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NEGATIVELY CHARGED MESOPOROUS SILICA NANOPARTICLES PENETRATE THROUGH THE ZEBRAFISH LARVAL BLOOD-BRAIN BARRIER

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This study aimed to investigate how mesoporous silica nanoparticles (MSNs), especially focusing on their surface functional groups, interacted with zebrafish embryos and performed the penetration into blood-brain barrier. Surface properties, such as size, charge and surface chemistry, are a particularly important determinant influencing the biological fate and actions between the nanoparticles and cells. Eight kinds MSNs were synthesized with the uniform and mesoporous structure in ranged from +35.7 to 46.8mV of zeta potential and in size of 50nm or 200nm. By pericardial injection into 72 hpf zebrafish embryos, results observed were plenty of N4-MSN@PEG/THPMP$_{50}$ entering into larval brain; N1-MSN@PEG$_{50}$ had some; however, particles in positively charged were hardly found in the brain. It indicated that particles in negatively charged can penetrate blood-brain barrier into larval brain area. The confocal image was also confirmed by the two-proton image. The confocal image of all three N2, N3 and N5-MSN@PEG/THPMP$_{50}$ particles clearly presented in the larval brain area in similar pattern as N4-MSN@PEG/THPMP$_{50}$. However, the N4-MSN@PEG/THPMP$_{200}$ had not shown the penetration effect in the brain. The results illustrated that the brain penetration effect is may due to a negatively charged dependent and size-dependent manner.

Biography

Chien-Tsu Chen is a Professor of Department of Biochemistry and Cell Biology at School of Medicine, Taipei Medical University. His academic and research expertise include Nanotechnology, genetic engineering, nanomedicine, protein therapeutics, health promotion, antibody therapy Nanotechnology, Genetic engineering, Nanomedicine, Protein therapy, Health promotion, Antibody therapy. He completed his PhD in 1993 from Brandeis University, Waltham MA and Visiting Scholar in 2006 at University of Washington, Seattle WA. He was the President of St. Mary Medicine, Nursing and Management College in 2007.

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LEUKOCYTES AND DRUG-RESISTANT CANCER CELLS ARE TARGETS FOR INTRACELLULAR DELIVERY BY ADENOVIRAL DODECAHEDRON

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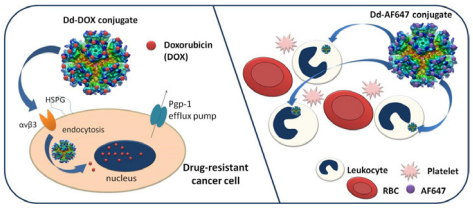
Virus-like particles (VLPs) are an attractive alternative to chemically synthesized nanoplatforms in development of novel carriers for targeted drug delivery. Adenoviral dodecahedron (Ad Dd), the symmetrical, small (28 nm) and non-infectious VLP, endowed with extraordinary ability for intracellular penetration through the receptor-mediated endocytosis followed by escape from the endosomes before reaching lysosomes. This results in cytoplasmic delivery of cargo molecules in a functional form. The chemical and genetic modifications allow for covalent attachment of cargo molecules or their insertion into particle structure without disrupting VLP integrity or penetration properties. The usefulness of Dd as a carrier for conjugated small molecules was already proven by targeted delivery of anticancer agents to hepatocellular carcinoma tumor in animal model, leading to inhibition of tumor growth. In the presented study we analysed delivery of doxorubicin conjugated to Dd (DdDOX). It was assessed in multidrug-resistant (MDR) human uterine sarcoma MES-SA Dx5 cells. The results of cytotoxicity tests attested the ability of Dd for circumvention of the Pglycoprotein 1 - mediated multidrug resistance mechanism. We demonstrated that efficient uptake of Dd-DOX conjugate in MDR cells leads to accumulation of drug in cell nucleus and significantly enhances doxorubicin cytotoxicity against target cancer cells. Furthermore, we demonstrated distinct Dd transduction efficiency for white blood cells, that could lead to the use of this vector for the transport of active molecules targeting leukocytes, in particular for *in vitro* testing of potential anticancer agents intended to treat leukaemia. The suitability of Dd for such application is supported by the lack of cytotoxicity of VLP in human peripheral blood mononuclear cells.

Images



The covalent attachment of doxorubicin to dodecahedron (Dd), enables efficient drug delivery and significantly enhances cytotoxicity in multidrug resistant cancer cells in vitro. Importantly, the affinity of Dd in human blood ex vivo is highly in favor of leukocytes, independently of their subtypes despite high representation of red blood cells and platelets. Thus, current results demonstrate that Dd is a promising vector targeting leukocytes and drug resistant cancer cells

Biography

Ewa Szolajska (Ph. D) an Assistant Professor at Institute of Biochemistry and Biophysics Polish Academy of Sciences (IBB PAS) Warsaw, Poland, is a Group leader in the Department of Protein Synthesis and Chief of Cell Culture Laboratory at IBB PAS. She got her Doctor's degree and habilitation in Biochemistry from IBB PAS. Currently Dr. Ewa Szolajskas' research focus on the development of an adenovirus derived virus-like particle as an intracellular delivery vector for the therapeutic applications.

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PHYTO-MEDIATED SYNTHESIS AND PHOTOCATALYTIC ACTIVITY OF NANOPARTICLES USING AQUEOUS EXTRACT OF BROCCOLI

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The intense interest in the study of nanoparticles arose by virtue of their unique physiochemical properties viz. mechanical, optical, electronic, magnetic, antimicrobial and catalytic. Among the metal oxide nanoparticles, CaO although rarely studied, has enormous use in the fields of catalysis, antimicrobial, detection, therapeutic and microelectronics. The green chemistry route is a pollution free synthetic method that employed the use of precursors, water and aqueous plant extract. It offers an alternative to chemical synthesis approach. In this work, we have synthesized CaO nanoparticles via green chemistry, using aqueous extract of broccoli as the capping and reducing agent and a combination of different calcium salts ($Ca(NO_3)_2$, $CaSO_4$, $CaCl_2$. $2H_2O$). The $Ca(OH)_2$ obtained were annealed at 750 °C to give CaO nanoparticles represented as C1, C2 and C3 from nitrite, sulphate and chloride sources sequentially. The nanoparticles were further investigated for their photocatalytic activities using bromocresol green (BG) and bromophenol blue (BP) as organic dyes and UV light as a radiation source. All the samples; C1, C2 and C3 exhibit significant degradation abilities against BG and BP. C2 and C3 revealed greater extent of photocatalytic degradation as they almost completely decolourised the organic dyes after a 180 mins of exposure to UV light. The degradation efficiency was found to be 73, 75 and 78% for C1, C2 and C3 respectively.

Biography

Jejenija Osuntokun obtained his PhD in Inorganic chemistry from University of Fort Hare, South Africa. He has wealth of experience in synthesis and characterization of metal complexes and subsequent use as a precursor for the synthesis of metal sulphide nanoparticles. He has worked on the synthesis of nanocomposite using synthesized metal sulphide nanoparticles as nano fillers. Presently, he is a Postdoctoral research fellow at North-west University in South Africa and his research focus is on the preparation of metal oxide nanoparticles via green chemistry. These metal oxides are further used for photocatalytic degradation of dyes with the ultimate application in environmental remediation especially in water purification.

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DEVELOPMENT OF HEPARINASE NANOSENSORS FOR METASTATIC CANCER CELLS DETECTION

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Heparin sulphate is closely related to heparan sulphate that plays a role in extracellular matrix modulation. Heparinase is a bacterial enzyme that breaks down heparin and heparan sulphate into small fragments. Heparanase is the mammalian enzyme that cleaves heparan and heparin sulphate but at more restricted regions. Detection of heparanase is quite important in early diagnosis of metastasis and cancer spreading. Herein, heparin sulphate was attached to the surface of silica nanoparticles (NPs) via intrachain carboxylate conjugation using cabodiimide chemistry. FRET based quenching was utilised to detect the fluorophore fluorescence changes using two different models. Model I adopted the attachment of fluorescein labelled heparin to the surface of black hole quencher 1-incorporated NPs. The achieved quenching efficiency was ~30% compared to fluorescent heparin attached to blank NPs as a control. In model II, BHQ 2 conjugated heparin was attached to the surface of core-shell NPs containing TAMRA in the shell and either blank core or 7-Methoxycoumarin incorporated core. NPs with different shell thicknesses were prepared ranging from 12 nm to 75 nm. The quenching efficiency of the 12 nm shell-NPs was ~ 42% using blank core. The quenching efficiency of 12 nm shell was also tested using Comarin incorporated core as internal standard to achieve more accurate results. In this system ratiometric method was developed, where the quenching efficiency was calculated based on the florescence ratio of TAMRA in the shell to coumarin in the core. The calculated quenching efficiency was ~ 10% which is lower than that predicted by blank core system. The developed system could be further employed as sensing tool for heparinase and heparanase to detect metastatic cancer cells.

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COATING MEDICAL DEVICES WITH ANTI-BACTERIAL NANOPARTICLES

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Sonochemistry is our deposition method for imparting unique properties to the desired substrates. It was applied first to coat a large variety of textiles (cotton, polyester, nylon wool and more) with anti-bacterial Nanoparticles (NPs). Excellent adherence to the textiles was demonstrated in withstanding 65 washing cycles in Hospital washing machines. In the current presentation its power will be demonstrated in the deposition of antimicrobial NPs on medical devices such as catheters, contact lenses, cochlear electrodes, and silicon-implants. It was also deposited on artificial teeth by the sonic irradiation and avoided the formation of biofilm of *s. mutans*. The NPs that have been used in this research are ZnO, CuO, $Cu_{0.89}Zn_{0.11}O$ and MgF_2. For the catheters too, *in vivo* experiments were conducted. The first experiment conducted in Israel where 5 coated and 5 uncoated silicon catheters were installed in rabbits (*Figure 1*) that were hanged during the 7 days of the experiment. The second was done in Synovo, Tubingen where 15 coated and 15 uncoated catheters were inserted in the rabbits which were free to run around. In both cases the coated catheters have avoided the formation of biofilm on the catheters in comparison with the appearance of urine contamination after day 4. Good results were obtained in all the above mentioned medical devices.

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ADVANCED NANOMEDICINES FOR MODULATION OF THE INTERACTION WITH BIOLOGICAL BARRIERS

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In drug delivery field, bioavailability and specificity are key challenges in the establishment of advanced products. Nanoparticles have been proposed by our group as valid approaches to provide successful systems to deliver drugs to their site of action, particularly to explore no-invasive, mucosal administration. Besides the proper control of nanoparticle matrix to provide a suitable release of drug payload, the surface of nanoparticles has a major impact on the interaction with biological barriers. We have studied thoroughly the interaction of nanoparticles with cells and mucus regarding their adhesive properties that modulates their mucoadhesive behaviour, ultimately related with passive targeting to mucosae. Understanding how nanosystems interact with individual mucin chains and the 3D structure of mucus is paramount, as a passive functionalization of nanoparticles may concern, exploring different biomaterials as mucus-modulators. Our active targeting approach for nanoparticles has been focused on ligand molecules attached to the surface of nanoparticles to increase the probability of binding to unregulated cell membrane receptors in key local effector sites. New and less-explored receptors are being targeted in engineered nanosystems, providing enhanced local and intracellular levels of drugs, without compromise the safety of the systems. In this talk, application of nanosystems for mucosal delivery of drugs with physiological and social impact, developed in our research group, will be presented.

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A NOVEL APPROACH TO THE THERAPY OF ALZHEIMER'S DISEASE BASED ON PEPTIDE NANOLIPOSOME INHIBITORS OF AMYLOID AND TAU AGGREGATION

David Allsop[1,2], Mark Taylor[1,2], Nigel Fullwood[1], Anthony Aggidis[1], Shoona Vincent[2] and Mark Dale[2]

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Considerable advances have been made over the last 30 years in understanding the neuropathology of Alzheimer's disease (AD) but this knowledge has not led to the successful development of new drugs. Currently available drugs only treat the symptoms of AD and many potential disease-modifying drugs have failed in clinical trials. Many of these drugs aim to reduce accumulation of β-amyloid (Aβ) in senile plaques and consist of inhibitors of β secretase or γ-secretase, which block Aβ production, or Aβ immunotherapy, which results in clearance of amyloid from the brain. These drugs have run into various problems and were probably given too late during the course of AD. Our proposed therapeutic candidates consist of modified peptides that inhibit the aggregation of Aβ or tau attached covalently to the surface of nanoliposomes. The latter contain a PEGylated lipid which has a maleimide group for covalent linkage to a thiol group (cysteine residue) on the peptide. The peptide developed against Aβ is retro-inverted (D-amino acids, with sequence reversal) and is stable against proteolysis. This is linked to a TAT sequence for targeting to the brain. Similar types of peptide-liposomes are under development for inhibition of tau aggregation (neurofibrillary tangle formation).Our approach is novel and specifically targets the early stages of aggregation of Aβ and tau. Multiple inhibitory peptides attached to the liposome surface create a potent, multivalent inhibitor that can cross the BBB and enter cells. Moreover, our peptide-liposomes hide from the immune system, and should not invoke an undesirable immune response. There is increasing recognition that combination therapies may be warranted to address the complex biology of AD and our development allows for Aβ or tau peptide inhibitors alone or in combination to be attached to the surface of the liposomes, resulting in a therapeutic with dual action against plaques and tangles.

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SYNTHESIS OF 2D FLATLANDS

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I will present our investigation of chemical vapor deposition (CVD)-growth, achieving localized, patterned, single crystalline or polycrystalline monolayers of TMDs, including MoS_2, WS_2, WSe_2 and $MoSe_2$, as well as their heterostructures. We study CVD-growth and perform extensive material characterization to illuminate the role of dissimilar 2D substrates in the prevention of interior defects in transition metal dichalcogenides (TMDs), thus uncovering the conditions for anti-oxidation. We further demonstrate the epitaxial growth of TMDs on hBN and graphene, as well as vertical/lateral heterostructures of TMDs, uniquely forming in-phase 2D heterostructures. This research provides a detailed observation of the oxidation and anti-oxidation behaviours of TMDs, which corroborate the role of underlying 2D layers in the prevention of interior defects in TMDs. If the technique could be developed to be highly reliable and high fidelity, it could have a large impact on the future research and commercialization of TMD-based devices. Furthermore, we develop flexible electrodes and energy storage toward wearable and multifunctional electronics. Here, we develop a facile fabrication technique utilizing vertically aligned carbon nanotubes (VACNTs), which enables high-throughput fabrication of flexible supercapacitors. We develop an innovative technique, which facilitates a stable charge/discharge under varied strains. Our structure shows a high flexibility and stability during stretching up to 20% and bending up to 180 degrees. These flexible supercapacitors are promising for various flexible electronics applications. Building on these previous results from 2D material growth and flexible electrodes, our next step is to combine 2D materials with flexible substrates toward next generation wearable detectors.

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PHYTOSYNTHESIS OF AG, AU AND AG-AU BIMETALLIC NANOPARTICLES USING GOLDEN ROD (SOLIDAGO CANADENSIS) PLANT AND ITS RELATED CYTOTOXICITY

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Silver, Gold and Silver-Gold bimetallic (BNP) nanoparticles have been synthesized in this work using golden rod leaf extracts. In typical biosynthesis reaction, the precursors $HAuCl_4.xH_2O$, $AgNO_3$ were differently and jointly mixed with aqueous extract of golden rod leaf and heated with stirring for 1 h. There were periodic changes in colour which reflects formation of plasmon bands as confirmed by UV-Vis spectroscopy. The different sizes of the nanoparticles were determined using XRD and TEM instruments while the bio reductants were examined using FTIR. In the BNPs, both Au and Ag were detected in the NP system. This observation reinforces the assertion that the plant biomolecules works to suppress the oxidation of Ag by Au and is key in forming Ag-Au alloy structures instead of hollow Au shells or core shells. Formation of Ag, Au and alloy Ag-Au bimetallic nanoparticles were evidenced by the appearance of bands at 420nm, 560nm and 530 nm respectively. The size and shape of Ag-Au bimetallic resemble the pure AuNPs more than the AgNPs. The cytotoxicity of the nanoparticles was equally studied using H4IIE-*luc* rat hepatoma cells by xCELLigence method and from the results, Ag and Ag-Au bimetallic showed little toxicity which may be due to the agglomeration experienced as shown by the TEM results while AuNPs recorded no toxicity.

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CHARACTERIZATION OF HPMC FILMS REINFORCED WITH CHITIN NANOMATERIALS AS PROMISING COMPOSITES FOR ECO-FRIENDLY APPLICATIONS

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Nanocrystals (NCchit) and nanofibers (NFchit) extracted from chitin are considered as nanomaterials of great innovative potential and promising biomaterial for academic and industrial fields. The research related to their production and application is still new. In the other hand, in recent years, many research programs have focused on developing more and more bio-degradable packaging of natural polymers. Among them, the one based on polysaccharide polymers is hydroxypropylmethylcellulose (HPMC). This is the first study to investigate the compatibility and reinforcement effect of nano-size chitin fillers (NCchit and NFchit) on hydroxypropyl methyl cellulose (HPMC). NCchit aqueous dispersions were prepared by acid hydrolysis of commercial shrimp shell α-chitin while NFchit were prepared by mechanical defibrillation using closed loop grinding. The average widths and lengths of NFchit were (8.7±3.17) nm (673.9±263.3) nm respectively while for NCchit were (9.7±3.2) nm and (243.5±55.1) nm. Composites of HPMC with different loadings of NCchit or NFchit were prepared by casting technique, using water as solvent. The effect of morphology and size of each nanomaterial on morphology, transparency, mechanical, thermal and barrier properties of the resulting nanocomposites were investigated using various techniques. The obtained results revealed the positive effect of both nanomaterials on HPMC by enhancing its mechanical properties. The nanocomposite films exhibited better oxygen and water barrier with slight decrease in transparency than control HPMC film. Overall, chitin NCchit offer superior reinforcing performance than chitin NFchit.

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BIOIMAGING AND NANOMEDICINE FOR CANCER THERANOSTICS

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Two fundamental and unsolved problems facing bioimaging and nanomedicine are nonspecific uptake of intravenously administered diagnostic and/or therapeutic agents by normal tissues and organs, and incomplete elimination of unbound targeted agents from the body. To solve these problems, we have synthesized a series of indocyanine near-infrared (NIR) fluorophores that varied systematically in net charge, conformational shape, hydrophilicity/lipophilicity, and charge distribution. Using 3D molecular modelling and optical fluorescence imaging, we have defined the relationship among the key independent variables that dictate biodistribution and tissue-specific targeting such as lung and sentinel lymph nodes, human prostate cancers and human melanomas. Recently, we have developed new pharmacophore design strategy structure-inherent targeting, where tissue- and/or organ-specific targeting is engineered directly into the non-resonant structure of a NIR fluorophore, thus creating the most compact possible optical contrast agent for bioimaging and nanomedicine. The biodistribution and targeting of these compounds vary with dependence on their unique physicochemical descriptors and cellular receptors, which permit 1) selective binding to the target tissue/organ, 2) visualization of the target specifically and selectively, and 3) provide curing options such as image-guided surgery or photo dynamic therapy. Our study solves two fundamental problems associated with fluorescence image-guided surgery and lays the foundation for additional targeted agents with optimal optical and *in vivo* performance.

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IN VITRO AND *IN VIVO* TOXICOLOGY OF SILICA AND DENDRITIC NANOPARTICLES

Hamid Ghandehari, Mostafa Yazdimamaghani, Pouya Hadipour, Raziye Mohammadpour and Zachary B Barber

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Advances in the fabrication of nanoparticles with exquisite control over shape, surface chemistry and three-dimensional architecture have not been matched by a detailed understanding of their biological fate. Recent efforts in our lab have focused on investigating the influence of particle size, core chemistry, porosity, shape, density, and surface functionality of silica and dendritic nanoparticles on interactions with macrophages, epithelial barriers and blood cells. A series of nonporous, mesoporous, spherical and rod or worm shaped silica nanoparticles were synthesized and characterized. Their cellular uptake, cytotoxicity, biodistribution, and hemocompatibility were investigated and compared to polymeric dendrimers with variations in size and surface functional groups. In the size regimes studied, results demonstrate that variations in geometry can influence mode of cellular uptake, surface functionality is a predominant factor in biological fate, cationic poly (amido amine) dendrimers result in disseminated intravascular coagulopathy, and particle density and porosity influence the rate of uptake and toxicity.

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APPLICATION OF NANOTECHNOLOGY AND STEM CELL TECHNOLOGY IN THE NEXT GENERATION CARDIOVASCULAR IMPLANTS

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Emerging advanced technologies such as nanotechnology offers great potential to overcome current issue in different fields including biomedical field. Development of novel diagnostics and nanobiosensors, new therapeutics based on smart delivery systems and nanocarriers as well as multifunctional theranostics has opened new horizons to current medical practice. Application of nanotechnology in the field of biomedical and cardiovascular devices has attracted research attention in recent years. Development of new nanomaterials and nanocomposite hybrids with enhanced bio and hemocompatibility and improved mechanical and physicochemical properties offers great advantages over conventional materials. Based on these advanced materials, development of next generation biomedical devices has become achievable. Merging nanotechnology with other advanced technologies such as stem cell technology and regenerative medicine principals in development of next generation viable or semi-viable devices is a new paradigm in biomedical research. In this paper we will report our findings of development of next generation cardiovascular devices such as heart valve, coronary stents and bypass grafts based on nanotechnology and regenerative medicine principles. The results of multiple tests to investigate bio and hemocompatibility, mechanical and surface properties and self-endothelialisation potential were very promising. This indicates that future prospect of the application of nanotechnology and stem cell technology in development of next generation cardiovascular devices is bright.

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RECENT APPLICATIONS OF NANOTECHNOLOGY IN ADVANCED DRUG DELIVERY SYSTEMS

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Nanotechnology is attracting great attention worldwide in biomedicine. Targeted therapy based on drug nanocarrier systems enhances the treatment of tumours and enables the development of targeted drug delivery systems. In recent years, theranostics are emerging as the next generation of multifunctional nanomedicine to improve the therapeutic outcome of cancer therapy. Polymeric nanoparticles with targeting moieties containing magnetic nanoparticles as theranostic agents have considerable potential for the treatment of cancer. The use of directed enzyme prodrug therapy (DEPT) has been investigated as a means to improve the tumour selectivity of therapeutics. Magnetic DEPT involves coupling the bioactive prodrug-activating enzyme to magnetic nanoparticles that are then selectively delivered to the tumour by applying an external magnetic field. Gene therapy is an attractive method for meeting the needs for curing brain disorders, such as Alzheimer's disease and Parkinson's disease. On the other hand, due to the fact that hepatocellular carcinoma (HCC) is resistant to standard chemotherapeutic agents, gene therapy appears to be a more effective cure for HCC patients. Ultrasound-mediated drug delivery is a novel technique for enhancing the penetration of drugs into diseased tissue beds noninvasively. This technique is broadly appealing, given the potential of ultrasound to control drug delivery spatially and temporally in a non-invasive manner.

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A MODULAR PLATFORM FOR TARGETED RNAI THERAPEUTICS USING BIOLOGICALLY-LIPIDATED ANTIBODIES

Itai Benhar, Ranit Kedmi, Nuphar Veiga, Limor Nahary, Edo Kon, Meir Goldsmith, Dan Rosenblum, Shani Leviatan-Ben-Arye and Dan Peer

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Small interfering (si) RNAs can be used to silence disease-causing genes. However, their development as drugs has been limited mainly in knocking down liver gene expression, since delivery to other tissues requires development of a targeted delivery carrier. Modulating immune cells function using siRNAs holds great promise in advancing targeted therapies to many immune-related disorders including cancer, inflammation, autoimmunity, and viral infections. However, the ability to effectively knockdown gene expression in leukocytes is still challenging. Here, we present a modular platform to target specific cell types, exemplified here with immune cells, using siRNA loaded lipid nanoparticles (LNP) coated with oriented, targeting antibodies noncovalently bound to a membrane-anchored lipoprotein that recognizes their Fc domain. Unlike chemically conjugated antibodies, these oriented antibodies maintain their high affinity and the LNPs avoid scavenging by Fc receptors on macrophages. A simple switch in 5 different targeting antibodies (against Ly6C, CD3, CD4, CD25 and Itgb7) redirected the LNP for exquisitely specific uptake in diverse leukocyte subsets *in vivo* and enabled specific knockdown in difficult-to-transfect $CD4^+$ cells. Intravenously injected anti-Ly6C-coated LNP encapsulating TNF siRNAs were taken up selectively by $Ly6C^+$ monocytes and activated tissue macrophages, suppressed TNF-α expression in the colon and ameliorated inflammatory bowel disease symptoms in a DSS-induced colitis mouse model, demonstrating the platform's potential therapeutic utility. This approach opens new avenues for studying cell biology *in vivo* and potentially for a wide range of therapeutic applications in a cell-specific manner.

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A 3D HUMAN LUNG-ON-A-CHIP MICRODEVICE FOR NANOTOXICITY TESTING

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The expansion of nanoparticles (NPs) and nanomaterials application has drawn increasing concerns about their impact on environment and human health. Lung is the major target organ during NP exposure. Establishing an *in vitro* lung model could promote nanotoxicity studies on pulmonary system and paratheatrical development. We proposed a new 3D human lung-on-a-chip that recapitulates the organ-level structure and functions of *in vivo* lung to investigate nanotoxicity during pulmonary NP exposure. The microdevice mimicked the alveoli niche including cellular components, 3D extra cellular matrix, and flow cue to reconstitute the alveolar capillary barrier, allowing tissue/organ level analysis of nanotoxicity during NP exposure (ZnO and TiO_2). The results exhibit decreased tight junction protein expression, increased permeability, dose dependent cytotoxicity under the treatment of NPs, and revealed their varied ability to drive ROS and apoptosis on lung epithelium. This *in vitro* 3D ACB model demonstrated great potential in the study of human pulmonary health, as well as safety assessment for nanoparticles, environment, food and drugs.

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DETECTION AND IDENTIFICATION OF CELL BOUND AND SOLUBLE ANTIGENS USING MAGNETIC LEVITATION: POC DETECTION

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Magnetic levitation is a technique for measuring the density and the magnetic properties of objects suspended in a paramagnetic field. Cells can be monitored and studied in an uninterrupted state. Here, we describe a novel magnetic levitation-based method that can specifically detect cell membrane-bound and soluble antigens by measurable changes in levitation height that result from the formation of antibody-coated bead and antigen complex. We demonstrate our method's ability to sensitively detect an array of membrane-bound and soluble antigens found in blood, including T-cell antigen CD3, eosinophil antigen Siglec-8, red blood cell antigens CD35 and RhD, red blood cell-bound Epstein-Barr viral particles and soluble IL-6, and validate the results by flow cytometry and immunofluorescence microscopy performed in parallel. Furthermore, extracellular vesicles (EVs) can be detected with the addition of anti-CR1 and anti-CD47 in an ELISA based complex. This may provide a quick yes or no EV/antigen answer. Finally, employing an inexpensive, single lens, manual focus, wifi-enabled camera (Melissa), we extend the portability of our method for its potential use as a point-of-care diagnostic assay with limitless applications. Future studies are incorporating the use of CCL11 for the prognosis of chronic traumatic encephalopathy. This could lend itself to immediate diagnosis of conclusions, Alzheimer's disease and dementia.

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TERMINAL COMPLEMENT COMPONENTS ARE CRITICAL IN THE RELEASE OF CELLULAR RNA IN CIRCULATION

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Despite over 10 years of intense research, the intimate mechanisms responsible for extracellular vesicles (EVs) formation (exosomes and microvesicles) and the release of cellular RNA species (ex RNAs) in circulation are currently known. The complement system is comprised of over 20 soluble and membrane bound proteins with critical roles in recognizing, binding, and removal of foreign particles as well as initiating and regulating innate and acquired immune responses. Activation of the complement system occurs during both, normal (circadian variation), and pathological conditions through either classical, alternative, or lectine pathways leading to the formation and transient insertion of C5b-9/Mac pore complex into cellular plasma membrane. We hypothesize that a) MAC-insertion promotes a sudden, significant and transient water and Ca++ influx, leading to: i) endocytosis of the affected area, followed by delivery of C5b-9/MAC-containing plasma membrane into the multi vesicular body (MVB) and its incorporation into exosomes or ii) exocytosis of the C9 channel/MAC-affected plasma membrane patch followed by micro vesicles (MVs) formation. In addition, the size of the MAC/C5b-9 pore, 12 nm, is large enough to: i) allow cytoplasmic RNA species to be transferred into the MVB following endocytosis of C5b-9/MAC-containing plasma membrane and ii) RNA species located near the plasma membrane to be released in the extracellular space upon C5b-9/MAC insertion. Our results, for the first time implicate MAC/C5b-9 as: i) a possible channel responsible for exosomes and microparticle biogenesis, and ii) loading of cytosolic RNAs into the exosomes, and iii) the direct release of cytoplasmic RNA species into the circulation (ex RNAs).

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MULTIFUNCTIONALIZED BIOCATALYTIC P22 NANOREACTOR FOR COMBINATORY TREATMENT OF ER+ BREAST CANCER

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Breast cancer is a leading cause of mortality in females worldwide. Tamoxifen continues to be the standard endocrine therapy, which requires metabolic activation by cytochrome P450 enzymes (CYP). However, the lower and variable concentrations of CYP activity at the tumour remain major bottlenecks for the efficient treatment, causing severe side-effects. Combination nanotherapy has gained much recent attention for cancer treatment as it reduces the drug-associated toxicity without affecting the therapeutic response. The principle of combination therapy in cancer is to use approaches that work by different mechanisms of action. Here we show the modular design of P22 bacteriophage virus-like particles for nanoscale integration of virus-driven enzyme prodrug therapy and photodynamic therapy. The estrogen receptors (ER) are the major role players in the initiation and progression of breast cancer and represent a potential site for directing receptor-mediated cellular uptake. Thus, in our approach we have functionalized biocatalytic P22 with the well-known photosensitizer, protoporphyrin IX (PpIX) and the estradiol derivative for achieving targeted inhibition of ER+ breast tumour cells. The final nanoparticles, P22CYP-PpIX-PEG(EST) are characterized by TEM, DLS, zeta potential and photo-physical analysis. These functionalized nanoparticles are recognized by and internalized into ER^+ breast tumour cells increasing the intracellular CYP activity and showing the ability to produce reactive oxygen species (ROS) upon UV_{365nm} irradiation. The generated ROS in synergy with enzymatic activity drastically enhanced the tamoxifen sensitivity *in vitro*, leading to a strong inhibition of tumour cells, which may allow the reduced toxicity owing to the lower drug concentration, and may overcome the tumour reoccurrence limitation. Thus, the targeted combinatory treatment using multifunctionalized biocatalytic P22 represents the effective nanotherapeutics for ER^+ breast cancer.

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PATTERN-GENERATING FLUORESCENT MOLECULAR PROBES FOR CHEMICAL BIOLOGY

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Fluorescent molecular probes have become a powerful tool in protein research. However, these probes are less suitable for analysing specific populations of proteins in their native environment. In this talk I will give an overview of a new class of fluorescent molecular probes that we have developed in recent years and show how they can be used to detect individual proteins, protein combinations, as well as binding interactions and dynamic changes that occur on their surfaces. In the second part of this talk, I will describe a new class of fluorescent molecular sensors that combines the properties of small molecule-based probes cross-reactive sensor arrays (the so-called chemical nose/tongue. On the one hand, the probe can detect different protein families by generating unique identification patterns, akin to the cross-reactive arrays. On the other hand, its unimolecular structure and selective binding allows identifying combinations of specific protein isoforms in complex mixtures and inside living cells, where macroscopic arrays cannot access.

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RECENT ADVANCES IN PROTEOTRONICS, THE SCIENCE OF PROTEIN-BASED ELECTRONIC DEVICES

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We investigate relevant electrical properties of several biomolecules like transmembrane proteins (opsins, olfactory receptors, etc) and DNA/RNA fragments (aptamers) which are of interest for the realization of a new generation of nanobiosensors. The investigation compares existing experiments, as obtained by the atomic force microscopic (AFM) shown in the figure, with the theoretical expectations obtained from an impedance network protein analogue, recently developed by the Lecce team. The changes in the electrical response due to the sensing action of the selected biomolecules are correlated with the conformational change undergone by them. The satisfactory agreement between theory and experiments points to a promising development of a new class of nanobiosensors based on the electrical properties of sensing proteins and aptamers.

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APPLICATION OF NUCLEIC ACID MIMICS IN THE TREATMENT OF BACTERIA

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The emergence of pathogenic bacteria resistant to most, if not all, currently available antimicrobial agents has become a critical problem in modern medicine. As we are apparently entering the post-antibiotics era, the development of alternative antibacterial therapies is of the utmost importance. One of the alternatives that have been exploring is the antisense technology that is based on the introduction of an oligonucleotide complementary to a given mRNA, thereby inhibiting translation. The development of a new generation of nucleic acid mimics (NAMs) with promising antisense characteristics together with several studies reporting successful modifications of gene expression has put the antisense technology in the spotlight. In fact, some of these molecules are already being developed for therapeutic applications *in vivo*, and protocols involving hybridization inside higher-order animals are available. The success of the antisense technique using NAMs obtained in eukaryotic cells has not been reproduced in microorganisms. In fact, all studies in microorganisms have so far showed limited ability to completely eliminate bacterial populations in a reproducible way. This might be due to a multitude of factors, and several studies have shown that the difficulty of these mimics to cross the bacterial cell envelope as one of the most critical factors. This work focus on the development of an integrated approach focused on the targeted delivery of different nucleic acid mimics using multiple delivery strategies into microorganisms. We will discuss and compare not only the influence of different nucleic acid mimics, such as peptide nucleic acids (PNA), locked nucleic acids (LNA) and 2´-O-Methyl-RNA, but also of different delivery strategies such as liposomes/nanoparticles and cell penetrating peptides (CPPs).

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INDUCTION OF IMMUNOGENIC CELL DEATH IN TUMOUR CELLS SENSITIZED BY CURCUMIN AND TREATED WITH PHOTODYNAMIC THERAPY MEDIATED BY ALUMINIUM-PHTHALOCYANINE CHLORIDE NANOEMULSION

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Cancer chemotherapy remains a challenge due to the mechanisms of resistance of tumour cells and the toxicity of anticancer drugs. Processes associated with immunogenic cell death may result in the emission of damage-associated molecular patterns (DAMPs), some are exposed on the plasma membrane, such as heat shock proteins and calreticulin. The curcumin executes mechanisms that can generate immunogenic cell death in cancer cells, because it can generate an increase of intracellular calcium that generates a stress in the endoplasmatic reticulum and possibly in the exposition of calreticulin in the plasma membrane. The photodynamic therapy leads to the generation of reactive oxygen species and thus leading to immunogenic cell death. In this context, the justification for this work is that combined anticancer therapy using curcumin and aluminium-phthalocyanine chloride nanoemulsion mediated by transcription factor decoy (TFD) can cause intense stress on cancer cells by promoting immunogenic cell death. The results presented in this study showed that: treatments containing curcumin and phthalocyanine nanoemulsion were the more toxic to CT26.WT cells after TFD than free curcumin; in 24 hours the lipid nanoparticles containing curcumin caused greater increase of granularity in CT26.WT cells; in 3 hours free curcumin produces greater accumulation of intracellular calcium than curcumin associated with lipid nanoparticles; in 3 and 24 hours free curcumin is more internalized than curcumin associated with lipid nanoparticles. Future studies to investigate the generation other DAMPs (calreticulin, HMGB1, ATP, HSP70 and 90) to prove that curcumin and phthalocyanine are capable of generating an immune response and are effective against tumours.

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DESIGN AND FABRICATION OF MICRO-PRESSURE SENSOR VIA CONDUCTING NANOPARTICLES

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Polydimethylsiloxane (PDMS) has played an important role in chip fabrication owing to its properties such as transparency, biocompatibility, and good flexibility. However, PDMS is a non-conducting polymer, on which patterning metallic structures during the fabrication is challenging due to the weak adhesion between metal and PDMS. Hence, the integration of conducting structures into bulk PDMS was a critical issue. Here, we introduce PDMS-based conducting composites synthesized by mixing conductive nano/micro-meter-sized particles with PDMS gel, with which the patterning of the conductive structure can be realized by soft lithographic approach. Experiments show that such composite material can be perfectly constructed into PDMS bulk material in all cases, thereby greatly enhancing their potential functionalities. By employing above conducting composite for the fabrication of electrodes, some applications for the on-chip signal control and monitoring becomes feasible, for example, micro pressure sensor has been designed and fabricated. The micro pressure sensor presented here is a deformable thin conducting membrane attached on the wall of a microfluidic channel and its line resistance can be tunable under the external pressure variations. The testing results show that a good linear relationship between line resistance of sensor and applied pressure can be obtained which is a desired property of pressure sensor.

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COATINGS BASED ON TIO_2 NANOPARTICLES AND BIOMACROMOLECULES AS A NEW FLAME-RETARDANT APPROACH FOR COTTON FABRICS

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A novel durable intumescent flame-retardant coating, based on metal oxide nanoparticles (NPs) and biomacromolecules, was designed and applied on cotton fabrics. This way, it was possible to combine the thermal insulating effect of the inorganic coating with the intumescent properties of the selected biomacromolecules, able to absorb the heat and oxygen from the atmosphere and blocking their transfer to the surrounding textile. Two peculiarities were exploited, namely: i) the ability of proteins and aminoacids to irreversibly cover NPs, according to protein corona theory and ii) the affinity of metal oxide NPs towards the natural hydrophilic fibers, for improving the washing fastness of the fire-resistant finishing. To this aim, different TiO_2 NPs/biomacromolecules systems were deposited by dip-pad-dry-cure process and the morphology of the resulting coating assessed by SEM analysis. The enhancement of the durability (i.e. the resistance to washing treatments) was verified by release tests carried out in static and dynamic conditions. Flammability and cone calorimetry tests were performed for evaluating the fire behaviour of the treated fabrics. More specifically, in horizontal flame spread tests, the different nanoparticles/biomolecules-based coatings provided an increase of the total burning time and the decrease of the burning rate. Furthermore, the residues at the end of the test were significantly higher with respect to untreated cotton fabric. Therefore, thanks to their high char-forming character, the combination of TiO_2 nanoparticles and biomacromolecules within coating may represent a valid durable fire-resistant finishing alternative to standard flame-retardant treatments for cotton.

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SENSING OF CANCER DNA USING RESONANCE FREQUENCY

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Lung cancer is one of the most common severe diseases driving to the death of human. Lung cancer can be divided into two cases of small-cell lung cancer (SCLC) and non-SCLC (NSCLC), and about 80% of lung cancers belong to the case of NSCLC. From several studies, correlation between epidermal growth factor receptor (EGFR) and NSCLCs has been investigated. Therefore, EGFR inhibitor drugs such as gefitinib and erlotinib have been used as lung cancer treatments. However, the treatments result showed low response (10~20%) in clinical trials due to EGFR mutations that cause the drug resistance. Patients with resistance to EGFR inhibitor drugs usually are positive to KRAS mutation. Therefore, assessment of EGFR and KRAS mutation is essential for target therapies of NSCLC patient. In order to overcome the limitation of conventional therapies, overall EGFR and KRAS mutations have to be monitored. In this work, only detection of EGFR will be presented. A variety of techniques have been presented for the detection of EGFR mutations. The standard detection method of EGFR mutation in ctDNA relies on real-time polymerase chain reaction (PCR). Real-time PCR method provides high sensitive detection performance. However, as the amplification step increases, cost effect and complexity increase as well. Other types of technology such as BEAMing, next generation sequencing (NGS), electrochemical sensor and silicon nanowire field-effect transistor have been presented. However, those technologies have limitations of low sensitivity, high cost and complexity of data analyzation. In this report, we propose a label-free and high-sensitive detection method of lung cancer using quartz crystal microbalance-based platform. The proposed platform is able to sense lung cancer mutant DNA with a limit of detection of 1nM.

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NOVEL DIAGNOSTIC SILICON NANOPARTICLES FOR TARGETED DELIVERY OF THIOUREA TO EGFR-EXPRESSING CANCER CELLS

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Conventional cancer chemotherapies have been associated with serious systemic toxicities and dose-limiting side effects that limit their clinical application. Targeted therapeutics on the other hand, by being more selective, can potentially minimize the side effects of these anticancer agents. In efforts to develop targeted anticancer drugs, it is essential to consider many different aspects of molecular biology, such as the interactions with cell surface receptors. Protein tyrosine kinases (PTKs) have been identified as major contributors in numerous signal transduction pathways within cell membranes and are implicated in cell proliferation. Epidermal growth factor receptor (EGFR) kinase is one of the most important PTKs and plays a key role in a wide diversity of biological processes, including cell proliferation, metastasis, and angiogenesis. The novel thiourea-functionalized silicon nanoparticles (SiNPs) have been successfully synthesized using allylamine and sulforaphane, an important anticancer drug, followed by a hydrosilylation reaction on the surface of hydrogen terminated SiNPs. Their physiochemical properties have been investigated by photoluminescence emission, FTIR and elemental analysis. MTT assay has been employed to evaluate *in vitro* toxicity in colorectal cancer cells (Caco-2) and primary normal cells (CCD). The results show significant toxicity of thiourea SiNPs after 72 h incubation in the cancer cell line and the toxicity is concentration dependent and saturated for concentrations above 100µg/mL. Confocal microscopy images have demonstrated the internalization of thiourea-functionalized SiNPs inside the cells. Flow cytometry data has confirmed receptor-mediated targeting in cancer cells. This nanocomposite takes advantage of the EGFR active targeting of the ligand in addition to the photoluminescence properties of SiNPs for bioimaging purposes. The results suggest that this novel nanosystem can be extrapolated for active targeting of the receptors that are overexpressed in cancer cells such as EGFR using the targeting characteristics of thiourea-functionalized SiNPs and therefore encourage further investigation and development of anticancer agents specifically exploiting the EGFR inhibitory activity of such nanoparticles.

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