SUMMARIES OF MAIN RESULTS AND SCIENTIFIC CONTRIBUTIONS of Assist. Prof. Dr. Tsvetelina Georgieva Foteva, for participation in the contest for the academic position of "Assoc. Prof." in the field of higher education Technical Sciences, professional field 5.11. Biotechnology, scientific specialty Technology of biologically active substances, announced in the State Gazette issue 96 / 17/11/2023

The reference to the scientific contributions was made based on the 37 publications submitted for participation in the competition for the academic position of "Associate Professor", of which 29 are in journals referenced in the global databases Web of Science and Scopus with an impact factor or SJR, and 8 are in non-referenced peer-reviewed journals or edited collective conference volumes. In addition, the candidate participated in the competition with more than 100 citations noticed, apart from those for obtaining the scientific degree "doctor". Scientific developments have been presented in the form of 40 poster presentations and 1 oral report at national and international conferences.

Assist. Prof. Dr. Foteva was actively involved in the implementation of 19 contracts, 4 of which were with the National Institute of Scientific Research. She was the head of 5 contracts at NIS-UCTM. These scientific indicators were achieved with an average annual study load (for the last 4 academic years) of more than 600 hours. Under her supervision, 13 theses were prepared and defended, of which 10 were for the Bachelor's degree and 3 for the Master's degree. Also, she was a consultant to 7 graduates.

The scientific contributions are grouped in the following thematic directions:

1. Antimicrobial activity of polymeric materials containing metal ions.

2. Biocompatibility and cytotoxicity of hybrid materials.

3. Synthesis, characterization, analysis, and activity research of biomolecules with the aim of their application in medicine, pharmacy, and diagnostics.

1. Antimicrobial activity of polymeric materials containing metal ions

In recent years, there has been an increased interest in the incorporation of metal nanoparticles into various hybrid polymer matrices to stabilize the nanostructures and obtain materials combining the properties of the hybrid and nanoparticles. This makes them an interesting candidate for application in both *in vivo* and *in vitro* biomedical research and biotechnology. The incorporation of metal nanoparticles in hybrid materials allows the formation of resistant substances possessing antibacterial and antifungal properties and their use both as antimicrobial agents against highly pathogenic and drug-resistant microorganisms, and also as carriers of drugs, in imaging diagnostics, in the regeneration of tissues and artificial implants.

Compared with organic antibacterial materials, inorganic-organic hybrids possess several advantages such as mechanical strength, chemical stability, and thermal resistance, and have surprisingly few side effects for humans. Hybrid polymer materials provide stabilization of metal nanoparticles, reducing the risk of rapid release of the active agent and prolonging the effect of these materials, making them applicable in biomedicine.

Three types of inorganic-organic hybrid materials based on silicon dioxide, hydroxypropyl cellulose (HPC), hydroxypropyl methylcellulose (HPMC), carboxymethyl cellulose (CMC) and included silver nanoparticles (AgNPs) were obtained by the sol-gel method. The structure and surface morphology of the obtained materials were characterized using infrared spectroscopy, atomic force microscopy (AFM), scanning electron microscopy (SEM), transmission electron microscopy (TEM), and Brunauer–Emmett–Teller (BET) analyses (**I.1, I.2**). The silver content of the materials varies from 0 to 2.5 wt. % leading to a change in surface morphology from smooth to rough with increasing silver content. Silver nanoparticles are uniformly distributed in the materials, spherical particles with a size of 10-50 nm are observed, and the highest silver content is 2.5 wt. % leads to particle aggregation (**I.1**). The small size of the particles as well as the irregular shape are essential for easier penetration through the cytoplasmic membrane and therefore for better antibacterial action. According to the UV–Vis analyses, silver is present in the materials in the form of both Ag^+ and nanoparticles (**I.2**). The microorganisms that were used are test cultures or also called control strains, provided by the National Bank for Industrial Microorganisms and Cell Cultures (NBPMKK) and serve to evaluate the antimicrobial effectiveness of the synthesized hybrid materials.

The antimicrobial properties of the hybrid materials against prokaryotic and eukaryotic microorganisms were investigated. *E. coli* K12 and *B. subtilis* 3562 were used as test cultures for Gram-negative and Gram-positive bacteria, respectively. The obtained results show a dose-dependent effect of microbial susceptibility about the silver content used *B. subtilis* 3562 was determined to be a more sensitive strain to the effects of the materials compared to *E. coli* K12

(**I.1, I.2**). Clinical strains of *Pseudomonas aeruginosa* were also used to evaluate the antimicrobial properties of the investigated hybrid materials (**III.2**). *Saccharomyces cerevisiae* 537 (**II.1**) and *Candida albicans* 74 (**I.4**) yeasts were used as test cultures for eukaryotic microorganisms. Cell growth of both strains was significantly reduced in the presence of the hybrid materials with high silver content. Morphological dimorphism of *C. albicans* 74 cells was observed in the presence of silver-containing materials. It is expressed in the transition of the cells from a pathogenic-mycelial form to an apathogenic-yeast form. In conclusion the studied materials can be used in biomedicine for the prevention of fungal diseases (**I.4**).

Analyzes focused on investigating the ant mycotic effect of the materials against *Asp. niger* 371 was carried out. (**III.3**). Based on the conducted experiments, a more pronounced sensitivity of *P. chrysogenum* 2303 to the tested hybrid materials was found compared to *Asp. niger* 371. The hybrids inhibited the growth of molds only in the initial stages of cultivation, after which an inability of the materials to show complete inhibition of the strains was reported at a later stage. For this reason, the effect that the hybrid materials have on the tested reference mold strains can be defined as fungistatic. Materials with a high silver content (2 wt. % and 2.5 wt. % Ag) were found to affect the sporulation process, which is a prerequisite for potential application as coatings.

Spherical silver nanoparticles included in porous collagen structures were also obtained by a reduction method with the participation of microwaves. Ammonia and ethanol play an important role in the reduction of silver ions to AgNPs. UV-Vis and TEM analyses were performed to confirm the presence and sizes of the nanoparticles. The bactericidal properties of the structures against *B. subtilis* NBIMCC 3562 and *E. coli* K12 NBIMCC 407 were evaluated and in conclusion it can be said that they may find medical applications as antibacterial implants (**II.11**).

Along with the obtained hybrid materials based on SiO_2, various cellulose derivatives, and silver nanoparticles, those containing other antimicrobial agents such as zinc were also synthesized by the sol-gel method. Their morphology was studied using UV-Vis, TEM, DTA/TG analysis, as well as their antibacterial and antifungal properties against reference strains *B. subtilis* NBIMCC 3562 and *E. coli* K12 (**II.3**), *Candida albicans* 74 (**III.6**) and clinical strains of *Salmonella* and *Shigella* (**III.8**), *Klebsiella* (**III.7**). The antimicrobial activity of the materials was tested against the molds *Asp. niger* strain 371 and *P. chrysogenum* strain 2303, where a good antifungal effect was demonstrated (**III.4**).

As a third class of synthesized hybrid materials, those obtained from SiO_2, HPC, and copper ions were studied. They were structurally characterized using XRD, IR, and UV-VIS analyses. The antimicrobial potential of the materials was investigated using reference strains for Gram-positive bacteria (*Bacillus subtilis* NBIMCC 3562), Gram-negative bacteria (*Escherichia coli* K12 NBIMCC 407) and yeast (*Candida albicans* NBIMCC 74) (**II.3, II.10**).

Nanocomposite materials with the participation of SiO2, ZnO, TiO_2, and reduced graphene oxide (RGO), as well as materials based on TiO_2 and SeO_2, were also investigated. All of them show good antibacterial properties, which makes them suitable and applicable in several fields (**I.5, II.2, II.14, II.17, II. 19**).

The toxic tolerance and ability of strain *Trichosporon cutaneum* R57 for the sorption of manganese ions (Mn^{2+}) from aqueous media was investigated. For manganese ions, the threshold of inhibition was 5 mM $MnSO_4.H_2O$, 10 mM concentration was moderately inhibitory as a change in cell morphology was observed, and 20 mM $MnSO_4.H_2O$ was lethal to the tested strain. The sorption efficiency decreases with increasing manganese concentration used. Sorption is the fastest in the first 30 minutes, as at a concentration of 5 mM $MnSO_4$ it is 61.67%, and at 10 mM $MnSO_4$ it is 42.04%. At the highest concentration used, the sorption is only 27.27%. As the incubation time increases, the sorption increases, but the increase is up to 3-4%. Sorption is most effective in the first 30 minutes (**III.1**).

Research has been carried out on the possibility of preserving wood by modifying it with alkali-activated hydrolyzed lignin, Cu_2S, and maleic anhydride by the infiltration method, and the influence of the process on some operational properties of the wood material has been investigated. All modified samples showed antibacterial resistance to G-negative bacteria (*E. coli* K12), with the sample modified with maleic anhydride (**I.9**) showing the greatest effect. The lignocellulosic waste materials produced after the enzymatic hydrolysis of plant raw biomass were doped with silver and proved to be highly effective in killing harmful bacteria such as G-positive *Bacillus subtilis* 3562 and G-negative *Escherichia coli* K12 in aqueous solutions (**I.8**).

A review article has been published about the application of nanotechnology in the cosmetic industry. Nanomaterials are classified based on their chemical nature as organic and inorganic. The organic ones include liposomes, nanoemulsions, solid-lipid particles, nanocapsules, nanocrystals, and the inorganic ones - gold, silver nanoparticles, fullerene, and nanotubes. The methods of penetration of nanomaterials through the skin barrier are analyzed, as is the issue of safety in their use (**II.18**).

2. Biocompatibility and cytotoxicity of hybrid materials

Biocompatibility is the ability of materials to be implanted in the body without inducing a cellular response to achieve an optimal therapeutic effect. The most important condition is the safety of the materials. They must not cause allergic reactions and have no toxic or carcinogenic effect. By meeting these requirements, materials are defined as biocompatible. Biocompatible materials are used in ceramic nanocoatings, bone implants, plastic surgery, etc. An important condition for the application of biomaterials is that they can be resorbed in the body.

For the application of nanoparticles in biology and medicine, it is first of all necessary to prove their biocompatibility. Testing the cytotoxicity of a material is the first step in determining its biocompatibility, essentially determining the biological reactivity of mammalian cell lines after contact with the material. Only then proceed to *in vivo* tests and clinical trials. Cytotoxicity refers to damage to individual cells, for example in cell cultures. The cell survival and adhesiveness of 3T3 mouse fibroblasts on the hybrid materials with silver nanoparticles were investigated. The obtained results revealed an increasing cell proliferation of the 3T3 cells, more pronounced after 48 hours of cultivation (**I.3, I.6, I.7**). Higher silver content resulted in lower cell survival. This can be explained on the one hand by the properties of silver as an antimicrobial agent, and on the other hand by the higher roughness and contact angle determined in the structural characterization of silver-containing materials (**I.7**). Cells entering apoptosis at high doses of silver undergo characteristic morphological changes. These include shrinkage and condensation of the cell, disruption of the cytoskeleton and nuclear envelope, and condensation of nuclear chromatin that breaks up into fragments, which we observed in materials with the highest silver content used (**I.6**).

Many clinically important pathogenic bacteria can colonize, forming biofilms that are difficult to respond to traditional antibiotics due to the resistance to them. Therefore, hybrid materials incorporating silver nanoparticles and their anti-biofilm efficacy are receiving increasing attention. The anti-adhesion ability of SiO_2/HPC/Ag and SiO_2/HPMC/Ag hybrid materials in biofilm formation by *P. aeruginosa* PAO1 was also studied (**I.7**). The results show a significant reduction of the biofilm formed, which is a prerequisite for these materials to be used in tissue engineering, in dentistry and in the preparation of catheters, where cell adhesion is not required. Ten strains of *P. aeruginosa* clinical isolates resistant to more than two antibiotics were also tested, defining them as polyresistant. A bactericidal effect was found for all three used hybrid materials with silver nanoparticles, with concentration-dependent activity being observed (**III.2**).

A series of zinc-containing SiO_2/HPC hybrid materials were successfully prepared by the sol-gel method. According to the infrared spectra, the hybrid materials showed typical SiO_2 network stripes and vibration of Zn-O bonds. UV-Vis spectroscopy and TEM analysis prove the existence of nanosized Zn particles (5-10 nm). The materials inhibited the growth of bacteria and the inhibitory effect corresponded to the concentration of zinc in the samples. Gram-positive bacteria are more sensitive and result in the formation of 17.5 mm inhibitory zones, while for Gram-negative the zone is 11 mm. The incubation period also affects bacterial growth. Materials containing zinc that show good antibacterial results are at the same time biocompatible, showing no changes in the actin cytoskeleton (**II.4**).

The biocompatibility and antifungal properties of hybrid materials containing SiO_2/pectin and silver were investigated. The MTT assay revealed good mouse fibroblast survival and satisfactory cytoskeletal organization in materials containing up to 1 wt. % silver. At the same time, the composites show an antifungal effect against the growth of *C. albicans* 74, which makes them a potential candidate material for *in vivo* implants (**II.6**).

An interesting topic of work is the study of the cytotoxicity of hybrid materials containing SiO_2/cellulose and copper from different sources. The results show that the cytotoxic effect of the materials used depends on the Cu source used (sulfate or nitrate), the amount of copper ions in the composite materials, the cell type, and the incubation time. Cell viability was affected by both types of composites, with the material obtained with $CuSO_4.5H_2O$ being more cytotoxic. Two cancer cell lines (Lung cells (A549) and Endothelial cells (HUVEC)) were investigated, with A549 cells exhibiting greater sensitivity. In this way, the synthesized composite materials can serve for further research and development, to apply them as a new and/or adjuvant cancer therapy (**II.15**).

3. **Synthesis, characterization, analysis, and activity research of biomolecules with the aim of their application in medicine, pharmacy, and diagnostics.**

Bioactive peptides have found application in various fields such as pharmacy, perfumery, cosmetics, food industry. In medical practice, they are mainly used in areas such as oncology, endocrinology, traumatology, diagnostics, etc. Antimicrobial peptides with antitumor activity are a large group of bioactive peptides that have found application in the so-called targeted therapy. It is one of the most reliable therapies for oncology, in which a large number of scientists and laboratories in the world are interested and work to improve the pharmacokinetics and pharmacodynamics of these peptides and their use for the delivery of pharmacophore molecules to certain cells or tissues.

Novel bioconjugates of monofluorinated analogs of BIM-23052 containing the second pharmacophore naphthalimide, caffeic acid, or the tripeptide Arg-Gly-Asp were investigated. The anticancer potential was tested against a panel of tumor cell lines including human mammary carcinoma cell lines MCF-7 (ER+, PR+, and Her-2); MDA-MB-231 (ER-, PR- and Her-2-), as well as BALB 3T3 (mouse embryonic fibroblasts) and MCF-10A (human breast epithelial cells) cell lines. The most active compound turned out to be a bioconjugate containing naphthalimide as the second active component in the molecule. It is selective for MCF-7 cells and has the least antiproliferative effect against the non-tumorigenic MCF-10 cell line. All three bioconjugates are practically non-toxic with IC_{50} values > 1000 μM. Unfortunately, hydrolytic stability studies show that this bioconjugate is most unstable to hydrolysis in model systems mimicking physiological conditions in the human body. Mathematical docking calculations show good selectivity results for some of the somatostatin receptors. The caffeic acid conjugate binds more strongly to SSTR1 and SSTR4, and this one contains a naphthalimide to SSTR2 compared to the parent compounds. A peptide conjugate with the tripeptide Arg-Gly-Asp had the best selectivity for binding to SSTR2. This is probably due to the optimal number of interactions that the additional amino acids allow to be realized with the receptor and the appropriate spatial arrangement relative to the binding center of the receptor (**I.10**).

Single and double sequence analogs of the antimicrobial peptide with additional antitumor activity (KLAKLAK)$_2$-NH$_2$ were designed synthesized and characterized by introducing the unnatural amino acids Nle and β-Ala into its primary structure. Bioconjugates of the truncated and doubled analogs of the single sequence of the antimicrobial peptide (KLAKLAK)$_2$-NH$_2$ with a second pharmacophore with proven antitumor activity 1,8-naphthalic anhydride and caffeic acid were designed, synthesized and characterized. Antibacterial properties, antiproliferative effect, and cytotoxicity of all newly synthesized analogs and conjugates of (KLAKLAK)$_2$-NH$_2$ were investigated and important structure-biological activity relationships were deduced (**II.8, II.9, II.12**). *Candida albicans* is an opportunistic pathogen, a type of dimorphic fungi with a pseudomycelial and yeast form that reproduces by budding, and is most prevalent in the gastrointestinal tract. It is a commensal microorganism, part of the resident microflora of healthy individuals of the human population. However, under certain circumstances, it can lead to intestinal, vaginal and digestive problems. When studying the antifungal properties of the synthesized bioconjugates, the peptides containing the second pharmacophore 1,8-naphthalic anhydride or caffeic acid showed the highest activity against *C. albicans* 74 (**II.9**).

In recent years, the popularity of herbal medicines and nutritional supplements has increased worldwide due to the many side effects associated with synthetic drugs. People are constantly looking for "all-natural" products. Undeclared drugs or synthetic compounds in botanicals can be not only toxic but often interact with other prescription medications and therefore, as a result, can cause adverse effects that could even be life-threatening. Adulteration consists of replacing the original raw herb or its extract partially or completely with another material that has no or inferior therapeutic value. This adulteration is done based on the similarity in the morphological characteristics of the added substance and the original herb. Adulteration with synthetic chemical drugs is also used to increase the effectiveness of the nutritional supplement, most often in those against overweight, obesity, antidiabetic preparations, potency, etc. This type of falsification can lead to serious health problems due to long-term intake of the food supplement.

A liquid chromatographic method with UV detection was developed and validated for the determination of six prohibited active substances yohimbine, vardenafil, sildenafil, tadalafil, dapoxetine, and sibutramine in dietary supplements for erectile dysfunction and weight loss (**II.7**). A liquid chromatography method with UV detection was developed and validated for the determination of thirteen substances with the steroid structure testosterone, testosterone propionate, testosterone enanthate, methyltestosterone, nandrolone, nandrolone propionate, nandrolone decanoate, methandienone, androstenedione, trenbolone, trenbolone acetate, trenbolone enanthate, boldenone undecylenate in nutritional supplements for sports. A confirmatory GC/MS method has been developed that can also be used for screening to determine the presence or absence of seventeen substances with the steroid structure testosterone, testosterone propionate, testosterone enanthate, methyltestosterone, nandrolone, nandrolone phenylpropionate, nandrolone decanoate, methandienone, androstenedione, trenbolone acetate, trenbolone enanthate, oxandrolone, stanozolol, drostaolone propionate, drostanolone enanthate, boldenone undecylenate in nutritional supplements for sports (**II.16**).

Structural analogs of memantine, which has an active action against Alzheimer's, have been studied. The obtained results show that analogs containing the unnatural amino acid β-Ala are active only against *E. coli* K12 407. Analogs with the aromatic amino acid Phe, as well as those with β-Ala, are not active against Gram-positive bacteria. The introduction of a bulky amino acid Phe containing an aromatic core and Val containing an aliphatic core results in good antifungal activity (**II.13**).

PERSPECTIVE FOR FUTURE WORK AND DEVELOPMENT

1. Synthesis, structurally characterized, and biological properties of new hybrid materials based on safe and accessible polymers and metal agents.

2. Research the biocompatibility and antibiofilm potential of new hybrid materials for application in medical practice.

3. Synthesis and research of new biomolecules based on modified amino acids, peptides, and glycopeptides with potential antitumor and antimicrobial properties.

4. Incorporation of biomolecules with antibacterial properties by various methods in hybrid matrices.