A Holistic Approach for Target Therapy of Cancer

Tamara Ivanova Pajpanova[1], Dessislava Borisova[2], Ivan Iliev[3], Inna Sulikovska[2], Emilia Naydenova[2], Tatyana Dzimbova[4], Spaska Yaneva[2], Dancho Danalev[2]
[1]Institute of Molecular Biology "Acad. Roumen Tsanev", Bulgarian Academy of Sciences, Sofia Bulgaria
[2]University of Chemical Technology and Metallurgy, Bulgaria, Sofia, Bulgaria
[3]Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria
[4]South-West University "Neofit Rilski", Blagoevgrad, Bulgaria

One of the major problems in cancer chemotherapy is that the applied drugs are not selective to cancer and normal cells. In recent years the concept called, targeted therapy have raised increasing interest as effective strategies to improve therapeutic efficiency, reduce the impact of adverse side effects, and overcome drug resistance.
It is known that some tumor cells may express on their surface integrins αvβ3 and αvβ5 able to recognize and to bond specifically the peptide sequence Arg-Gly-Asp (RGD). Many examples of RGD peptide-drug conjugates have been proposed as targeted drug delivery systems. These molecular hybrids incorporate high-affinity ligands targeting over expressed integrin receptors or specific integrin-related pathways within diseased cells / tissues and associated with recognized therapeutic units, ultimately control stability, physicochemical properties, timing, and localization of drug release.
We present a series of new structural analogues of RGD and SST, showing enhanced metabolic stability; a new hybrid structures of RGD/SST, as well as the establishment by biological investigations the reasons for the change in the biological activity - whether and how certain modifications in SST- and RGD-monomer units, as well as in the conjugates will alter the cytotoxic activity of the parent molecules.

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Keywords: RGD, SST, peptide conjugate, target therapy

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Beneficial microbiota of traditional Bulgarian cheeses – from old technology to new functional food

Svetla Trifonova Danova[1], Lili Ivailova Dobreva[1], Miglena Angelova Koprinarova[2], Kristina Svilenova Kostova[1], Veronika Pencheva Nemska[3]
[1]The Stephan Angeloff Institute of Microbiology, BAS, 26, Acad. G. Bonchev, str. 1113, Sofia, Bulgaria
[2]Institute of Molecular Biology "Roumen Tsanev", 25, Acad. G. Bonchev, str. 1113, BAS, Sofia, Bulgaria
[3]Faculty of Chemical and System Engineering, UCTM, 8 Blvd. Kliment Ohridski; 1756, Sofia, Bulgaria

Bulgaria is a country with a centuries-old tradition of consuming fermented dairy products. However, limited information on biodiversity and beneficial properties of lactic acid microbiota of some of them, exists. With this aim several samples of white-brined cheeses, homemade from ovine, goat, buffalo and caw-milk were collected, from different rural regions. High viability of various LABs was shown during the study. The polyphasic taxonomy approach allowed to identify *Lactiplantibacillus plantarum*, in predominance as autochthonous non-starter microbiota. A broad spectrum of antimicrobial activity of 24 LAB strains, including Gram (+) and Gram (-) defines their potential as promising bioprotective cultures. The active LAB postmetabolites (10% v/v) or live cells, during the co-cultivation in milk or in model systems, significantly inhibit *Salmonella Tiphimurium*, *Staphylococcus aureus* and other pathogens. Bacteriocinogenic lactobacilli were selected and characterized as candidate – probiotics, according to EFSA's in vitro criteria for functionality, safety and technological relevance. Established high transit tolerance to simulated gastric conditions is a predisposition of LAB's viability and stable gut colonization. Two of them were applied as probiotic adjuncts in new formulas of white brined and cream cheeses, prepared without preservatives.

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Keywords: Cheeses, Lactic acid bacteria, Probiotics, *Lactiplantibacillus plantarum*, Functional food

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Ultrasound-stimulated Brownian ratchet enhances diffusion of molecules retained in hydrogels

Faezeh Gerayeli[1], Nawel Khalef[2], Aziz Bakri[2], Philippe Benech[3], Donald K Martin[1]
[1]Univ. Grenoble Alpes, CNRS, Grenoble INP, TIMC / SyNaBi (UMR 5525), 38000 Grenoble, France
[2]Univ. Grenoble Alpes, CNRS, Grenoble INP, TIMC / Pharmaceutical Engineering (UMR 5525), 38000 Grenoble, France
[3]Univ. Grenoble Alpes, Grenoble INP, G2ELab, 38000 Grenoble, France

This presentation highlights the application of nanotechnology to a biomedical issue. We demonstrate that low-frequency ultrasonic stimulation applied directly to a hydrogel, at energy levels below the cavitation threshold, can control the release of a therapeutic molecule. The hydrogel that contained the molecules was enclosed within a hollow acoustic horn. The harmonic modes in the acoustic horn combined with the physical gel structure to induce a flashing ratchet that released all of the retained molecules in less than 90 seconds at an intensity of 1.5 W cm^{-2} (applied energy of 135 J cm^{-2}, ultrasound center frequency of 27.9±1.5 kHz). In contrast, ultrasound is used currently as a remote stimulus for drug-delivery systems, at energy levels above the cavitation threshold. The low-energy flashing ratchet approach that we describe is applicable to drive the diffusion of molecules in a range of gels that are ubiquitous in biomedical systems, including for example in drug delivery, molecule identification and separation systems.

Keywords: Hydrogel structure, directed diffusion, flashing ratchet, ultrasound, harmonic modes, acoustic horn

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How cells respond to magnetic field? Magnetic nanoparticles and hyperthermia for cancer treatment

Rumiana Dimitrova Tzoneva[1], Veselina Plamenova Uzunova[1], Aikaterini Tsiapla[2], Eirini Myrovali[2], Irina Iordanova Georgieva[1], Maria Zdravkova Popova[1], Sonia Nikolova Apostolova[1], Makis Angelakeris[2], Teodoros Samaras[2], Orestis Kalogirou[2]
[1]Transmembrane signalling Lab., Institute of Biophysics and Biomedical Engineering, BAS, Sofia, Bulgaria
[2]School of Physics, Faculty of Sciences, Aristotle University, Thessaloniki, Greece

Cells can sense magnetic forces as they affect and control cellular functions such as cell proliferation, cell cycle, gene expression, ion channels and cell morphology. Nowadays, the use of magnetic fields and magnetic materials in medicine is already routine (transcranial magnetic brain stimulation, MRI, magnetically guided catheters), and some promising approaches are undergoing preclinical trials: magnetic hyperthermia, magnetic targeting of stem cells and drugs, etc. In the last decade there has been an enormous development of magnetic nanoparticles (MNPs) for biomedical applications, such as magnetic carriers for drug delivery aided by external magnetic fields, magnetic resonance imaging contrast agents or cancer therapy compounds for hyperthermia. In particular, iron oxide nanoparticles are being actively investigated to achieve highly efficient carcinogenic cell destruction through magnetic hyperthermia treatments. Magnetic hyperthermia is a process which induces local heat when a radiofrequency magnetic field is applied, provoking a temperature increase in those tissues and organs where the tumoral cells are present.
This presentation examines the main biological changes in cancer cells observed after magnetic hyperthermia, which lead to cancer cell death. Among them are cytoskeleton remodeling, oxidative stress and expression of heat shock proteins.

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Keywords: magnetic nanoparticles, hyperthermia, cancer cell death

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Application of nanomembrane separation for isolation of natural biologically active components

Dimitar Peshev[1], Yoana Stoyanova[2], Nevena Lazarova Zdravkova[2], Veronika Nemska[2], Neli Georgieva[2]
[1]Department of Chemical Engineering, University of Chemical Technology and Metallurgy, Sofia, Bulgaria
[2]Department of Biotechnology, University of Chemical Technology and Metallurgy, Sofia, Bulgaria

Recent development of new solvent-stable materials has permitted the application of membrane filtration techniques to different processes in organic solvents and solvent mixtures. The biologically active constituents of plants are traditionally isolated trough solid-liquid extraction and further evaporation of the obtained dilute liquid extracts. Due to their susceptibility to thermal degradation and oxidation, traditional thermal separation technologies result in poor product quality. The presented experimental data and process simulations illustrated that nanofiltration reduces the specific energy consumption while preserving product activity during the concentration or fractionation of liquid extracts.
Particular attention is drawn on the current results of a project for fundamental research dealing with application of nanofiltration for valorisation of the waste streams from the essential oil industry. The possibility to fractionate extracts (residual waters) from the steam and hydrodistillation of essential oil plants using nanofiltration was investigated. The separation performance of five commercial nanofiltration membranes with respect to key biologically active components of the studied waste aqueous fractions was predicted based on regression models. The results clearly demonstrated the viability of nanofiltration for isolation of refined polyphenolic fractions from the extracts effluents.

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Keywords: nanofiltration, essential oil, biological activity, valorisation, residual water, COSMO-RS

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Magnetic nanoparticle-based fluorescent immunoassays for determination of biotoxins in foods

Tzonka Ivanova Godjevargova, Zlatina Rumenova Chengolova, Yavor Lukanov Ivanov
Department of Biotechnology, Prof. Dr A. Zlatarov University, Burgas, Bulgaria

Biotoxins are small (MW ~700), toxic chemical products formed as secondary metabolites by a different pathogenic microorganisms. Foods with high protein content (milk, meat and salads) and agriculture products (wheat, peanuts) have been frequently incriminated in staphylococcal enterotoxin A (SEA), aflatoxin B1 (AFB1), ochratoxin A (OTA) outbreaks. Aflatoxin M1 (AFM1) may be found in the milk of animals that are fed with AFB1-contaminated food. Potency of this toxins require that the methods of analysis in food must be sensitive enough to measure very low concentrations. Magnetic nanoparticle-based competitive fluorescent immunoassays (FIA) for determination of SEA, OTA and AFM1 in milk, and AFB1 in peanuts, were developed. Magnetic nanoparticles (MNPs) were prepared and then modified to introduce amino groups. $F(ab')_2$ fragments of the anti-SEA and anti-OTA monoclonal antibodies (mAbs) were obtained by pepsin hydrolysis of mAbs. Corresponding mAbs and their $F(ab')_2$ fragments were immobilised on MNPs. Each kind of biotoxin was conjugated with fluorescent dyes. The detection limits and linear ranges of all developed immunoassays were determined. The obtained results were compared. The use of MNPs in developed immunoassays resulted in easier separation of the bound conjugates by applying a magnetic field, high mass transfer rate, faster and more sensitive assay.

Keywords: aflatoxin B1, aflatoxin M1, enterotoxin A, ochratoxin A, magnetic nanoparticles, immunoassay

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Lactic acid bacteria in the oral cavity – assessment of the probiotic potential

Nikola Nikolov Atanasov, Yana Yordanova Evstatieva, Dilyana Petrova Nikolova
Sofia University "St. Kliment Ohridski", Faculty of Biology, department of Biotechnology

The oral microbiome is highly complex, it's the most colonized area and it's the second most diverse with microorganisms in the human body after the intestinal microbiome. The oral cavity has a number of different environments on its own and each one of them is colonized with microorganisms and has a different microbial diversity. The microbial composition is not homogenous and is susceptible to change by anatomical and physiological conditions. The table shows the main bacterial and nonbacterial representatives in the oral cavity. The microorganism growth depends on factors like temperature, acidity, redox potential, nutrient availability, water content, structural morphology of the oral cavity, the flow of the saliva and the presence of antimicrobial compounds. Each of these factors selectively affects the oral ecosystem and help maintain a balance between the microorganism populations.

The synergism and interaction between the changing oral microorganisms can help against invasions of unwanted microflora outside the body, and the imbalance of the microbial flora can cause diseases in both the oral cavity and also systemic diseases. Therefore, the oral microbiome plays an important role in the human microbial community and human health.

Lactic acid bacteria are one of the prevalent Gram-positive oral representatives. Present on the hard dental tissues, mainly in the dental biofilm, and in the saliva, they express their probiotic properties in a balanced microbiome environment. There are number of microorganisms in the biofilm's composition that help keeping pathogenic species from colonizing the oral tissues. Growing on the oral tissues, LAB express characteristics such as competitive interactions for adhesion places, nutrients and growth factors, aggregation and production of antimicrobial substances, which include acids, hydrogen peroxide and bacteriocins. Introduced like this, LAB can inhibit pathogenic adhesion, growth and colonization in the oral environment. Another way is to affect the local and systemic immune response by inhibiting collagenases and reduction of inflammatory associated molecules, production of IgA and defensins and also prevention of cytokine-induced apoptosis. These characteristics can lead to antagonistic effects against pathogens and reduced inflammation and destruction of the oral tissues. So the presence as a residential microflora and the addition as oral probiotic supplements, LAB can lead to prevention of diseases like caries and periodontal diseases and can have a role in maintaining the health of the dental biofilm and the overall oral and systemic health.

We have isolated twelve strains from the oral cavity of healthy individuals. Analyses for peroxidase and catalase activities were held and the results showed negative enzymatic activity, therefore showing their belonging to LAB. Every new isolate with a probiotic potential must be fully characterized to be used in probiotic substances. All strains were analyzed to define their affiliation by determining their biochemical profile using the API system, analysis of the 16S rDNA gene sequence and using their protein profile with the MALDI-TOF MS. The results show that ten of the strains belong to the genera Lactobacillus and the other two were identified as Weissella confusa.

It is known that pathogens are opportunistic and survive even in unfavourable conditions and can spread relatively easy. Therefore, it is important to study the ability of LAB to inhibit their growth. Screening for antimicrobial activity against microbial test pathogens was held. Analysis with eight bacterial and one yeast test pathogens was evaluated. Two of them common oral pathogens and the rest associated with their possible transition from the outside environment. The results show that the native supernatant of the majority of the LAB strains inhibit the growth of *B. subtilis*, *B. cereus* and *E. coli*. Growth inhibition against the most common oral pathogen *S. mutans* was shown only from the two Weissella confusa strains. The inhibition effect against the test-pathogens is from the produced acids from the LAB strains. Neutralized supernatants were also analyzed which had no positive results.

The evaluation of the antibiotic susceptibility is important for LAB with probiotic potential for selecting strains to be used as regulators of the microbiota in the human body and mainly in the oral cavity. Thirteen test antibiotics were used. Most of the strains show resistance to Kanamycin, Neomycin, Streptomycin, Vancomycin, Ciprofloxacin, Trimethoprim and Gentamicin. The established variety of resistances from the used range of antibiotics is a prerequisite for further study for establishment of the genetic determinants. The aggregation capability is connected with the LAB's probiotic properties and is important for the formation of the dental biofilm. Autoaggregation analysis was held to determine the percentage of cell aggregation of the newly isolated strains. From the results, all strains show low self-aggregating capability. From all tested strains *L. rhamnosus* shows the highest autoaggregation.

Further analyses will be made to evaluate the potential of the LAB strains as a model probiotic product for oral health. Survivability in the GIT analysis will be held, focusing mainly on the survivability in the oral cavity, a co-aggregation with the test pathogens E. coli and S. mutans, the survivability assessment drying technological processes (freeze drying and ets.) and the potential for including in a model oral probiotic product.

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Keywords: Lactic acid bacteria, probiotics for oral health, *Lactobacillus*, Weissella

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Co-cultivation of red yeasts and microalgae – a tool to effective production of high valuable biomass and metabolites

Ivana Marova, Martin Szotkowski, Jiri Holub, Samuel Simansky, Klara Hubacova, Dana Byrtusova
Department of Food Chemistry and Biotechnology, Faculty of Chemistry, Brno University of Technology, Brno, Czech Republic

Carotenogenic yeasts utilize various C-sources including agricultural by-products and in a short time produce large amount of biomass enriched by carotenoids, sterols and lipids (30–50 %). Autotrophic microalgae produce the same value added metabolites as red yeast, usually are unable to utilize organic carbon and wastes. However, they process CO_2 and have low demands on medium.
Co-cultivation of yeasts and microalgae works with the idea of the natural transport of gases between specific microorganisms to ensure their essential vital functions. Microalgae produce oxygen, which stimulates yeast growth. Yeasts produce CO_2, which is beneficial for algae growth. Moreover, specific elements have released during cell lysis. Thus, the symbiotic effect can achieve maximal biomass and metabolites yields.
The aim of present study was to evaluate the ability of selected strains of microalgae and carotenogenic yeasts to grow and metabolize in co-culture. The effect of media composition on growth and metabolism of yeasts was assessed and, simultaneously, microalgae adaptation to organic carbon and mixotrophy was tested. Cultivations were transferred gradually from small-scale flasks to aerated bottles and 3L bioreactor. Pilot experiments have confirmed that carotenogenic yeasts and microalgae are capable of effective symbiotic coexistence.

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Keywords: carotenogenic yeasts, microalgae, co-cultivation, carotenoids, lipids

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Conservation of rare, threatened and endemic halophytes in Mediterranean salt marshes affected by global warming

Ricardo Mir[1], Monica Boscaiu[2], Oscar Vicente[1]
[1]Institute for the Conservation and Improvement of Valencian Agrodiversity (COMAV), and
[2]Mediterranean Agroforestry Institute (IAM). Universitat Politècnica de València, Valencia, Spain

Mediterranean salt marshes are extremely threatened ecosystems, affected by human activities and very sensitive to the effects of climate change, with predicted increases in temperatures and soil salinity, and longer, more frequent, and intense drought periods and heatwaves. Over the last years, we have studied the responses to salt stress and water deficit of several abundant, widely distributed 'structural' halophytes inhabiting these specialised habitats, combining field work in salt marshes of the Valencia Community and controlled stress treatments in the greenhouse. These halophytic species seem to possess built-in mechanisms that will allow them to adapt and survive the expected increase in the level of environmental stress in their natural habitats. More recently, we have focused on less abundant, 'differential' halophytes, the most interesting taxa from the ecological and biodiversity points of view, for which little information is available. They include the most vulnerable taxa, rare, threatened species and narrow endemics, with few populations of a limited number of individuals, even some locally extinct. We will present the analysis of stress tolerance thresholds and mechanisms of some of these species, showing how the obtained results can help the design and implementation of salt marsh conservation/regeneration programmes in progress in our region.

Keywords: climate change, biodiversity, salt marsh habitats, drought, salt stress, regeneration programmes

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Prospecting a new wave of commercialization of PHA biopolyesters

Martin Koller[1], Anindya Mukherjee[2]
[1]University of Graz, Office of Research Management and Service, c/o Institute of Chemistry, NAWI Graz, Heinrichstrasse 28/VI, 8010 Graz, Austria
[2]Global Organization for PHA (GO!PHA), Amsterdam, The Netherlands; PHAXTEC, Inc., Wake Forest, North Carolina, USA

Microbial polyhydroxyalkanoates (PHA) biodegrade in soil and aquatic environments, and are both industrial- and home-compostable. Starting from renewables, many natural microbes natively produce PHA homo- and heteropolyesters of 3-hydroxybutyrate (3HB), 3-hydroxyvalerate (3HV), 3-hydroxyhexanoate (3HHx), and 4-hydroxybutyrate (4HB), plus numerous other types of natural PHA. However, only some of them are thoroughly researched, and an even smaller number reliably emulate properties of established petro-plastics: slowly but still, these typed of PHA are becoming commercially relevant, because consumers, brand owners, converters, waste managers and policy makers start appreciating the positive attributes of PHA related to their biodegradability, resulting in the currently witnessed first wave of serious commercialization efforts. The next, eagerly expected wave consisting of commercialization of PHA in durable applications would potentially make the decisive quantum leap in reducing plastics pollution, depletion of fossil resources, and greenhouse gases, according to current activities to fight Climate Change.
The product portfolio of most companies commercializing PHA is restricted to the "top-selling" types of PHA P(3HB), P(3HB-co-3HV), P(3HB-co-4HB) and P(3HB-co-3HHx), plus Tepha´s surgically important P(4HB). Therefore, this presentation focuses on past and current global industrial production of these PHA types, their properties and market potential.

Keywords: Biopolymers, Biopolyesters, Commercialization, Polyhydroxyalkanoates

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Microbial lipids from renewable resources

Mirela Ivancic Santek, Marina Grubisic, Suncica Beluhan, Bozidar Santek
Department of Biochemical Engineering, Faculty of Food Technology and Biotechnology, University of Zagreb, Zagreb, Croatia

Oleaginous microorganisms are known for their ability to accumulate substantial amounts of lipids inside their cells or mycelia under specific growth conditions. These diverse group of microorganisms include different genera of microalgae, bacteria, fungi, and yeasts with lipid content exceeding 20 % in dry cell weight. The most abundant class of lipids in oleaginous microorganisms are neutral triacylglycerols (TAGs). Fatty acids (FAs) profile of TAGs is characteristic of specific microbial species but can vary depending on the growth conditions, carbon source and growth phase. Oleaginous yeasts such as *Rhodosporidium toruloides*, Yarrowia lipolytica and *Cryptococcus curvatus* have been considered for biodiesel production due to their plant-like FAs profile and high lipid productivity. Several fungi (genus *Mortierella*) and microalgae (*Crypthecodinium cohnii*) strains have been used in the large-scale production of long-chain polyunsaturated fatty acids (e.g. docosahexaenoic and arachidonic acid), which are used as nutraceuticals and food additives. Production of microbial lipids is still commercially unfeasible due to the high production cost. Using biomass resources such as agricultural and forestry residues, industrial wastes and byproducts, food and municipal wastes as a substrate for microorganism growth and lipid production could significantly reduce biodiesel production costs.

Keywords: microbial lipids, biodiesel, polyunsaturated fatty acids, renewable substrate

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Bioinspired Nanotechnology: Peptide Based Molecular LEGO

Adam Mechler
Department of Chemistry and Physics, La Trobe University, Melbourne, Australia

Peptides are best known for their bioactivities but they can also serve as building blocks of complex nanostructure. Natural peptides however often aren't stable enough metabolically and chemically to be used in technological applications. This limitation can be overcome by using bioinspired systems, e.g. substituted oligoamides (SOs) that are artificial analogues of natural peptides. SOs are synthesized residue-by-residue using established peptide synthesis methodology; however virtually any molecule can be coupled, provided that it has an amine and a carboxyl moiety. Oligoamides formed from β3 amino acids have a stable helical fold with almost exactly 3 amino acids per turn, that is stable with as little as 4 residues. Chemical modification implements a self-assembly motif that allows these helical units to assemble into nanorods head-to-tail, where each monomer retains the helical fold; these supramolecular nanorods can grow up to millimeters in length. This three point hydrogen bonding motif essentially turns the helical SO units into molecular LEGO bricks, allowing co-assembly of chemically distinct units. This platform allows the design of geometrically and chemically well defined nanostructured materials, opening new avenues to build molecular machines.

Keywords: bioinspired material, nanostructure, spontaneous assembly, molecular machines

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Does DL-Sulforaphane Induce Oxidative Stress in Breast Cancer Cell Lines in High Glucose Media?

Sule Ozdas[1], Gunnur Demircan[1], Ummugulsum Guzalsoy[2], Demet Akin[3]
[1]Demiroglu Bilim University
[2]Yildiz Technical University
[3]BAU Batumi International University

Impaired glucose metabolism is an important risk factor for cancer formation by increasing oxidative stress at the cellular level. Therefore, the diabetes is an important risk factor in the progression of cancer. In many studies, it has been shown that sulforaphane (SFN) has anticancer properties and reduces cancer risk. In this study, we aimed to demonstrate the antioxidant and anticarcinogenic effects of SFN in breast tissue healthy and cancer cell lines incubated in a high glucose media.
Cells were incubated for 24 and 48 hours in high glucose medium using different SFN concentrations. Oxidative stress responses in the cell lysates were evaluated with total oxidant and antioxidant measurement kits. Anti-apoptotic (Bcl2) and apoptotic protein(Apaf1,Caspase3)expressions were evaluated by Western Blot analysis. At the same time, intracellular insulin levels were shown by ELISA.
According to our study, SFR induces apoptosis via a reactive oxygen species (ROS)-dependent mechanism. The production of ROS has been postulated to be a key mechanism by which SFN induces apoptosis. SFR acts strengthening the cellular defenses against oxidative damage and promoting the removal of carcinogens. There is substantial and compelling evidence for SFN mediated apoptosis in both of breast cancer cells, but this may depend on SFN dose.

Keywords: sulforaphane, breast cancer, oxidative stress

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Peptides - molecules with different biological activities

Dancho Danalev[1], Sirine Jaber[1], Spaska Yaneva[1], Ivan Iliev[2], Lyubomir Vezenkov[1], Adriana Bocheva[3], Stanislava Vladimirova[1], Hristina Nocheva[4], Tamara Pajpanova[5], Atanas Shopov[1], Nelly Georgieva[1], Emilia Naydenova[1]
[1]University of Chemical Technology and Metallurgy, 8 Kliment Ohridski blvd., 1756 Sofia, Bulgaria
[2]Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Acad. G. Bonchev str., bl. 25, 1113 Sofia, Bulgaria
[3]Medical University-Pleven, Faculty of Medicine, Department of Physiology and pathological physiology
[4]Medical University-Sofia, Faculty of Medicine, Department of pathophysiology
[5]Institute of Molecular Biology "Roumen Tsanev", Bulgarian Academy of Sciences, Sofia, Bulgaria

Some of the widely studied biologically active substances are bioactive peptides. They could play a diverse role in the human body. They can be neurotransmitters, neuromodulators, hormones, enzymes as well as inhibitors of key enzyme systems. Some species of frogs secrete neuroparalytic peptides, whose main function is to protect them against enemies or provide them with the food they need to survive. Different types of insects such as ticks and leeches produce peptides with anticoagulant properties, the main role of which is to ensure their even feeding over time with the blood they ingest. Tyr-MIF is a tetrapeptide well know from the literature with its opioide activity and good selectivity to the μ-receptors. Somatostatin and its analogues are example for application of peptides as anticancer agents. Herein, we will present some of our investigations on biologically active peptides with different activities as possible alternative of conventional medical drugs with lower or missing side effects.

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Keywords: biologically active peptides, anticoagulants, anticancer peptides, opioide peptides

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Artificial intelligence applications in sleep medicine

Osman Erogul
Department of Biomedical Engineering, Tobb University of Economics and Technology, Ankara, Turkey

The most basic definition of the sleep is related with organism's response to environmental stimuli. Sleep can be defined as a reversible, less-than-awake response to the environment, which recurs on a daily cycle. All problems that affect sleep quality, duration and subject's daily life are named as sleep disorder. Polysomnography is the gold standard for the evaluation of sleep signals. A polysomnography device records electroencephalography, electrooculography, and electromyography activities at the same time. A polysomnographic measurement can be extended with recording additiona lphysiological signals, like electrocardiography and body position, oxygen level in the blood, and snoring. These additional signals are important for the diagnosis of sleep disorders. The diagnosis of sleep disorder is done with analyzing laborious overnight polysomnography recording. Nowadays, to reduce duration and increase accuracy of diagnosis, artificial intelligence applications are used. These applications developed by using feature extraction-based machine learning or deep learning algorithms that generally apply to one or two polysomnography signals. By using artificial intelligence in sleep studies, duration of diagnosis reduces from hours to minutes and increases accuracy of diagnosis to over 90%. This study gives examples about artificial intelligence applications used in sleep studies.

Keywords: Sleep, Artificial intelligence, Polysomnography, Physiological signals

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Copper dyshomeostasis in synucleinopathies: a potential role for PLK2 kinase as a counter for a-synuclein aggregation and oxidative stress

Michele Maffia, Debora Musarò, Luca Giulio Cossa, Alessandra Inguscio, Antonio Danieli, Marco Greco
Dept of Biological and Environmental Science and Technology, Univ of Salento, Lecce, Italy

Parkinson's Disease (PD), see the accumulation of α-synuclein and alterations in its phosphorylation pathways, which give rise to the formation of aggregates defined Lewy's bodies. In this context, the PLK2 kinase-mediated Ser-129 phosphorylation, is mainly involved in the protein clearance. Attention in recent years has been equally divided among the study of conditions promoting aggregation and the elucidation of alterations of proteolytic pathways. Conditions of impaired unfolded protein response, and alterations in proteasomal and autophagic processes are widely associated to PD onset. Copper accumulation in the central nervous system can act in a manifold manner, seeding of proteins aggregation, triggering redox cycles that generates reactive oxygen species and inhibiting degradatory pathways. SHSY5Y neuroblastoma cells were differentiated to obtain an in vitro model capable of assuming a dopaminergic neuron-like phenotype. Treatments with increasing concentrations of copper in the culture medium were used to replicate what observed in the pathology. In this context, PLK2 protein shows to be modulated as a consequence of our treatments, increasing α-synuclein phosphorylation, and countering the oxidative stress in neuronal cells. A better understanding of the copper-dependent alterations may therefore represent a potential route for the development of innovative clinical strategies against α-synuclein aggregation

Keywords: copper, α-synuclein, PLK2 kinase, SHSY5Y neuroblastoma cells

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Rare genetic variants in lipedema

Stefano Paolacci[1], Sandro Michelini[2], Karen L. Herbst[3], Maria Rachele Ceccarini[4,5], Tommaso Beccari[4,5], Vincenza Precone[6], Elena Manara[6], Paolo Enrico Maltese[1], Elisa Sorrentino[6], Giuseppe Marceddu[6], Astrit Dautaj[7], Matteo Bertelli[1,6,7]
[1]Magi's Lab, Rovereto, Italy
[2]Vascular Diagnostics and Rehabilitation Service, Marino Hospital, Marino, Italy
[3]Department of Endocrinology and Research, Total Lipedema Care, Los Angeles, USA
[4]Department of Pharmaceutical Sciences, University of Perugia, Italy
[5]C.I.B., Trieste, Italy
[6]Magi Euregio, Bolzano, Italy
[7]Ebtna-Lab,Rovereto, Italy

Lipedema is a disabling disease characterized by a symmetric excessive accumulation of subcutaneous fat in the lower and/or upper limbs. The aim of this study was to find the genetic etiology of lipedema and eventually implement a genetic test for the identification of genetic variants associated with lipedema.
We developed an NGS gene panel comprising genes possibly associated with isolated lipedema. One hundred and sixty-two patients with lipedema enrolled by Italian and American clinical centers were sequenced. The panel consisted of 549 genes involved in lipid metabolism and/or disorders characterized by fat accumulation.
In 41 patients, we identified 42 variants (in 23 genes) possibly linked with lipedema. To interpret those variants, we used the online tool VarSome. According to VarSome, 6 variants were classified as pathogenic, 20 variants were likely pathogenic, and 13 variants were of uncertain significance.
This NGS-based sequencing approach may represent a useful tool for the genetic analysis of patients with lipedema, find possible therapeutic targets and possibly predict the outcome of the treatments.

Keywords: Lipedema, subcutaneous fat accumulation, next generation sequencing, genetic test

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Study of the components of Lemna minor

Kyrylo Anpilogov[1], Elisabetta Miraldi[2], Maria Rachele Ceccarini[3,4], Giulia Baini[2], Elena Manara[1], Kristjana Dhuli[1], Francesco Ria[5], Gabriele Di Sante[5], Andrea Zanlari[6,7], Claudia Catelani Cardoso[6], Pietro Chiurazzi[8], Tommaso Beccari[3,4], Matteo Bertelli[1,9,10]
[1]Magi Euregio, Bolzano, Italy
[2]Department of Physical, Earth and Environmental Sciences, University of Siena, Italy
[3]Department of Pharmaceutical Science, University of Perugia, Italy
[4]C.I.B., Trieste, Italy
[5]Department of Translational Medicine and Surgery, Università Cattolica del Sacro Cuore, Rome, Italy
[6]SSICA, Parma, Italy
[7]Università Mercatorum, Parma, Italy
[8]Dipartimento Scienze della Vita e Sanità Pubblica and UOC Genetica Medica, Policlinico Universitario A. Gemelli, Università Cattolica del Sacro Cuore, Rome, Italy
[9]EBTNA-LAB, Rovereto, Italy
[10]MAGI'S LAB, Rovereto, Italy

Lemna minor is a plant with a huge repertoire of secondary metabolites. In the present work, we extracted polyphenols, flavan-3-ols and triterpens and discussed their therapeutic potentials.
We grew L. minor on a standard medium containing Gamborg B5 and vitamins. We performed the extraction of compounds from L. minor through maceration and decoction and the phytochemical profile was characterized through chromatographic, spectrophotometric and spectroscopic techniques.
Maceration allowed for the extraction of triterpens, whereas polyphenols and flavan-3-ols were obtained after decoction. In literature, it is reported that extracts of dried L. minor leaves inhibit bacterial and fungal growth. Furthermore, extracts of Lemna minor exhibit antioxidant, antiradical, immunomodulatory and anti-inflammatory properties. These properties could be exploited for the development of therapeutic agents for autoimmune diseases, graft rejection and organ transplantation.

Keywords: Lemna minor, metabolites, immunomodulation

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Rare genetic variants in Anorexia Nervosa

Kristjana Dhuli[1], Maria Rachele Ceccarini[2,3], Erika Granese[2], Elena Manara[1], Stefano Paolacci[4], Paolo Enrico Maltese[4], Kevin Donato[1], Matteo Bertelli[1,4,5], Laura Dalla Ragione[6], Tommaso Beccari[2,3]
[1]Magi Euregio, Bolzano, Italy
[2]Department of Pharmaceutical Science, University of Perugia, Perugia, Italy
[3]C.I.B., Trieste, Italy
[4]Magi's Lab, Rovereto, Italy
[5]Ebtna-Lab, Rovereto, Italy
[6]Food Science and Human Nutrition Unit, University Campus Biomedico of Rome, Rome, Italy

Anorexia nervosa (AN) is a psychiatric disease difficult to treat with high mortality rates. AN results by a complex interaction between environmental risk factors and genetic predisposition. The genetic association was revealed in twin studies and the heritability for AN is around 48-74%.
We analyzed 68 AN patients (63 female and 5 male) with familiarity (one or more than one relatives affected) recruited in Italy. Blood samples of all patients were collected in the Department of Eating Disorder, Palazzo Francisci (USL 1 Umbria, Todi, Italy). The body mass index values were 14.3 ± 1.98. The whole coding region of 164 genes were investigated using Next Generation Sequencing (NGS). Genetic variants in different genes coding for proteins involved in leptinergic system, dopaminergic system, endocannabinoid metabolism pathway and calcium channel subunit were found.
To date, the main criteria for the diagnosis of AN is the clinical evaluation of low body weight. This study suggests that NGS-based genetic testing may have a relevant role in the evaluation and in the diagnosis of AN.

Keywords: Anorexia nervosa, Rare genetic variants, Next Generation Sequencing

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Identification of Mendelian obesity and related genetic fat disorders in bariatric obesity

Paolo Enrico Maltese[1], Vincenza Precone[2], Elena Manara[2], Giuseppe Marceddu[2], Giacinto Abele Donato Miggiano[3], Maria Rachele Ceccarini[4,5], Tommaso Beccari[4,5], Barbara Aquilanti[3], Matteo Bertelli[1,2,6]
[1]Magi's Lab, Rovereto, Italy
[2]Magi Euregio, Bolzano, Italy
[3]UOC Nutrizione Clinica, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy
[4]Department of Pharmaceutical Science, University of Perugia, Perugia, Italy
[5]C.I.B., Trieste, Italy
[6]Ebtna-Lab, Rovereto, Italy

Obesity is a complex and often disabling disease involving abnormal fat accumulation, which also causes a higher risk of developing comorbidities for its patients. In this study, our aim was to evaluate the diagnostic yield of a molecular approach using a customized next-generation sequencing (NGS) panel focusing on variants in bariatric patients.
We designed a targeted NGS gene panel, comprising 49 diagnostic genes and 190 pre-diagnostic genes, and we used it to analyze 247 Italian patients affected by obesity (BMI >= 30).
From this sample, we identified patients carrying variants in genes related to the leptin/melanocortin signaling pathway, in genes usually associated with syndromic obesity, and in genes already associated with unsuccessful weight loss after bariatric surgery.
Our study demonstrated that out NGS-based panel including diagnostic and pre-diagnostic genes can improve the molecular diagnosis of genetic obesity and, consequently, it could play a relevant role in the clinical management of these patients, in particular in suggesting a close follow-up in bariatric patients.

Keywords: obesity, next generation sequencing, genetic test

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Reliable techniques for auto-antibody detection in autoimmune retinopathy

Maria Rachele Ceccarini[1,2], Tommaso Beccari[1,2], Elena Manara[3], Matteo Bertelli[3,4]
[1]Department of Pharmaceutical Science, University of Perugia, Perugia, Italy
[2]C.I.B., Trieste, Italy
[3]Magi Euregio, Bolzano, Italy
[4]Ebtna-Lab, Rovereto, Italy

Autoimmune retinopathy (AR) is a rare blinding retinal disorder characterized by the presence of auto-antibodies (AAbs) against retinal proteins. AR is difficult to diagnose because the clinical features are not distinctive and usually overlapped with other conditions. For this reason, the presence of AAbs in the serum is an essential parameter for the diagnosis, but unfortunately, AAbs can also be found in patients with other auto-immune disorders and sometimes in healthy controls (CTRs). The aim of the project is to develop a reliable assay for the detection of serum AAbs in AR patients. Using Western Blotting assay and Dot Blot technique the serum of 20 patients and 10 CTRs were analyzed for ten different recombinant retinal proteins: Enolase 1, Recoverin, Rodopsin, Heat Shot Protein 27, Carbonic anhydrase II, Glyceraldeide-3-phosphate dehydrogenase, Arrestin 3, G protein subunit alpha transducin 2, member RAS oncogene family and Bestrophin 1. The results indicate that Recoverin, Rodopsin, and G protein subunit alpha transducin 2 are good candidates for the diagnosis. Understanding if age, gender, and other kinds of diseases could modify the presence and the detection of AAbs is a crucial point that must be elucidated. The diagnosis of AR patients is fundamental for therapeutic approaches.

Keywords: Autoimmune retinopathy, auto-antibodies, Western Blot, Dot Blot

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Long read PacBio sequencing Strategy in Rpe65 gene analysis

Elisa Sorrentino[1], Giuseppe Marceddu[1], Matteo Bertelli[1,2,3]
[1]Magi Euregio, Bolzano, Italy
[2]Magi's Lab, Rovereto, Italy
[3]EBTNA-Lab, Rovereto, Italy

Bi-allelic mutations in the RPE65 gene are associated to retinal dystrophies, such as Leber congenital amaurosis (LCA) and Retinitis Pigmentosa (RP), inherited in autosomal recessive manner. Diagnostic panel-based NGS analysis is usually performed on coding regions and intron/exon junctions of genes. The aim of the study was to identify on RPE65 a second hit in LCA or RP patients where a single heterozygous variant was found with NGS, therefore reaching a more conclusive diagnosis. Here, we used the PacBio technology to sequence the full gene and identify putative second-hits in intronic, problematic and in promoter region. Moreover, PacBio sequencing allows to determine the allelic phase in a single analysis.
The analysis workflow begins with DNA extraction, library preparation, sequencing, and bioinformatics analysis. For the latter, we developed an automated pipeline that includes quality control, alignment, SNV, small indels, structural variants calling, phasing, annotation and variant interpretation. All variants identified in the diagnostic setting were correctly detected by the pipeline. Furthermore, a previously undetected frameshift pathogenic variant was found in a RP patient and confirmed by Sanger sequencing.
We thank Novartis Farma for providing economical funding with this study (CLTW888A1IT01T).

Keywords: RPE65, PacBio sequencing, Retinitis Pigmentosa, Leber congenital amaurosis

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Detecting AKR1C1 structural alterations via molecular dynamics

Jurgen Kaftalli[1], Andrea Bernini[2], Giuseppe Marceddu[1], Matteo Bertelli[1,3,4]
[1]Magi Euregio, Bolzano, Italy
[2]Department of Biotechnology, Chemistry and Pharmacy, University of Siena, Siena, Italy
[3]Magi's Lab, Rovereto, Italy
[4]EBTNA-Lab, Rovereto, Italy

The purpose of this study is to evaluate the impact of molecular dynamics simulations and other bioinformatics tools for the prediction of pathogenicity of variants of the AKR1C1 gene, with focus on the structural alteration of the gene product caused by missense mutations. Mutations were extracted by Varsome and filtered on a MAF minor of 0.05 and functional predictors such as Polyphen2 and SIFTH, then tested for impact by position entropy on multiple sequence alignment and for stability effect on the three-dimensional structure of the enzyme - using the mCSM predictor of change in free energy of the folding. The availability of the structure of the enzyme bound to substrates (steroids and NADP(H)) allowed for the comparison of molecular dynamics simulation of both the free and bound form of the wildtype and the missense mutants, and subsequent estimation of change in biological activity, also in a high throughput fashion by in-house programming of the workflow. In conclusion, we were able to quantify the structural alteration of the protein caused by VUS variants in AKR1C1 by using molecular dynamics simulation, providing an additional tool to predict the pathogenicity of new mutations and a valuable tool for the design of future therapeutics.

Keywords: Molecular Dynamics, AKR1C1, VUS

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Could in vitro propagation compete cultivation of aromatic-medicinal plants for specific chemical composition of essential oils?

Ariola Bacu[1], Valbona Sota[2]
[1]Department of Biotechnology, Faculty of Natural Sciences, University of Tirana, Albania; Unit for Bioscience, Biotechnology & Genetics, Academy of Sciences of Albania
[2]Department of Biotechnology, Faculty of Natural Sciences, University of Tirana, Albania;

Aromatic plants grown naturally in Albania, are also cultivated massively for export. As trade expands, so does the scientific interest in improving the quality and quantity of essential oils through biotechnology. Molecular markers based interpopulation diversity screening was accompanied by the control of the diversity of monoterpene synthase coding genes, and have demonstrated the presence of quite distinct ecotypes of Salvia officinalis L., Oreganum vulgare L., Rosemarinus officinalis L, Myrtus communis L.
GC of essential oil content has shown high variation, and has enabled the determination of the most valuable ecotypes. However, the composition and content of essential oils remains highly influenced by environmental factors, raising the need on plant production away from their impact. In this context, special attention is being paid to finding appropriate micropropagation protocols to stably modify the terpenoid content. Lateral buds cultured in different cytokine / auxin ratios produced plantlets whose leaves were compared with those of in vivo grown plants, via histological methods. Obtained results show differences in the density and size of glandular trichomes. Challenge remains the production of plants with standard quality and quantities of essential oils, as well as the possibility of their preferential use by the industry over cultivated plants.

Keywords: essential oils, terpenoids, GC, molecular markers, micropropagation

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Molecular mechanisms of ovulation: the role of local prostaglandins

Bajram Berisha[1,2]
[1]Department of Animal Biotechnology, University of Prishtina, Prishtina, Kosovo
[2]Animal Physiology and Immunology Weihenstephan, TUM, Munich, Germany.

Ovulation in mammals occurs as a result of interaction between the LH surge and local follicular factors, especially prostaglandins. Therefore the objective of this study was to characterize the regulation pattern of local produced prostaglandin F2alpha (PGF), prostaglandin E2 (PGE), their receptors (PGFR, PGER2, PGER4), COX-2 and PGF synthases (PGFS, PGES) in the bovine follicles during preovulatory period and early corpus luteum (CL). Ovaries containing preovulatory follicles or CL were collected by transvaginal ovariectomy before and after GnRH treatment. The COX-2 mRNA increased significantly 4h and again 20h after GnRH, followed by a significant decrease after ovulation. The mRNA of PGFS in follicles before GnRH was high followed by a continuous and significant downregulation afterwards. In contrast, PGES mRNA increased significantly in follicles 20h after GnRH and remained high afterwards. The mRNA of PGFR, PGER2, and PGER4 before GnRH was high, followed by down regulation afterwards and significant increase only after ovulation. The low concentration of PGF and PGE in follicular fluid before GnRH, increased continuously and significantly before and especially around ovulation. In conclusion, our results indicate that the examined prostaglandin family members are involved in the local mechanisms regulating final follicle maturation and ovulation in the cow.

Keywords: Prostaglandins, gene regulation, follicle, ovulation, corpus luteum, cow

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Nanobubble ozone stored in hyaluronic acid-decorated liposomal solution: a new strategy for antibacterial & antiviral effect

Sehime Gulsun Temel[1], Perihan Erkan Alkan[2], Cem Müjde[3], Hivda Polat[4], Cemre Örnek Ergüzeloğlu[5], Atıl Biskin[6], Cüneyt Özakın[7], Ahmet Ümit Sabancı[8]
[1]Bursa Uludağ University, Faculty of Medicine, Department of Medical Genetics,
[2]Bursa Uludağ University, Vocational School of Health Services. Medical Laboratory Technician Department, Bursa, Turkey
[3]Cukurova University AGENTEM (Adana Genetic Diseases and Treatment Center), Adana, Turkey
[4]TUBITAK, Marmara Research Center, Genetic Engineering and Biotechnology Institute, Gebze, Kocaeli, Turkey
[5]Bursa Uludag University, Institute of Health Sciences, Department of Translational Medicine, Bursa, Turkey.
[6]Çukurova University, Faculty of Medicine, Department of Medical Genetics, Adana, Turkey
[7]Bursa Uludağ University, Faculty of Medicine, Department of Infectious Diseases and Microbiology, Bursa, Turkey
[8]Bursa, Turkey Çekirge State Hospital, Orthopedics and Traumatology Clinic, Bursa, Turkey

Our world is currently struggling with a viral pandemic caused by SARS-CoV-2, which resulted in a huge burden on healthcare services.The rapid spread of the virus among the individuals has posed an enormous challenge for outbreak control. For this reason, supportive, preventive actions should be taken immediately. SARS-CoV-2 is a respiratory virus, and COVID-19 affects mainly the respiratory tract.An agent that limits viral replication in the upper airways may be promising for preventing the progression of disease into a severe lower respiratory tract infection, and it may restrict person-to-person transmission. We have characterized the anti-microbial and anti-viral activity of our novel NOHAL solution, developed by using nanotechnology. NOHAL solution prevented bacterial growth in a dose/time dependent manner. SARS-CoV-2 was rapidly inactivated by NOHAL, and viral infection of the VERO76 cells was inhibited. NOHAL solution did not cause any toxicity in in vivo or in vitro experiments. the levels of viral RNA in the nasopharyngeal and lung samples were reduced effectively. Our results suggest that NOHAL solution may be a potential agent for reducing or preventing the spread of the virus through nose and oral cavity. Further clinical studies are needed to evaluate this solution in the early phase of COVID-19.

Keywords: Nanobubble, Ozone, Hyaluronic Acid, liposome, anti-microbial, anti-viral

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Clinical studies on the efficacy of alpha-cyclodextrin and hydroxytyrosol against SARS-CoV-2 infection: North Cyprus Experience

Mahmut Çerkez Ergören[1], Stefano Paolacci[2], Elena Manara[3], Gulten Tuncel[4], Kristjana Dhuli[5], Giorgio Camilleri[5], Huseyin Kaya Suer[6], Nazife Sultanoglu[4], Murat Sayan[7], Munis Dundar[8], Matteo Bertelli[1], Tamer Sanlidag[4]
[1]Department of Medical Genetics, Faculty of Medicine, Near East University, Nicosia, Cyprus
[2]MAGI'S LAB, Rovereto (TN), Italy
[3]MAGI EUREGIO, Bolzano, Italy
[4]DESAM Research Institute, Near East University, Nicosia, Cyprus
[5]EBTNA-LAB, Rovereto (TN), Italy
[6]Department of Infectious Diseases and Clinical Microbiology, Faculty of Medicine, Near East University, Nicosia, Cyprus
[7]PCR Unit, Clinical Laboratory, Kocaeli University, Kocaeli, Turkey
[8]Department of Medical Genetics, Erciyes University Faculty of Medicine, Kayseri, Turkey

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel coronavirus responsible for the coronavirus disease 2019 (COVID-19), leading to the current pandemic. This virus attacks cells of the airway epithelium by binding to the transmembrane angiotensin-converting enzyme 2 (ACE2). Hydroxytyrosol has anti-viral properties, and it is found that, it has the ability to reduce the serum lipids in mice fed with high-cholesterol diet. Alpha-cyclodextrin can deplete sphingolipids and phospholipids from the cellular membranes. The aim of the present experimental study was to evaluate the efficacy of alpha-cyclodextrin and hydroxytyrosol in improving defenses against SARS-CoV-2 infection in humans. 225 volunteers at a higher risk of SARS-CoV-2 infection from Northern Cyprus were included in the treatment group and 76 healthy volunteers, who did not use the spray containing alpha-cyclodextrin and hydroxytyrosol, were included in the control group. The 149 of 225 volunteers did not become positive after 30 days for SARS-CoV-2 RT-qPCR assay after the administration of the compounds for four weeks, despite they were at higher risk of infection than the general population. Overall, based on the results of this study, it was reported that the alpha-cyclodextrin and hydroxytyrosol may improve defenses against SARS-CoV-2 infection and reducing viral endocytosis.

Keywords: SARS-CoV-2, ACE2, hydroxytyrosol, α-cyclodextrin

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Biotechnology in pandemic resilient governance

Lejla Pojskic[1], Haris Vranic[2], Jasmin Ramic[1], Nikolina Tomic[1], Belmina Saric[1], Ademir Spahic[2], Ednan Drljevic[3]
[1]University of Sarajevo-Institute for Genetic Engineering and Biotechnology, Zmaja od Bosne 8, 71000, Sarajevo, Bosnia and Herzegovina
[2]Ministry of Health, Canton Sarajevo, Reisa Dz. Čauševica 1, 71000 Sarajevo, Bosnia and Herzegovina
[3]Opšta bolnica „Prim.dr. Abdulah Nakaš", Kranjčeviceva 14, 71000 Sarajevo, Bosnia and Herzegovina

COVID-19 led to global crisis with more than 195 million COVID cases reported worldwide with average global death rate of 4,1M and total of 3,8 billion vaccine doses administered according to WHO by the end of July 2021. Biotechnologies are being widely adopted and incorporated in daily use for isolation, detection and identification of virus. Many countries use daily monitoring of SARS-CoV-2 VoC for public health risk assessment and informed escalation of epidemiological measures.
In a retrospective analysis of SARS-cOV-2 ocurrence,relative change in frequency and their impact on epidemiological parameters suggest that combining viral genomics and classical epidemiology is possible solution for detection and timely management of public healthcare emergencies of local, regional and global significance.
Epidemiological measures applied to reduce the negative impact of pandemics to public health need to be revised and improved to everchanging genome of SARS-COV-2 and its consequences on spreading pace and impact to health at individual level. This approach could be incorporated as a model for evidence based measures to protect population health but also preserve economies.

Keywords: SARS-CoV-2, VoC, epidemiology, genomics biotechnology, resilient society

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Development and study of an everolimus coated endovascular peripheral balloon for the treatment of peripheral arterial disease

Anargyros N Moulas[1], Arsen A Semertzioglou[2], Athanasios Vratimos[2], Panagiotis Kitrou[3], Michael Theofanis[3], Christos Papageorgiou[3], Fotios Anagnostopoulos[3], Ioannis Spyridonidis[3], Georgios Karpetas[4], Georgia Karanasiou[5], Dimitrios Fotiadis[5], Dimitrios Karnabatidis[3]
[1]General Department, University of Thessaly, Larissa, Greece
[2]Rontis SA, Greece
[3]Department of Radiology, University Hospital of Patras, School of Medicine, University of Patras, Greece
[4]Department of Anesthesiology, University of Patras, School of Medicine, Patras, Greece
[5]Department of Biomedical Research, Institute of Molecular Biology and Biotechnology, FORTH, Ioannina, Greece

Drug Coated Balloons (DCBs) are endovascular balloons coated with anti-proliferative drugs. DCBs are inflated in narrowed arteries or in implanted arterial stents with restenosis (re-narrowing) in order to restore blood flow. Existing DCBs mainly use paclitaxel, an antineoplastic drug that has been associated with increased mortality by some meta-analyses. Here we describe the development and study of a novel peripheral everolimus coated balloon (ECB) with a proprietary coating based on cellulose derivatives. The ECB was studied in silico and in vivo. The in silico study included simulation of balloon expansion and drug delivery. The safety and effectiveness of the balloon was studied in vivo in peripheral rabbit and swine experimental models and in an in-stent model in swine peripheral arteries for periods up to three months. Everolimus was detectable in treated rabbit arteries 1 month after treatment. ECB treated arteries showed minimal neointimal formation, comparable with paclitaxel coated DCBs and reduced arterial reaction compared to paclitaxel coated and uncoated balloons. These results indicate that the developed ECB is biocompatible and safe in the tested models.
This research has been co- financed by the European Regional Development Fund of the EU and Greek national funds under the call Research-Create-Innovate (T2EDK-03677).

Keywords: Drug eluting balloon, drug coated balloon, everolimus, peripheral arterial disease, in silico, simulation

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Development and study of an everolimus coated endovascular coronary balloon for the treatment of coronary arterial disease

Anargyros N Moulas1, Eleftheria Katsioti1, Evangelos Andreakos2, Maria Salagianni2, Ioanna Samara3, Christos Katsouras3, Grigorios Tsigkas4, Georgios Vasilagkos4, Alexandros Tousis4, Leonidas C Goudas5, Periklis Davlouros4, Lambros Michalis3
1General Department, University of Thessaly, Larissa, Greece
2Department of Immunology, Center for Translational and Clinical Research, Biomedical Research Foundation, Academy of Athens, Athens, Greece.
32nd Department of Cardiology, University Hospital of Ioannina, and Faculty of Medicine, School of Health Sciences, University of Ioannina, Greece
4Department of Cardiology, University of Patras, School of Medicine, Patras, Greece
5Department of Radiology, University of Patras, School of Medicine, Patras, Greece

Angioplasty, endovascular intervention with the use balloons and stents, is used for treatment of coronary arterial disease. Angioplasty may result in proliferation of arterial cells and restenosis, secondary narrowing of the treated arteries. The use of Drug Coated Balloons (DCBs) coated with anti-proliferative drugs, can reduce restenosis. Currently most DCBs use the drug paclitaxel. A novel everolimus coated balloon (ECB) using a proprietary coating with cellulose derivatives was developed and studied.
The effect of the ECB components on the proliferation of coronary arterial smooth muscle cells was studied in vitro. Safety and effectiveness were studied in an in-stent swine coronary experimental model. Bare metal stents (Rontis, Switzerland) were expanded in coronary arteries of domestic pigs followed by expansion of ECB or bare balloons or commercially available sirolimus eluting balloons. After 4 weeks treated arteries were explanted and examined. Everolimus and sirolimus eluting balloons showed lower intimal area and thickness than bare balloons. Our results indicate that the developed ECB is safe in the tested model and results in reduced stenosis compared to uncoated balloons.
This research has been co-financed by the European Regional Development Fund of the EU and Greek national funds under the call Research-Create-Innovate (T2EDK-03677).

Keywords: Drug coated balloon, drug eluting balloon, everolimus, coronary arterial disease, angioplasty

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Advances in medical biotechnologies transforming drug development and healthcare

Nikolai Zhelev1, Alexander Vladimirovich Kilchevsky3, Marianna Murdjeva4
1University of Dundee, Dundee, UK
2Venrize Life Sciences, Sofia, Bulgaria
3Institute of Genetics and Cytology, Minsk, Belarus
4Medical University of Plovdiv, Plovdiv, Bulgaria

Novel advances in medical biotechnologies have a substantial impact on the way drugs are developed and used in clinical practice. We review the use of Next Generation Sequencing (NGS), biosensors, stem cell technologies, biomarkers, and systems pathology in drug development and clinical practice with examples from our joint work in the field of cancer and cardiovascular diseases at the School of Medicine at University of Dundee, Medical University of Plovdiv and Institute of Genetics and Cytology, Minsk. Results from the development and validation of live-cell biosensors are presented. Recent advances in stem cell biology and 3D cultures and their use in drug development are illustrated with examples. Novel approaches for more efficient management of innovations in the drug discovery and development process employed by Venrize Life Sciences are presented.

Keywords: drug discovery, drug development, innovations, medical biotechnology

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