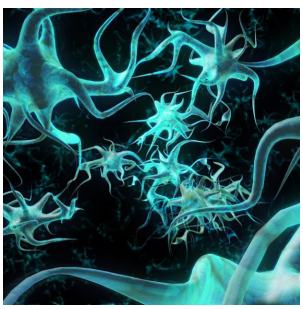
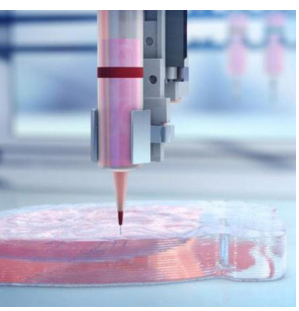
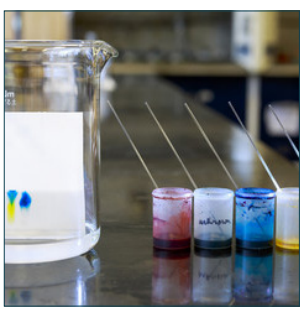
Video Presentation

Tissue Science 2019
Molecular Biology 2019
Separation Techniques 2019




Joint Event on

2nd International Conference on

Tissue Science and Molecular Biology, Stem Cells & Separation Techniques

June 06-07, 2019 | London, UK

Jayita Goswami, Biomed Res, Volume 30
ISSN: 0976-1683

Clinical Separation Techniques, Drugs and Nanotechnology

Jayita Goswami
Independent Researcher and Writer, USA

Molecules in nature stay in complex form. To analyze those molecules various separation techniques have evolved. The separation can be based on size, affinity or towards a particular bonding. This is the era of nanotechnology and nanomedicine. Along with former cell-based treatment, now various nanoparticle helps to activate specific drugs. Various separation techniques will be discussed in this talk. MALDI-TOF-ESI helps to separate and analyze bacterial and viral proteins. We use ESI to add extra ions in the reaction chamber. During drug production, molecules can be in isomer and enantiomer form. To make it more effective, the same structure gets separated during drug production.

Clinical laboratories use a lot of liquid chromatography. HPLC-MS, UPLC is very frequently used. Nanoparticles and Nanomedicine are taking the market. Efficiency, effectivity and the cost cutting are important in the clinical world and in various separation techniques. Some chemicals are toxic for environmental disposal. Combination of liquid chromatography and mass spectrometry brought the different level of dimension in molecular separation techniques. Liquid chromatography combined with Mass Spectrometry and the microfluidic device gives the ultimate sensitivity to detect single nucleotide polymorphism and an early stage of disease detection. Drug delivery purposes various new molecules are getting explored. Nested molecules help with the slow release of drugs and a keeps a long-term dosage activity.

Speaker Biography

Jayita Goswami is a researcher and faculty for several years. She taught in the colleges and performed research in various laboratories. She got a fellowship from NIH (USA) right after her graduation. Her graduate research was based on bioenergetics and molecular biochemistry. The model organism was *Chlamydomonas reinhardtii*. This organism is getting explored for biofuel research purposes by various research institutions. Molecular, biochemical and biophysical techniques were used to address my research questions. I explored the importance of the hydrogen bond between molecules. She was involved with cancer drug discovery research. She worked with various breast, prostate and blood cancer cell lines and designed drugs. She got the opportunity to explore molecular forensic world too. She gained the expertise to make various molecular tags that has many fold applications. One of her works got patented. She worked in the food microbiology and infectious disease field as well. Although she started my career with botany, my passion for the biomedical science brought me here.

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Notes:

Poster Presentation

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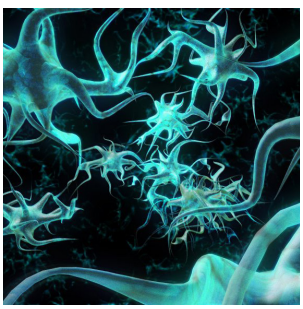

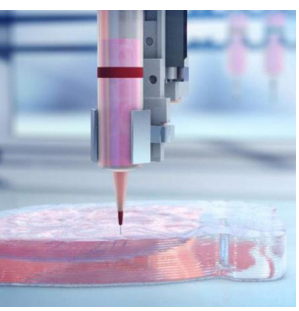
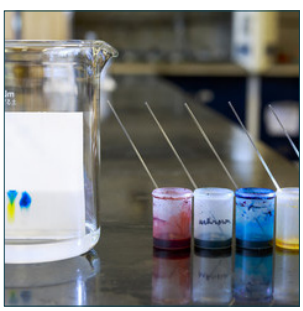


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Noura Alzahofi, Biomed Res, Volume 30
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Spire: A new Rab27a-effector characterizing Rab27a interaction-sites within spire

Noura Alzahofi
University of Nottingham, UK

Rab27a is a small GTPase and a member of the RAS oncogene family. Rab27a governs different Kinds of intracellular trafficking through interaction with distinct effectors. The extent of known Rab effectors has contributed to enlightening the molecular mechanism and dysfunctions that lead to a variety of human diseases. Recently, we identify spire, an actin nucleation protein, as a new Rab-27a effector. Spire interacts with formin-1 (an actin elongation factor) to nucleate linear actin-filaments that are used as a track for myosin to transport intracellular cargo, including melanosome in skin melanocytes. Using melanosome distribution as an indicator, in modified-nanoscale pulldowns assay, we found that spire is able to interact with Rab-27a on the cytoplasmic face of the melanosome via its C-terminal membrane- binding region. In addition, the results highlight a crucial role of Spire-Box domain in this interaction. Interestingly, a point mutation within Spire-Box (K419W) blocked the said interaction. This mutation corresponding to that of R35W in melanophilin/ Slac2 (Rab27a-effector) that causes Griscelli syndrome type-3 in humans.

Speaker Biography

Noura Alzahofi is currently pursuing her PhD in the University of Nottingham, UK. She has few publications in international journals.

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Notes:

Exploring the effect of buffer strength on the retention time of weak acids, neutral and weak bases in Hydrophilic Interaction Liquid Chromatography (HILIC) mode

Naser Al-Tannak, Sami Bawazeer and **David G Watson**
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Hydrophilic interaction liquid chromatography (HILIC) orthogonal to conventional reversed phase high-performance liquid chromatography (HPLC) mode allowing separation of polar compounds, yet the separation mechanisms reported in HILIC are much more complicated. Therefore, this study was designed to investigate the effect of water layer thickness on silica gel and the amount of ammonium ions present within the buffer on HILIC retention mechanism. Thus, a test system was designed which used weak acids, neutrals and weak bases as probes with three different strengths (5, 10 and 20 mM) of ammonium acetate, formate and propionate as the counter-ions to compete with the test probes with ionised silanol groups and water present in the stationary phase. A Kromasil 60-5 SIL column (150 mm × 4.6 mm × 4 µm, pore size 60Å) was used as stationary phase. As a result, retention times were examined for the test probes at 90% acetonitrile (ACN) with 10% of 5, 10 and 20 mM of ammonium acetate, formate and propionate. As the buffer strength increases, the thickness of the water layer on the surface of the silica gel increases and also the repulsion between ionized silanol groups and acidic test probes will decrease. On the other hand, such increase in buffer strength will increase the competition between the ammonium ions and basic test probes. In conclusion, At 20 mM buffer strength acidic probes with low log P values retain more due to reduced repulsion by silanol groups. However, in 5 mM buffer strength basic probes with low log P value will be retained longer.

Speaker Biography

Naser Al-Tannak has completed his PhD at the age of 31 years from Strathclyde University and postdoctoral studies from Strathclyde Institute of Pharmacy & Biomedical Sciences, Strathclyde University, United Kingdom. He is an assistant professor in Faculty of Pharmacy-department of pharmaceutical Chemistry-Kuwait University. He has published more than 12 papers in reputed journals.

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Notes:

E-Poster

Tissue Science 2019
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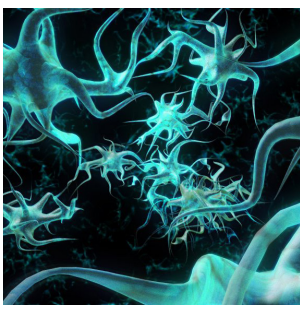

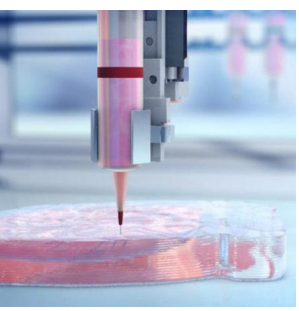
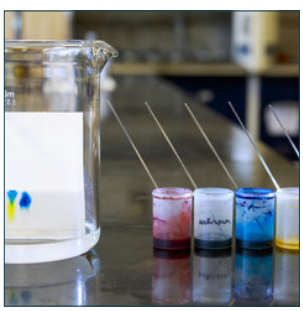


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Ayyub Patel, Biomed Res, Volume 30
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Biochemical characterization of crucial domain RNA dependent helicase of dengue virus (DENV)

Ayyub Patel
King Khalid University, Saudi Arabia

Dengue infection (DENV) is the reason for dengue fever. It is a mosquito-borne single positive-stranded RNA virus of the family Flaviviridae. Dengue infection has expanded drastically during the recent most 20 years and is getting to be one of the most exceedingly awful mosquito-borne human pathogens tropical nations need to manage. Current assessments demonstrate that upwards of 390 million diseases happen every year and it is progressively comprehended that various dengue contaminations are asymptomatic or sub-clinical. DEAD-box proteins are associated with an arrangement of metabolic procedures that regularly include RNAs. However, now and again additionally other nucleic acids are also included. DEAD-box RNA helicases assume critical jobs in RNA digestion, for example, grafting, ribosome biogenesis, RNA transport, debasement and interpretation. In the present study, we report that dengue virus contains RNA dependent ATPase activity and RNA unwinding activities.

Conclusion: The biochemical studies revealed in in this original copy are the important initial step to obtain new insights into enzyme function and regulation. Overall, this investigation is the main direct proof to demonstrate the RNA helicase action of HABD protein has a place with DEAH family. The HABD protein demonstrates the ATPase activity in presence of RNA. Maximum energy provided in the presence of ATP and dATP. This energy helps to unwind the RNA duplex. This HABD protein may be useful for mitochondrial RNA splicing, translation and genome maintenance.

Speaker Biography

Ayyub Patel currently working as an assistant professor in the department of clinical biochemistry, King Khalid University. He does research in biochemistry, spectroscopy, e-learning and medical education. His current projects include: Promoting active learning in medical students; Zamzam water and acid reflux, anti-cancer activity of natural herbs and spices like qist albahri qist al Hindi, tumeric, gum arabic, moringa seeds etc.

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Notes:

Mohd Farhan, Biomed Res, Volume 30
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A prooxidant mechanism of cancer chemopreventive properties of plant polyphenols

Mohd Farhan
King Faisal University, Saudi Arabia

Introduction: In the last couple of decades there has been some interest in alternative mechanisms of apoptosis induction which do not involve caspases. This is particularly of interest in relation to cancer cells. We have hypothesized that mobilization of endogenous copper ions by plant polyphenols such as EGCG and consequent oxidative degradation of cellular DNA could be an important mechanism of their anticancer properties.

Objectives: Over the years we have validated our hypothesis to a considerable degree. We further confirm the hypothesis by using analogues of EGCG to identify the structural features of tea catechins important for mobilizing endogenous copper and breakage of cellular DNA in cancer cells.

Methodology: Comet assay to study DNA breakage, MTT assay for cell proliferation and Histone/DNA Elisa for apoptosis induction was used to examine the catechin mediated oxidative breakage of cellular DNA in various cancer cell lines.

Results & Conclusion: Catechins have been shown to inhibit cell proliferation and induce apoptosis in different cancer cell lines and that such cell death is prevented to a significant extent by copper chelator neocuproine. Further, normal breast epithelial cells (MCF-10A), cultured in a medium supplemented with copper (MCF-10A-Cu), become sensitized to EGCG induced growth inhibition. Copper transporters Ctr1 and ATP7A are found to have an increased expression in MCF-10A-Cu cells and EGCG inhibits the expression of both the copper transporters in such cells. Moreover, silencing of copper transporter Ctr1 by siRNA reduces the sensitivity of MCF-10A-Cu cells to EGCG. We conclude that the position and the number of hydroxyl groups in various catechins determine their capacity to mobilize endogenous copper and degrade cellular DNA.

Speaker Biography

Mohd Farhan is currently working as an assistant professor in King Faisal University, Saudi Arabia. He has many publications in the international journals.

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Shokria Ehsani, Biomed Res, Volume 30
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Proliferation of human endometrial stem cells are experimented by C0-culture of mouse fallopian stem cells

Shokria Ehsani
Ghalib University, Afghanistan

Introduction: Endometrial stem cells like other adult stem cells are rare undifferentiated cells present in most adult tissue. Concerning to the valuable application of human endometrial stem cells in clinic and tissue engineering, the proliferation of human endometrial stem cells is going to be one of the important issues among researcher. Recently, it has been reported that growth factors like leukemia inhibitory factor in medium culture of adult stem cells is necessary to maintain MSC self-renewal and undifferentiated. However, the effect of leukemia inhibitory factor on proliferation and pluripotency of human endometrial stem cells, which have very important, has not been explored. In this study we tried to investigate the effects of LIF on ESCs proliferation and pluripotency.

Materials and Methods: The endometrial cells were collected from the uterus of hysterectomies samples. Then the isolated cells were cultured in the DMEM+F12 with LIF. In experimental group study 10 ng/ml LIF was added to culture and at the end of forth subculture and after treatment with LIF the CD90 positive cells were evaluated using flow cytometry. The proliferation rate of both experimental study and control group using MTT assay were done. The expression of Nanog, Oct4, PCNA and LIFr genes was evaluated using real time-PCR in high proliferation are (LIF treated) group.

Results: The proliferation rate of treated and control groups were 1/61 ± 0/06 and 1/1 ± 0/01. The rate of CD90 positive cells before treatment with LIF was %94 ($P<0.05$) and after treatment was %98 ($P<0.05$). The expression rate of all target genes to housekeeping was higher in LIF treated group than other group ($P<0.05$).

Conclusion: 10 ng/ml LIF in medium culture has a great impact on proliferation and pluripotency of human endometrial stem cells LIF also increased the CD90 positive endometrial stem cells and the expression of Oct4, Nanog, PCNA and LIFr. Co-cultured groups have no significance effect on proliferation of endometrial stem cells

Speaker Biography

Shokria Ehsani is a head of anatomy department in Ghalib University, Afghanistan and she has many publications in the international journals.

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Notes:

Accepted Abstracts

Tissue Science 2019
Molecular Biology 2019
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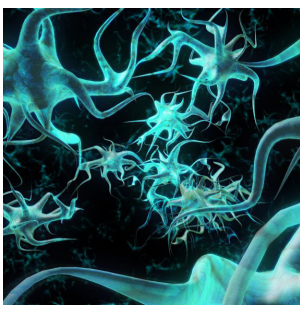

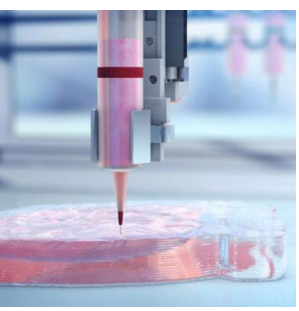
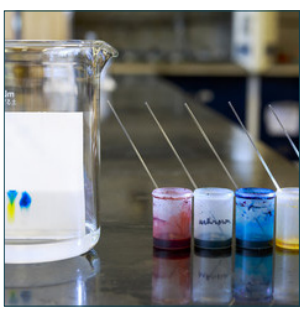


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Say goodbye to hospitals and hello to implantable nanosensors

Thomas J Webster
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There is an acute shortage of organs due to disease, trauma, congenital defects and most importantly, age related maladies. While tissue engineering (and nanotechnology) has made great strides towards improving tissue growth, infection control has been largely forgotten. Critically, as a consequence, the Centers for Disease Control have predicted more deaths from antibiotic-resistant bacteria than all cancers combined by 2050. Moreover, there has been a lack of translation to real commercial products. This talk will summarize how nanotechnology can be used to increase tissue growth and decrease implant infection without using antibiotics but using sensors (while getting regulatory approval). Our group has shown that nanofeatures, nano-modifications, nanoparticles and most importantly, nanosensors can reduce bacterial growth without using antibiotics. This talk will summarize techniques and efforts to create nanosensors for a wide range of medical and tissue engineering applications, particularly those that have received FDA approval and are currently being implanted in humans.

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Therapeutic potential of primitive mesenchymal stem cells to treat degenerative diseases

G Rasul Chaudhry
Oakland University, USA

Perinatal tissues are non-invasive, abundant and rather primitive sources of mesenchymal stem cells (MSCs) in comparison to MSCs isolated from adult tissues. They have received increasing attention since they do not pose ethical or moral concerns. We have developed a robust, reproducible and high yielding method for isolation of highly proliferative MSCs from umbilical cord/placenta tissue. MSCs isolated from all other sources stop growing after a few passages. However, irrespective of the source, all MSCs exhibit fibroblastoid morphology, express $CD29^+$, $CD44^+$, $CD73^+$, $CD90^+$ and $CD105^+$ and differentiate into adipogenic, chondrogenic and osteogenic lineages and some into neural lineage as well. However, the cord/placenta MSCs display higher colony forming efficiency and express even some pluripotent genes. They can also be maintained for self-renewal and potency for extended period of time; therefore, we call them primitive MSCs. We have investigated the therapeutic potential of these primitive MSCs to treat degenerative diseases including degenerative disc disease (DDD) and retinal degenerative disease (RDD) using animal models. When MSCs and their chondrogenic derivatives were injected into the IVDs of a rabbit model of DDD, they significantly improved the histology, cellularity, extracellular matrix protein and water and glycosaminoglycan contents. The IVDs receiving chondroprogenitor or nucleus pulposus (NP) like cells derived from MSCs exhibited higher expression of NP specific markers. The transplanted cells were functionally active in rabbit IVDs as they expressed human genes and proteins, SOX9, ACAN, COL2, FOXF1, KRT19, PAX6, CA12 and COMP implicated in NP biosynthesis. These studies suggested involvement of TGFβ1 pathway in regulating NP regeneration in rabbit IVD. Likewise, primitive MSCs and their neural derivatives have shown efficacy to improve vision in rd12 mice, a model of RDD. Latest findings of these translational studies as well as challenges and new opportunities will be discussed in the presentation.

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Translation of basic research into cell-based therapies in tissue regeneration

Madhu Dhar, David E Anderson, Henry S Adair, James Schumacher and **Dennis Geiser**
University of Tennessee, USA

The large animal regenerative medicine program, started in 2010 conducts research in the use of mesenchymal stem cells, biomaterials and other forms of cell-based therapies that will translate to both veterinary and human medicine. Our ultimate goal is to make basic discoveries and to expand these discoveries into the development of diagnostic modalities and treatment protocols to solve complex medical problems related to musculoskeletal, and nerve injuries. We carry out specific *in vitro* assays to evaluate cell adherence, proliferation and potential for differentiation into osteocytes, chondrocytes or neural-like progenitors. We then conduct controlled studies, using rodents, to confirm the biocompatibility and efficacy of mesenchymal cells used alone or in combination with biomaterials. Finally, we translate these findings into controlled, preclinical studies using large animals, including goats, sheep, pigs and horses. We perform the *in vitro* and rodent studies to improve clinical outcomes for large animal and human patients.

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Similar processes affect cancer and atherosclerosis

Abdallah Alameddine
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Cancer and Cardiovascular diseases are the leading causes of mortality worldwide with increasing new cases every year. Both illnesses are characterized by uncontrolled cellular proliferation and progressive growth and based on the recent advent in cell biology and cancer metabolism in addition to epidemiologic observations, the critical roles of immune system have been revealed in patients with those conditions. We have thus learned that the inflammatory response in arteries does not differ from that in cancer tissue or from those in chronic inflammatory states. In this presentation, we shed light on the characteristic parallels between the neoplastic response and atherosclerosis. As we highlight, ostensibly both processes are interlinked by an inflammatory response as has been predicted by Rudolph Virchow who first made the connection between inflammation and cancer over 150 years ago. Important for our discussion, an example of epidemiologic data suggesting increased cancer rates in adults after cardiac interventions as compared with the state of Massachusetts general population will be underscored. Based on these remarks, strategies for reducing cancer risk may be implemented that could positively affect outcome in cardiovascular patients and the screening process for asymptomatic malignancies.

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Mesenchymal stem cells-based therapy for glaucoma through regeneration of the trabecular meshwork

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Centre de recherche Hôpital Maisonneuve-Rosemont (CRHMR), Canada

In open angle glaucoma, dysfunction of the trabecular meshwork (TM) leads to elevated intraocular pressure (IOP) and concomitant optic nerve damage. Consequently, regeneration of the TM cells may represent an effective therapeutic option for many cases of glaucoma. We previously demonstrated the regenerative effects of mesenchymal stem cells conditioned media (MSC-CM) in tissue regeneration in laser damaged TM. This process led to a decrease in IOP in a rat model of glaucoma. This mechanism is depended solely on macrophage recruitment. In this study, we have investigated the mechanism of MSC-CM educated macrophages on tissue regeneration. To better understand the role of macrophages in the TM regeneration process, rats were pretreated with clodronate liposomes resulting in a reduction in the number of macrophages within the damaged TM. This culminated in the attenuation of the effect of MSC-CM on the IOP. In addition, reintroduction of *in vitro* MSC-CM educated macrophages into macrophage depleted eyes restored the healing effect. Furthermore, to elucidate the mechanistic basis of MSC-CM educated macrophages-mediated decrease in IOP, we injected the supernatant from MSC-CM educated macrophages into glaucomatous eyes. Surprisingly, this resulted in a decrease in the IOP, thus indicating that MSC-CM educated macrophages mediate TM regeneration and a decrease in IOP through paracrine factor secretion. In addition, we have identified one factor which we call RPF1 (Regenerative paracrine factor 1) produced by MSC-CM educated macrophages as a potent mediator of this regenerative effect. This finding provides a novel cellular therapeutic approach for glaucoma treatment.

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Investigation into the mechanism regulating mitotic DNA synthesis (MiDAS)

Jedrzej J Jaworski and **Fumiko Esashi**
University of Oxford, UK

All multicellular organisms develop via proliferation-dependent growth, which requires full genome duplication for each mitotic division. Cells with unreplicated DNA fragments may occasionally proceed to mitosis by bypassing canonical checkpoint activation. The resulting under-replicated regions are particularly prevalent following replication stress, as seen for instance, in cancer cells. They can be fixed by a recently characterized mechanism-mitotic DNA synthesis (MiDAS). Here, we investigate the upstream regulation of this process in osteosarcoma cells following induction of aphidicolin-mediated replicative stress and cell synchronisation. Candidate components of the cell-cycle regulating machinery were ablated using RNAi and MiDAS was quantified using EdU incorporation during mitosis. Collectively, our results expose a vital role of BRCA2 and the UBR5 complex in regulating MiDAS, which facilitates a last-resort protective response to unreplicated genome regions in mitosis. Mechanistically, we propose that BRCA2-mediated RAD51 phosphorylation and UBR5-dependent chromatin clearance promote MiDAS. Our results uncover new potential factors that could be exploited therapeutically in cancer treatment.

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Maintaining physiological fidelity during *in vitro* stem cell culture and expansion; a role for oxygen control

Nicholas R Forsyth
Keele University, UK

Hypoxia or physiological normoxia, plays a key role in determining stem cell behaviour in the *in vivo* niche. In spite of this little attention is payed to the role of reduced oxygen levels during *in vitro* culture generating a risk of forced paradigm and artefactual norms. Bone marrow-derived human mesenchymal stem cells (hMSC), due to the sinusoidal blood vessel architecture found within their niche, are particularly vulnerable to oxygen tension fluctuations. We and others, have now described fundamental, artefactual, alterations in hMSC biology as a consequence of air oxygen exposure. These include reduced colony forming unit-fibroblastic isolation; dysregulated epigenome, transcriptome and proteome; altered biochemical volatile footprints during culture; and counter intuitive alterations in reactive oxygen species management. This lecture will discuss the fundamental biology underpinning these biological differences, their potential impact on regenerative medicine and what we can do to transform biological understanding into therapeutic application.

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Total tissue regeneration in necrosed diabetic foot with new biomedical strategy

Debora Nino-Laffont
Iberoamerican Institute of Bioregenerative Medicine (IBIOMER), Mexico

The present exposes a medic protocol to treat endothelial dysfunction and clinical and subclinical inflammation, whose therapeutic approach is based on the principle of extended mitochondrial hormesis, as previous physiological conditioning *ex vivo*/*in vivo*, to promote the angiogenic power of autologous stem cell transplantation mesenchymal (MSCs) from bone marrow and whithout culture. Mitohormesis behaves as a subtle extrinsic disturbance that triggers a series of nuclear signaling events with biochemical and metabolic changes that induce a cytoprotective state, which can be obtained with oxygen-Ozone (O^2/O^3) applications at low doses, through different routes of administration for 15 continuous days before and 15 continuous days after autologous transplant MSCs, producing a cytoprotective, anti-oxidant and anti-inflammatory microenvironment that synergizes the angiogenic power and immunoregulatory of MSCs. Diabetes Mellitus (DM) is an inflammatory pathology, where there is a chronic hyperglycemic state alters the molecular/cellular structures of vessels and nerves, generating hyperplasia, endothelial dysfunction and inflammation with cellular hypoxia. It has as a complication diabetic foot (DF) in 10-15% and that it occurs with ulceration, infection and destruction of deep tissues of the lower extremity. I present a clinical case of male of 47 years, with Dx of DM of 18 years and with DF of 3 years of evolution that degenerated in a state of necrosis, DF grade III/IV, indicating partial amputation that rejected to be submitted to combined protocol O^2/O^3-MSCs, giving results of total tissue regeneration, 30 days after the first autologous MSC with previous *ex vivo*/*in vivo* physiological conditioning, which was evidenced clinically by signs of trophism and functional recovery. After 12 months, the cycle was repeated and there was improvement in the protective sensibility of both lower limbs (Semmens-Weinstein monofilament) and improvement of erectile dysfunction (IIEF-5), as an expression of microvascular regeneration and systemic and autonomic nervous connections. Prospective studies are carried out evaluating clinical correlation of the angiogenic power with the use of the combined O^2/O^3-MSCs protocol and if the periods between cycles could be shortened.

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Prospecting, development, optimization and clinical trial of new heterologous fibrin sealant derived from snake venom: From bench to the bedside

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To bridge the GAP between basic and applied sciences is needed to push forward disease research and therapeutics. What types of drug leads are truly "druggable", sit in "patented bioproducts space" and can be pushed towards clinical trials? We present one successful translational case of bioproduct from laboratory bench to the bedside. Although animal toxins present excellent candidate molecules because they have high specificity for a cellular receptor without side effects, few drugs are approved for human use. Considering that infectious diseases could be transmitted via human blood a new heterologous fibrin sealant (HFS) is proposed, whose components are a serine protease (a thrombin-like enzyme) extracted from the venom of *Crotalus durissus terrificus* snakes and a fibrinogen-rich cryoprecipitate extracted from the blood of *Bubalus bubalis* buffaloes. This new bioproduct has been used as a coagulant, sealant, adhesive and recently as a scaffold candidate to bone and cartilage repair using mesenchymal stem cells. Thus, we show its pre-clinical applications aiming at repairing nervous system traumas and bone regeneration. Also, we have finished an innovative safety trial phase I/II to treat chronic venous ulcers concluding that the product is safe and clinically promising candidate for this purpose due its preliminary effectiveness.

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From tissues to cells and back: Microfluidics-enabled solutions for parallel and time-resolved multi-parameter characterization of individual cells across a population

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A set of techniques to separate, analyse and manipulate single cells provided from a suspension, e.g. as obtained from tissue samples will be presented. Taking advantage of effects specific to microstructures or microconfinement, the cellular response of exposure to a sequence of stimuli and stains can be monitored for all cells within a cohort in parallel and even in real time. Cells of interest may be selected and forwarded to further analysis, e.g. for content screening or propagation. The presented microfluidic systems have proven to work with label-based methods, typically for validation, as well as direct photonic "fingerprinting" and to resolve valuable detail on population statistics and dynamics way beyond averages based on end-point assays. Examples are studies on glycosylation, secretion and inflammation, for instance to relate affected tissue to the onset of cardiovascular disease.

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The combined use of fat graft enriched with stromal vascular fraction cells and platelet-rich plasma in the treatment of breast soft tissue defect

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The use of autologous fat grafting is ideal in breast reconstruction. However, published data on long-term outcomes and instrumental results of fat grafting to the breast are lacking. The purpose of this study was to review the authors' experience of fat grafting, evaluating the effects related to the use of enhanced stromal vascular fraction (e-SVF) and fat grafting with platelet-rich plasma (PRP) in the maintenance of fat volume in breast reconstruction, comparing the results with a control group. Twenty-three patients aged 19–60 years affected by breast soft tissue defects were analyzed at the Plastic and Reconstructive Department of the University of Rome Tor Vergata. 10 patients were treated with SVF-enhanced autologous fat grafts and 13 patients were treated with fat grafting mixed with platelet-rich plasma. The patients in the control group (n=10) were treated with centrifuged fat grafting injection according to Coleman's procedure. The patients treated with SVF-enhanced autologous fat grafts showed a 63% maintenance of the contour restoring and of three-dimensional volume after 1 year compared with the patients of the control group treated with centrifuged fat graft, who showed a 39% maintenance. In those patients who were treated with fat grafting and PRP, we observed a 69% maintenance of contour restoring and of three-dimensional volume after 1 year. As reported, the use of either e-SVF or PRP mixed with fat grafting produced an improvement in maintenance of breast volume in patients affected by breast soft tissue defect.

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Generation of 3d organoids of human fetal biliary tree stem cells (hbtscs) as innovative tool for the regenerative medicine of liver and pancreas

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3D organoids represent an advanced culture technology in the field of stem cells and regenerative medicine, recapitulating embryonic organ development. Adult or fetal biliary tree represent ideal cell sources of stem/progenitor cells to be used for the regenerative medicine of liver and pancreas. The aim of our study was to generate 3D organoid cultures of hBTSCs and differentiate them toward hepatocyte cells which are suitable for cell therapy and regenerative medicine of liver. The fetal biliary tree (N=3, obtained from elective pregnancy termination) was digested, mechanically and enzymatically, to isolate *EpCAM/LGR5*-enriched hBTSCs, we also used the fragments of undigested bile duct to cultivate the organoids. Cells and bile duct fragments were then embedded in Matrigel and cultured in an expansion organoid medium containing soluble factors typical of the stem cell niche (e.g. EGF, FGF, Noggin, R-Spondin1) that represent LGR5 ligands and Wnt agonists and favor the expansion of stem cells and maintenance of stemness. Culture medium was also supplemented with Forskolin, a cAMP activator and with a TGFβR inhibitor to induce cell proliferation and arrest of differentiation. After 7 days the medium was changed to differentiation medium for a period of 10 days. We analyzed colony formation efficiency, organoid size and morphology, cell proliferation and gene expression by RT-qPCR. An average of 85 ± 7 million (N=3) *EpCAM/LGR5* enriched fetal hBTSCs were obtained. The cells isolated from fetal biliary tree showed a high tendency to generate organoids with high colony formation efficiency (> 60%). After 5 days in culture, the organoids were microscopically detected as spherical structures and after 7 days, they reached a macroscopically visible size. Cell proliferation and population doubling in organoids was significantly higher compared to 2D conditions ($p < 0.05$). Fetal biliary tree organoids were composed of single layered cuboidal epithelium and inner cell masses. RT-qPCR analysis demonstrated that organoids in expansion condition expressed multipotency stem cell markers (SOX2, NANOG, OCT4), endodermal stem/progenitor cell markers (LGR5, EpCAM, PDX1, SOX17), hepatic progenitors and ductal markers (CK19, CK7) and stem/progenitor surface genes (NCAM, CD133, CD44), recapitulating major processes of self-organization during embryonic development, whereas the differentiated organoids expressed high level of mature hepatocyte marker like CYP3A and ALB. Interestingly, LGR5 Expression reduced notably in organoids in differentiation condition compared to expansion condition ($p < 0.01$). Moreover, differentiated organoids acquired a hepatocyte morphology, including polygonal cell shape and secreted significant high level of albumin into medium respect to the same cells in 2D culture. We have demonstrated that organoids expand clonogenically stable *in vitro* for at least two months, maintaining a stable phenotype of multipotent stem cells and they can differentiate toward mature functional hepatocyte. This system has potential applications in regenerative medicine of liver and pancreas and in disease modelling.

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Liver tissues regenerated from human tooth treats liver failure of rat cirrhosis model and swine NASH model

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Cadaveric or live-donor liver transplant is only the treatment for severe liver condition. However, the number of transplantations is very limited because of fewer available organs than number of the patients on the waiting list. The liver regeneration might be one of the alternatives. Several clinical studies employed mesenchymal stem cells from blood, adipose tissue or others to transplant without differentiating the cells. However, transplantation of these cells can only slow decline of hepatic function, but they cannot treat the conditions of the liver. The objective of adult stem cell transplantations might be to launch "bridge to transplant" strategy rather than treating liver condition. We have shown that human dental pulp stem cell demonstrates huge potential to treat lethal liver conditions. We have previously reported we treated the biliary liver cirrhosis and acute liver injury in nude rats with transplanting the regenerated liver tissues originated from human dental pulp. One of the most prevailing liver conditions is non-alcoholic steatohepatitis (NASH). Hence the objectives of the research are to evaluate the clinical possibility of our transplantation protocols using swine model of progressive liver failure developed from NASH. After four weeks of transplantation of hepatocytes described from human tooth into the spleen of 6 swine with the failure under immune suppression, we found the secondary liver in the spleen was produced, as well the regenerated liver was produced using the original liver as scaffold. Biliary ducts are reproduced with human tissues only 4 weeks after the transplantation. Serum albumin level recovered from 1.5 g/dL to over 3.0 g/dL. HPT, choline esterase, collagen type IV, ALT and others have been dramatically improved. But any of the positive control has shown no change. Following above we also treated rat cirrhosis model.

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Advances in separation science and bioinformatics for analysing glycosylation in manufacturing biologics

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Controlling glycosylation is a major issue for ensuring te safety and efficacy of Biologics. Traditional challenges for glycan analysis can be addressed by a range of technologies that are automated, high throughput, sensitive and quantitative. To give a more complete detailed analysis of intact glycoforms a combination of technologies such as LC/MS/CE are required. These enable the analysis of intact glycoproteins, glycopeptides, released glycans and glycolipid head groups. The bottleneck now lies in data interpretation so this talk focuses on the application of four new software programmes: (i) GlycopeptideGraphMS (for the detailed identification of glycopeptides which is data base and platform independent) (ii) GlycoStore (an international resource including experimental glycan data bases and metadata) (iii) Glycoanalyser (software to aid interpretation of exoglycosidase array digestions) and (iv) MAGMap (a programme giving a confidence score to an assignment based on multiple attributes).

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Pseudobioaffinity ligands coupled to high throughput support matrix for purification of proteins from preparative to analytical validation important aspect in DSP of biopharma

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The term "Pseudobiospecific affinity chromatography" was coined by Prof. M A Vijayalakshmi in 1989 (TIBTECH 1989), which cover classes of ligands which have both structural and functional recognition of proteins based on their aminoacid sequence and three dimensional structure. Pseudobiospecific AC systems (e.g. amino acids, metal-chelates and triazine dyes) are highly economic and robust, can be fine-tuned to excellent specificities and medium dissociation constants (10-7 - 10-5). Ligand and Matrix are the two chromatography components that guide molecular interactions in any AC system. The ligand governs thermodynamic aspect of the chromatographic system which includes binding specificity, binding strength, ligand concentration etc. The support matrix governs the high-throughput-hydrodynamic aspect which includes the porosity of the support, particle size, etc. "MONOLITHS" are new stationary phase materials introduced in 1990's as "non-particulate homogeneous methacrylate material with high pore interconnectivity and lack of interestial voids" containing mega pores. They can be prepared in different forms like disks (CIM®: Convective Interactive Media), radio flow columns, capillaries and microfluidics. Due to the high pore interconnectivity, the flow is convective which results in efficient mass transfer of molecules and without any diffusion limitation like in the agarose based system. Thus a flow independent binding system gives very high capacity and binding, even at very high flow rates like 5 column volumes per minute. The CIM systems are hydrophilic and versatile such that any ligand can be coupled as is being done with agarose matrices and with same chromatographic buffer systems. Chromatographic runs are done seconds to minutes not in hours and days. Apart from these, monoliths possess other advantages like ease of preparation, low dead volumes, chemical and mechanical stability and compatibility to get hyphenated with conventional chromatography equipment's.

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The combination of photodynamic therapy with anti-angiogenic therapy for the effective control of local prostatic tumor and distant metastasis

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The Combination of anti-angiogenic therapy with cytotoxic therapy has shown positive outcome in various preclinical studies. However, these promising results did not always translate to clinical efficacy, demonstrating the complexity of combination strategies. As a result, there are ongoing efforts to combine existing cytotoxic therapy with anti-angiogenic therapy to enhance the efficacy of cancer treatment. However, the optimal scheduling of anti-angiogenic therapy with cytotoxic therapy, although crucial for maximizing treatment efficacy remains unclear. The aim of this study is to investigate VEGF regulation following cytotoxic therapy as a basis for the efficacy of combination anti-angiogenic therapy. Materials and methods: Orthotopic prostate tumors were implanted in the prostate of 6-week-old male severe combined immunodeficient mice. In particular, we investigated the effect of the combination treatment strategy on the two major patterns of metastasis: hematogenous as well as lymphatic metastasis. Here, we investigated an optimal protocol for combining Avastin anti-angiongenic therapy with photodynamic therapy (PDT), a cytotoxic therapy for various diseases including cancer. We demonstrate that PDT leads to a temporally transient regulation of vascular endothelial growth factor (VEGF) following treatment. More importantly, combination Avastin therapy was most effective in inhibiting lung metastasis when delivered around the peak of VEGF response following PDT. Considering that temporally transient VEGF regulation was observed following PDT, radiotherapy and chemotherapy. In conclusion, PDT lead to a temporally transient regulation of vascular endothelial growth factor (VEGF) following treatment. Considering that temporally transient VEGF regulation was observed following PDT, radiotherapy and chemotherapy, optimal scheduling of combination anti-angiogenic therapy based on temporal dynamics of the VEGF response has implications in a wide range of cancer treatments.

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Extraction of basic nitrogen-containing compounds from gasoline using cholinechloride/ glycerol deep eutectic solvent: Insight from phase equilibrium data

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In response to stringent regulations aimed at preventing environmental pollution, nitrogen- containing impurities have to be removed from transportation fuels, including gasoline. In this context, deep eutectic solvents (DES) have been recently identified as credible green candidates for denitrogenating liquid fuels. In this work, the ability of choline chloride/ glycerol DES as a denitrogenation solvent for model gasoline was investigated at 295.15 K and atmospheric pressure. Experimental liquid-liquid equilibrium data for heptane + pyridine or quinoline + choline chloride/glycerol DES were determined using the equilibrium cell method. Heptane served as a model for gasoline while nitrogen-containing impurities consisted of pyridine and quinoline. Choline chloride/glycerol DES showed greater extraction potential for pyridine than quinolone. However, calculated selectivities and distribution coefficients indicated that the studied DES is a promising denitrogenation agent in both cases. Subsequently, the experimental data were successfully correlated by means of the non-random two-liquid (NRTL) model. The root mean square deviations (RMSD) between calculated and experimental compositions were 0.0457 and 0.0458 for heptane + pyridine + choline chloride/glycerol DES and heptane + quinoline + choline chloride/glycerol DES, respectively.

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