# Biological properties of nanoparticles

Eliška Daďová

Bachelor's thesis
2019

Tomas Bata University in Zlín
Faculty of Technology

Univerzita Tomáše Bati ve Zlíně
Fakulta technologická
Ústav technologie tuků, tenzidů a kosmetiky
akademický rok: 2018/2019

# ZADÁNÍ BAKALÁŘSKÉ PRÁCE
(PROJEKTU, UMĚLECKÉHO DÍLA, UMĚLECKÉHO VÝKONU)

| | |
|---|---|
| Jméno a příjmení: | **Eliška Daďová** |
| Osobní číslo: | **T16348** |
| Studijní program: | **B2901 Chemie a technologie potravin** |
| Studijní obor: | **Technologie výroby tuků, kosmetiky a detergentů** |
| Forma studia: | **prezenční** |
| Téma práce: | **Biologické vlastnosti nanočástic** |

Zásady pro vypracování:

**Nanočástice se staly běžnou součástí kosmetických přípravků. Jejich biologické vlastnosti jsou však velmi obtížně stanovitelné a vysoce závislé na konkrétních nanočásticích. Cílem bakalářské práce je proto získat znalosti o typech nanočástic, typech jejich syntézy a aplikačním potenciálů s důrazem na kosmetické prostředky. Zároveň by student/ka měl pochopit klíčové vlastnosti determinující interakci mezi částicemi a živými organismy ať již na úrovni organismu, orgánů či buněk a mezibuněčné hmoty. Předmětem studia jsou nejen eukaryotické organismy, ale také organismy prokaryotické. Student/ka zároveň provede experimenty stanovení biologických vlastností nanočástic pomocí in vitro technik.**

Rozsah bakalářské práce:

Rozsah příloh:

Forma zpracování bakalářské práce: **tištěná/elektronická**

Seznam odborné literatury:

**SNUSTAD, D.P., SIMMONS, M.J., RELICHOVÁ, J. et al. Genetika. Brno: Masarykova univerzita, 2009.**
**ALBERTS B. et al. Molecular Biology of the Cell 5th ed. Garland Science.**
**DAVID A.P. BIZIOS R. Biological Interactions on Material Surfaces. ISBN 978-0-387-98160-4**

| | |
|---|---|
| Vedoucí bakalářské práce: | **doc. Ing. Petr Humpolíček, Ph.D.**<br>Centrum polymerních materiálů |
| Datum zadání bakalářské práce: | **2. ledna 2019** |
| Termín odevzdání bakalářské práce: | **20. května 2019** |

Ve Zlíně dne 12. března 2019

L.S.

doc. Ing. Roman Čermák, Ph.D.
*děkan*

doc. Ing. Marián Lehocký, Ph.D.
*ředitel ústavu*

Příjmení a jméno: ……. Obor: …….

# P R O H L Á Š E N Í

Prohlašuji, že

- beru na vědomí, že odevzdáním diplomové/bakalářské práce souhlasím se zveřejněním své práce podle zákona č. 111/1998 Sb. o vysokých školách a o změně a doplnění dalších zákonů (zákon o vysokých školách), ve znění pozdějších právních předpisů, bez ohledu na výsledek obhajoby [1)];
- beru na vědomí, že diplomová/bakalářská práce bude uložena v elektronické podobě v univerzitním informačním systému dostupná k nahlédnutí, že jeden výtisk diplomové/bakalářské práce bude uložen na příslušném ústavu Fakulty technologické UTB ve Zlíně a jeden výtisk bude uložen u vedoucího práce;
- byl/a jsem seznámen/a s tím, že na moji diplomovou/bakalářskou práci se plně vztahuje zákon č. 121/2000 Sb. o právu autorském, o právech souvisejících s právem autorským a o změně některých zákonů (autorský zákon) ve znění pozdějších právních předpisů, zejm. § 35 odst. 3 [2)];
- beru na vědomí, že podle § 60 [3)] odst. 1 autorského zákona má UTB ve Zlíně právo na uzavření licenční smlouvy o užití školního díla v rozsahu § 12 odst. 4 autorského zákona;
- beru na vědomí, že podle § 60 [3)] odst. 2 a 3 mohu užít své dílo – diplomovou/bakalářskou práci nebo poskytnout licenci k jejímu využití jen s předchozím písemným souhlasem Univerzity Tomáše Bati ve Zlíně, která je oprávněna v takovém případě ode mne požadovat přiměřený příspěvek na úhradu nákladů, které byly Univerzitou Tomáše Bati ve Zlíně na vytvoření díla vynaloženy (až do jejich skutečné výše);
- beru na vědomí, že pokud bylo k vypracování diplomové/bakalářské práce využito softwaru poskytnutého Univerzitou Tomáše Bati ve Zlíně nebo jinými subjekty pouze ke studijním a výzkumným účelům (tedy pouze k nekomerčnímu využití), nelze výsledky diplomové/bakalářské práce využít ke komerčním účelům;
- beru na vědomí, že pokud je výstupem diplomové/bakalářské práce jakýkoliv softwarový produkt, považují se za součást práce rovněž i zdrojové kódy, popř. soubory, ze kterých se projekt skládá. Neodevzdání této součásti může být důvodem k neobhájení práce.

Ve Zlíně .....

.....

---

I [1)] *zákon č. 111/1998 Sb. o vysokých školách a o změně a doplnění dalších zákonů (zákon o vysokých školách), ve znění pozdějších právních předpisů, § 47 Zveřejňování závěrečných prací:*

*(1) Vysoká škola nevýdělečně zveřejňuje disertační, diplomové, bakalářské a rigorózní práce, u kterých proběhla obhajoba, včetně posudků oponentů a výsledku obhajoby prostřednictvím databáze kvalifikačních prací, kterou spravuje. Způsob zveřejnění stanoví vnitřní předpis vysoké školy.*

*(2) Disertační, diplomové, bakalářské a rigorózní práce odevzdané uchazečem k obhajobě musí být též nejméně pět pracovních dnů před konáním obhajoby zveřejněny k nahlížení veřejnosti v místě určeném vnitřním předpisem vysoké školy nebo není-li tak určeno, v místě pracoviště vysoké školy, kde se má konat obhajoba práce. Každý si může ze zveřejněné práce pořizovat na své náklady výpisy, opisy nebo rozmnoženiny.*

*(3) Platí, že odevzdáním práce autor souhlasí se zveřejněním své práce podle tohoto zákona, bez ohledu na výsledek obhajoby.*

II [2)] *zákon č. 121/2000 Sb. o právu autorském, o právech souvisejících s právem autorským a o změně některých zákonů (autorský zákon) ve znění pozdějších právních předpisů, § 35 odst. 3:*

*(3) Do práva autorského také nezasahuje škola nebo školské či vzdělávací zařízení, užije-li nikoli za účelem přímého nebo nepřímého hospodářského nebo obchodního prospěchu k výuce nebo k vlastní potřebě dílo vytvořené žákem nebo studentem ke splnění školních nebo studijních povinností vyplývajících z jeho právního vztahu ke škole nebo školskému či vzdělávacího zařízení (školní dílo).*

**III** *[3)] zákon č. 121/2000 Sb. o právu autorském, o právech souvisejících s právem autorským a o změně některých zákonů (autorský zákon) ve znění pozdějších právních předpisů, § 60 Školní dílo:*

*(1) Škola nebo školské či vzdělávací zařízení mají za obvyklých podmínek právo na uzavření licenční smlouvy o užití školního díla (§ 35 odst. 3). Odpírá-li autor takového díla udělit svolení bez vážného důvodu, mohou se tyto osoby domáhat nahrazení chybějícího projevu jeho vůle u soudu. Ustanovení § 35 odst. 3 zůstává nedotčeno.*

*(2) Není-li sjednáno jinak, může autor školního díla své dílo užít či poskytnout jinému licenci, není-li to v rozporu s oprávněnými zájmy školy nebo školského či vzdělávacího zařízení.*

*(3) Škola nebo školské či vzdělávací zařízení jsou oprávněny požadovat, aby jim autor školního díla z výdělku jím dosaženého v souvislosti s užitím díla či poskytnutím licence podle odstavce 2 přiměřeně přispěl na úhradu nákladů, které na vytvoření díla vynaložily, a to podle okolností až do jejich skutečné výše; přitom se přihlédne k výši výdělku dosaženého školou nebo školským či vzdělávacím zařízením z užití školního díla podle odstavce 1.*

## ABSTRAKT

Bakalářská práce je zaměřena na teoretické poznání nanočástic s důrazem na jejich využití v kosmetickém průmyslu. Pro správné pochopení nanočástic je důležité si uvědomit, že se jedná o širokou skupinu materiálů, jejichž využití a vlastnosti se značně odlišují. Mezi faktory, které ovlivňují chování nejvíce, patří velikost, poměr povrch/objem, tvar, materiál, ze kterého jsou vyrobeny a způsob syntézy. Vymezení těchto parametrů hraje důležitou roli pro stanovení jejich biologických vlastností, interakcí s biomolekulami a míry toxicity. Praktická část bakalářské práce je zaměřena na osvojení různých technik biologického testování materiálů v laboratořích. Dále se zabývá testováním toxicity materiálů obsahující nanočástice. Toxicita vzorků byla stanovena pomocí cytotoxicity a proliferace za použití eukaryotických buněk.



Klíčová slova:

Nanočástice, biologické vlastnosti, biokompatibilita



## ABSTRACT

The Bachelor's thesis is focused on theoretical knowledge about nanoparticles (NPs) in general and their usage in the cosmetic industry. For the right understanding of NPs, it is important to realize that NPs are a wide group of particles with different usage and properties. Factors influencing their behavior the most are size, surface/volume ratio, shape, used material and type of synthesis. Defining of these parameters plays a crucial role in the determination of biological properties, interaction with biomolecules and amount of toxicity. The practical part of the thesis continues with the determination of the toxicity of materials containing nanoparticles. The toxicity of samples was determined by cytotoxicity and proliferation using the eukaryotic cells.



Keywords:

Nanoparticles, biological properties, biocompatibility

I would like to thank doc. Ing. Petr Humpolíček, Ph.D. for helpfulness and patience in leading my Bachelor’s thesis and for valuable advice during consultations.

I hereby declare that the print version of my Bachelor's/Master's thesis and the electronic version of my thesis deposited in the IS/STAG system are identical.

# CONTENT

# INTRODUCTION

There are a number of definitions of nanoparticles (NPs) in the scientific literature. For the purpose of this thesis, the most appropriate definition is as followed „Nanoparticles are particles that exist on a nanometer scale (i.e., below 100 nm in at least one dimension).“ [1] Recent interest in nanotechnology rises from nanoparticle's unique properties connected to their size, surface, photochemical activity, and high surface/volume ratio. Despite the fact that NPs are nowadays widely used in various commercial and domestic applications, their biological properties and impact on human health are still uncertain.

Due to the scope of the theme of this thesis, it was not always possible to clarify exact NPs mentioned in different applications. It became clear that every type of NPs is highly specific in its purpose and properties. To keep this thesis in the required length, in most cases NPs are presented in general.

NPs have been used for a long period of time. As they can be produced by weathering, volcano eruptions, wildfires or microbial processes, non-intended using NPs by the humanity can be traced back to ancient times. From the scientific point of view, as an important step forward, the work of Michael Faraday might be considered. He studied the interaction between light and metal materials in 1857, as several literary sources declare. In the upcoming years, mostly observation of their side effects and looking for their possible applications had taken place. [2] More recently, nanotechnology is a rapidly developing branch of science. In 2014, there were about 1814 consumer products with nano-base. [3] It is obvious that its influence will grow up even more in the future. The unique properties of NPs offer uncountable opportunities for surprising discoveries and represent great challenges to the scientists.

# I. THEORY

# 1 BIOLOGICAL PROPERTIES OF NANOPARTICLES

Biological properties and thus their possible applications of NPs are based on their physical and physicochemical properties. Among these properties, size, photochemical activity, specific surface chemistry, energy and roughness, and unique surface/volume ratio are included.

## 1.1 Size and shape

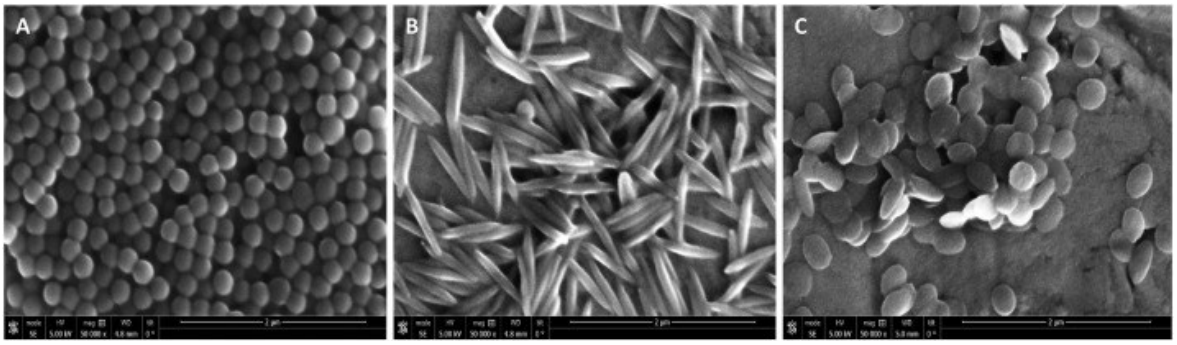


Fig. 1 Different shapes of NPs [4]

Size and morphology of NPs determine, how they can be used. [5] These two parameters influence their toxicity and targeting ability in living organisms. It also has an impact on how living cells are able to see and recognize NPs. Small size and appropriate shape determine some NPs to be used as very small probes to explore and even heal cells without introducing too much damage. [6]

## 1.2 Surface/volume ratio

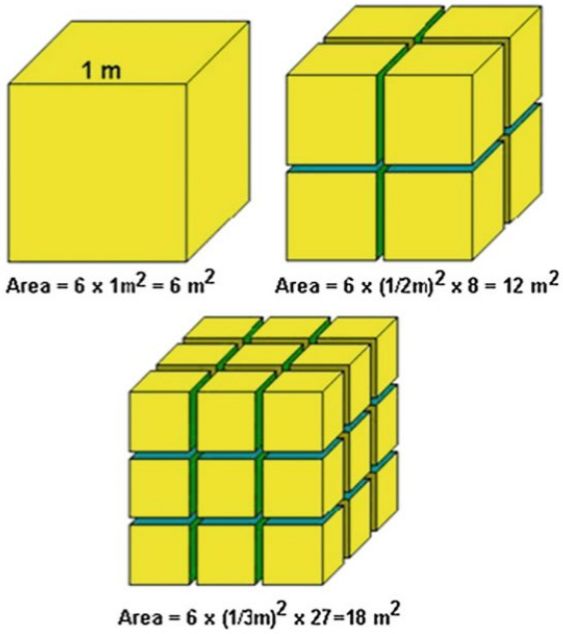


Fig. 2 Surface/volume ratio [7]

The surface area defines the quantity of exposed area of solid objects. One of the crucial properties of NPs is, related to their small size, high surface/volume ratio. This ratio is the amount of surface area per unit volume of an object. With the decreased size, amount of atoms on NPs surface increases. The higher percentage of atoms on the surface leads to rapid growth in the reaction rate for the chemical reaction. [7]

## 1.3 Surface

The essential property of NPs and then their following applications is their ability to create interactions with biomolecules. For interaction between NPs and biomolecules, the surface charge is the most crucial parameter. It can be described through zeta potential[1]. Thanks to zeta potential, it is possible to visualize the level of stability of bonds between NPs and biomolecules. [8]

[1] Zeta potential is a term for electro-kinetic potential in colloidal systems, and a measure of surface charge. [63]

## 1.4 Optical properties

The optical properties of NPs are important for biosensing, bioimaging, and detection of foreign compounds in the human body. Especially one group of NPs, called quantum dots, are well-known for their extreme fluorescent properties[2]. They can absorb and emit light at different wavelengths depending on a core diameter. The other optical property is resistance to photobleaching and a long fluorescence lifetime compared with organic dyes. [9] The importance of QDs is described later in this thesis.

[2] Fluorescence in generally is the ability of a material to absorb light at one wavelength (color) and emit it in a different one. [71]

# 2 APPLICATIONS OF NPS IN BIOLOGY

Particular NPs have become universal tools for a whole range of biological applications, such as controlled drug release, detection of pathogens, separation of cells and biomolecules, bioimaging or in tissue engineering. They also gain importance in the curing of tumor diseases. [5] To demonstrate how important NPs become, let me introduce you a few of these applications.

## 2.1 NPs as sensors

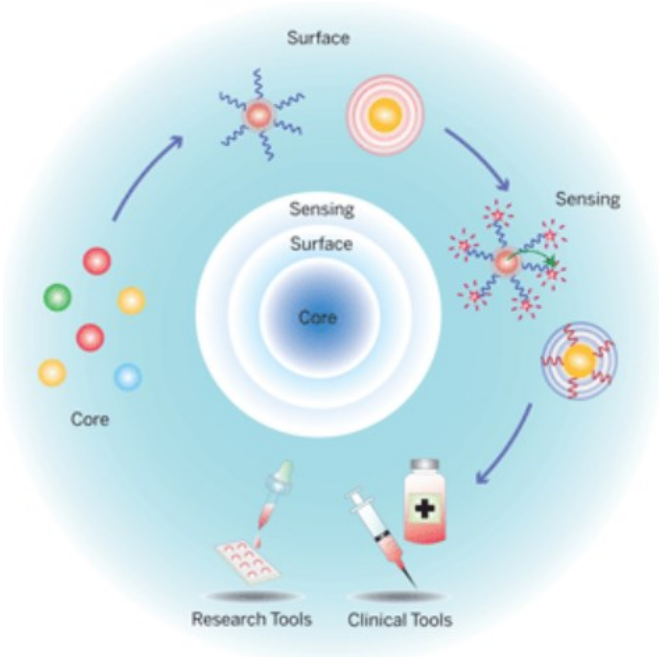


Fig. 3 Optimization of NPs for becoming biosensors [64]

NPs as sensors have been used for a variety of applications including detection of analytes at very low concentrations, detection of pathogens, capturing cells, and detecting molecular functions. [10] In particular, gold NPs and semiconductor NPs (e.g. quantum dots) have been largely exploited for colorimetric and fluorescence detection of oligonucleotides, proteases, and other molecular species. [11]

## 2.2 NPs for pathogen detection

The optical and magnetic properties of metallic NPs are utilized for the detection of foreign molecules as well as undesirable microorganisms in the human body. Microorganisms can be detected through the use of metal core NPs with a molecule of Fe in their center. [10]

## 2.3 NPs for cell detection and separation

For biological research, isolation of certain cells from complex mixtures is an important issue. NPs have been discovered as sensitive tools for the detection of specific cell types in low concentrations. Magnetic NPs, meaning NPs coated with a thin layer of gold or special types of dendrimers, are used e.g. for the identification of circulating tumor cells, which are responsible for creating metastasis. [10] Detection and separation functions of NPs can be used also for molecules not only for cells. For example, nanotube membranes can act as a channel for selective transport of molecules and ions between separated solutions. [12]

## 2.4 NPs as imaging agents

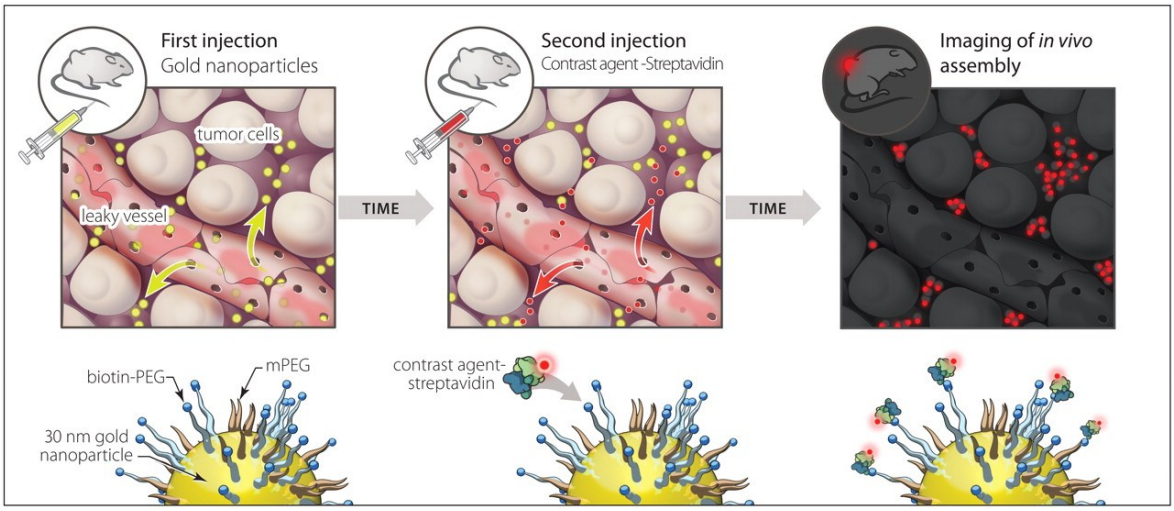


Fig. 4 Gold NPs assembling with contrast agent *in vivo* [13]

NPs have been studied as contrast agents in molecular imaging. Some kinds of NPs are enabled to create sensitive and specific monitoring of molecular targets, which allows the detection of diseases such as cancer and cardiovascular diseases. [10] That can be achieved in several different ways:

1) long term lasting imaging signals
2) using passive or active targeting
3) multiple ligands presence on the surface of NPs
4) using both imaging and the therapeutic ability of NPs
5) using different types of imaging technique per one type of NPs
6) detection of more targeted molecules simultaneously. [14]

## 2.5 Tissue engineering

A high level of precision and high quality has been achieved in current tissue engineering. Despite this fact, the uncertain response of the immune system on foreign substances in the body is still a tremendous problem. It has been shown that specific NPs are able to solve this issue. Precisely modified NPs increase lasting implants and reduce the risk of causing inflammation and body rejection of implants. The effect of enhancing the adoption of implants by the immune system was demonstrated on implants of a hip or knee. The addition of nano-sized structures on the surface of the prosthesis has reduced the chance of rejection by the immune system and also stimulated the production of its own osteoblasts. Types of NPs using in coating the prosthesis depend on a kind of replaced tissue. For bone implants, ceramic NPs or nanostructured apatite film can be used. [5]

## 2.6 NPs as drug carries

Studies have shown that nanoparticle-drug complexes, using, for example, organic NPs or quantum dots, have the ability to reduce toxicity and side effects of e.g. chemotherapy drugs. Reducing toxicity of raw drugs is allowed through various methods such as encapsulation, micellization, and protein cage architecture using carbon nanotubes, graphene oxide, polymeric based NPs, liposomes, dendrimers or polymeric micelles. [15]

# 3 BIOCOMPATIBILITY NPS WITH THE HUMAN BODY

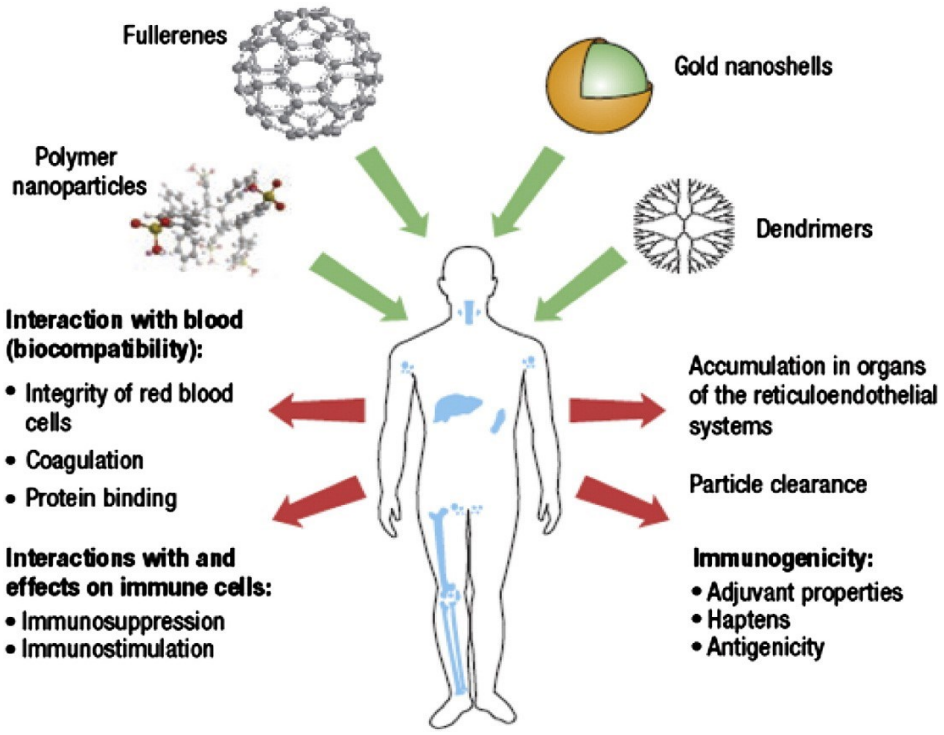


Fig. 5 Possible impact of NPs on human body [15]

NPs have been used for a long time. It is necessary to be interested in their possible toxic effects on the environment (accumulation of NPs in water and soil), plants, animals and human health so that they will only have positive effects on our lives. In this case, it is important to talk about the potential danger of NPs in the human body.

## 3.1 Entry of NPs into the human body

The potential entrances for NPs to the human body are at least four: The skin, the respiratory route, the gastrointestinal system, and the nasal epithelium. There is also a possibility of penetration by injection of drugs containing NPs. [16]

### 3.1.1 The skin

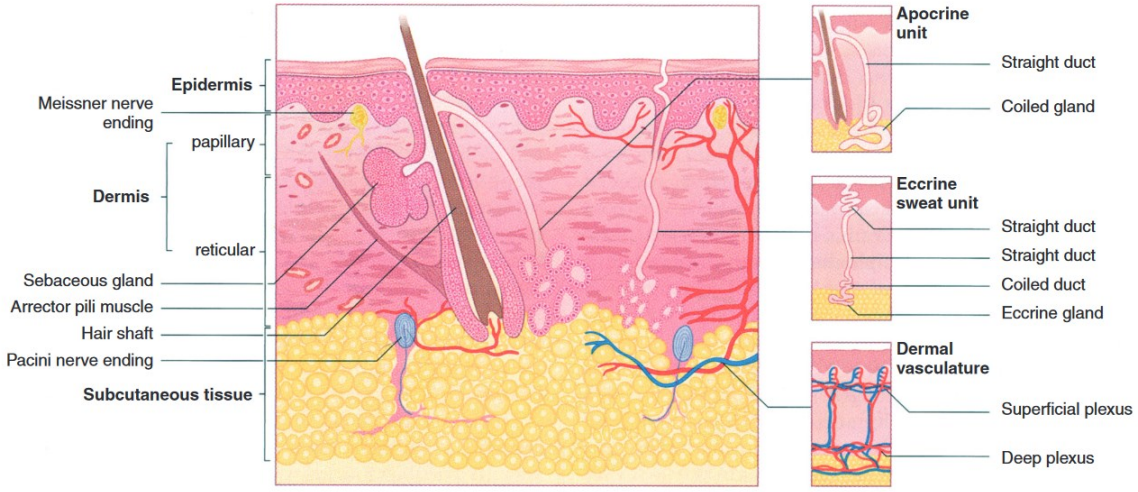


Fig. 6 Structure of human skin [65]

The human skin and epithelium of respiratory and gastrointestinal tracts are the first and biggest barriers against the outer environment. The skin is composed of three parts: epidermis, dermis and subcutaneous. The outer part of the epidermis is called *Stratum corneum* and consists of keratinized dead cells. The layer of fully keratinized cells should provide sufficient protection against the surrounding environment. The second part of the skin, dermis, contains blood vessels, nerves, sweat glands, and hair follicles. It has been shown that NPs can remain in hair follicles. [12] However, penetration of NPs through the skin is rare as the outer part of the skin, *Stratum corneum*, is able to protect the human body against any NPs scattered in dust made of manufacturing or combustion. [17] On the other hand, the effect of a few substances, such as titanium dioxide, has been discussed. Titanium dioxide is used as UV-absorber in a wide range of cosmetics products and its nano-scaled particles are suspected for causing serious diseases. [18] Toxic effects of NPs on the skin are determined by the length of their exposition. That is the reason why *SC* is able to protect skin against NPs scattered in the air. However, NPs of titanium dioxide are able to stay and even penetrate through *SC* because of its long term using in sunscreens and other types of cosmetics, which may be used for several years. Nevertheless, the concerns about the penetration of NPs through the skin and resulting toxic effects are highly debatable topics among scientists. The fact is that NPs are still not fully characterized from this point of view. [19]

### 3.1.2 Inhalation

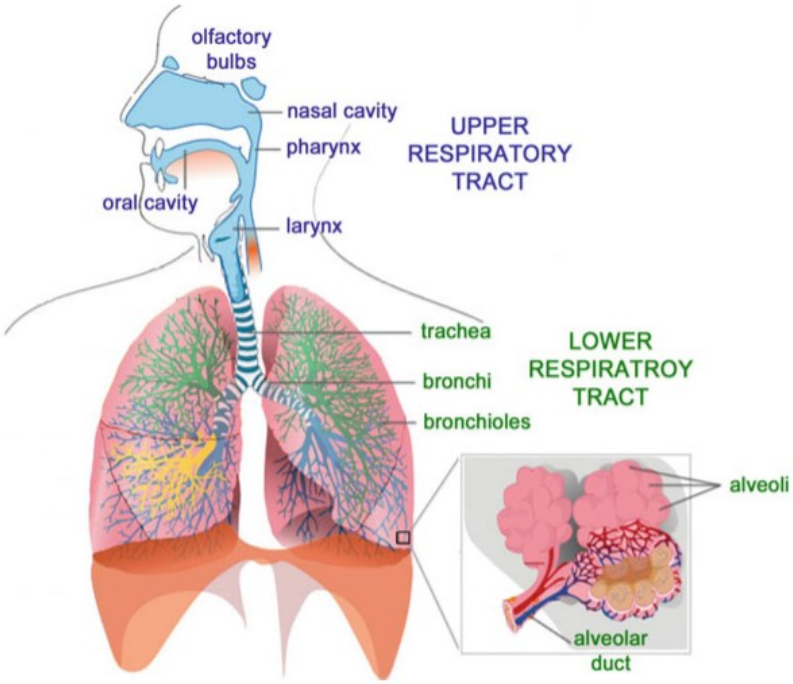


Fig. 7 The respiratory system [66]

The respiratory system provides a major doorway for particulate materials scattered in the air like asbestos, quartz, and carbon. The effect of NPs on the respiratory tract depends on their size and type. [16] Particles on the nanoscale level have a high probability of passing through the thoracic zone and have a definite probability of reaching the pulmonary alveoli, where gaseous exchange occurs. [17] The way how NPs influence the respiratory system also depends on the anatomic structure of the breathing tract, airflow patterns, and the flowing characteristics of particles. If NPs are able to penetrate deeper into the lungs, they interact with epithelium and can cause inflammation and have chronic effects. [19]

### 3.1.3 The gastrointestinal system

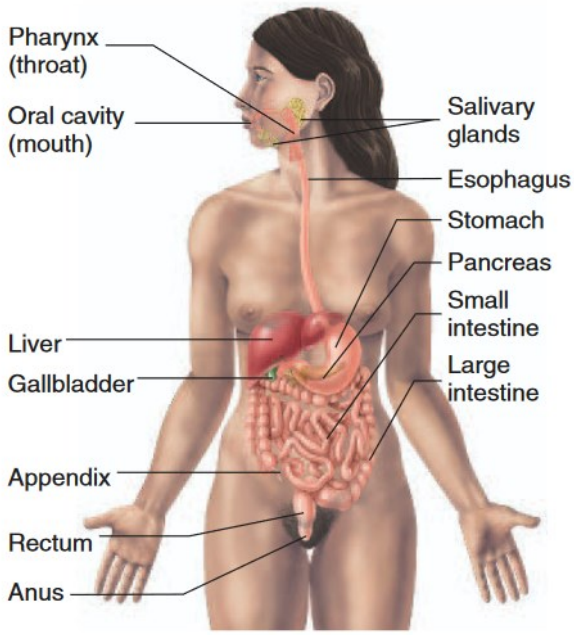


Fig. 8 The structure of gastrointestinal system [20]

Nanomaterials can get into the gastrointestinal tract by ingesting them directly in water, food, drugs, and drug delivery devices or after mucociliary clearance from the respiratory tract. In fact, studies, about the toxicity of nanomaterials after oral ingestion, are insufficient and very little studies have been done until now on how the gastrointestinal tract reacts to NPs. The gastrointestinal tract is a highly complex environment and understanding the efficiency of ingested NPs requires control of multiple factors. [21] However, it is known that permeability of the gastrointestinal lining may be changed due to a high concentration of ingested NPs. This condition can result in ulcers, weakening of the epithelium, cause metaplasia or dysplasia of the epithelium, malabsorption of nutrients, or it may lead to chronic bleeding. [19]

### 3.1.4 Lymphatic system and blood

NPs reach the lymphatic system in macrophages or as free particles, due to detection and drug delivery against tumor diseases. After NPs enter the lymphatic system, they aggregate in lymph nodes. The health effects of NPs against the lymphatic system are not examined enough yet. Oxidative stress caused by NPs can lead to damage of lymphocytes, lymph nodes, and spleen. [12]

There are three different types of cells in human blood and it is clear that NPs interact with each of them differently. The effect of NPs on blood cells depends on their size and surface charge. Thrombosis and cardiovascular malfunctions were observed after the translocation of NPs. On the other hand, NPs used as drug delivers are in direct contact with blood cells and so their toxicity against cells has to be minimalized. [12]

## 3.2 Biocompatibility of NPs with the human body

In the first place, it is important to define what biocompatibility means. This concept presents the interaction between implants or every foreign object with the human body. One of the definition was made in 2010 by Kohane and Langer and they explained biocompatibility in the context of drug delivery and defined biocompatibility as "an expression of the benignity of the relation between a material and its biological environment". It is clear, that biocompatibility presents both beneficial and harmful interactions. [15] Specific reactions between NPs and the human body include for example immunostimulation, immunosuppression or the creation of oxidative stress.

### 3.2.1 Immunostimulation

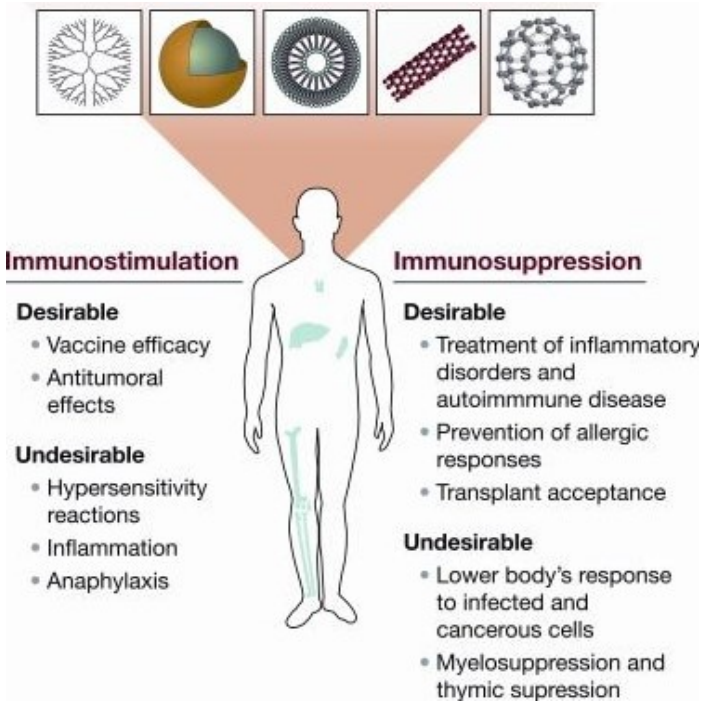


Fig. 9 The impact of NPs on human body [22]

When the immune system recognizes foreign materials it elicits the immune response. It leads to enhancing an activity of one or more components of the immunoregulatory complexes. In relation to immunostimulation, some NPs can behave as adjuvants. Adjuvants are molecules that are able to increase the body's immune response to an antigen. It has been shown, that using NPs, specifically poly(methyl methacrylate) NPs, against human immunodeficiency virus HIV, led to enhancing an antibody response in mice which was 100 times stronger than the traditional aluminum hydroxide or aqueous control vaccine. [15] On the other hand, many immunostimulatory reactions caused by NPs are mediated by the production of inflammatory cytokines. Several studies have reported cytokine induction by various types of nanomaterials (gold colloids, dendrimers, polymers, lipid nanoparticles, etc.). [22] This can be prevented by modifying nanoparticles by adding special ligands on their surface. [15]

### 3.2.2 Immunosupression

Immunosuppression is described as a reduction or prevention of the activation of the immune system. [15] Two types of immunosuppression are known: intended and unintended. Intended immunosuppression means that inhibition of immune response is expected because of relieving immune-mediated pathologies. Unintended immunosuppression occurs when a decrease in immune function is unplanned and can be beneficial. Unintended immunosuppression can be beneficial when it helps in inhibiting inflammatory and autoimmune conditions, e.g. to reduce the rejection of transplanted organs. [23] NPs are able to have the immunosuppressant function. In studies, it has been found out that a type of water-soluble fullerene on nanoscale level suppressed arthritis-induced inflammation, inhibited autoimmune diabetes as well as allergies type I and type II to common environmental and food allergens. [15]

### 3.2.3 Oxidative stress

Various types of NPs can cause the creation of reactive oxygen species (ROS) as it has been shown *in vivo* as well as *in vitro* studies. Free radicals are typical for containing one or more unpaired electrons. Substances with missing electrons are highly reactive with a wide range of different molecules, for example with DNA, proteins and with lipids in a cell membrane. [24] ROS can affect DNA in several ways, e.g. degradations of bases, DNA breaks, purine or pyrimidine modifications or mutations. DNA damages are

relevant to carcinogenesis, aging, cardiovascular or autoimmune diseases. ROS are responsible for disrupting the membrane lipid bilayer, as well as for protein fragmentations, cross-linking or oxidation of amino acids. [25] It is clear that a high concentration of ROS in living organisms may have a fatal impact on their health.

### 3.2.4 Cytotoxicity

Various types of NPs (NPs of $TiO_2$, ZnO, $Fe_3O_4$, $Al_2O_3$, $CrO_3$, and others… ) are connected with toxicity, which affects cell morphology, mitochondrial function and damages membranes. NPs with positive charge occurring in the cell are also undesirable. The cell membrane has a negative charge and if positively charged NP come into contact with the cell, it is able to block cell communication. Loss of possibility of a cell's communication leads to its death. [26]

### 3.2.5 Genotoxicity

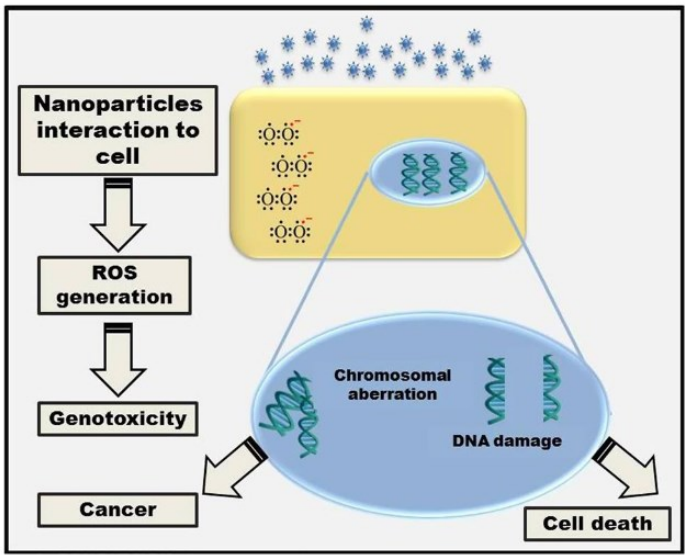


Fig. 10 Genotoxicity of NPs [26]

It has been already proven that NPs based on metals are able to interrupt the cell replication process and damage DNA. They are responsible for cell death and toxic effects on a genome. Changes in the cell genome can lead to chromosomal aberration, which may even cause tumor diseases. [26]

### 3.2.6 Toxic effect of NPs on the reproductive system

NPs can have toxic effects on any stage of reproduction and pregnancy in the human reproductive cycle. NPs may be responsible for damaging the reproductive organ function and physiological structure of germ cells. The toxic impact of NPs on the reproductive system differs at female and male reproductive organ systems. [27]

# 4 SYNTHESIS OF NPS

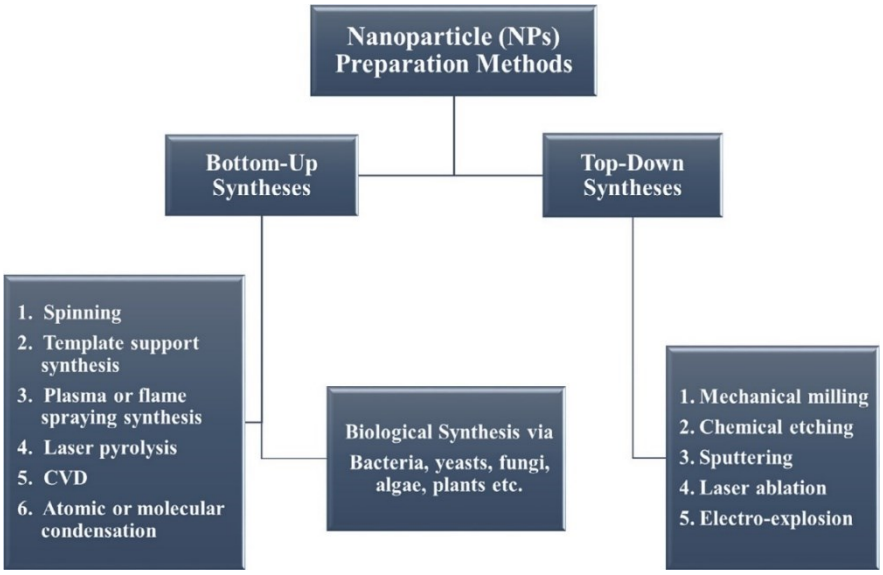


Fig. 11 Types of NPs synthesis [37]

There are many ways of synthesizing NPs. Usually, they are divided into two main classes: the bottom-up approach and the top-down approach. These approaches divide into various subclasses based on a different process of production.

## 4.1 The top-down syntheses

This method has a destructive character. It starts from a larger molecule, which is transferred into smaller units. These units are converted into suitable NPs. This can be done e.g. by grilling/milling or by physical vapor deposition. [28]

## 4.2 The bottom-up syntheses

In this method, NPs are formed from relatively simpler substances, therefore this approach is also called the “building up” approach. NPs are made by using sedimentation or reduction techniques. [28]

# 5 CLASSIFICATION OF NPS

As a tremendous amount of different kinds of NPs are known, it is in place to divide them into a few classes. NPs can be classified in many ways. Depending on the material used to produce them, they can be classified as follows:

## 5.1 Organic NPs

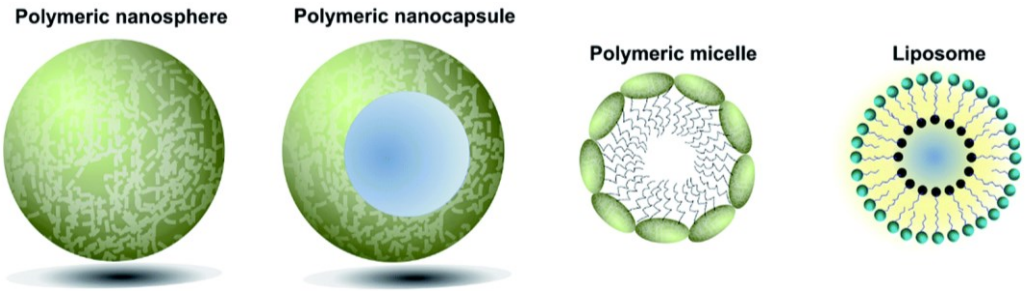


Fig. 12 Types of organic nanoparticles [31]

**Liposomes**

Liposomes are spherical vesicles of phospholipids bilayer. [29] They belong to the most developed nanocarriers for targeted drug delivery. Their synthesis is based on the hydration of dry phospholipids. [8]

**Polymeric NPs**

Polymeric NPs are made up of biocompatible and biodegradable polymers. Due to their ability of slow and controlled drug release, they are used as therapeutic carriers. [30]

**Polymeric micelles**

Polymeric micelles are colloidal structures composed of block copolymers. They are formed of hydrophobic fragments that form the inner core of the sphere, while hydrophilic fragments create the outer shell. They are used as drug delivery. Polymeric micelles also exhibit low toxicity, like other organic NPs. [29]

**Solid lipid nanoparticles (SLNs)**

They have been produced as an alternative carrier system to liposomes and polymeric NPs for controlled drug release. [8] They are composed of highly stable lipids that remain solid even at body temperature. Their properties depend on the condition of the medium in which they are located. [29]

## 5.2 Inorganic NPs

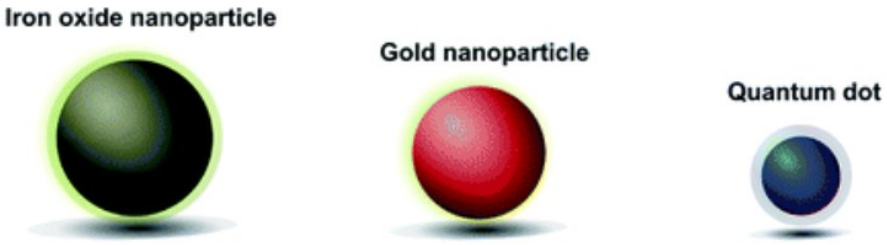


Fig. 13 Types of chosen inorganic NPs [31]

Inorganic NPs are composed of different inorganic oxides. They differ in size, shape, solubility and long-term stability. They are smaller than organic NPs. The inorganic NPs are prepared by the reduction of metallic salts with reducing agents. Their characteristics can be modified by temperature, pH, reduction time or concentration of reducing agent. The metallic NPs are toxic in higher concentrations for cells. Their interaction with proteins can result in skin or liver damage. They also might stimulate reactive oxygen species and inflammatory cytokines. [29]

**Iron oxide nanoparticles**

These NPs are composed of iron oxide core with a hydrophilic coat usually made of dextran or another biocompatible compound to enhance their stability. Because of their superparamagnetic properties, they are studied as imaging agents. [30]

**Gold nanoparticles**

Thanks to their flexible chemical and physical properties, they are suitable candidates for a large number of biomedical applications. They are usable as detectors of DNA, aminoglycoside antibiotics or even for detecting cancer stem cells. [32]

**Silver nanoparticles**

AgNPs have gained prime importance for biomedical applications across other metallic NPs. They have a wide range of purposes, from bioassays, detection, imaging to drug delivery and antimicrobial properties. [8]

**Quantum dots (QDs)**

These are metallic NPs with a core made of semiconductor materials like cadmium or zinc. However, the potential toxicity resulting from the presence of heavy metal ions in inorganic quantum dots may impede their medical applications. Therefore, a new class of quantum dots has recently been synthesized. They are called graphene quantum dots. [33]

**Graphene quantum dots (GQDs)**

**Definition of GQDs**

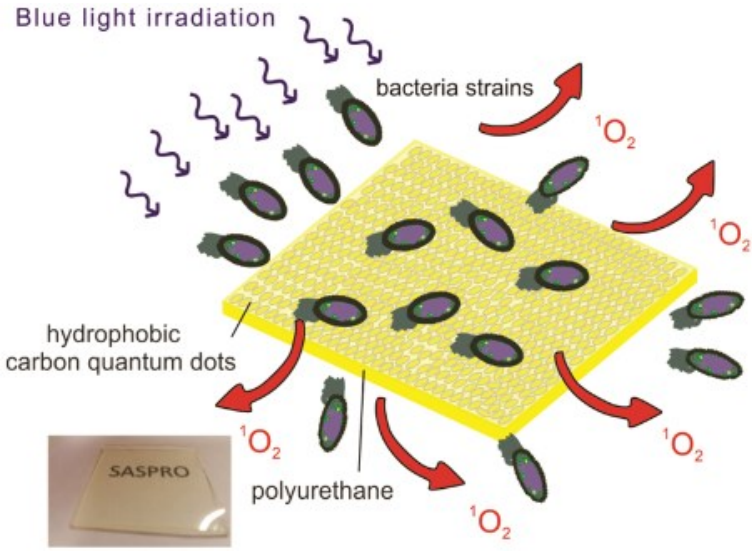


Fig. 14 The principle of photoexcitation [34]

They are made of a single layer of carbon atoms in a honeycomb structure, with large surface area and excellent thermal/chemical stability. GQDs, due to different electronic structure, possess some unique physicochemical and biological properties. In suspension, GQDs are able to generate reactive oxygen species upon photoexcitation. That factor makes GQDs potential candidates for photodynamic therapy. GQDs can target microbial pathogens or cancer cells. [33]

**Biological use of GQDs:**

<u>GQDs as immunosensors</u>

Immunosensors are used to detect many clinical diseases and biochemical compositions. It is based on classical antibody-antigen interactions. GQDs are supposed to be useful materials for fabricating various immunosensing platforms. [35]

GQDs for *in vivo* imaging

High photostability and a small number of particles needed to generate the signal make from GQDs candidates in bioimaging and for the imaging deeper tissue samples. Applying GQDs as contrast agents for *in vivo* imaging has been an area of high expectations in medicine. [35]

QDs as antibacterial agents

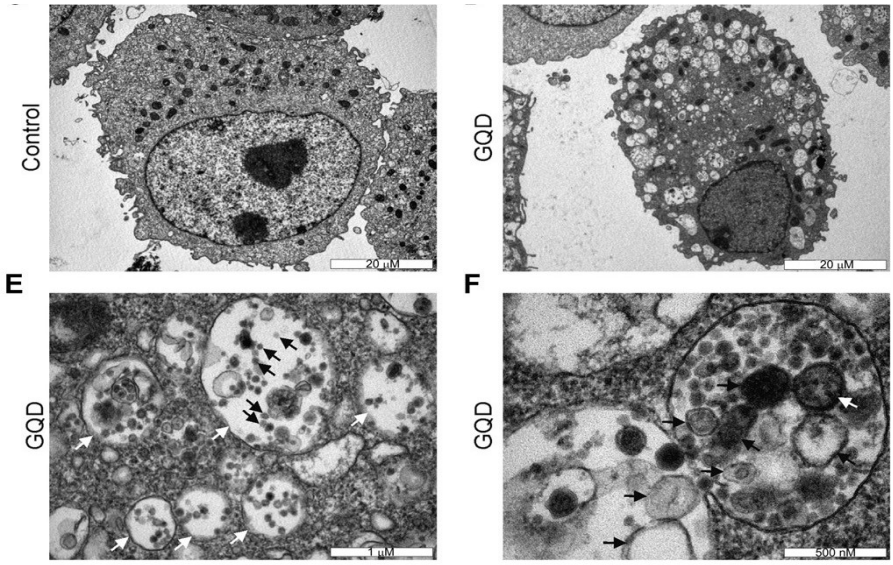


Fig. 15 The phototoxicity of GQDs [36]

The electrochemically produced GQDs irradiated with blue light generate reactive oxygen species. It has been found out that cell membrane permeability changes due to oxidative stress. Intracellular ROS causes loss of membrane's integrity, attacks proteins and enzymes that are crucial for cell morphology as well as for maintaining normal physiological processes for the life of cells. [36]

GQDs as a drug delivery system

GQDs are able to increase drug's solubility in water and improve the controlled release of therapeutics in the right places. Targeted drug delivery system is important in a matter of not damaging healthy cells when drugs with high toxicity are used to cure attacked cells. The possibility of using GQDs against tumor cells to improve drug efficacy and prolong the time of cytotoxic efficiency has been explored. [35]

# 6 APPLICATIONS OF NPS

## 6.1 Applications in manufacturing and material engineering

Different NPs possess various electrical, mechanical, optical and imaging properties. In general, they may be used in certain applications within the medical, commercial, and ecological sectors. The marketable products comprise microelectronics, aerospace and pharmaceutical industries. The wide category of consumer products includes health fitness products, followed by the electronic and computer category. The optical properties of noble metals NPs make them suitable for a wide variety of applications such as chemical sensors and biosensors. [37]

## 6.2 Application in environmental protection

Most of the environmental applications of nanotechnology fall into three categories:

1. Environmentally benign sustainable products
2. Removal of hazardous substances from contaminated materials
3. Sensors for environmental stages

Superparamagnetic iron oxide NPs are an effective sorbent material for heavy metals such as mercury, lead, thallium, cadmium or arsenic in water. [37] QDs are used for microbial monitoring and detecting microorganisms in an aqueous environment. [38]

## 6.3 Application in electronics

Semiconductor and metal-based NPs are the key structural block for a new generation of electronics, sensors and photonic materials. Their properties are responsible for a wide range of usage in electronic applications. [37] For example, gold NPs can be applied on the surface of appropriate substrates to enhance their luminescence. [39]

## 6.4 Application in mechanical industries

As revealed from their mechanical properties, some NPs may offer many utilizations in mechanical industries especially in coating, lubricants and adhesive applications. If they are used for coating different kinds of surfaces, they enhance toughness and protect products from damage. [37] Companies, such as Mercedes-Benz, use nanoparticle-

based coating onto their series production to increase resistance for scratching and improve the gloss of the surface on their products. [2]

## 6.5 Application in Biology

Three major areas of using NPs in Biology are drug and gene delivery, biosensing, and bioimaging. Nanoparticle-biomolecule interactions are crucial points for their successful effects on living organisms. The conjugation of NPs and biomolecules (e.t. proteins, DNA,..) is possible due to two different approaches. Direct covalent linkage can be accomplished either through chemisorption of the biomolecule to the particle surface or through the use of heterobifunctional linkers. Non-covalent interactions between NPs and biomolecules include electrostatic interactions, intercalation or groove binding. The fact, that NPs can recognize biomacromolecules surface, makes them a potential tool for controlling cellular and extracellular processes. These processes may include the transcription regulation, enzymatic inhibition or drug delivery and sensing. [40]

### 6.5.1 NPs as delivery systems

Appropriate NPs compounded with drugs or specific ligands have been used in nanomedicine. They should be able to minimize or avoid the side effects of the active drugs on healthy tissue and deliver therapeutics on target places. The targeted delivery can be done by using two different ways: [41]

a) Passive targeting

Passive targeting is based on taking advantage of the ability of some NPs to recognize targeted tissues. For example, tumor tissue differs from healthy tissues in a variety of signs. Tumor tissue has higher vascular density, a disorganized structure of tumor cells and irregular branching between cells. [10] Specific NPs are able to recognize and target affected tissue due to its different structure. The efficiency of NPs in targeting different kinds of tissues can be enhanced by modifying exact properties, e.g. particle composition, size, shape and surface characteristics. [10]

a) Active targeting

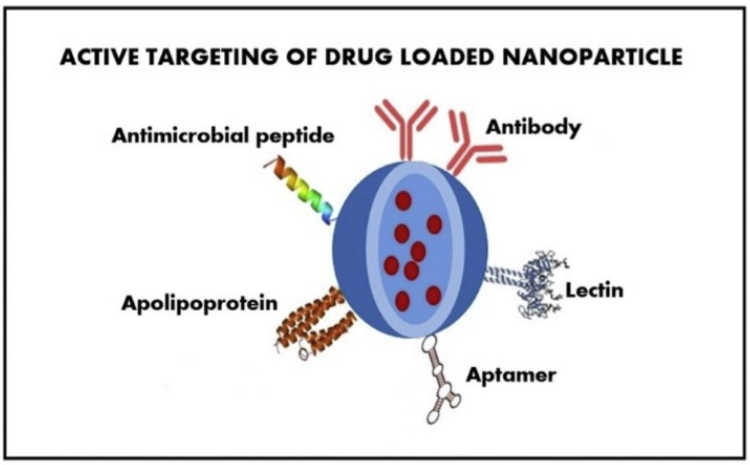


Fig. 16 Active targeting of NPs using different types of ligands [29]

The active targeting is based on using specific ligands on the surface of NPs. Targeting ligands are usually small molecules, peptides, antibodies, and their fragments or nucleic acids. They are bonded to NPs by non-covalent or covalent bonds. Active targeting can be used for therapeutics delivery. It increases the concentration of therapeutic agents in targeted places. [10]

#### *6.5.1.1 Delivering hydrophobic compounds without solvent or excipients*

The main biological processes of cells are done in an aqueous environment, the problem is that many biologically active compounds are hydrophobic and are poorly soluble in water. One solution about how to overcome the challenge of delivering these hydrophobic molecules is by using specialized NPs. There are many NPs with amphiphilic structure. Their non-polar core can encapsulate hydrophobic agents and their polar surface enhances solubility of hydrophobic compounds in water. Encapsulation also protects the agent from the environment until it is released from the NPs in a targeted area. [10]

#### *6.5.1.2 Delivering drugs and therapeutics*

Most of the current drugs have side effects and some of them have low efficiency. To reduce side effects and even improve drug´s efficiency, using NPs is an effective solution as it was discussed earlier in this thesis (page 15). [15] The ability of controlled drug release is based on a starter in a form of certain signals. These signals can have physical

or physiological character. Physical signals can involve e.g. ultrasound, electric field, temperature or magnetic field. The physiological signals are acidic or basic pH, the ionic strength of the medium, redox potential or enzymatic activity. NPs also can be used to keep drugs dormant until they reach the site of infection. After various signals, they are able to release drugs in targeted sites. [29]

### 6.5.2 Biosensing

For biomedical diagnosis, forensic analysis, and environmental monitoring, recognizing the origin of the caused problem is crucial. For sensing biological agents, diseases and toxic materials, special sensors consisting of two components are used. The components are a recognition element and an element for signaling the binding event. The fact, that metallic and semiconductor NPs possess unique physicochemical properties (optical and electronic properties), leads to using them in sensing applications. Colorimetric sensing is based on changing color after using sensors. This method is used for the diagnosis of genetic and pathogenic diseases and quantifying the number of products generated by polymerase chain reaction. [40]

### 6.5.3 NPs for bioimaging

Nowadays, the numbers of molecular imaging[3] are well-known. These methods are optical imaging (OI), magnetic resonance imaging (MRI), ultrasound imaging or positron emission tomography. The development of luminescent and magnetic NPs improves imaging technology. For imaging, two different types of NPs have been widely used: luminescent nanoprobes for OI and magnetic nanoparticles for MRI. [40]

a) Optical imaging

Major nanoparticle-based groups with optical imaging agents are quantum dots (QDs) and dye-doped NPs. Quantum dots are stable photochemically as well as metabolically. Mainly, they possess optical properties appropriate for optical imaging. Nevertheless, there are issues with toxicity, phototoxicity, and water solubility. To solve these problems, silica nanoparticles have been synthesized. They are able to encapsulate organic dyes, which are normally rapidly photo-bleaching. Silica NPs are less toxic and provide

[3] Molecular imaging is process of visualizing and measuring the function of biological and cellular processes *in vivo*. [70]

better biocompatibility. They have a huge range of usage, one of them is the detection of cancer cells in conjugation with magnetic particles. [40]

b) Magnetic resonance imaging

MRI is a diagnostic technique using for imaging soft tissues. This technique is based on using a strong and uniform magnetic field together with radiofrequency waves. The biggest advantages of MRI are various types of contrasts the image. [42] To improve this technique, cross-linked iron oxide NPs are using for targeted imaging with high cellular imaging. [40]

### 6.5.4 NPs for cell detection and separation

For biological researches and applications, isolation of specific cells is necessary. To detect specific cell types, distinct NPs can be used. The biggest interest in cell detection, it is to recognize circulating tumor cells (CTCs). [10] CTCs escape the primary tumor and then travel through the bloodstream. Their danger is in the ability, to cause secondary malignant tumor colonies, called metastasis. [43] The key role of NPs is an ability to create links with specific ligands, which are able to recognize CTCs with high specificity. That makes CTCs ready to be detected, characterized and isolated thanks to other properties of NPs. [44]

# 7 NPS IN COSMETICS

## 7.1 Barrier function of the skin

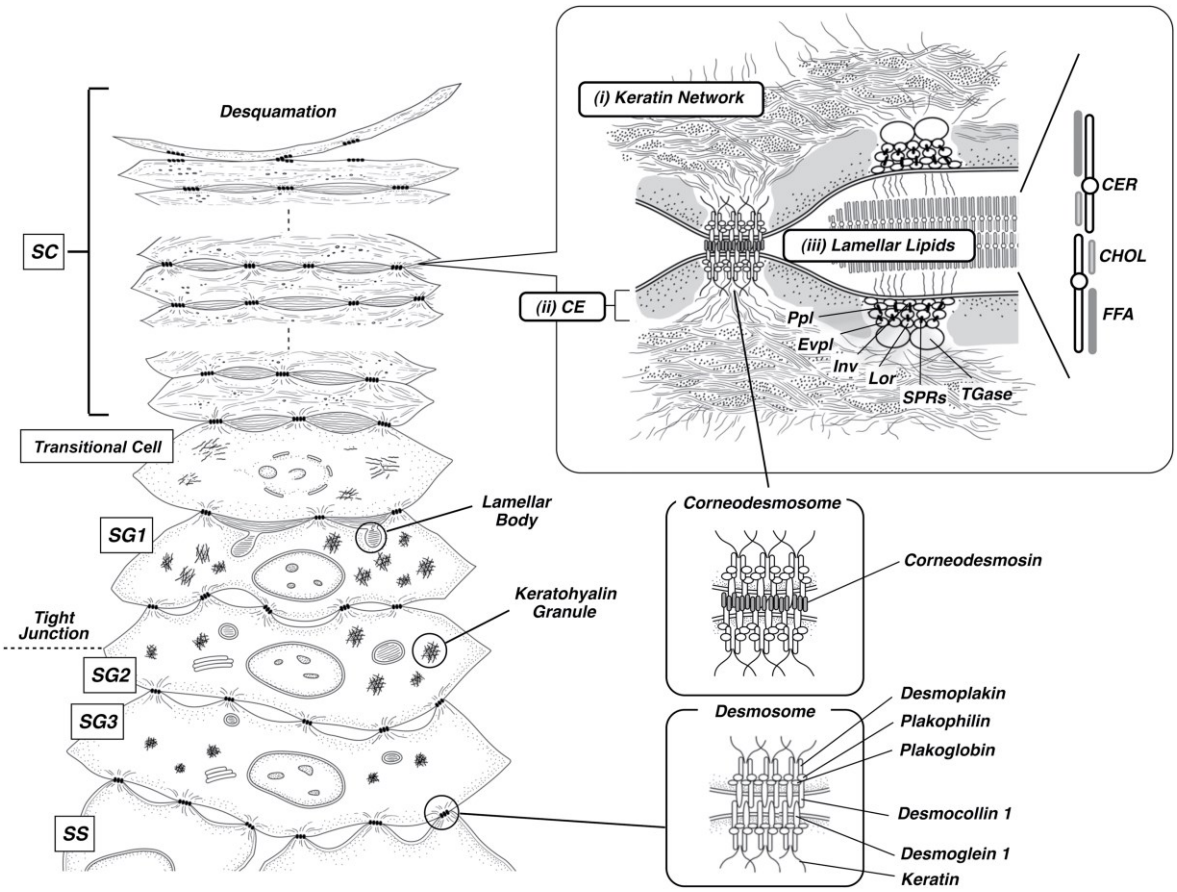


Fig. 17 Major components of *Stratum corneum* [45]

The skin is the largest organ of mammalian organisms. The main function of the skin is to protect the body in many different ways. It provides UV-protection, anti-oxidant and antimicrobial activity. Skin also acts as a sensory organ and the primary regulator of the body temperature. After the living conditions changed from water to dry land, it was needed to create a system, which protects the body from losing water and keeps homeostasis at the same level. The skin barrier function is also responsible for controlling the water and electrolytes movement. The skin barrier is situated in the outer layer of the skin called *Stratum corneum*. [46] *SC* is built up of two different parts: corneocytes and intracellular lamellar lipid bilayers. [47] Corneocytes are important for the mechanical resistance and lipid bilayers protect skin from transepidermal water loss. [46]

### 7.1.1 Lipid matrix

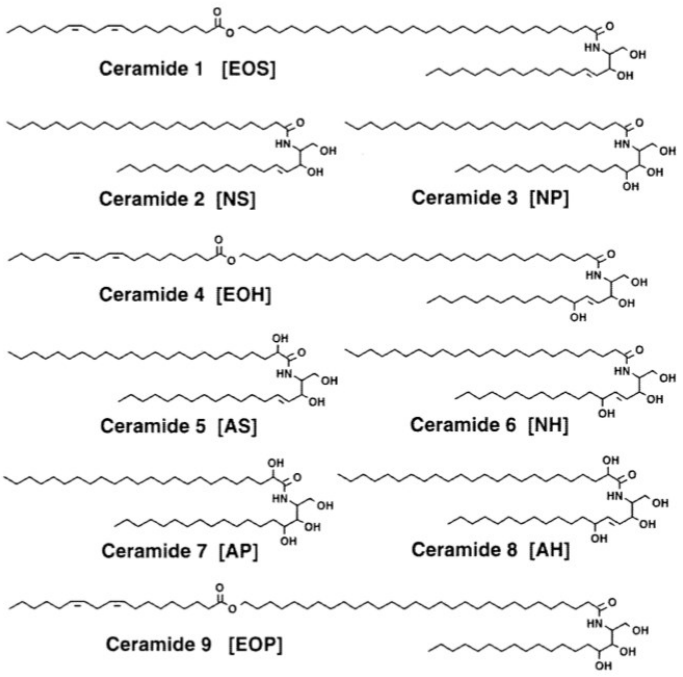


Fig. 18 Structure of ceramides [67]

Lipids forming skin barrier comprise of ceramides, cholesterol, and cholesteryl esters and free fatty acids. In the human skin, 9 types of ceramides occur. They differ from each other by the head-group architecture and bounding fatty acids. Free fatty acids mainly consist of saturated acids. The third lipid-based structure in the skin is cholesterol, especially the cholesterol-sulfate. The cholesterol-sulfate is responsible for the desquamation process in *SC*. [48]

### 7.1.2 Corneocytes

Corneocytes are dead flat cells filled with keratin. They are surrounded by "cell envelope" made of cross-linked proteins. [48] Corneocytes provide strengthening, UV protection of underlying mitotic active cells and hydration. The organized network of corneocytes and lipid matrix forms a "bricks and mortar" system. [49]

### 7.1.3 Protiens

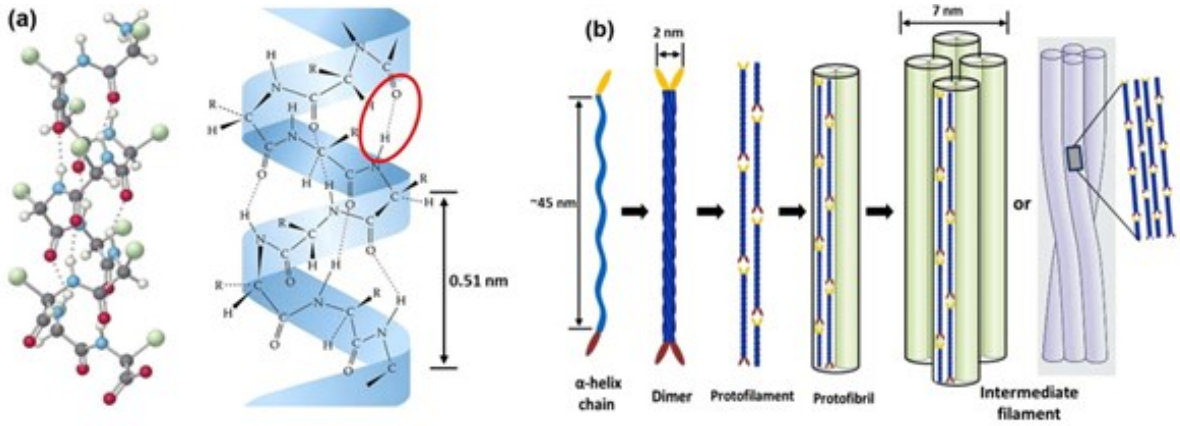


Fig. 19 Structure of α-keratin [68]

Several proteins (keratin, loricrin, involucrin, filaggrin, and corneodesmosin) build the corneocyte cytosol, the envelope, and the linkage between the corneocytes. The keratins build the intermediate filaments of the corneocyte cytoskeleton. Filaggrin is responsible for the formation of keratin filaments into macrofibrils and it is also an important source for maintaining skin moisture. Loricrin is a major component of the cell's envelope. Involucrin is considered as the primary precursor for the envelope. [46]

## 7.2 Mechanisms of regulation of the skin barrier

As it was written above, the skin barrier accomplishes various defensive functions: protection from environmental factors (physical, chemical, biological); antimicrobial protection, regulating water transport and the exchange of substances with the environment (excretion, secretion, resorption). Different mechanisms and signaling systems ensure the formation and maintenance of the epidermal barrier. [46]

### 7.2.1 Hydratation of *Stratum corneum*

Because water is essential for the right function of the physiological processes in mammalian organisms, there is a need for controlling the passage of water to the outer environment. The amount of water loss depends on the integrity of the *Stratum corneum*. Also, many physiological processes depend directly on the hydration of *SC*. [46]

### 7.2.2 Calcium ions gradient in the epidermis

It has been explored that calcium gradient plays a crucial role in skin barrier function. After damaging the skin, the concentration of calcium ions decreases. [47]. The reduction of $Ca^{2+}$ ions stimulates recovery systems. It speeds up the secretion of lamellar bodies at the border between *Stratum corneum* and *Stratum granulosum*. A high concentration of $Ca^{2+}$ slows down the recovery of the skin barrier. [46]

### 7.2.3 Skin surface acidity as a regulatory mechanism

Slightly acidic disposition of the skin is crucial for the epidermal antimicrobial barrier and controlling *Stratum corneum* integrity and cohesion. The level of skin pH ranges from 4,5 to 5,5. The acidic potential of *SC* is done by releasing free fatty acids from phospholipid hydrolysis or the emergence of free fatty acids by the effect of bacterial lipase, free fatty acids derived from sebum or eccrine gland-derived products. The acid mantle has diverse functions on human skin. It supports for example growth of normal microflora, inhibits the growth of skin pathogens and it also affects the desquamation process. [47]

## 7.3 Nanocosmetics

Nanotechnology has great potential in the field of medicine, personnel and health care. The characteristic properties of the material on a nanoscale are different from those of both individual molecules and bulk. Cosmetics enriched by different kinds of NPs gain benefits, which normal materials used in cosmetics do not possess. [50] Cosmetic product is according to Regulation No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products, any substance or mixture intended for contact with outer parts of human body (skin, hair, nails, lips…) or teeth and oral cavity only for their cleaning, perfuming, changing their appearance, protecting, keeping them in good conditions and adjusting body odors. Nanomaterial is insoluble

or bioperzistent and intentionally made material of one or more outer extent or with inner structure in size from 1 to 100 nm. Using NPs in cosmetics is defined by European Parliament in Regulation No 1223/2009. Nanocosmetics is a concept commonly used in scientific articles, reviews and also in EU definitions and represents any cosmetics containing NPs.

### 7.3.1 Nanomaterials in cosmetics

Incorporating certain NPs in cosmetics increases cosmetics efficiency and transparency and provides better texture and protection of active ingredients. Cosmetics products containing nanomaterials are sold all over the world. The most commonly used nanoscale materials are nanoemulsions, nanosomes, nanopigments or carbon-based nanomaterials. [50]

**a) Nanoemulsions**

Emulsions possess particular tactile and texture properties and are highly used in cosmetics. They are dispersions with droplets of one liquid in another. Because it is a system when one liquid is not soluble in other, their stability is limited. It has been shown that the smaller the size of droplets, the higher the stability of the emulsion is. The structure and size of droplets can be modified by different methods of preparation. Nanoemulsions differ from normal emulsions in the size of the dispersed phase. The size ranges from 1 – 100 nm. Due to their nano properties, nanoemulsions are transparent or translucent systems and have larger surface area owing to reduced dimension. [51] Nanoemulsions are used as carriers for active substance and to improve skin hydration. [50]

**b) Nanosomes**

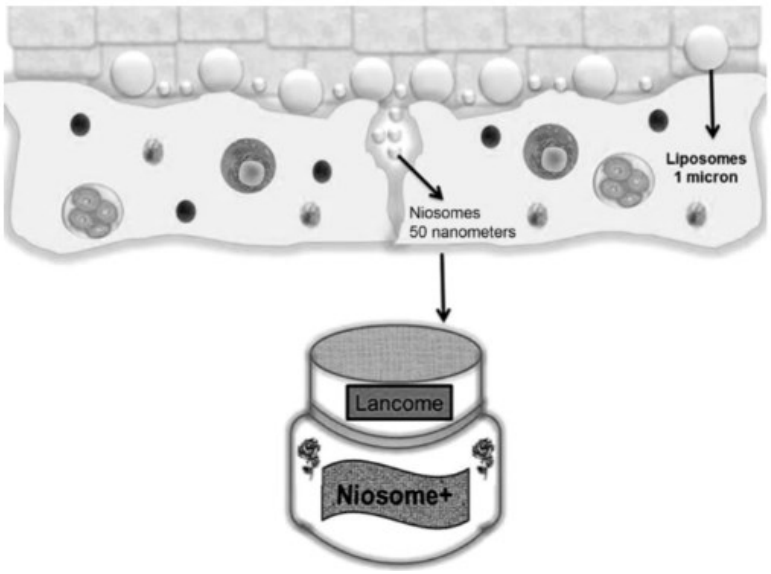


Fig. 20 Penetration of nanosomes through the skin [51]

Nanosomes are structures with a hydrophobic lipid bilayer surrounding the aqueous core. The lipid bilayer can infuse in the cell membrane and can release therapeutic material stored in the hydrophilic core. This property is used for improving the transport of polar substances through the non-polar environment. Liposomes and niosomes represent this category. They are able to penetrate to *Stratum corneum* and release drugs. Moreover, liposomes are made of phosphatidylcholine, which is able to improve the softness of the skin. [51]

**c) Nanopigments**

Nanopigments are used as labile carriers of cosmetic agents. They are used as UV rays blocking agents in sunscreens, as components of beauty soaps, gold facial masks, and creams. Nowadays, the safety of nanopigments in sunscreen applications is highly discussed. [50]

**d) Nanostructured lipid carriers**

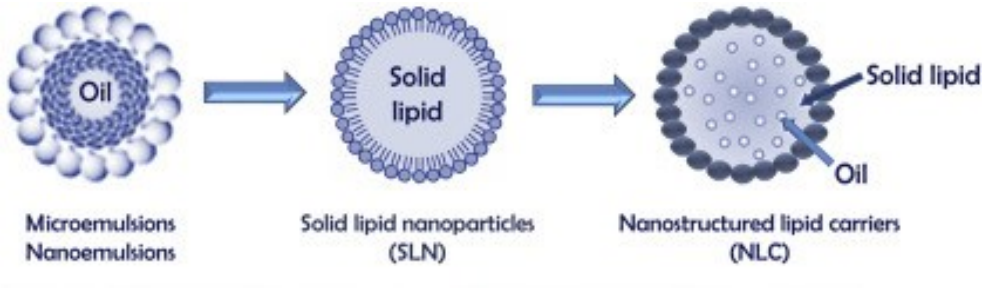


Fig. 21 Structure of SLNs [52]

Solid lipid nanoparticles (SLNs) are lipid droplets of nanosized dimensions. SLNs have similar properties as liposomes and niosomes. These structures are able to protect encapsulated active ingredients. They can be also used for controlled cosmetics delivery and improving skin penetration. SLNs enhance skin hydration and sunscreen efficiency as well. [53]

**e) Nanoclays**

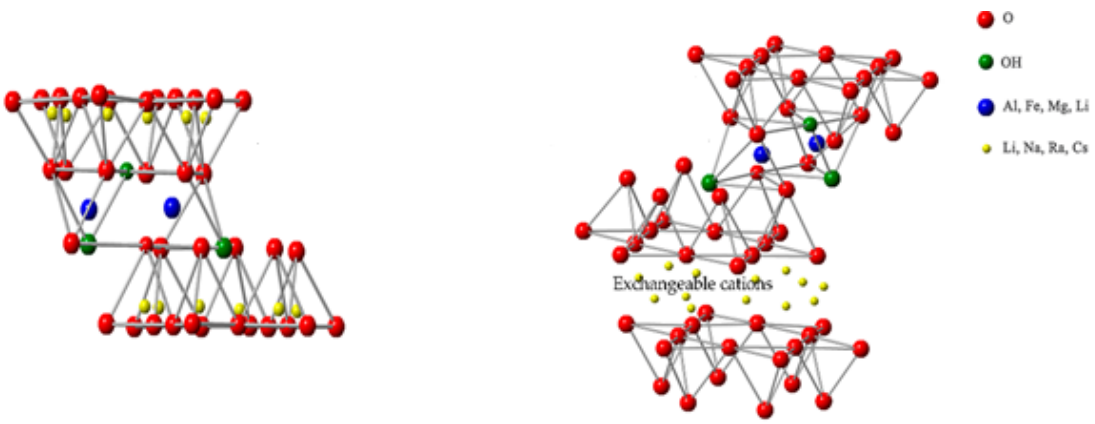


Fig. 22 Example of crystal structures of nanoclays [69]

Nanoclays are layers of mineral silicates about 1 nm thick, including kaolinite, smectite, sepiolite, chlorite, bentonite, saponite, etc. Organically modified nanoclays are created by the chemical reaction between layered silicates and ammonium or phosphonium salts. Nanoclays are used for color retention and coverage properties to nail paints or eye shadows. [50]

**f) Carbon nanomaterials**

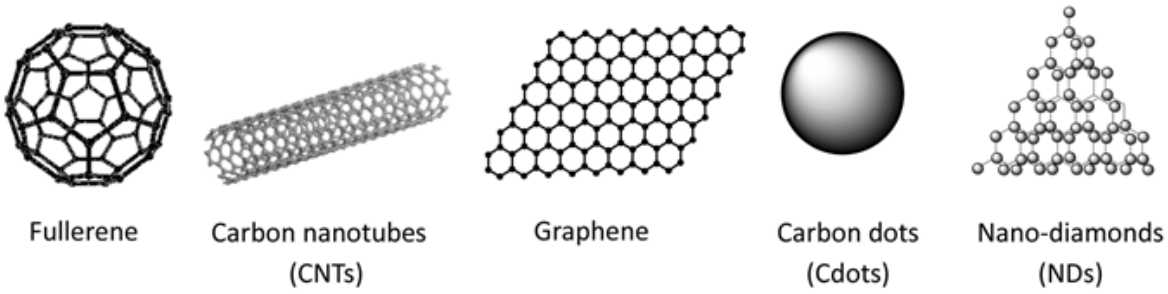


Fig. 23 Types of carbon nanomaterials [54]

Many divergent carbon-based NPs are synthesized. Carbon nanotubes act as promising pigments for hair coloring when they are chemically functionless and physically modified. Nanodiamond particles are known for absorption properties. They enhance mechanical properties, improve bonding of certain biological materials, and protect a subject from UV radiation. They are also able to reduce and prevent the formation of free radicals. In 2013 a sunscreen composition containing functionalized nanodiamonds for protection against skin cancer has been patented. [50]

### 7.3.2 Advantages and disadvatages of nanocosmetics

Generally, materials containing NPs provide unique properties and effects, which differ from those without NPs. On the other hand, the toxicity of NPs against the human body is still unclear. Certain toxic effects have been already described, but finding how deeply NPs can truly affect human body and environment, is still a question of many researches. Especially using NPs in cosmetics, where they are in direct contact with human body and time of exposition is long. It is needed to ensure their medical harmlessness.

**Advantages:**

- Improving the penetration of active ingredients through *SC*
- Stabilization of active ingredients (vitamins and anti-oxidants) delivery to the skin
- Improving UV protection due to enhancing sunscreens properties
- Rejuvenation of the skin, anti-oxidant effect
- Stabilization of cosmetics, anti-septic effects [55]

**Disadvantages:**

- Higher chemical reactivity, and producing a greater amount of reactive oxygen species
- NPs may cause inflammation, damaging proteins, membranes, and DNA
- Increased level of oxidative stress in living cells
- Provided toxicity of titanium dioxide in sunscreens
- Inhalation of nanoparticles induces pulmonary inflammation [56]

# 8 TOXICITY OF NPS AND THEIR IMPACT ON ENVIRONMENT

NPs occur in every part of the environment, in water as well as in soil and air during various human activity and as a product of weather and climate effects. Despite many industrial and medical application, there is a possibility of certain toxicities which are associated with using NPs. Studies have shown that NPs can enter the human body in few different ways. They may be responsible for protein damage, fibrillation, thiol cross linking, and loss of enzymatic activity. The toxic impact of NPs depends on their size, shape, characteristic charge, functionalized groups, and free energy. Also, it is necessary to bear in mind, the effects of NPs on the surrounding environment. Due to the fact that NPs are now used for many applications and it is necessary to find out how to make NPs more convenient and environmentally friendly. [28]

# II. ANALYSIS

The main aim of the practical part of this Bachelor thesis was to clarify biological effects of NPs and required properties of samples containing NPs. For testing, classical methods such as MTT test or fluorescent microscopy were used. To extend possibilities for biological testing, new methods were applied. These new methods included ATP bioluminescence assay and determination of hemolysis effects.

# 9 MATERIALS AND METHODS

## 9.1 Chemicals

For cell's cultivation Dulbecco Modified Eagle Medium (Biosera), calf serum (Biosera) and penicillin-streptomycin (Biosera) were used, according to ATCC standards. The essential material for MTT testing was MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Duchefa biochemie) and solution of DMSO (dimethylsulfoxide, Duchefa biochemie). DMSO was used also in hemolysis testing. Fluorescent microscopy required using of 4% formaldehyde (Penta chemicals), 0.5% Triton (Sigma Aldrich), PBS (phosphate buffered saline, Biosera) and fluorescent colours, specifically Hoechst (Sigma Aldrich) for staining cell's nuclei. ATP assay (Invitrogen) was done through using oxidation of D-luciferin catalyzed by firefly luciferase. This reaction was possible thanks to other different chemicals, but these two were crucial. For hemolysis, PBS, DMSO, $dH_2O$, and Triton were used to determine positive and negative control.

## 9.2 Cell lines

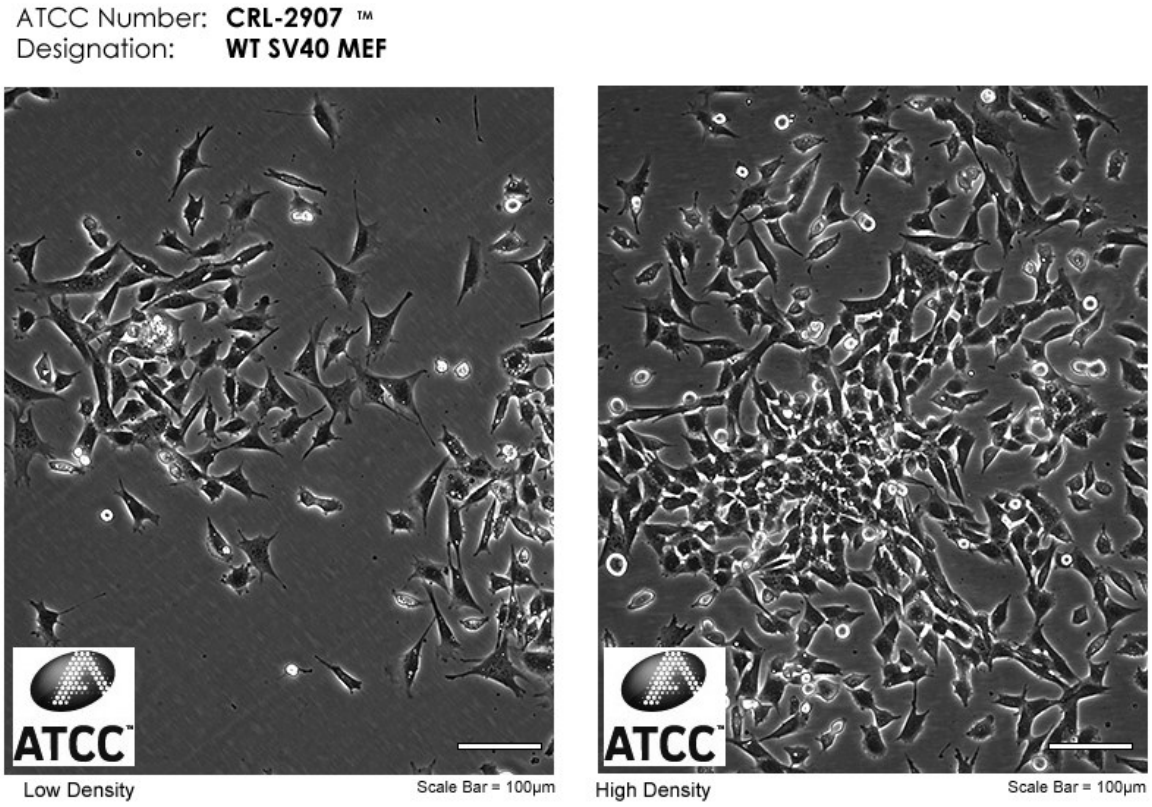


Fig. 24 NIH/3T3 cells [57]

The mouse embryonic fibroblast cell line (NIH/3T3) was used in the experiments according to ISO 10993 standards. This type of cells is suitable, due to their easy accessibility, rapid growth rates and a wide range of possible experiments. Fibroblasts are a group of heterogeneous resident cells of mesenchymal origin. [58] Line A549 was used for MTT tests as well. It is a cell line obtained from human cancerous lung cells.

The cells were cultivated in Dulbecco Modified Eagle Medium. The medium was mixed with calf serum and penicillin-streptomycin. Penicillin-streptomycin is used to protect cells against infection. Cells were incubated at 37 °C in 5% $CO_2$ in humidified air. When the cells were grown enough, they were ready to be used for experiments. Before testing, it was needed to remove cells from the surface of a culture dish. That was achieved by adding 7.5 ml of trypsin (Biosera). Trypsin was left to react with cells for about 10–15 minutes. After this period of time, 7.5 ml of pure medium was added. This mixture was removed into a 15 ml tube and vortexed for 3 min in 37°C 1100 rpm. The medium with trypsin was moved out from the tube and 2 ml of pure medium was put in. The mixture of cells and medium was diluted in required volumes.

## 9.3 Samples

Carbon quantum dots are NPs smaller than 10 nm and have very interesting properties such as high chemical stability, endurance against photobleaching, very good solubility in water, high photoluminescence and simple way of preparation. Two types of CQDs have been synthesized: hydrophilic and hydrophobic CQDs. In these experiments, hydrophobic CQDs (hCQDs) were used for filling two types of polymers (silicone and polyurethane). [34] Samples were prepared and used in cooperation with the Slovak Academy of Science. Samples were tested on cytotoxicity of their extracts and the proliferation of cells grown on their surface was observed. These methods are described below.

## 9.4 Determination of viability

### 9.4.1 MTT assay

MTT is a colorimetric assay, based on reduction MTT by enzymes of living cells. It is a classic method for determining the cell's viability in which testing substance is added directly to cells. For this kind of testing, cells were grown on microtitration plates in concentration $10^5$ in 100 µl of the medium. Extracts from samples were made by cutting down 3 $cm^2$ big

squares to smaller pieces. One ml of pure medium was added. Tubes with this mixture were incubated for 24 hours at 37°C with stirring. After that time, solutions were filtrated and added in different concentrations to cells prepared on microtitration plates. Samples were put into an incubator for another 24 hours. Another day, MTT testing followed. MTT was dissolved in deionized water in ratio 5 mg/1ml $H_2O$. Ten µl of this solution was added to examined samples and mixed with 90 µl of the pure medium. These samples were put back into the incubator for at least four hours. After four hours, yellow tetrazolium dye MTT changed colour, due to reducing itself into purple crystals of formazan. The absorbance was measured at 570 nm and the reference wavelength was 690 nm.

### 9.4.2 ATP assay

ATP bioluminescence assay is based on measuring cell cytotoxicity through the amount of luminescence. ATP (adenosine triphosphate) is the main donor of energy in metabolically active cells. Any damage of cells is accompanied by a decreasing level of ATP. For the determination of ATP, a lot of methods have been used. The most sensitive method is measuring bioluminescence. Bioluminescence is a phenomenon presented in many animal species. It has been observed that releasing of light emission by fireflies is based on oxidation of the substance called D-luciferin. Oxidation can be achieved only by consuming ATP. The reaction is catalyzed by enzyme luciferase. The amount of emitted light is linear related to ATP concentration. [59] Diagram of this reaction is shown below.

$$luciferin + ATP + O_2 \xrightarrow[luciferase]{} AMP + CO_2 + light$$

To make a standard curve, a standard reaction solution was prepared according to the manual for the ATP determination kit (A22066). Different concentrations of ATP were added to the standard reaction solution and luminescence was measured. ATP assay was used for determining the cytotoxicity of silica NPs, which were prepared at the Center of Polymer Systems in Zlín.

## 9.5 Proliferation

Proliferation was determined on the surface of the samples. Squares at size 1 $cm^2$ were cut down from samples. They were sterilized by UV light for 30 minutes from both sides and put on a microtitration plate. After sterilization, 250 µl of cells suspension in concentration $2 \cdot 10^5$ was added on each square. Samples were put into the incubator for an hour to ensure sticking cells to the surface. Then aliquot part of the medium was added to complete 1 ml volume.

Proliferation is usually observed by using fluorescent microscopy. Cells are not able to be seen on their own and they have to be coloured with fluorescent colours. Firstly, it was needed to fix the cells. That was achieved by removing the medium carefully, trying not to damage the surface of the samples. About 1 ml of 4% formaldehyde was added and let it to react for 15 minutes. Then samples were washed by 1 ml of PBS. One ml of 0.5% Triton was put on samples and left reacting for 5 minutes, followed by washing samples by PBS three times through. The colouring of cells followed. At that moment it was necessary to work without light to protect colours against fading. One ml of pure PBS and 25 µl of Hoechst stain was added to samples. Samples were left to incubate for 30 minutes in the dark. An inverted Olympus phase contrast microscope was used to visualize the cell morphology.

## 9.6 Hemolysis

Hemolysis is the process of the damaging the red blood cells membrane. The breakdown can be caused by a wide range of different factors. Clarifying positive and negative controls was made in the first step. As a negative control, DMSO (dimethylsulfoxide) and PBS (phosphate-buffered saline) were used. The negative control is characterized by almost no toxicity. On the other side, positive control should damage most of the cells. As positive control $dH_2O$, solutions of Triton in water and Triton in PBS were used. [60] After damaging the cell membrane, haemoglobin is released. The amount of released haemoglobin indicates the toxicity of the added substance.

From a sample of fresh human blood, serum was removed by centrifugation at 200 rpm for 5 minutes. Remaining cells were washed with through PBS five times. After the last wash, cells were diluted ten times with PBS again. 0.2 ml of red blood cell solution was combined with 0.8 ml testing sample (PBS, DMSO, diluted triton, $dH_2O$). Mixtures were vortexted for 2 hours at room temperature. Then red blood cells in samples were separated by centrifugation. The amount of haemoglobin was indicated by measuring the absorbance of supernatants left in tubes after centrifugation. The absorbance was measured at 560 nm.

# 10 RESULTS AND DISCUSSION

## 10.1 ATP assay

ATP bioluminescent method was successfully introduced into the laboratory practice at Cell biology laboratory at Centre of Polymer Systems of Tomas Bata University in Zlín (CPS). The standard curve was made from the results of a known amount of ATP. First of all, linear dependence of luminescence on the concentration of ATP is demonstrated on Fig. 25.

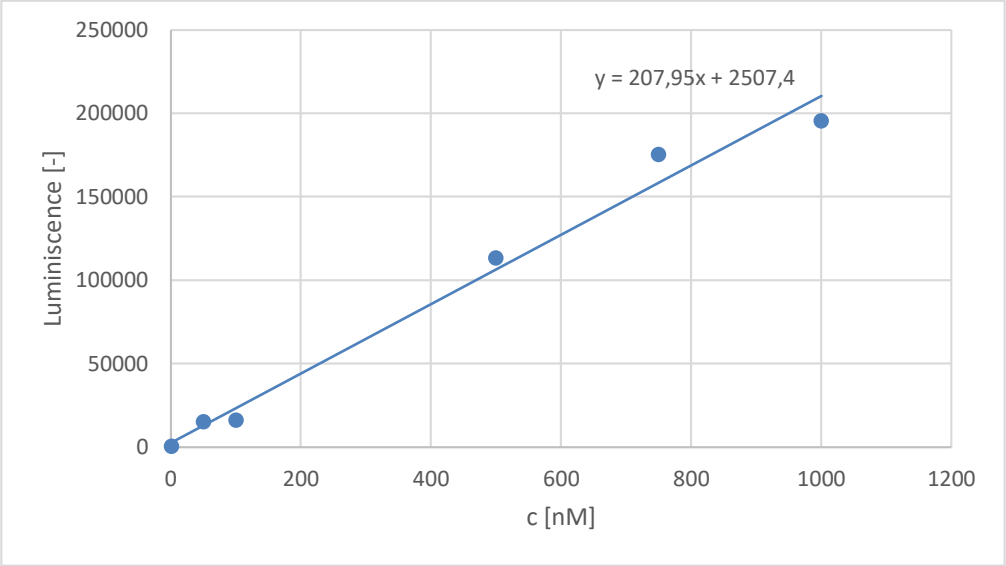


Fig. 25 Graph of dependence of luminescence

on the concentration of ATP

The introduction of ATP assay enables to use this method for testing of cytotoxicity of silica NPs mentioned earlier. For the purpose of this thesis, the results of just three samples are shown to declare the ATP methods was successfully used. The total amount of 21 samples was tested till the bachelor thesis was finalized. Fig. 26 illustrates the cytotoxic effects of samples 15,17 and 21 which were prepared by colleagues at CPS. None of these three samples indicate a toxic effect in comparison to the reference. Therefore, there is a possibility of using them in direct contact with living cells.

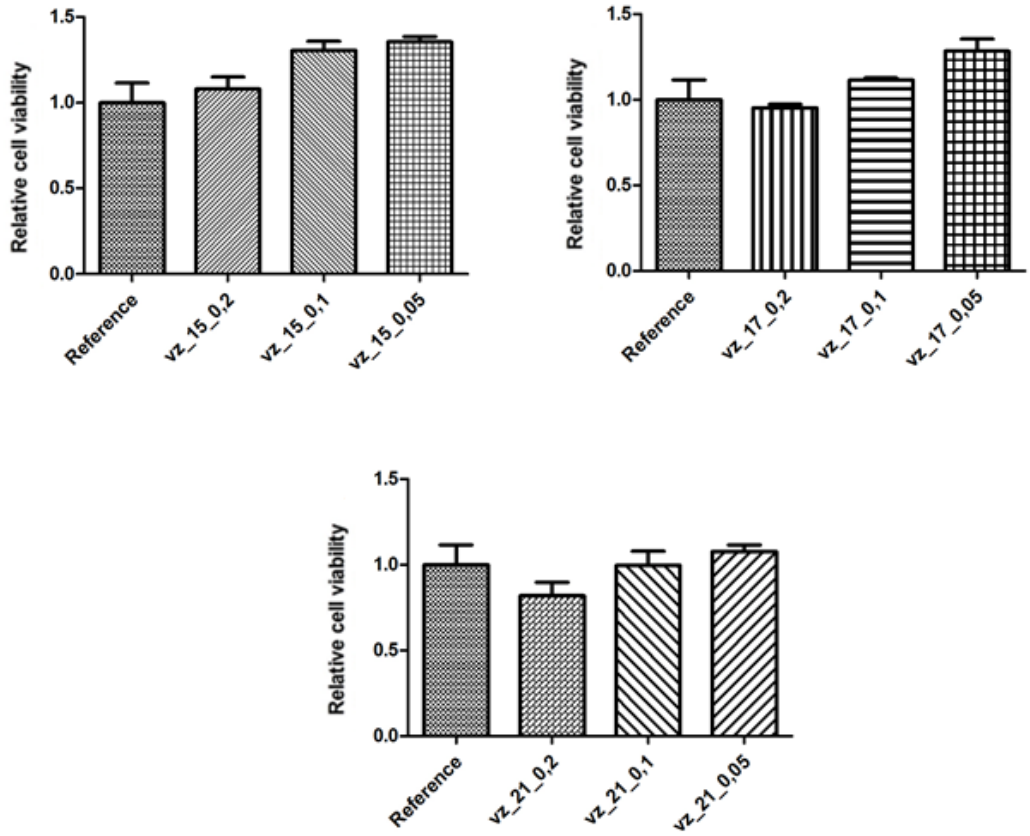


Fig. 26 Determination of cell viability using ATP assay

It is worth discussing the use of ATP and MTT tests and their negative and positive aspects. Both of them require specific metabolic conditions (pH changes and glucose deficiency). Using ATP assay instead of the MTT test is more appropriate for testing samples with lower concentration. Due to the high sensitivity of ATP, results are then more precise. Another problem may be caused due to the non-specific reduction of formazan by compounds presented in the culture medium. MTT testing is also more time-consuming. While ATP assay can be done immediately after adding a standard reaction solution to samples, the MTT test requires reaction time at least four hours. However, an important parameter is the price of testing assay. MTT test is cheaper than the ATP assay kit. For measuring the luminescence in ATP testing it is also needed to possess the device able to measure luminescence. Both of these methods are easy to perform and have their own advantages. The choice of the proper testing assay depends on testing conditions and the decision of researchers. [61] Due to my own experience with both testing methods, the ATP seems to be more sensitive and less time-consuming one. Nevertheless, higher price of this method does not allow

to use this method for bigger number of samples repeatedly. MTT assay does not required preparation of reactive solution and it is based on using just one chemical dissolved in water, so it is a lot cheaper than full ATP assay kit and easier for preparation. The lower sensitivity, however does not allow to use this method in case of small amount of samples or cells.

## 10.2 Hemolysis

The determination of the hemolytic effect is crucial for materials, which are in direct contact with blood cells. Drugs, NPs used as sensors, imaging agents or drug carriers, materials used for implants have to have a minimal toxic effect on blood if their use is required in medicine or biological application. In this thesis, the determination of negative and positive control was accomplished. Measured absorbance was higher in samples of blood with $dH_2O$ and solutions of Triton. The level of hemolytic effect is presented in Figure 28, where differences among negative (DMSO, PBS) and positive ($dH_2O$, SDS and Triton solutions) control are shown. The supernatant colour of negative control should be transparent, light red colour indicates a low hemolysis effect as well. Damage might have been caused by less gentle vortexing or inaccurate temperature through testing. Testing of particular samples on their hemolytical properties is a matter of future research.

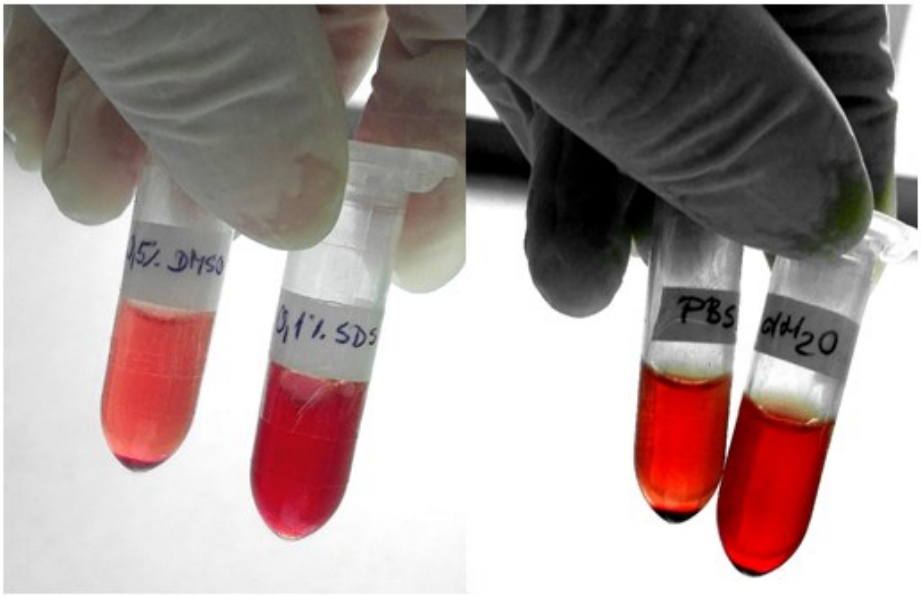


Fig. 27 Comparison of negative (0.5% DMSO, PBS)
and positive (0.1% SDS and $dH_2O$) control

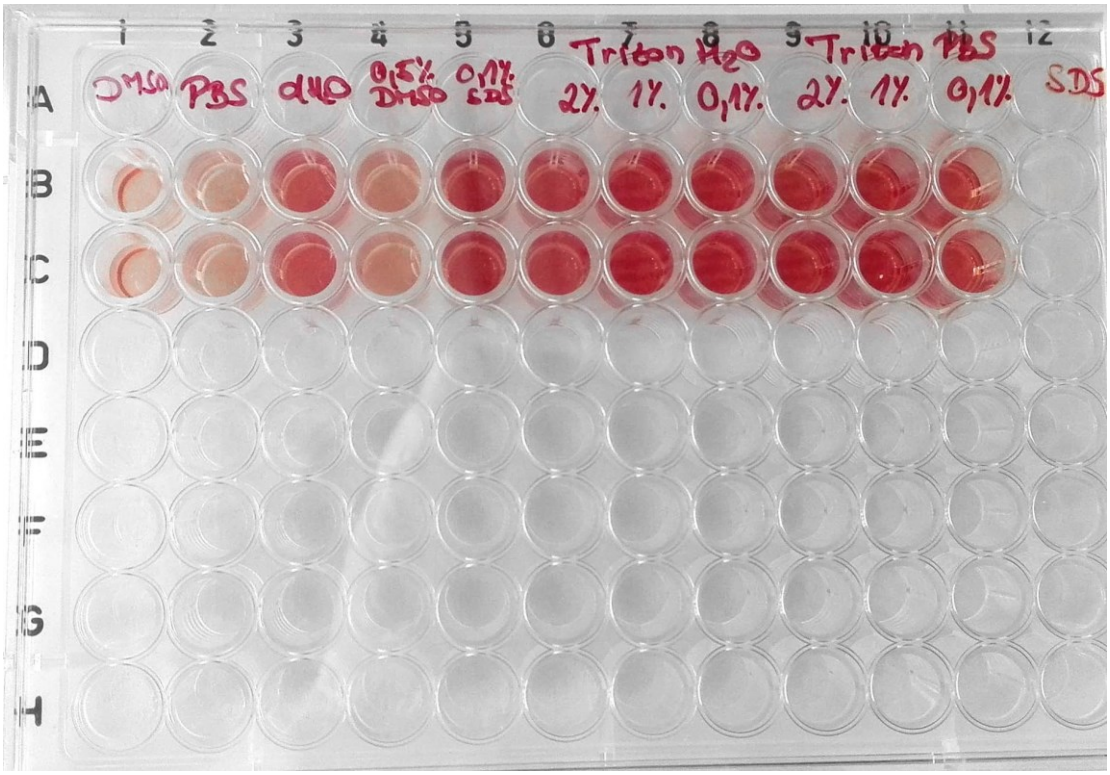


Fig. 28 Microtitration plate with supernatants

## 10.3 Cytotoxicity

Cytotoxicity is one of the important parameters of biocompatibility of biomaterials. It is based on measuring the toxic effects of any substances at the cellular level. Cytotoxic assays are highly used because of their low price, easy reproducibility, and quantification. Type of used assay varies from target cells, required response and studied agent. Cytotoxic assays may be focused on cell's viability, survival, transformation, irritancy or on measuring cell's metabolic response. Cytotoxicity can be done through several different assays such as assays based on cell proliferation, metabolic assays or microtitration assays. Proliferation determinates the effects of various compounds on the cell's growth. Metabolic assays are based on determining the metabolic activity of cells. Metabolic activity includes e.g. reduction of tetrazolium salts (MTT) or the synthesis of DNA and proteins. Microtitration assays provide the possibility of testing a higher amount simultaneously. The viability is then determined by measuring metabolic products such as ATP or NADH concentration. [62] For the purpose of this thesis, cytotoxicity was determined by the proliferation and metabolic activity assay (MTT test and ATP assay).

### 10.3.1 Cytotoxicity of samples with hCQDs

Used materials were tested according to ISO 10993-5, focused on testing of medical devices. From the results of cytotoxicity testing, it is possible to make a conclusion and decision, for which application sample is useful. Low cytotoxicity is prerequisite for applications of samples as drug carriers, drugs, implants and in other ways when direct contact with cells is necessary. In this thesis, cytotoxicity was determined by using the MTT method. Results are shown on Fig. 29 and 30. It was tested on two types of cells, NIH/3T3 and A549 according to ISO standards. Testing has shown, that all concentrations of extracts, except 100% extract, were nontoxic for NIH/3T3 cells as cell viability was higher than 0.8. The 100% extract was on the edge of low toxicity and nontoxic effect as cell viability was exactly at 0.8 point. For cell line A549, 100% extract had middle toxic effect, 75 and 50% extract indicated a low toxic effect for the cells. Results lead to the conclusion that samples are not dangerous for cells of normal tissue and can be used in direct contact with it. In higher concentrations sample indicated cytotoxicity against tumor cells. This fact may be interesting in future researches. These results were used in the article I cooperate on. This article has been already published in ACS Biomaterials journal (DOI: 10.1021/acsbiomaterials.8b00582).

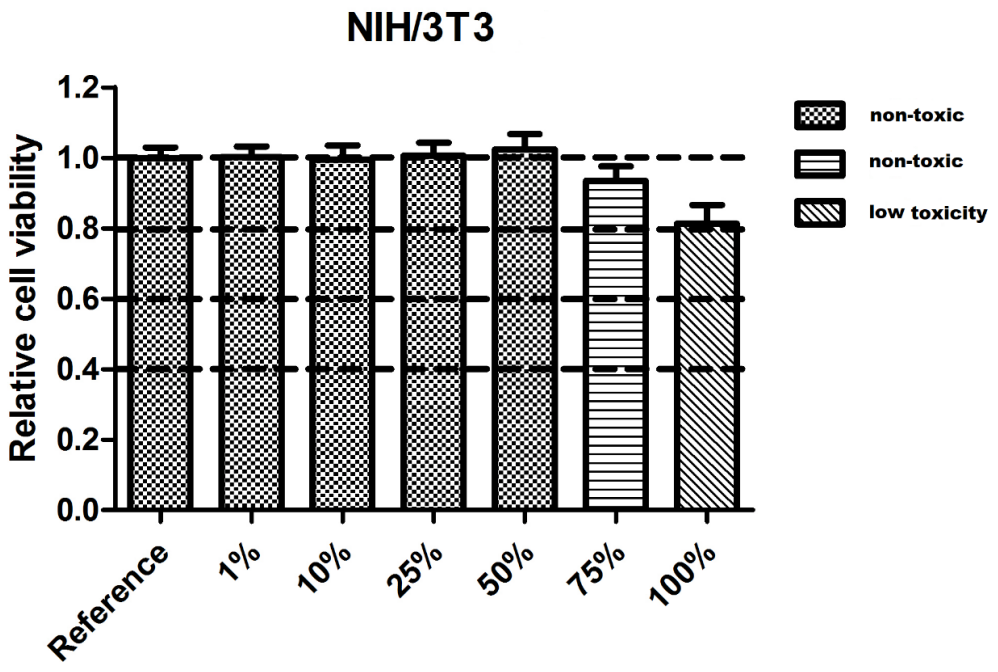


Fig. 29 Graph of cell viability of cell line NIH/3T3

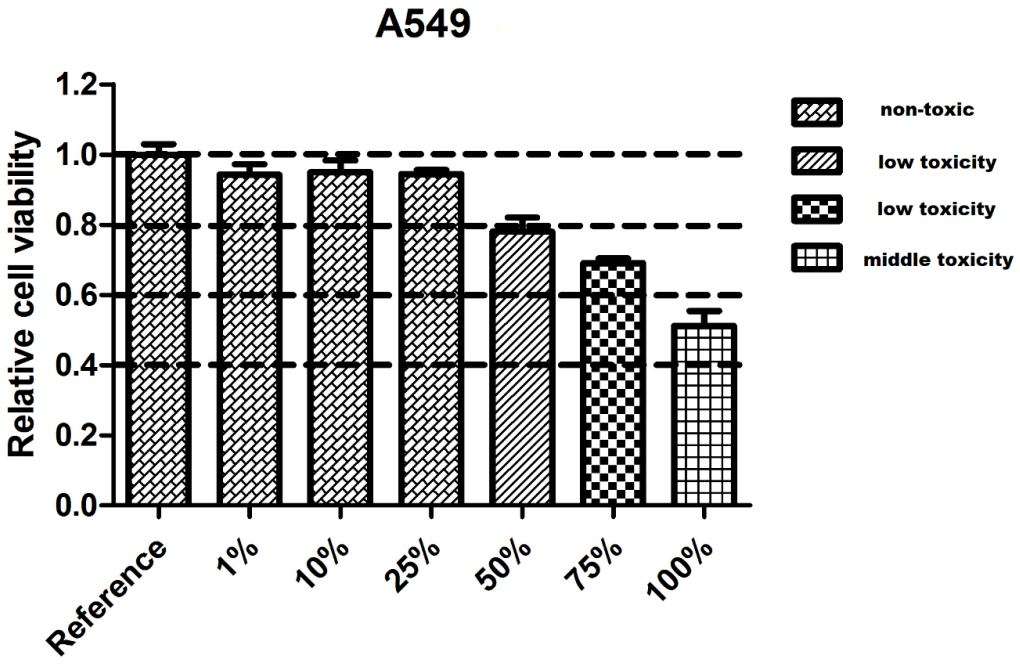


Fig. 30 Graph of cell viability of cell line A549

### 10.3.2 Proliferation

Proliferation and morphology of cells is also a useful tool for the determination of biocompatibility of samples. Fluorescent microscopy represents an easy way how proliferation can be observed. Stained cell's nuclei were detected on the surface of a polyurethane film with hCQDs. Cells, in general, are not able to grow properly on highly hydrophobic surfaces. In this case, the material was hydrophobic plus contained hydrophobic CQDs. Due to this fact, it was challenging to keep the cells on the surface. The proliferation was influenced by high hydrophobicity as it can be seen in Figure 31. The cells grew unequally on the surface of tested sample and they created clumps. Fig. 31 represents sample with the lowest concentration of hCQDs therefore the cells were able to stay and proliferate of the surface. With increasing concentration of hCQDs in samples, proliferation and adhesion of the cells decreased rapidly. On the sample with the highest amount of hCQDs, the cells were not able to grow and adhere at all. In many possible applications, good adhesion and proliferation are not always required. For production of thin medical tools, where blockage by adhered cells can occur, the material with low adhesion of cells is preferred.

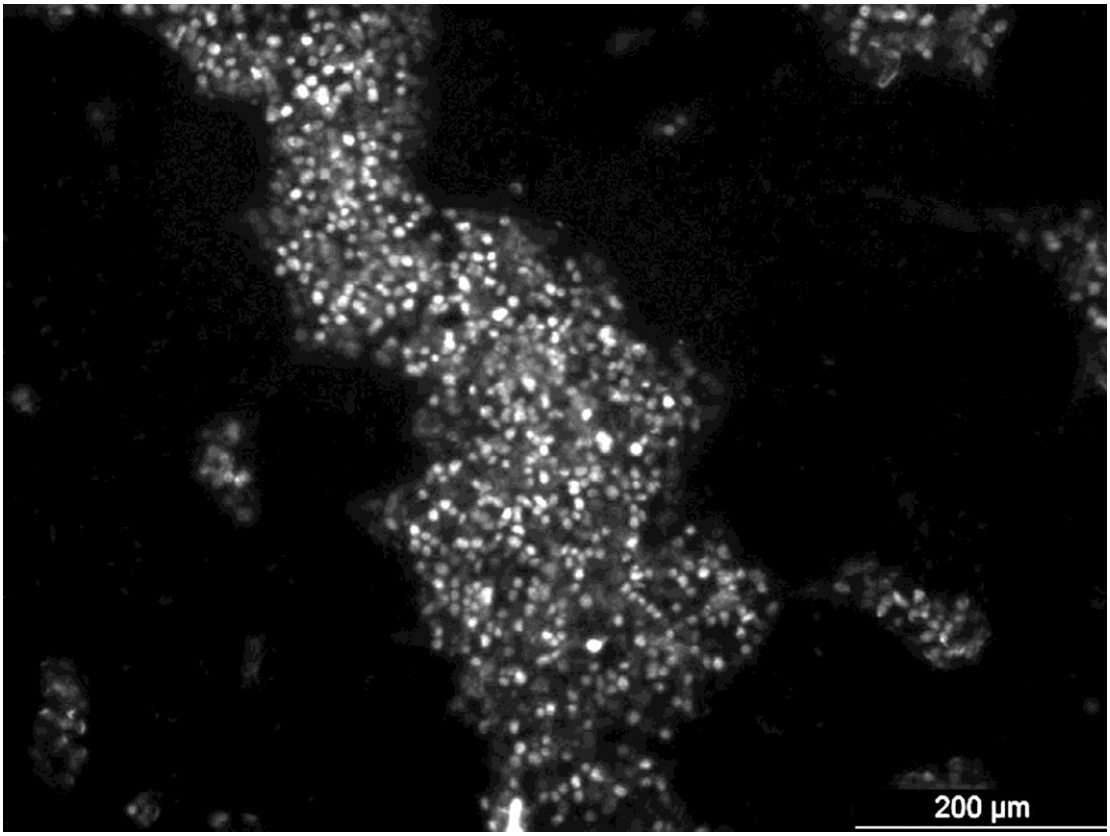


Fig. 31 Cell's growth on sample of hCQDs/PU

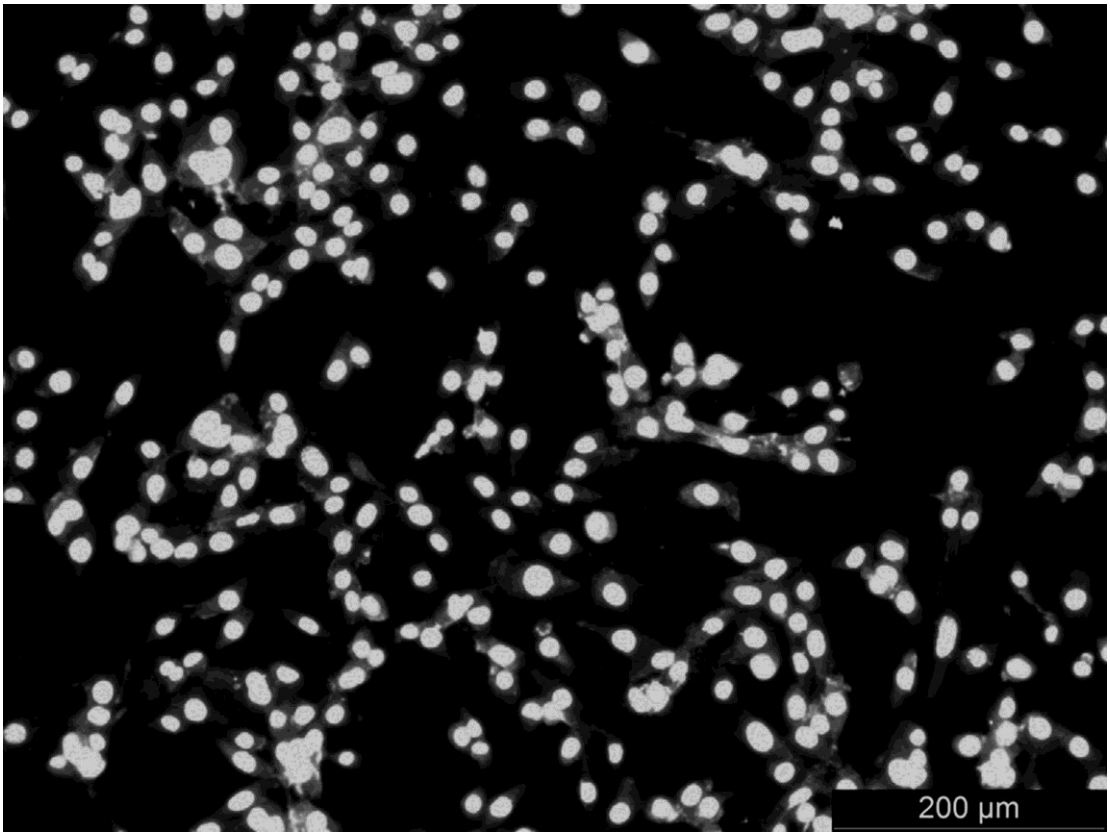


Fig. 32 The reference

# CONCLUSION

The aim of the theoretic part of the Bachelor thesis was to understand the concerns related to nanomaterials and their biological properties. The main characteristic of NPs influencing their properties and behavior in relationship with the human body is the tremendous variability in size, shape, surface, and materials from which NPs can be made. Therefore, their impact on human health varies from different kinds of NPs. Their properties might be useful and suitable for medical or biological applications. Their use depends mostly on biocompatibility with the human body. NPs in direct contact with live tissue can have beneficial as well as harmful effects. Some kinds of NPs may be used as drug carriers, pathogen detectors or imaging agents. They can also be able to enhance the efficiency of cosmetics products or drugs. On the other hand, the effects of a long time using NPs are still questionable and highly discussed topics in the scientific community. NPs are suspected for affecting the human immune system, reproductive system, DNA, causing inflammatory or oxidative stress and others. Determining the toxicity of NPs is complicated and can be influenced by a whole range of different signals.

The practical part of the thesis was focused on gaining knowledge and skills necessary for working in biological laboratories. These techniques were then used for biological testing of substances and materials. Most of the performed testing was based on the determination of cytotoxicity. Cytotoxicity was determined by using standard testing methods (MTT assay) as well as by introducing a new method, ATP assay. Measuring cytotoxic effects is based on a change in a cell's viability after direct contact with samples. The other part of biological testing was using the fluorescent microscopy to determine the proliferation and morphology of the cells on the surface of testing samples. Toxic effects may be tested by using different kinds of cells. In most testing, standard cell lines (NIH/3T3, A549) were used. To extend the knowledge about different types of biological testing, a method based on hemolysis of red blood cells was also introduced. This test indicated the hemolytic effect of samples, which is determined by the amount of released heamoglobin.

# BIBLIOGRAPHY

[1] *Nature: Nanoparticles* [online]. b.r. [cit. 2019-03-29]. Available from: https://www.nature.com/subjects/nanoparticles

[2] HEILIGTAG, F. and M. NIEDERBERGER. The fascinating world of nanoparticle research. *Materials Today* [online]. 2013, 16(7-8), 262-271 [cit. 2018-09-25]. DOI: 10.1016/j.mattod.2013.07.004. ISSN 13697021. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1369702113002253

[3] JEEVANANDAM, J., A. BARHOUM, Y. CHAN, A. DUFRESNE a M. DANQUAH. Review on nanoparticles and nanostructured materials: history, sources, toxicity and regulations. *Beilstein Journal of Nanotechnology* [online]. 2018, 9, 1050-1074 [cit. 2018-09-18]. DOI: 10.3762/bjnano.9.98. ISSN 2190-4286. Available from: https://www.beilstein-journals.org/bjnano/articles/9/98

[4] BANERJEE, A., J. QI, R. GOGOI, J. WONG a S. MITRAGOTRI. Role of nanoparticle size, shape and surface chemistry in oral drug delivery. *Journal of Controlled Release* [online]. 2016, 238, 176-185 [cit. 2019-03-29]. DOI: 10.1016/j.jconrel.2016.07.051. ISSN 01683659. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0168365916304977

[5] SALATA, OV. Applications of nanoparticles in biology and medicine. *Journal of Nanobiotechnology* [online]. 2004, 2(1), 16 [cit. 2018-09-25]. DOI: 10.1186/1477-3155-2- 3. ISSN 14773155. Available from: http://jnanobiotechnology.biomedcentral.com/articles/10.1186/1477-3155-2-3

[6] RIZVI, S.A.A. and Ayman SALEH. Applications of nanoparticle systems in drug delivery technology. *Saudi Pharmaceutical Journal* [online]. 2018, 26(1), 64-70 [cit. 2018- 09- 25]. DOI: 10.1016/j.jsps.2017.10.012. ISSN 13190164. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1319016417301792

[7] SANJAY, S. and A. PANDE. A Brief Manifestation of Nanotechnology. SHUKLA, Ashutosh Kumar, ed., Ashutosh SHUKLA. *EMR/ESR/EPR Spectroscopy for*

*Characterization of Nanomaterials* [online]. 1. New Delhi: Springer India, 2017, 47–63 [cit. 2019-03-22]. Advanced Structured Materials. ISBN 978-81-322-3653-5.

[8] Nanoparticles Types, Classifi cation, Characterization, Fabrication Methods and Drug Delivery Applications. BHATIA, Saurabh. *Natural Polymer Drug Delivery Systems* [online]. 1. Cham: Springer International Publishing, 2016, 1-6 [cit. 2018-09-25]. ISBN 978-3-319-41128-6.

[9] MASSADEH,S. Biological applications of Semiconductor Nanoparticles. *Nanomedicine* [online]: One Central Press, 2014, s. 357-373 [cit. 2018-10-13]. ISBN 978-1-910086-01-8. Available from: http://www.onecentralpress.com/wp- content/uploads/2014/11/CHAPTER-14-NM-05-LATEST2.pdf

[10] WANG, E. and A. WANG. Nanoparticles and their applications in cell and molecular biology. *Integr. Biol*[online]. 2014, 6(1), 9- 26 [cit. 2018- 09- 18]. DOI: 10.1039/C3IB40165K. ISSN 1757- 9694. Available from: http://xlink.rsc.org/?DOI=C3IB40165K

[11] COLOMBO, M., S. CARREGAL-ROMERO, M. CASULA et al. Biological applications of magnetic nanoparticles. *Chemical Society Reviews* [online]. 2012, 41(11), 129 [cit. 2018- 09- 25]. DOI: 10.1039/c2cs15337h. ISSN 0306- 0012. Available from: http://xlink.rsc.org/?DOI=c2cs15337h

[12] BUZEA, C., I. PACHECO and Kevin ROBBIE. Nanomaterials and nanoparticles: Sources and toxicity. *Biointerphases* [online]. 2007, 2(4), 17-71 [cit. 2018-10-13]. DOI: 10.1116/1.2815690. ISSN 1934- 8630. Available from: http://avs.scitation.org/doi/10.1116/1.2815690

[13] PERRAULT, S. D. and W. CHAN. In vivo assembly of nanoparticle components to improve targeted cancer imaging. *Proceedings of the National Academy of Sciences* [online]. 2010, 107(25), 11194- 11199 [cit. 2019- 04- 28]. DOI: 10.1073/pnas.1001367107. ISSN 0027- 8424. Available from: http://www.pnas.org/cgi/doi/10.1073/pnas.1001367107

[14] THAKOR, A., J. JOKERST, P. GHANOUNI, J. CAMPBELL, E. MITTRA and S. GAMBHIR. Clinically Approved Nanoparticle Imaging Agents. *Journal of Nuclear Medicine* [online]. 2016, 57(12), 1833- 1837 [cit. 2019- 02- 08]. DOI: 10.2967/jnumed.

116.181362. ISSN 0161- 5505. Available from: http://jnm.snmjournals.org/cgi/doi/10.2967/jnumed.116.181362

[15] NAAHIDI, S., M. JAFARI, F. EDALAT, K. RAYMOND, A. KHADEMHOSSEINI and P. CHEN. Biocompatibility of engineered nanoparticles for drug delivery. *Journal of Controlled Release* [online]. 2013, 166(2), 182-194 [cit. 2018-09-18]. DOI: 10.1016/j.jconrel.2012.12.013. ISSN 01683659. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0168365912008553

[16] CASELLA, M. Bionanotechnologies: positive aspects and risks for health. CASELLA, M. *Nanomedicine - Overview of a new science* [online]. 1., 2015, [cit. 2018-10-13]. ISBN 9786050364224.

[17] OSTIGUY, C., G. LAPOINTE, L. MÉNARD, Y. CLOUTIER, M. TROTTIER, M. BOUTIN, M. ANTOUN and Ch. NORMAND. Nanoparticles Actual Knowledge about Occupational Health and Safety Risks and Prevention Measures. *Institut de recherche RobertSauvé en santé et en sécurité du travail* [online]. 2016, 1-70 [cit. 2018-10-13]. Available from: https://www.irsst.qc.ca/media/documents/PubIRSST/R-470.pdf

[18] RAAB, C. and A. GAZSÓ. How Nanoparticles Enter the Human Body and Their Effects There. *Nanotrust dossiers*. 2011, 2, 1-3. ISSN 1998-7293. Available from: http://epub.oeaw.ac.at/ita/nanotrust-dossiers?frames=yes

[19] SAJID, M., M. ILYAS, Ch. BASHEER, M. TARIQ, M. DAUD, N. BAIG and F. SHEHZAD. Impact of nanoparticles on human and environment: review of toxicity factors, exposures, control strategies, and future prospects. *Environmental Science and Pollution Research* [online]. 2015, 22(6), 4122-4143 [cit. 2018-09-25]. DOI: 10.1007/s11356-014-3994-1. ISSN 0944-1344. Available from: http://link.springer.com/10.1007/s11356-014-3994-1

[20] SEELEY, R., T. STEPHENS and P. TATE. *Anatomy & physiology*. 6th ed. Boston: McGraw-Hill, 2003. ISBN 9780072351132.

[21] VISWANATH, B. and S. KIM. Influence of Nanotoxicity on Human Health and Environment: The Alternative Strategies. DE VOOGT, Pim, ed., Pim DE VOOGT. *Reviews of Environmental Contamination and Toxicology Volume 242* [online]. Cham: Springer International Publishing, 2017, s. 61-104 [cit. 2018-09-25]. Reviews of

Environmental Contamination and Toxicology. DOI: 10.1007/398_2016_12. ISBN 978-3-319-51242-6. Available from: http://link.springer.com/10.1007/398_2016_12

[22] ZOLNIK, B., Á. GONZÁLEZ-FERNÁNDEZ, N. SADRIEH and M. DOBROVOLSKAIA. Minireview: Nanoparticles and the Immune System. *Endocrinology* [online]. 2010, 151(2), 458-465 [cit. 2018-09-25]. DOI: 10.1210/en.2009-1082. ISSN 0013-7227. Available from: https://academic.oup.com/endo/article-lookup/doi/10.1210/en.2009-1082

[23] ILINSKAYA, A and M DOBROVOLSKAIA. Immunosuppressive and anti-inflammatory properties of engineered nanomaterials. *British Journal of Pharmacology* [online]. 2014, 171(17), 3988-4000 [cit. 2018-09-25]. DOI: 10.1111/bph.12722. ISSN 00071188. Available from: http://doi.wiley.com/10.1111/bph.12722

[24] BURTON, G. and E. JAUNIAUX. Oxidative stress. *Best Practice & Research Clinical Obstetrics & Gynaecology* [online]. 2011, 25(3), 287-299 [cit. 2018-11-27]. DOI: 10.1016/j.bpobgyn.2010.10.016. ISSN 15216934. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1521693410001392

[25] BIRBEN, E., U. SAHINER, C. SACKESEN, S. ERZURUM and O. KALAYCI. Oxidative Stress and Antioxidant Defense. *World Allergy Organization Journal* [online]. 2012, 5(1), 9-19 [cit. 2018-11-27]. DOI: 10.1097/WOX.0b013e3182439613. ISSN 1939-4551. Available from: http://www.waojournal.org/content/5/1/9

[26] SARDOIWALA, M., B. KAUNDAL and S. CHOUDHURY. Toxic impact of nanomaterials on microbes, plants and animals. *Environmental Chemistry Letters* [online]. 2018, 16(1), 147-160 [cit. 2019-01-29]. DOI: 10.1007/s10311-017-0672-9. ISSN 1610-3653. Available from: http://link.springer.com/10.1007/s10311-017-0672-9

[27] BROHI, R., L. WANG, H. TALPUR et al. Toxicity of Nanoparticles on the Reproductive System in Animal Models: A Review. *Frontiers in Pharmacology* [online]. 2017, 8(), 1–22 [cit. 2019-02-09]. DOI: 10.3389/fphar.2017.00606. ISSN 1663-9812. Available from: http://journal.frontiersin.org/article/10.3389/fphar.2017.00606/full

[28] KHAN, I., K. SAEED and I. KHAN. Nanoparticles: Properties, applications and toxicities. *Arabian Journal of Chemistry* [online]. 2017, 2017(1), 124 [cit. 2018-09-25].

DOI: 10.1016/j.arabjc.2017.05.011. ISSN 18785352. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1878535217300990

[29] ZAIDI, S., L. MISBA and A. KHAN. Nano-therapeutics: A revolution in infection control in post antibiotic era. *Nanomedicine: Nanotechnology, Biology and Medicine* [online]. 2017, 13(7), 2281-2301 [cit. 2018-09-25]. DOI: 10.1016/j.nano.2017.06.015. ISSN 15499634. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1549963417301235

[30] HAROON ANWAR, S. A Brief Review on Nanoparticles: Types of Platforms, Biological Synthesis and Applications. *Research & Reviews Journal of Material Sciences* [online]. 2018, 06(02), 1–8 [cit. 2018-11-26]. DOI: 10.4172/2321-6212.1000222. ISSN 23216212. Available from: http://www.rroij.com/open-access/a-brief-review-on-nanoparticles-types-of-platforms-biologicalsynthesis-and-applications.php?aid=86995

[31] RICHARDS, D., A. MARUANI and V. CHUDASAMA. Antibody fragments as nanoparticle targeting ligands: a step in the right direction. *Chemical Science* [online]. 2017, 8(1), 63-77 [cit. 2019-03-22]. DOI: 10.1039/C6SC02403C. ISSN 2041-6520. Available from: http://xlink.rsc.org/?DOI=C6SC02403C

[32] HASAN, S. A Review on Nanoparticles: Their Synthesis and Types. *Research Journal of Recent Sciences* [online]. 2014, (4), 1–4 [cit. 2018-11-26]. ISSN 2277-2502. Available from:https://www.researchgate.net/publication/273203342_A_Review_on_Nanoparticles_Their_Synthesis_and_Types

[33] RISTIC, B., M. MILENKOVIC, I.R. DAKIC et al. Photodynamic antibacterial effect of graphene quantum dots. *Biomaterials* [online]. 2014, 35(15), 4428-4435 [cit. 2018-09-25]. DOI: 10.1016/j.biomaterials.2014.02.014. ISSN 01429612. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0142961214001549

[34] KOVÁČOVÁ, Mária, Zoran MARKOVIć, Petr HUMPOLÍČEK et al. Carbon Quantum Dots Modified Polyurethane Nanocomposite as Effective Photocatalytic and Antibacterial Agents. *ACS Biomaterials Science & Engineering* [online]. 2018 [cit. 2018-11-07]. DOI: 10.1021/acsbiomaterials.8b00582. ISSN 2373-9878. Available from: http://pubs.acs.org/doi/10.1021/acsbiomaterials.8b00582

[35] CHEN, F., W. GAO, X. QIU, H. ZHANG, L. LIU, P. LIAO, W. FU and Y. LUO. Graphene quantum dots in biomedical applications: Recent advances and future challenges. *Frontiers in Laboratory Medicine* [online]. 2017, 1(4), 192-199 [cit. 2018-10- 13]. DOI: 10.1016/j.flm.2017.12.006. ISSN 25423649. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2542364917301358

[36] MARKOVIC, Z., B. RISTIC, K. ARSIKIN et al. Graphene quantum dots as autophagy-inducing photodynamic agents. *Biomaterials* [online]. 2012, 33(29), 7084-7092 [cit. 2018-09-25]. DOI: 10.1016/j.biomaterials.2012.06.060. ISSN 01429612. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0142961212007107

[37] KHAN, I., K. SAEED and I. KHAN. Nanoparticles: Properties, applications and toxicities. *Arabian Journal of Chemistry* [online]. 2017, 5–7 [cit. 2018-10-13]. DOI: 10.1016/j.arabjc.2017.05.011. ISSN 18785352. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1878535217300990

[38] LIU, W. Nanoparticles and their biological and environmental applications. *Journal of Bioscience and Bioengineering* [online]. 2006, 102(1), 1-7 [cit. 2018-09-18]. DOI: 10.1263/jbb.102.1. ISSN 13891723. Available from: http://linkinghub.elsevier.com/retrieve/pii/S138917230670620X

[39] RAMOS, A.P., M. CRUZ, C. TOVANI and P. CIANCAGLINI. Biomedical applications of nanotechnology. *Biophysical Reviews* [online]. 2017, 9(2), 79-89 [cit. 2018-11-26]. DOI: 10.1007/s12551-016-0246-2. ISSN 1867-2450. Available from: http://link.springer.com/10.1007/s12551-016-0246-2

[40] DE, M., P. GHOSH and V. ROTELLO. Applications of Nanoparticles in Biology. *Advanced Materials* [online]. 2008, 20(22), 4225-4241 [cit. 2018-09-18]. DOI: 10.1002/adma.200703183. ISSN 09359648. Available from: http://doi.wiley.com/10.1002/adma.200703183

[41] DE CROZALS, G., R. BONNET, C. FARRE and C. CHAIX. Nanoparticles with multiple properties for biomedical applications: A strategic guide. *Nano Today* [online]. 2016, 11(4), 435-463 [cit. 2018-09-25]. DOI: 10.1016/j.nantod.2016.07.002. ISSN 17480132. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1748013216300743

[42] ILANGOVAN, S., B. MAHANTY and S. SEN. Biomedical Imaging Techniques. SANTHI, V., ed., D. P. ACHARJYA, ed. and M. EZHILARASAN, ed., V. SANTHI, D. ACHARJYA, M. EZHILARASAN. *Emerging Technologies in Intelligent Applications for Image and Video Processing* [online]. 1.: IGI Global, 2016, s. 401-421 [cit. 2018-09-25]. Advances in Computational Intelligence and Robotics. DOI: 10.4018/978-1-4666-9685-3.ch016. ISBN 9781466696853. Available from: http://services.igi-global.com/resolvedoi/resolve.aspx?doi=10.4018/978-1-4666-9685-3.ch016

[43] MYUNG, J., K. TAM, S. PARK, A. CHA and S. HONG. Recent advances in nanotechnology-based detection and separation of circulating tumor cells. *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology* [online]. 2016, 8(2), 223-239 [cit. 2018-09-25]. DOI: 10.1002/wnan.1360. ISSN 19395116. Available from: http://doi.wiley.com/10.1002/wnan.1360

[44] BHANA, S., Y. WANG and X. HUANG. Nanotechnology for enrichment and detection of circulating tumor cells. *Nanomedicine* [online]. 2015, 10(12), 1973-1990 [cit. 2018-09-25]. DOI:10.2217/nnm.15.32. ISSN 1743-5889. Available from: https://www.futuremedicine.com/doi/10.2217/nnm.15.32

[45] MATSUI, T. and M. AMAGAI. Dissecting the formation, structure and barrier function of the stratum corneum. *International Immunology* [online]. 2015, 27(6), 269-280 [cit. 2019-03-29]. DOI: 10.1093/intimm/dxv013. ISSN 0953-8178. Available from: https://academic.oup.com/intimm/article-lookup/doi/10.1093/intimm/dxv013

[46] DARLENSKI, R., J. KAZANDJIEVA and N. TSANKOV. Skin barrier function: morphological basis and regulatory mechanisms. *Journal of clinical medicine* [online]. 2011, 4(1), 36– 45 [cit. 2018- 10- 19]. Available from: https://www.researchgate.net/publication/285876351_Skin_barrier_function_Morphological_basis_and_regulatory_mechanisms

[47] LEE, S.H., S. JEONG and S. AHN. An Update of the Defensive Barrier Function of Skin. *Yonsei Medical Journal* [online]. 2006, 47(3), 293–306 [cit. 2018-12-23]. DOI: 10.3349/ymj.2006.47.3.293. ISSN 0513-5796. Available from: https://synapse.koreamed.org/DOIx.php?id=10.3349/ymj.2006.47.3.293

[48] BOUWSTRA, J. and M. PONEC. The skin barrier in healthy and diseased state. *Biochimica et Biophysica Acta (BBA) - Biomembranes* [online]. 2006, 1758(12), 2080-2095 [cit. 2018-12-23]. DOI: 10.1016/j.bbamem.2006.06.021. ISSN 00052736. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0005273606002410

[49] MURPHEY, M. and P. ZITO. Histology, Stratum Corneum. *StatPearls* [online]. 2018, 2018, [cit. 2018- 12- 23]. Available from: https://www.statpearls.com/as/integument/29520/

[50] AJAZZUDDIN, M., G. JESWANI and A. JHA. Nanocosmetics: Past, Present and Future Trends. *Recent Patents on Nanomedicine* [online]. 2015, 5(1), 3-11 [cit. 2018-10-19]. DOI: 10.2174/1877912305666150417232826. ISSN 18779123. Available from: http://www.eurekaselect.com/openurl/content.php?genre=article&issn=1877-9123&volume=5&issue=1&spage=3

[51] NANDA, S., A. NANDA, S. LOHAN, R. KAUR and B. SINGH. Nanocosmetics: performance enhancement and safety assurance. *Nanobiomaterials in Galenic Formulations and Cosmetics* [online]. 10.: Elsevier, 2016, s. 47-67 [cit. 2018-12-23]. DOI: 10.1016/B978-0-323-42868-2.00003-6. ISBN 9780323428682. Available from: https://linkinghub.elsevier.com/retrieve/pii/B9780323428682000036

[52] MONTENEGRO, L., F. LAI, A. OFFERTA, M. SARPIETRO, L. MICICCHè, A. MACCIONI, D. VALENTI and A. FADDA. From nanoemulsions to nanostructured lipid carriers: A relevant development in dermal delivery of drugs and cosmetics. *Journal of Drug Delivery Science and Technology* [online]. 2016, 32, 100-112 [cit. 2019-03-29]. DOI: 10.1016/j.jddst.2015.10.003. ISSN 17732247. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1773224715300368

[53] SHOKRI, J. Nanocosmetics: benefits and risks. *BioImpacts* [online]. 2017, 7(4), 207-208 [cit. 2018-10-19]. DOI: 10.15171/bi.2017.24. ISSN 2228-5660. Available from: http://bi.tbzmed.ac.ir/Abstract/bi-17532

[54] LIU, Z. and X. LIANG. Nano-Carbons as Theranostics. *Theranostics* [online]. 2012, 2(3), 235-237 [cit. 2019-03-29]. DOI: 10.7150/thno.4156. ISSN 1838-7640. Available from: http://www.thno.org/v02p0235.htm

[55] BECK, R., ed., S. GUTERRES, ed. and A. POHLMANN, ed. *Nanocosmetics and Nanomedicines* [online]. Berlin, Heidelberg: Springer Berlin Heidelberg, 2011 [cit. 2019-03-29]. DOI: 10.1007/978-3-642-19792-5. ISBN 978-3-642-19791-8. Available from: https://www.academia.edu/19997919/Nanocosmetics_and_Nanomedicines_New_Approaches_for_Skin_Care

[56] SHOKRI, J. Nanocosmetics: benefits and risks. *BioImpacts* [online]. 2017, 7(4), 207-208 [cit. 2018-12-23]. DOI: 10.15171/bi.2017.24. ISSN 2228-5660. Available from: http://bi.tbzmed.ac.ir/Abstract/bi-17532

[57] *ATCC® CRL-1658™* [online]. b.r. [cit. 2019-03-29]. Available from: https://www.lgcstandards- atcc.org/products/all/CRL- 2907.aspx?geo_country=cz#characteristics

[58] QIU, L., W. LAI, D. STUMPO and P. BLACKSHEAR. Mouse Embryonic Fibroblast Cell Culture and Stimulation. *BIO-PROTOCOL* [online]. 2016, 6(13), [cit. 2019-03-08]. DOI: 10.21769/BioProtoc.1859. ISSN 2331- 8325. Available from: https://bio- protocol.org/e1859

[59] CHOLLET, R. and S. RIBAULT. Use of ATP Bioluminescence for Rapid Detection and Enumeration of Contaminants: The Milliflex Rapid Microbiology Detection and Enumeration System. LAPOTA, David, ed., David LAPOTA. *Bioluminescence - Recent Advances in Oceanic Measurements and Laboratory Applications* [online]. 1. France: InTech, 2012, 1–22 [cit. 2019-03-08]. DOI: 10.5772/37055. ISBN 978-953-307-940-0. Available from: http://www.intechopen.com/books/bioluminescence-recent-advances-in-oceanic-measurements-and-laboratory-applications/use-of-atp-bioluminescence-for-rapid-detection-and-enumeration-of-contaminants-the-milliflex-rapid-m

[60] LI, Hung-Cheng, Feng-Jen HSIEH, Ching-Pin CHEN et al. The hemocompatibility of oxidized diamond nanocrystals for biomedical applications. *Scientific Reports* [online]. 2013, 3(1), [cit. 2019-03-08]. DOI: 10.1038/srep03044. ISSN 2045-2322. Available from: http://www.nature.com/articles/srep03044

[61] PETTY, R., L. SUTHERLAND, E. HUNTER and I. CREE. Comparison of MTT and ATP-based assays for the measurement of viable cell number. *Journal of Bioluminescence and Chemiluminescence* [online]. 1995, 10(1), 29-34 [cit. 2019-03-22].

DOI: 10.1002/bio.1170100105. ISSN 0884- 3996. Available from: http://doi.wiley.com/10.1002/bio.1170100105

[62] FRESHNEY, R. *Culture of animal cells: a manual of basic technique and specialized applications*. 6th ed. Hoboken, N.J.: Wiley-Blackwell, 2010. ISBN 978-0470528129.

[63] GUMUSTAS, M., C. SENGEL-TURK, A. GUMUSTAS, S. OZKAN and B. USLU. Effect of Polymer-Based Nanoparticles on the Assay of Antimicrobial Drug Delivery Systems. *Multifunctional Systems for Combined Delivery, Biosensing and Diagnostics* [online]. Elsevier, 2017, s. 67-108 [cit. 2019-01-28]. DOI: 10.1016/B978-0-323-52725-5.00005- 8. ISBN 9780323527255. Available from: https://linkinghub.elsevier.com/retrieve/pii/B9780323527255000058

[64] HOWES, P., R. CHANDRAWATI and M. STEVENS. Colloidal nanoparticles as advanced biological sensors. *Science* [online]. 2014, 346(6205), 1247390-1247390 [cit. 2019-02-08]. DOI: 10.1126/science.1247390. ISSN 0036-8075. Available from: http://www.sciencemag.org/cgi/doi/10.1126/science.1247390

[65] JAMES, W., D. ELSTON and T. BERGER. *Andrews' Diseases of the skin: clinical dermatology*. Twelfth edition. Philadelphia, PA: Elsevier, 2016. ISBN 978-0323319676.

[66] TU, J., K. INTHAVONG and G. AHMADI. The Human Respiratory System. TU, J., K. INTHAVONG a G. AHMADI. *Computational Fluid and Particle Dynamics in the Human Respiratory System* [online]. 1. Dordrecht: Springer Netherlands, 2013, s. 19-44 [cit. 2019-03-22]. Biological and Medical Physics, Biomedical Engineering. DOI: 10.1007/978-94-007-4488-2_2. ISBN 978-94-007-4487-5. Available from: http://www.springerlink.com/index/10.1007/978-94-007-4488-2_2

[67] MADISON, K. Barrier Function of the Skin: "La Raison d'Être" of the Epidermis. *Journal of Investigative Dermatology* [online]. 2003, 121(2), 231-241 [cit. 2019-03-22]. DOI: 10.1046/j.1523-1747.2003.12359.x. ISSN 0022202X. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0022202X15303560

[68] WANG, B., W. YANG, J. MCKITTRICK and M. MEYERS. Keratin: Structure, mechanical properties, occurrence in biological organisms, and efforts at bioinspiration. *Progress in Materials Science* [online]. 2016, 76, 229-318 [cit. 2019-01-29]. DOI:

10.1016/j.pmatsci.2015.06.001. ISSN 00796425. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0079642515000596

[69] GUO, F., S. ARYANA, Y. HAN and Y. JIAO. A Review of the Synthesis and Applications of Polymer–Nanoclay Composites. *Applied Sciences* [online]. 2018, 8(9), [cit. 2019-03-29]. DOI: 10.3390/app8091696. ISSN 2076-3417. Available from: http://www.mdpi.com/2076-3417/8/9/1696

[70] PYSZ, M.A., S.S. GAMBHIR and J.K. WILLMANN. Molecular imaging: current status and emerging strategies. *Clinical Radiology* [online]. 2010, 65(7), 500-516 [cit. 2019-01-29]. DOI: 10.1016/j.crad.2010.03.011. ISSN 00099260. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0009926010001789

[71] SAUER, M., J. HOFKENS and J. ENDERLEIN. *Handbook of fluorescence spectroscopy and imaging: from single molecules to ensembles*. 1. Weinheim: Wiley-VCH, 2011. ISBN 978-3-527-31669-4.

# LIST OF ABBREVIATIONS

| | |
|---|---|
| A549 | Line of human cancerous lung cells |
| AgNPs | Silver nanoparticles |
| ATP | Adenosine triphosphate |
| CQDs | Carbon quantum dots |
| CTCs | Circulating tumor cells |
| DMSO | Dimethylsulfoxide |
| GQDs | Graphene quantum dots |
| hCQDs | Hydrophobic carbon quantum dost |
| MRI | Magnetic resonance imaging |
| MTT | Tetrazolium dye |
| NADH | Nicotinamide adenine dinucleotide |
| NIH/3T3 | Mouse embryonic fibroblast cell line |
| nm | Nanometer |
| NPs | Nanoparticles |
| OI | Optical imaging |
| PBS | Phosphate buffered saline |
| PU | Polyurethane |
| QDs | Quantum dots |
| ROS | Reactive oxygen species |
| rpm | Revolutions per minute |
| *SC* | *Stratum corneum* |
| SDS | Sodium dodecyl sulfate |
| SLNs | Solid lipid nanoparticles |

# LIST OF FIGURES