) 33, 553-558
How to counteract protein misfolding with nanoparticles?

- Nanoparticle
- Neuronal Death
- Aβ fibrils
- Aβ oligomers
- Aβ binding site
- β-sheet breaking site
- Protease
- Neuronal Death
- β-sheet rich aggregates of Aβ
- Aβ oligomers
Molecular Design for Abeta-Targeting and Binding

19 interactions between D3 and Aβ
modeled by Laura Akkari, UDE

Aβ nonamer
Anionic glutamate side chains of the Aβ peptide

50 ns (simulation)

Funke et al., ACS Chem. Neurosci. (2010), 1, 639–648
Müller-Schiffmann et al., Angew. Chem. Int. Ed. 2010
Inhibition of Alzheimer protein aggregation

- Abundance of Aβ oligomers
- Fluorescence [RFU]
- Relative size of Aβ aggregates

C. Streich, L. Akkari….T. Schrader, S. Barcikowski. ACS Nano, 2016, 10, 7582-7597
Inhibition of Alzheimer protein aggregation

C. Streich, L. Akkari…T. Schrader, S. Barcikowski. ACS Nano, 2016, 10, 7582-7597
Endosomal Release by “Photodispersion”

- Incubation of AuNP-NLS agglomerates
- Endosomal uptake
- Laser irradiation of enclosed agglomerates
- Intracellular release
Endosomal Release by Photodispersion

Krawinkel et al. J Nanobiotechnol (2016) 14:2
Implants for Parkinson Therapy
Deep Brain Stimulation (DBS)

H. Fernandez, *Cleveland Clinic Journal of Medicine* 2012, 79, 28

Andrew Johnson, on: youtu.be./uBh2LxTW0s0
Nanoparticle Coating of Neural Electrodes (Parkinson Disease)

Unwanted electrode encrustation. Coronal cross-section of rat brain with glial reaction around electrode tip.

Neural Pt\textsubscript{9}Ir electrode coated with monolayer of laser-generated Pt\textsubscript{9}Ir nanoparticles after annealing.

J. Jakobi et al. Nanotechnology (2011)
Clinical Testing of Neural Electrodes

in vitro  in vivo  post mortem

Zbc  Zac  OP (Implant.)  Z0  Z1  Z2  Z3  LFP  Histology

2 w. recovery  1st w.  2nd w.  3rd w.  stim.  stim.  stim.

Presently, situation for such a field of research, i.e., „Nanoparticle Generation by Lasers in Liquids”, is becoming a much hotter topic due to availability of picosecond and femtosecond lasers. Shorter time for energy deposition causes less problems with high temperature, narrowing the size distribution, with possible applications in biological systems.

Prof. Stephan Barcikowski, University Essen-Duisburg Germany
Prof. Vincenzo Amendola, University of Padova, Padova, Italy
Thank you for your attention