

VŠB-TECHNICAL UNIVERSITY OF OSTRAVA

Nanotechnology Centre

The summary of dissertation thesis

**Antimicrobial properties
of engineered nanomaterials**

Ostrava 2015

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Study program: Nanotechnology

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ANNOTATION

The dissertation thesis deals with the study of antimicrobial properties of engineered nanomaterials. Considerable part of metal and metal oxide based nanomaterials exhibit antibacterial properties. There are several methods for evaluation of antibacterial activity; however a number of issues need to be overcome for instance the difficulty of comparison of results obtained via different experimental methods. It also shows that not all established techniques are suitable for assessing the antibacterial activity of nanomaterials. Researchers do not have a reproducible low cost method that could be successfully used for rapid screening of antibacterial properties of developed nanomaterials.

The aim of the thesis was to design a method for evaluation which fulfills these attributes even though respects specifics of nanomaterials. Further step was application of the designed method to nanomaterials developed at Nanotechnology Centre (VSB-Technical University of Ostrava) for a purpose of evaluation of their possible antibacterial properties. Based on the obtained results deduce the ways how these nanomaterials cause bacterial inhibition.

Standard microdilution method was used as a basic microbiological method and some modifications were done in the respect of photocatalytically active nanostructured composite materials. The first modification was using the lamp simulating daylight for induction of photocatalytic reaction. The second one was using the inoculation hedgehog for transfer of living bacterial cells from reaction plate to the pure media to reduce inaccuracies during determination of the minimum inhibitory concentrations (MIC) caused by the turbidity in the reaction plate. The modified method was applied for assessing the antibacterial potency of nanocomposites kaolinite/TiO₂, kaoline/ZnO, ZnO/kaoline, graphite/TiO₂ and ZnO/graphite. The performed experiments confirmed the suitability of the method for determining the MIC of the nanostructured composite materials. Discussion about differences among tested materials and proposed mechanisms of antibacterial activity of these materials is also part of this work.

Key words: nanostructured composite materials, kaolinite, graphite, TiO₂, ZnO, antibacterial activity.

1 INTRODUCTION

Nanotechnology is an interdisciplinary field of science which associates the knowledge from different sciences such as physics, chemistry, biochemistry, quantum mechanics, materials science. Nanomaterials can be defined as materials with the size in the range of 1-100nm in one dimension at least. Nanoscale world does not obey the classic laws of science and therefore nanomaterials have unique physical, chemical and physicochemical properties and exhibit unique behavior. Due to these properties, nanomaterials may be used for creation of new products and applications [1].

A diverse series of nanomaterials containing metal oxides, polymeric materials, composite materials, ceramics and broad range of processes are already being used on commercial scale, e.g. semiconductor chips, lighter-weight alloys, coatings, paints and plastics. A special part of nanotechnological research is dedicated to promising medical applications including diagnostic systems, drug delivery systems or selective targeting of cancer cells. There is also an environmental implication of nanotechnology. It has to be pointed out that this issue has two opposite sides. On one hand, the positive features with new technology for example for waste water cleaning or oil remediation, helping also to improve life quality. On the other hand there are potentially negative aspects where several environmental and health impacts have not been studied, described and well understood yet [1].

Nanoparticles which are mobile in environmental media constitute a potential threat for ecosystems. There are several methods how to decrease this threat and eliminate negative effects. One of them is anchoring nanoparticles on specific matrices. If nanoparticles are for instance chemically bonded to a suitable matrix, their mobility is limited but they still exhibit unique properties for instance photodegradation or antibacterial activity.

The aim of this work

1. To develop a method for evaluation of the antibacterial activity of nanomaterials.
2. To apply the developed method on antibacterial assessment of nanocomposites designed and prepared at Nanotechnology centre (VŠB-Technical University of Ostrava).
3. From the obtained results discuss the influence of selected matrices and chemical origin of active compounds on the resulting biological properties in the frame of biomedical applications.

2 THEORETICAL PART

2.1 Nanomaterials

Materials such as metals, metal oxides, polymers, composite materials or materials based on carbon are currently being prepared in nanoscale intensively. High surface to volume ratio of these materials leads to unique material properties in comparison with traditional bulk materials. For example, nanomaterials have improved electrical and thermal conductivity, photonic behavior, chemical and catalytic reactivity, self-cleaning and antimicrobial properties [1].

Metal-based nanomaterials have significantly improved properties contrary to the materials with standard (micro or larger) particle size. Among these properties are quantum effects, the ability to sinter at lower temperatures, increased catalytic activity and more rapid chemical reaction rates can be mentioned. If metal nanoparticles are consolidated into larger structures, they often display increased strength, hardness and tensile strength. [1].

A wide range of methods for the preparation and the production of nanomaterials have been developed. Methods can be divided into two major groups - chemical and physical methods. Chemical methods can be based on decomposition of metal carbonyls, reduction of metal ions or synthesis of nanopowders from aqueous solution precursors. Grinding, vaporization techniques and electroexplosion belong to the group of physical methods. It is challenging to develop production methodologies which are effective, low-cost and can allow for production of nanomaterials with defined particle size, particle size distribution, purity, morphology and uniformity in composition or structure. All these mentioned properties have an effect on the resulting applications of prepared nanomaterials [1].

2.2 Antimicrobial properties of nanomaterials

During the past few years there has been a remarkable growth of research activities exploring properties and applications of nano-sized materials. Size, shape, size distribution, morphology, surface functionalization and stability of nanomaterials have an influence on resulting biological or chemical effects of nanomaterials. Nanoparticles may occur in different shapes such as spheres, plates [2], nanorods [2,3], hexagonal discs [4], nanoflowers [5], nanocubes and nanobars [6]. One study described [7] shape-dependent antibacterial activity of

copper oxide nanoparticles. Authors found the plate-like CuO nanoparticles displayed more antibacterial activity than grain or needle shaped CuO nanoparticles, however the authors did not proposed the mechanism.

Antibacterial activity of nanomaterials has already been demonstrated against several gram negative and gram positive bacterial strains [8]. Metals, metal oxides, metal salts, metal hydroxides, hybrid materials, polymers, nano-carbon based materials or organic nanocarriers loaded with antibacterial agents may create nanoparticles or nanostructured materials exhibiting antibacterial properties [9].

Due to the unnecessary overuse of antibiotics in the second half of the 20th century, mankind must face the incidence increase of resistant and/or multiresistant bacterial strains, which may significantly decrease the efficiency of current medical treatment, contribute to the development of various chronic diseases and even increase mortality. Therefore, a number of research groups all over the world are aiming to find and develop new antibacterial agents which could help to solve this persistent issue. Nanoparticles or nanomaterials could potentially represent the new medicines because no acquired bacterial resistance after application of nanomaterials has been observed yet [10].

The antibacterial activity of the engineered nanomaterials can be evaluated qualitatively or quantitatively [11] using model bacterial strains due to the determination of the following parameters: minimum inhibitory concentration (MIC) [12] or minimum bactericidal concentration (MBC) [13] via disc diffusion method [11], growth inhibition method [14], colony-counting procedure [14], halo test [15], agar [16] or broth dilution technique [14], turbidity assay [17] or microdilution method [18]. Each method has its advantages and disadvantages; therefore a best suitable technique has to be carefully selected for every single tested nanomaterial.

The representative group of usually pathogenic microorganisms including *Staphylococcus aureus*, *Escherichia coli* or *Pseudomonas aeruginosa* is commonly being used to determine antibacterial activity of synthetic nanomaterials. These pathogens are responsible for various human or animal infections and represent serious issues in medical area, drinking water quality or food industry, therefore the development of materials with enhanced properties such as activity leading to the decrease of bacterial colonization is important for human and animal disease prevention and disease treatment [18,19].

Several proposed mechanisms of antibacterial activity of nanomaterials have already been published but it should be noted that some of these mechanisms are still not clear and they need further examination. In general, nanomaterials exhibit antimicrobial properties because of their large surface area to volume ratio which enables appropriate contact with microbial cells [20].

2.2.1 Proposed mechanisms of the antibacterial activity of titanium dioxide

The first proposed killing mechanism implies an oxidation of intracellular coenzyme A, which leads to the inhibition of cell respiration and it causes cell death as a result of a direct contact between the target cell and titanium dioxide [21,22].

The next proposed killing mechanism suggests that a death of bacteria is caused by a disorder in cell permeability and decomposition of cell walls [23]. The cell wall might be destroyed primarily than the cytoplasmic membrane. Photocatalytic treatment increases the cell permeability rapidly and it allows free efflux of intracellular constituents. Particles of titanium dioxide may also access damaged cells and attack intracellular structures directly, which causes alternation of protein structure [24,25]. Electron microscopy was used for the observation that the outer membrane is decomposed first, followed by the disordering of the plasmatic membrane. The cells were completely destroyed after further illumination [26].

2.2.2 Proposed mechanisms of the antibacterial activity of zinc oxide

The antibacterial behavior and mechanism of this phenomenon was also studied for other nanomaterials based on zinc oxide (ZnO) [27]. Two possible mechanisms of antibacterial activity of ZnO nanoparticles were proposed. The first one is based on chemical interactions between membrane proteins and hydrogen peroxide, which is generated due to the presence of ZnO particles. The second one is based on the presence of unknown chemical species produced by ZnO nanoparticles and their interaction with lipid bilayer.

2.3 Interaction of nanomaterials at cellular level

Due to the unique physico-chemical properties and size in nano-scale, nanomaterials possess the ability to modernize medical diagnostics and therapeutics. However, one has to think about the fact that the unique properties may also underlie their biotoxicity. Therefore, to maintain clinical relevance, the toxicity of nanomaterials has to be studied in detail [28].

The size, coating, molecular properties of nanoparticles and their intracellular destination as well as the type of a cell may determine the preferred endocytic pathway for their uptake.

Cytotoxic effect of titanium dioxide nanoparticles is highly dependent on the cell type. One study published that no cytotoxic effect was observed in human blood endothelial cells, but for rat lung alveolar macrophage cells, cytotoxicity of titanium dioxide nanoparticles was given. Pulmonary inflammation could be caused by inhaled ultrafine titanium dioxide particles in contrast to fine TiO₂. Ultrafine nanoparticles of TiO₂ can also cause pulmonary fibrosis and lung tumors in rats. These particles are photogenotoxic [29].

2.4 Applications of engineered nanomaterials

Due to the significant progress of synthesis and characterization of nanomaterials, the development of applications of the nanomaterials has begun. Nanomaterials have been already applied in cosmetics, burn dressing and medical devices, food preservation, water treatment and other range of commercially available products [9].

As it has been stated before, TiO₂ can be used in different domains of human life. Self-cleaning cover glasses for highway tunnel lamps were one of the first commercialized products. This kind of cover can decompose exhaust compounds and the cover glass can stay clear and transparent for a long time. The efficiency of self-cleaning materials depends on the relative rates of contamination/decontamination. This material can work effectively when decontamination rate is greater than the contamination one. Water flow and number of incident photons can enhance the efficiency of this material, therefore it was suggested that the best use of this self-cleaning material should be at exterior construction materials because these materials will be exposed to sunlight and natural rainfall. New innovative materials such as plastic films, tent materials, glasses, etc. have already been commercialized. Applications of the self-cleaning technology are also under examination [30].

In general, it can be stated that properly selected synthetic nanomaterials have a potential to help controlling some undesired events, such as bacterial colonization of various surfaces, animal or human bacterial diseases or biodegradation of building materials by algae or lichens. However, these materials have to be utilized with a special care to avoid their spreading into the environment and the subsequent increase of environmental risks due to their adverse effects on other organisms within the ecosystem.

2.5 Environmental risks of nanomaterials

Nanomaterials are applied in more than 800 consumer products nowadays including cigarette filters, antimicrobial fabrics, ski waxes, self-cleaning windows or cosmetics. It has to be pointed out that, although some of the physico-chemical properties make nanomaterials unique and useful in the applications, they also may pose environmental and health risks through the potential ecotoxic, genotoxic and cytotoxic effects, the induction of inflammatory reaction or even cancer. Nanomaterials with high surface to volume ratio have increased surface reactivity, changes in melting points or electrical conductivity and also the difference in the crystallite structure of the materials [31].

Man must face the question of the association between toxic effects and size-related properties (particle number, size distribution, specific surface, etc.), because this question is specific to nanomaterials. In general toxicology, a dose is related to the amount of material involved in the exposure, which is a quantity linked to the mass. Nevertheless, focused on inhalation exposition of aerosols, particle number and surface area have entered the discussion about the correct metrics. Additionally, growing attention has been focused on the nanotechnology-biology interface which is still not well understood. To make the issue of safety and risk assessment more complicated, nanomaterials are not uniform but there is a great diversity of substances and their morphology [31].

Numerous nanomaterials are known to be much more reactive than their bulk but chemically identical corresponding items, known to have a potential to form reactive oxygen species in this manner rendering them a priori harmful to living systems [31].

According to the design and functional properties of nanoproducts, different kinds of particles may be released: single nanoparticles, aggregated or agglomerated nanoparticles or nanoparticles embedded in a nano- or micrometer scale matrix material. External impacts such as abrasion, pressure or heat may cause unintended release of nanoscale particles from commercially available products. Therefore the product life cycle and the design of a product play significant roles in the understanding of what exposure situations are relevant and which environmental and health risks may occur. For instance, a T-shirt made from nanoAg-modified fibers gets to direct contact with human body and its material is strongly affected by the washing process and therefore unintentionally released nanoparticles/ions would end up on human skin or in wastewaters. Contrary to that geotextiles tuned up with nanoparticles will probably not get into direct contact with human bodies. Nevertheless, if

nanoparticles are released from this material they may pose toxicity on plants and terrestrial organisms [32].

TiO₂ in nanoscale is a relatively stable material that does not subject to dissolution but surface properties and modification can affect the reactivity and thereby the toxicity. Photostable nano-TiO₂ was found to be less toxic to water organisms than photocatalytic nano-TiO₂. The fate of nano-TiO₂ in the environment is mainly governed by agglomeration processes. These properties cause elimination of TiO₂ nanoparticles from the water column and sedimentation. Toxic effects on water organisms occur at concentrations lower or equal to 1mg/L depending on the physico-chemical properties of tested nanoparticles. Photostable TiO₂ nanoparticles and non-illuminated nanoparticles did not exhibit toxic effect in concentrations up to several grams per liter [32].

Nanoparticles of ZnO are soluble in water and therefore, one can expect they will be rapidly dissolved in natural waters. Nevertheless, surface properties can retard or inhibit the dissolution. One study described the occurrence of developmental toxicity in the embryo-larval stages of zebrafish exposed to nano-ZnO. The developmental toxicity tests uncovered that nano-ZnO significantly retarded the embryo hatching and increased malformation after the 96-hpf exposure. Acute induced DNA damage through excessive ROS due to the presence of ZnO nanoparticles changed the activities of the defense enzymes. Obtained results entail that the exposure to ZnO nanoparticles promotes oxidative stress and DNA damage responses in zebrafish larvae. Comparative experiments showed that the toxicity of ZnO nanoparticles to zebrafish was significantly higher than in the case of Zn²⁺, suggesting that dissolved Zn²⁺ only partially contributed to the toxicity of ZnO nanoparticles [33].

Finally, it should be summarized that nanomaterials have several unique physico-chemical properties which made them useful and applicable in a wide range of products. If nanomaterials are under control, they may create better life for humans. However, people should care about the negative side which is created by the toxic effect. If nanomaterials are not under control, they may cause irreversible changes in the environment or may affect not only human health.

3 EXPERIMENTAL PART

Several approaches how to evaluate the antibacterial activity of nanomaterials can be applied. Every method has its advantages and disadvantages; hence a fitting technique has to be carefully chosen for each tested nanomaterial. There is also an issue with slight difference in the experimental results obtained from different methods used for the antibacterial activity evaluation due to the different experimental arrangement. Therefore one has difficulties with the comparison of the obtained results with the results already published.

Conventional microbiological methods may sometimes not be used for the evaluation of the antibacterial activity of nanomaterials. Sedimentation of a tested nanomaterial or how to keep the tested nanomaterial suspended in whole reaction media for the entire experiment duration make the testing irreproducible. Conventional microbiological methods also do not respect the specificity of the evaluation of the antibacterial activity of photoactive nanomaterials whose biological activity is based on photocatalytic reaction. Using lamps for induction of photocatalytic reaction is not common in standard microbiological methods.

Moreover, there is also another challenging aspect. Fast, cheap and user-friendly method for the evaluation of antibacterial activity suitable for rapid screening of antibacterial potency of a single material among a number of developed nanomaterials needs to be designed. After this fast appreciation where the basic information about the antibacterial activity of the nanomaterial is obtained, researchers can subsequently focus on some nanomaterials without wasting time and money with unpromising ones.

With regard to the above mentioned, a modification of the standard microdilution method was designed and the antibacterial activity of photoactive composite kaolinite/nanoTiO₂ was evaluated under the daylight irradiation as is presented in the following paper:

K. Dědková, K. Matějová, J. Lang, P. Peikertová, K.M. Kutláková, L. Neuwirthová, et al., Antibacterial activity of kaolinite/nanoTiO₂ composites in relation to irradiation time, J. Photochem. Photobiol. B Biol. 135 (2014) 17–22.

Based on the results published in the paper, it can be concluded that, the modification of the standard microdilution method can be used for in vitro screening of antibacterial activity of photoactive nanocomposite materials. The issue with photoactivation of the tested

material was solved using a lamp with a bulb simulating daylight. This modification also eliminated issues with the determination of MIC caused by turbidity of dead cells, or caused by the presence of the sample in microtitration plate, because only living bacterial cells can be captured by needles of the inoculation hedgehog. Nanostructured composites kaolinite/TiO₂ exhibited antibacterial activity under daylight irradiation and the lowest MIC values equal to 0.41 mg/ml (*Tab. 1*) were determined.

Table 1 Experimental MIC values (mg/ml) determined for KATI composites

Sample	Bacterial strain	1 day after irradiation	2days before irradiation	2days after irradiation	3days before irradiation	3days after irradiation
KATI12	<i>S.aureus</i>	>100	>100	3.7	3.7	1.2
KATI12	<i>E.faecalis</i>	>100	>100	0.41	0.41	0.41
KATI12	<i>E.coli</i>	>100	>100	0.41	0.41	0.41
KATI12	<i>P.aeruginosa</i>	>100	>100	1.2	0.41	0.41
KATI14	<i>S.aureus</i>	>100	>100	11.1	11.1	11.1
KATI14	<i>E.faecalis</i>	>100	>100	3.7	3.7	3.7
KATI14	<i>E.coli</i>	>100	>100	3.7	3.7	3.7
KATI14	<i>P.aeruginosa</i>	>100	>100	1.2	1.2	1.2
KATI62	<i>S.aureus</i>	>100	100	100	11	3.7
KATI62	<i>E.faecalis</i>	>100	100	100	11	3.7
KATI62	<i>E.coli</i>	>100	100	100	11	3.7
KATI62	<i>P.aeruginosa</i>	>100	100	33.3	33.3	11.1
KATI64	<i>S.aureus</i>	>100	33.3	33.3	11	3.7
KATI64	<i>E.faecalis</i>	>100	33.3	33.3	11	3.7
KATI64	<i>E.coli</i>	>100	33.3	33.3	11	3.7
KATI64	<i>P.aeruginosa</i>	>100	33.3	33.3	3.7	1.2

Therefore it was decided to further apply the modified evaluation method to other prepared nanocomposite materials. Next studies were focused on daylight induced antibacterial activity of ZnO/kaoline and kaoline/ZnO nanocomposite where the developed screening method was utilized for another nanocomposite material designed at Nanotechnology Centre by Vlastimil Matějka's and Kateřina Mamulová-Kultáková's research teams.

K. Dědková, B. Janíková, K. Matějová, P. Peikertová, L. Neuwirthová, J. Holešinský, et al., Preparation, characterization and antibacterial properties of ZnO/kaoline nanocomposites, J. Photochem. Photobiol. B Biol. 148 (2015) 113–117.

Experiments revealed that nanostructured composites ZnO/kaoline exhibited antibacterial activity under natural daylight irradiation. The lowest MIC values equal to 0.41 mg/ml (Tab. 2) were determined.

Table 2 Experimental MIC values (mg/ml) determined for ZinKa composites

Sample	Exposure time					
	180min	240min	300min	1 day	2 day	3 day
<i>Staphylococcus aureus</i>						
ZinKa51	100	11.1	11.1	1.2	0.41	0.41
ZinKa55	100	11.1	11.1	1.2	0.41	0.41
<i>Escherichia coli</i>						
ZinKa51	>100	>100	100	100	33.3	33.3
ZinKa55	>100	>100	100	100	33.3	33.3
<i>Enterococcus faecalis</i>						
ZinKa51	>100	>100	>100	33.3	33.3	33.3
ZinKa55	>100	>100	>100	>100	>100	>100
<i>Pseudomonas aeruginosa</i>						
ZinKa51	>100	>100	>100	100	100	100
ZinKa55	>100	>100	>100	>100	>100	>100

In comparison with previous study, the onset of the antibacterial activity was faster however the sensitivity of *P. aeruginosa* to Zinka composites was lower than to KATI composites. Generally lower values of MIC were determined for KATI. The reason of the difference in the obtained MIC values could be caused by using the lamp with wide spectrum bulb in the case of KATI experiments instead of using natural daylight in ZinKa experiments. There might be also difference in the proposed mechanism of daylight induced antibacterial activity and additional role of Zn²⁺ ions.

***K. Dědková, K. Mamulová Kutláková, K. Matějová, J. Pištora, J. Kukutschová,
The study of the antibacterial activity of kaolinite/ZnO composites – submitted to Advanced
Science Letters***

The study addresses laboratory preparation, characterization and in vitro evaluation of antibacterial activity of kaolinite/ZnO nanocomposites. Composites kaolinite/ZnO with

various amount of ZnO particles (30 wt.%, and 50 wt.%) were prepared using a simple hydrothermal method, and subsequently dried at 105°C or calcined at 500 °C. The prepared samples were characterized by using several phase-analytical methods. A developed modification of standard microdilution test was used for in vitro evaluation of daylight induced antibacterial activity using four common human pathogens (*Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis* and *Pseudomonas aeruginosa*). Antibacterial activity of kaolinite/ZnO composites could be based on photocatalytic reaction. During the antibacterial activity experiments the kaolinite/ZnO nanocomposites exhibited antibacterial activity, where differences in the onset of activity and activity against bacterial strains were observed (*Tab. 3*). The highest antibacterial activity was observed against *S.aureus*.

Table 3 Experimental MIC values (mg/ml) determined for KAZN composites

Exposure time									
Sample				1 day	1 day	2 day	2 day	3 day	3 day
	180min	240min	300min	before irradiation	after irradiation	before irradiation	after irradiation	before irradiation	after irradiation
<i>Staphylococcus aureus</i>									
KAZN13	100	100	100	11.1	1.2	1.2	1.2	1.2	1.2
KAZN15	100	100	100	11.1	1.2	1.2	1.2	1.2	1.2
KAZN53	100	100	100	11.1	1.2	1.2	1.2	1.2	1.2
KAZN55	100	100	100	3.7	1.2	1.2	1.2	1.2	1.2
<i>Escherichia coli</i>									
KAZN13	>100	>100	>100	>100	>100	33.3	33.3	33.3	33.3
KAZN15	>100	>100	>100	>100	>100	11.1	11.1	11.1	11.1
KAZN53	>100	>100	>100	100	33.3	33.3	33.3	33.3	33.3
KAZN55	>100	>100	>100	100	33.3	33.3	33.3	33.3	33.3
<i>Enterococcus faecalis</i>									
KAZN13	>100	>100	>100	>100	>100	>100	>100	33.3	33.3
KAZN15	>100	>100	>100	>100	>100	>100	>100	33.3	33.3
KAZN53	>100	>100	>100	>100	>100	>100	>100	100	100
KAZN55	>100	>100	>100	>100	>100	>100	>100	100	100
<i>Pseudomonas aeruginosa</i>									
KAZN13	>100	>100	>100	>100	>100	>100	100	33.3	33.3
KAZN15	>100	>100	>100	>100	100	100	100	33.3	33.3
KAZN53	>100	>100	>100	>100	>100	>100	>100	100	100
KAZN55	>100	>100	>100	>100	>100	>100	>100	100	100

In our study previous the daylight induced antibacterial activity of ZnO/kaolinite (ZinKa) activity was studied. However, KAZN and ZinKa are not same materials how it seems to be at the first sight. Both materials have the same clay matrix but they are prepared via different methods from different precursors. ZnO in KAZN composite is originated from the reaction of zinc chloride with sodium hydroxide, but in the case of ZinKa is originated from the reaction of zinc chloride with sodium carbonate. The antibacterial activity of the KAZN and ZinKa composites is different. KAZN composites cause faster and stronger inhibition of bacterial growth against *E. coli* and *P. aeruginosa*. However *E. faecalis* and *S. aureus* are more efficiently inhibited by ZinKa composites. From this point of view it has to be concluded that nanostructured composited materials do not cause inhibition of bacterial growth in the wide range as several antibiotics and there are several factors affecting the resulting biological properties.

Kaoline matrix in nanostructured composite materials is proven to be useful for anchoring on TiO₂ and ZnO nanoparticles. Vlastimil Matějka's research team tried to find another suitable matrix and prepared analogue nanocomposites GrafTi and ZinGra. Following papers submitted to Elsevier's journals present the study of the daylight induced antibacterial activity of these nanocomposites.

K. Dědková, J. Lang, K. Matějová, P. Peikertová, J. Holešinský, V. Vodárek, J. Kukutschová, Nanostructured composite material graphite/TiO₂ and its antibacterial activity under visible light irradiation –under review after revisions in Journal of Photochemistry and Photobiology B: Biology

The study presents laboratory preparation, characterization and in vitro evaluation of antibacterial activity of graphite/TiO₂ nanocomposites. Composites graphite/TiO₂ with various ratio of TiO₂ nanoparticles (30 wt.%, and 50 wt.%) to graphite were prepared using a thermal hydrolysis of titanylsulphate in the presence of graphite particles, and subsequently dried at 80°C. Several physico-chemical methods were used for characterization of prepared nanostructured composite materials. A developed modification of the standard microdilution test was used for in vitro evaluation of daylight induced antibacterial activity, using four common human pathogenic bacterial strains (*Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis* and *Pseudomonas aeruginosa*). Antibacterial activity of the graphite/TiO₂ nanocomposites could be based mainly on photocatalytic reaction with subsequent potential interaction of reactive oxygen species with bacterial cells. During the antibacterial activity experiments, the graphite/TiO₂ nanocomposites exhibited antibacterial activity, where differences in the onset of activity and activity against bacterial strains were observed. The highest antibacterial activity evaluated as minimum inhibitory concentration was observed against *P. aeruginosa* after 180min of irradiation (Fig. 1, 2).

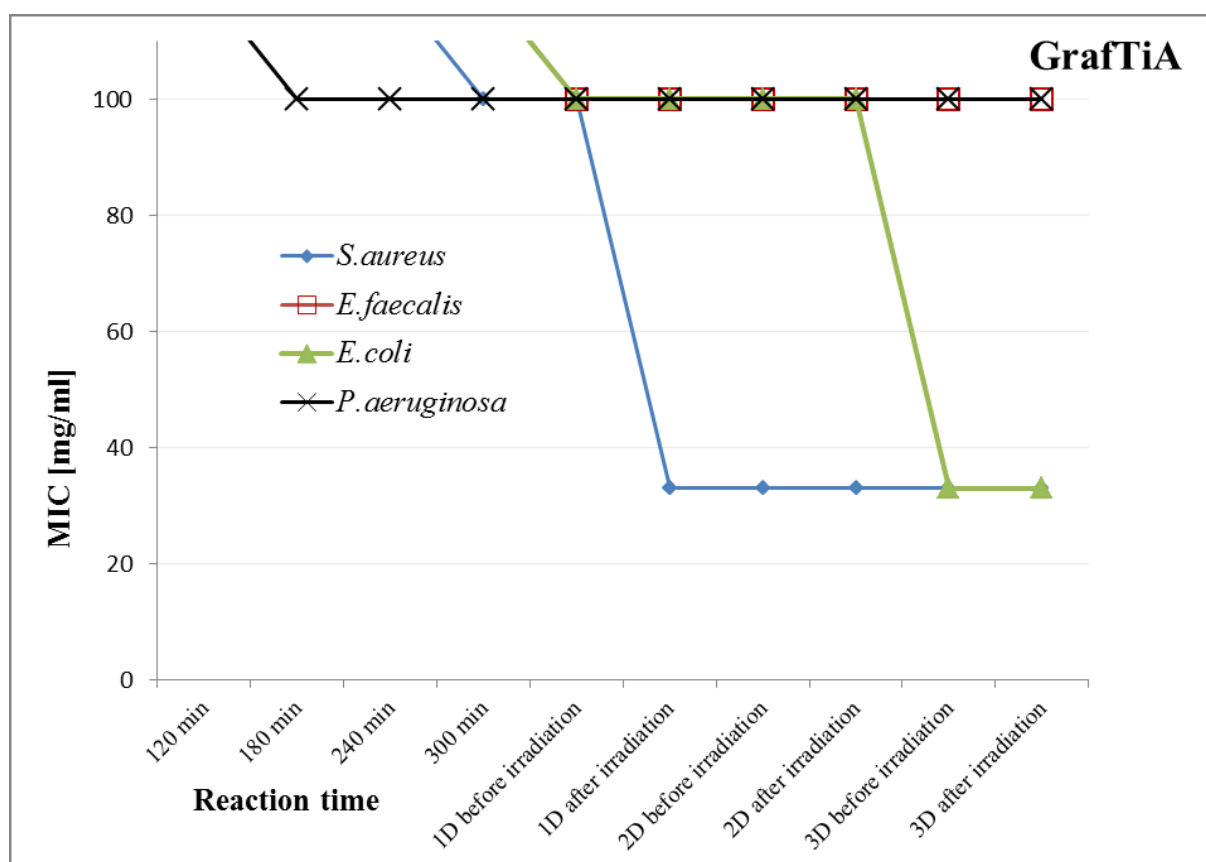


Fig. 1 A graphical representation of MIC-reaction time progress of the antibacterial activity of GrafTiA composite (D – day).

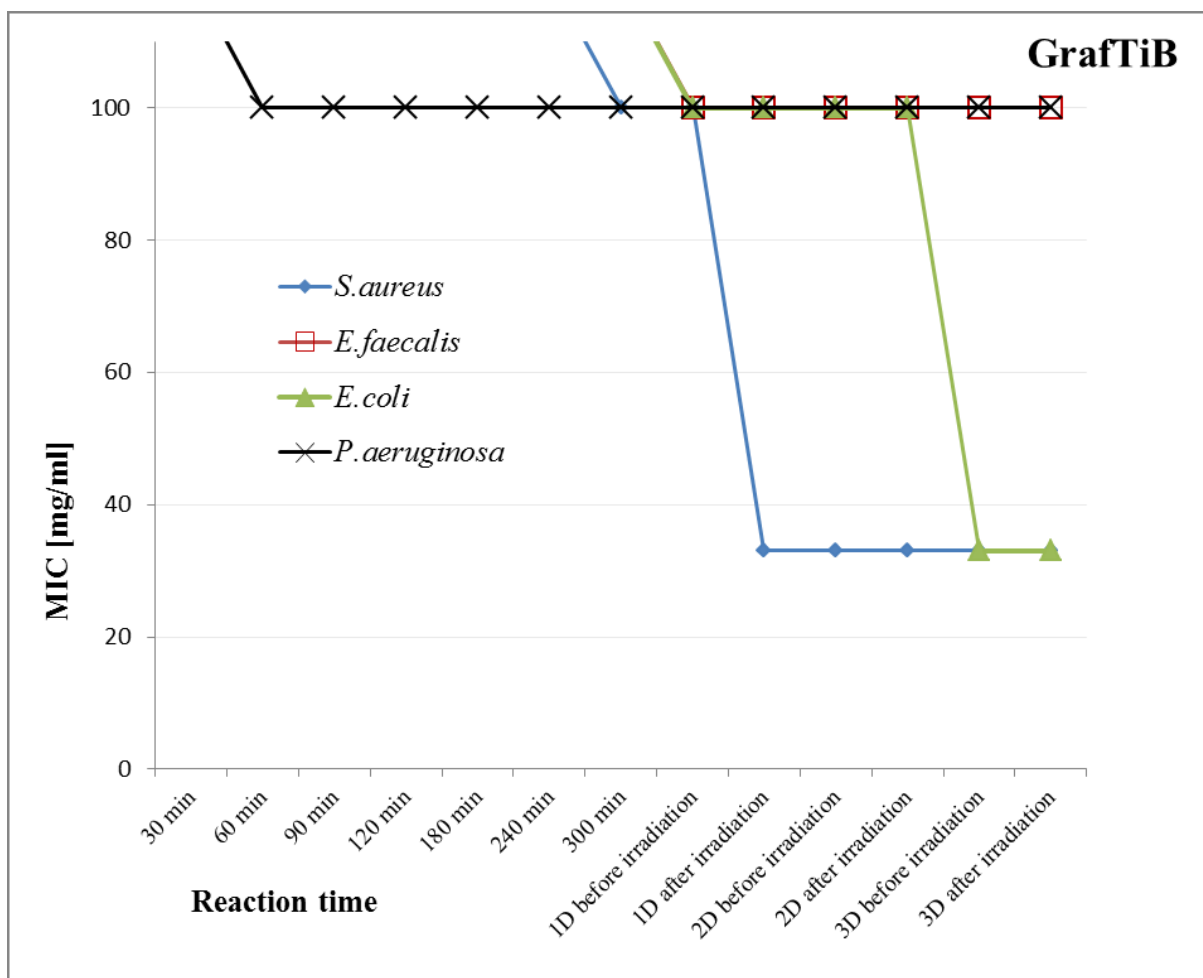


Fig. 2A graphical representation of MIC-reaction time progress of the antibacterial activity of GrafTiB composite (D – day).

In comparison with previous work where daylight induced antibacterial activity of kaolinite/nanoTiO₂ (KATI) composites was studied, we can say that the onset of antibacterial activity of the GrafTi composites is faster than KATI, especially against *P. aeruginosa*, however generally lower MIC values were determined for KATI composites. Therefore, it has to be pointed out that the matrix in nanostructured composite material could play a role in resulting biological activity.

K. Dědková, B. Janíková, K. Matějová, K. Čabanová, R. Váňa, J. Kukutschová, ZnO/graphite composites and its antibacterial activity at different conditions – under review at Journal of Photochemistry and Photobiology B: Biology

The paper reports laboratory preparation, characterization and in vitro evaluation of antibacterial activity of ZnO/graphite nanocomposites. Zinc chloride and sodium carbonate served as precursors for synthesis of zinc oxide, while micromilled and natural graphite were

used as the matrix for ZnO nanoparticles anchoring. During the reaction of ZnCl₂ with saturated aqueous solution of Na₂CO₃ a new compound is created. During the calcination at the temperature of 500°C this new precursor decomposes and ZnO nanoparticles are formed. Composites ZnO/graphite with 50 wt.% of ZnO particles were prepared. X-ray powder diffraction and Raman microspectroscopy served as phase-analytical methods. Scanning electron microscopy technique was used for morphology characterization of the prepared samples and EDS mapping for visualization of elemental distribution. A developed modification of the standard microdilution test was used for in vitro evaluation of daylight induced antibacterial activity and antibacterial activity at dark conditions. Common human pathogens served as microorganism for antibacterial assay. Antibacterial activity of ZnO/graphite composites could be based on photocatalytic reaction; however there is a role of Zn²⁺ ions on the resulting antibacterial activity which proved the experiments in dark condition. There is synergistic effect between Zn²⁺ caused and reactive oxygen species caused antibacterial activity.

Table 4 Experimental MIC values (mg/ml) determined for ZinGra composites.

Reaction time	ZinGra(1)51						ZinGra(1)55					
	<i>S.aureus</i>		<i>E.coli</i>		<i>P.aeruginosa</i>		<i>S.aureus</i>		<i>E.coli</i>		<i>P.aeruginosa</i>	
	DARK	LIGHT	DARK	LIGHT	DARK	LIGHT	DARK	LIGHT	DARK	LIGHT	DARK	LIGHT
30 min	>100	>100	>100	>100	>100	>100	100	>100	>100	>100	>100	>100
60min	>100	>100	>100	>100	>100	>100	100	100	>100	>100	>100	100
90 min	>100	>100	>100	>100	>100	>100	100	100	>100	>100	>100	100
120 min	>100	>100	>100	>100	>100	>100	100	33.3	>100	100	>100	100
180 min	>100	100	>100	>100	>100	>100	100	33.3	>100	100	>100	100
240 min	>100	100	>100	>100	>100	>100	33.3	33.3	>100	33.3	100	100
300 min	>100	100	>100	>100	>100	100	33.3	33.3	>100	33.3	100	33.3
1day before irradiation	33.3	33.3	100	100	100	100	11.1	11.1	33.3	33.3	100	33.3
1day after irradiation	33.3	11.1	100	100	100	100	11.1	3.7	33.3	33.3	100	33.3
2day before irradiation	33.3	11.1	100	100	100	100	11.1	3.7	33.3	11.1	100	33.3
2day after irradiation	33.3	11.1	100	33.3	100	100	11.1	3.7	33.3	11.1	100	33.3
3day before irradiation	33.3	11.1	100	33.3	100	100	11.1	3.7	33.3	11.1	100	33.3
3day after irradiation	33.3	11.1	100	33.3	100	100	11.1	3.7	33.3	11.1	100	33.3
Reaction time	ZinGra(2)51						ZinGra(2)55					
	<i>S.aureus</i>		<i>E.coli</i>		<i>P.aeruginosa</i>		<i>S.aureus</i>		<i>E.coli</i>		<i>P.aeruginosa</i>	
	DARK	LIGHT	DARK	LIGHT	DARK	LIGHT	DARK	LIGHT	DARK	LIGHT	DARK	LIGHT
30 min	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
60min	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
90 min	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	100
120 min	>100	>100	>100	>100	>100	>100	>100	>100	>100	100	>100	100
180 min	>100	33.3	>100	>100	>100	>100	>100	11.1	>100	100	>100	100
240 min	>100	33.3	>100	>100	>100	>100	100	11.1	>100	100	100	100
300 min	>100	33.3	>100	100	>100	>100	100	11.1	>100	33.3	100	100
1day before irradiation	11.1	11.1	100	100	100	100	11.1	11.1	33.3	33.3	100	11.1
1day after irradiation	11.1	11.1	100	100	100	100	11.1	0,41	33.3	33.3	100	11.1
2day before irradiation	11.1	11.1	33.3	33.3	100	100	11.1	0,41	33.3	11.1	100	11.1
2day after irradiation	11.1	11.1	33.3	33.3	100	100	11.1	0,41	33.3	11.1	100	11.1
3day before irradiation	11.1	11.1	33.3	33.3	100	100	11.1	0,41	33.3	11.1	100	11.1
3day after irradiation	11.1	11.1	33.3	33.3	100	100	11.1	0,41	33.3	11.1	100	11.1

Previous study described the daylight-induced antibacterial activity of composite ZnO/kaoline (ZinKa). The obtained MIC values are nearly similar to the ZinGra composites; however the antibacterial activity of the ZinGra composites to *P. aeruginosa* is

slightly higher than in the case of ZinKa. It should be mentioned that the matrix in a nanostructured composite material could play a role in resulting biological activity. Our studies proved that kaoline or graphite can be used for anchoring of ZnO nanoparticles to decrease their possible mobility in the environment. From this perspective it has to be concluded that the nanocomposites including ZnO do not cause inhibition of bacterial growth in the wide range as several antibiotics and there are several factors affecting the resulting biological properties. The outcome of the day light-induced antibacterial activity of the composites ZinGra is relevant in terms of potential applications of these nanocomposites for antibacterial modification of various surfaces. These nanocomposites would be used in future for surface treatment for reduction of potential infection.

4 CONCLUSIONS

The aim of this work was to develop a suitable method for the rapid evaluation of the antibacterial activity of nanomaterials and its application for nanomaterials designed and prepared at Nanotechnology Centre of VŠB-TUO. Standard microdilution method served as a default microbiological method and a modification of it was designed. First of all, the issue with the turbidity caused by the presence of nanomaterials was removed due to the reinoculation of the bacterial cells from the reaction plates to the pure media and the subsequent incubation of the reinoculated plate. After one day of incubation, values of MIC were determined via visible appreciation of bacterial growth.

The problem of antibacterial activity evaluation of photoactive nanomaterials whose antibacterial activity is based on the photocatalytic reaction was solved using a lamp for induction of the photocatalytic reaction. With respect to the practical applications of the prepared nanomaterials, a bulb with wide spectrum was chosen for the experiments due to the fact that using UV lamp in practical, for example biomedical applications is difficult, expensive and partly harmful. It was observed that all tested nanostructured composite materials exhibited antibacterial activity under artificial or natural day light.

It was demonstrated that the modification of the standard microdilution method may serve as a fast, cheap and user friendly screening method and therefore it was applied on all the composites mentioned above. The differences in the onset of the antibacterial activity were observed, where faster onset was observed in the case of the composites with the graphite matrix. Composites with ZnO demonstrated faster onset of antibacterial activity than composites with TiO₂. It can be attributed to the role of Zn²⁺ ions in proposed mechanism of the antibacterial activity. Experiments with ZinGra composites, where the antibacterial activity under dark conditions and under the irradiation were done, showed the synergistic effect of photocatalytic-based reaction and the Zn²⁺. The most sensitive bacterial strain to all nanocomposites was *S. aureus*; however, it has to be pointed out, that the prepared nanocomposites cannot replace antibiotics taken orally. Their antibacterial effect was not observed in broad spectrum. However, they have a potency to reduce bacterial contamination of surfaces and can be used as a surface modification to control bacterial growth. Also their combination in the respect of potential bacterial contamination of the product where they could be applied is the way of success; nevertheless, whether it is feasible is a question for the researchers dealing with preparation of the nanocomposites.

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Dědková, K., Biologické znehodnocení staveb, Příčiny a možná řešení, Silesian University in Opava, Czech Republic 13. 3. 2014

Dědková, K., Antimicrobial properties of engineered nanomaterials, Beijing University of Chemical Technology, Beijing, China, 6.6.2014

Fellowship

National Cheng Kung University, Taiwan – foreign research fellowship, 5 months

Preparation of nanocomposites based on TiO₂ and evaluation of their photodegradation properties

Education

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Bachelor thesis review: Marta Natšínová, Aplikace biosyntetizovaných nanocástic a nanokompozitů, Bachelor thesis, 2012, Program – Nanotechnologie

Supervising: Ľubomíra Kuzníková, Akutní akvatická toxicita nanočástic vybraných oxidů lanthanidů pro sladkovodní zelené řasy, Bachelor thesis, 2014, Program – Nanotechnology

Participation in projects

SGS SP2012/45 “Oxidické systémy – metody jejich charakterizace a environmentální aspekty” – researcher

SGS SP2013/83 “Environmentální a zdravotní aspekty přítomnosti nanomateriálů v životním prostředí” - researcher

SGS SP2014/52 “Nanočástice a nanostrukturované kompozitní materiály na bázi oxidů kovů a jejich biologická aktivita” – research leader

SGS SP2015/54 “Zdravotní a environmentální rizika nanomateriálů” – research leader

CZ.1.07/2.3.00/20.0074: “Nanotechnologie – báze pro mezinárodní spolupráci” – researcher

LH12184 “Nová nanostrukturovaná plniva pro polymerní kompozity – researcher

LO1203 “Regional Materials Science and Technology Centre - Feasibility Program“ - researcher

TA04010819 “Sloučeniny titanu pro automobilové frikční kompozity” – researcher

CZ.1.07/2.3.00/45.0021 “ Zlepši si techniku - lecturer of motivational seminars and workshops

CZ.1.07/1.1.24/01.0084 “ Ambasadoři přírodovědných a technických oborů“ - lecturer of motivational seminars and workshops