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Invited Talk  

**Quantitative Point-of-Need Diagnostics**  
Scott T. Philips  
*Pennsylvania State University, USA*

This talk will describe strategies for achieving highly selective and sensitive quantitative point-of-need assays that limit the use of electronics for achieving the quantitative result. One strategy, in particular, combines paper microfluidics with phase-switching polymers to enable quantitative assays where the readout for the assay is the “time to appearance of color”.

Invited Talk  

**Paper-Based Fluidic Devices: From Fabrication to Assay Quantification**  
Andres W. Martinez, Haydn T. Mitchell, Cory A. Chaplan, Kevin M. Schilling and Connor K. Camplisson  
*California Polytechnic State University, USA*

Paper-based fluidic devices, also known as microPADS, offer a promising platform for the development of point-of-care diagnostic assays for use in remote, resource-limited settings. Paper-based fluidic devices are inexpensive to fabricate, portable, simple to operate, and can complete an assay without relying on electrical power or supporting equipment. This presentation will describe our work on the development of new methods of fabricating paper-based devices, methods for controlling wicking in paper-based devices and methods for calibrating the results of assays, all of which provide important building blocks for the development of future paper-based diagnostic devices. Our fabrication methods rely primarily on the use of commercially available printers, and are developed specifically for lab-scale prototyping of devices. Our interest in controlling the wicking of fluids in paper-based devices stems from the observation that the time it takes for a fluid to wick across a channel in a paper-based fluidic device is typically on the order of minutes for short distances (< 3 cm), but can be on the order of hours for longer distances (> 5 cm). We have developed methods for fabricating “fast channels” that reduce the time it takes for fluids to wick across longer distances and enable new functions to be incorporated into paper-based devices. Finally, paper-based diagnostic devices frequently rely on colorimetric assays to detect analytes. These types of assays are ideal for on-site diagnostics because they can be read by the eye. It is often proposed that the results of the assays could also be quantified using a digital camera. We have developed a paper-based standard addition assay that provides quantitative results from colorimetric assays that are independent of the lighting conditions under which the devices are imaged.
Recent progress in nanoscale science and technology has resulted in a number of nanomaterials with interesting optical, electrical, magnetic properties that can be ideal choices for cellular delivery and signal transductions for nanomedicine and nanoassay. However, most of these materials lack the selectivity required for these applications. Functional DNA, a new class of DNA with functions similar to either antibodies (known as aptamers) or enzymes (called DNAzymes), can provide high selectivity for a wide range of molecules, including small molecules such as metal ions and small organic molecules that antibodies do not bind with high selectivity. We have been able to use a combinatorial method called in vitro selection to obtain functional DNA that can bind targets of choice strongly and specifically, and used negative selection strategy to improve the selectivity. By labeling the resulting functional DNA with gold nanoparticles, upconversion nanoparticles, quantum dots, and supermagnetic iron oxide nanoparticles, we have developed new classes of colorimetric and fluorescent sensors, and smart MRI contrast agents for metal ions, organic and biomolecules. A novel approach of using an inactive variant of functional DNA to tune the detection range of the sensors is also demonstrated. For even more straightforward field applications, these sensors have been converted into simple “dipstick” tests for qualitative detection. The application of functional DNA nanotechnology has also been expanded to include targeted drug delivery. These, and other recent results, will be presented.
Interleukin 8 (IL-8) has been recognized as an inflammatory factor and a pro-angiogenic factor for years. IL-8 can be secreted by many inflammatory cells and highly aggressive cancer cells. Using our metastasis model, we found that IL-8 strongly promotes metastasis of nasopharyngeal carcinoma (NPC) cells via autocrine and paracrine means, involving activation of AKT signaling and inducing EMT in NPC cells. Tissue micro-array analyses coupled with multivariate survival analyses further confirmed that elevated expression of IL-8 in the primary tumor was an independent prognostic factor for overall survival, distant metastasis-free survival, and disease free survival of NPC patients. IL-8 can be secreted into circulation. Simultaneous detection of serum IL-8 together with 38 other circulating cytokines is feasible using multiplex bead-based Luminex technology. When we explored the 39 cytokines in two cohorts of serum samples from colorectal cancer (CRC) patients, we found that IL-8 was one of the 17 cytokines which formed a powerful cytokine-based prognostic classifier (CBPC) for the overall survival of CRC patients in both the training cohort and the validation cohort. Upon further investigation using the serum samples of hepatocellular carcinoma (HCC) patients, IL-8 was again one of the six cytokines and three clinical characteristics consisting of a CBPC, which had a significant predictive power for predicting tumor recurrence and overall survival. In conclusion, IL-8 is an important prognostic marker in multiple malignancies, and targeting IL-8 signaling is a promising therapeutic strategy for inhibiting cancer progression.

For sustainable green synthesis processes, it is still rather difficult to simultaneously achieve satisfactory conversion and selectivity. Among the various methods exploited to meet this challenge, the design and synthesis of well-defined bimetallic or alloyed catalysts is one of the most effective ways. Herein, for the first time, we designed Au alloyed Pd single-atom catalysts and succeeded in tuning the activity/selectivity of several organic synthesis processes (e.g. Ullmann reaction of aryl chlorides, oxidative coupling of alcohols and amines, and selective hydrogenation of acetylene in excess ethylene.). The design and synthesis of alloyed single-atom catalysts could lead to a new way of developing efficient catalysts for green synthesis of fine chemicals.
Highly Efficient Chemical Process to Bio-Adipic Acid
Xiukai Li, Ting Lu, Guangshun Yi and Yugen Zhang
Institute of Bioengineering and Nanotechnology, Singapore

The production of bulk chemicals and fuels from renewable bio-based feedstock is important for a sustainable society. Adipic acid, one of the most sought after chemicals from bioresource, is used primarily for the large volume production of nylon-6,6 polyamide. In this work, we have demonstrated the highly efficient synthetic protocol for the conversion of mucic acid to adipic acid through oxorhenium complex catalyzed deoxydehydration (DODH) - Pt/C catalyzed transfer hydrogenation sequence. Quantitative yields (99%) were achieved from mucic acid to muconic acid and adipic acid either in separate sequences or in one-step process. The acidity of the oxorhenium catalysts determined the selectivity to the free acid products in the DODH reactions. With modified rhenium catalysts, mucic acid was converted to muconic acid and then to adipic acid in 98% selectivity, and similarly tartaric acid was converted to maleic acid and then to succinic acid at more than 96% selectivity.

Recyclable, Strong Thermosets and Organogels via Paraformaldehyde Condensation with Diamines
James L. Hedrick
IBM Research, USA

Nitrogen-based thermoset polymers have many industrial applications (for example, in composites), but are difficult to recycle or rework. We report that a simple one-pot, low-temperature polycondensation between paraformaldehyde and 4,4’-oxydianiline (ODA) that forms hemiaminal dynamic covalent networks (HDCNs) which can further cyclize at high temperatures producing poly(hexahydrotriazine)s (PHTs). Both materials are strong thermosetting polymers, and the PHTs exhibited very high Young’s moduli (up to ~14.0 gigapascals and up to 20 gigapascals (GPa) when reinforced with surface-treated carbon nanotubes), excellent solvent resistance, and resistance to environmental stress cracking. However, both HDCNs and PHTs could be digested at low pH (<2) to recover the bisaniline monomers. When poly(ethylene glycol) diamine monomers were used to form HDCNs, elastic organogels formed that exhibited self-healing properties.
Magnetic nanoparticles are promising in applications where magnetic separation is intended, although material loss via leaching mechanisms are often inevitable. Magnetic separations with widely available permanent magnets can effectively trap particles, leading to a complete removal of used or waste particles. In this report, we first demonstrate the synthesis of the thinnest (112.7 ± 16.4 nm) and most magnetic (71.96 emu g⁻¹) barium hexaferrite (BaFe₁₂O₁₉, BHF – fridge magnet) via an organic solvent-free electrospinning procedure. When the fibers are then packed into a column, they clearly remove 12 nm magnetite (Fe₃O₄) nanoparticles quantitatively. The same BHF cartridge also removes more than 99.9% As-treated magnetite nanoparticles at capacities up to 70 times of its weight. As a result, one liter of 150 μg L⁻¹ As-contaminated water can be purified rapidly at a materials cost of less than 2 US cents.
Molecular Self-Assembly of Biological and Bio-Inspired Building Blocks: From Biological Association to Novel Materials
Ehud Gazit
Tel Aviv University, Israel

The formation of ordered amyloid fibrils is the hallmark of several diseases of unrelated origin. In spite of grave clinical consequence, the mechanism of amyloid formation is not fully understood. We have suggested, based on experimental and bioinformatic analysis, that aromatic interactions may provide energetic contribution, as well as order and directionality in the molecular-recognition and self-association processes that lead to the formation of these assemblies. This is in line with the well-known central role of aromatic-stacking interactions in self-assembly processes. A significant part of the activity in our lab is related to the development of new therapeutic agents based on this notion. Our works on the mechanism of aromatic peptide self-assembly, have led to the discovery that the diphenylalanine recognition motif self-assembles into peptide nanotubes with a remarkable persistence length. Other aromatic homodipeptides could self-assemble in nano-spheres, nano-plates, nano-fibrils and hydrogels with nano-scale order. We demonstrated that the peptide nanostructures have unique chemical, physical and mechanical properties, including ultra-rigidity as aramides, semi-conductive, piezoelectric and non-linear optic properties. We also demonstrated the ability to use these peptide nanostructures as casting mould for the fabrication of metallic nano-wires and coaxial nano-cables. The application of the nanostructures was demonstrated in various fields including electrochemical biosensors, tissue engineering, and molecular imaging. Finally, we had developed ways for depositing of the peptide nanostructures and their organization. We had used inkjet technology, as well as vapor deposition methods to coat the surface and from the peptide “nano-forests”. We recently demonstrated that even a single phenylalanine amino-acid can form well-ordered fibrilar assemblies of distinct electron diffraction pattern and toxic properties.
**Synthetic Water Channels for Water Reclamation and Desalination**

Huaqiang Zeng  
*Institute of Bioengineering and Nanotechnology, Singapore*

Toward the creation of channel molecules with well-defined conformations and functions for selective transports of ions, water or other molecules across the lipid membrane, we have been investigating the use of directional, inward-pointing intramolecular H-bonding forces to fold aromatic backbones into helical or circular shapes. For the folding helices requiring 5 repeating units to form a helical turn, an additional covalent constraint using intramolecular macrocyclization produces a unique class of cavity-containing pentagon-shaped macrocycles that are rarely found in both natural and unnatural worlds. Our macrocyclic pentameric system is characterized by a small lumen size of ~ 1.45 Å in radius that is suitable for cation recognition, and further by their modular nature in backbone. These notable traits enable a combinatorial production of an enriched family of macrocyclic pentamers, functioning as modularly engineerable, scalable, cavity-forming systems, wherein precise pin-point modifications with variable functionalities in both the interior and exterior can be readily attained. Among their possibly diverse applications in chemistry, materials sciences, biology and medicine, I will highlight their ability to selectively recognize metal ions, and their medical relevance in building up synthetic species-transporting channels.

**Biochemical Modulation of Biomimetic Semi-Interpenetrating Network Hydrogels**

Sushmitha Sundar and Yen Wah Tong  
*National University of Singapore, Singapore*

Semi-interpenetrating network hydrogels (S-IPN) comprising two polymeric networks carry the ability to possess two distinctly different properties. In our study, we have designed S-IPN hydrogels comprising poly (ethylene glycol) diacrylate (PEGDA) as one component and peptide amphiphile (PA) nanofiber as the second component, thus, imparting mechanical and biochemical cues respectively for the scaffold. The biochemical cue is varied by the PA design as collagen mimetic or surface charge. The whole system is formed as a photo-cross linked system thus, has in-situ gelling capability. The combined effect of various biochemical and mechanical cues is investigated for skin tissue engineering application with the growth of human dermal fibroblast cells. The results indicate the presence of surface charge along with collagen mimetic biochemical cue necessary for the growth of fibroblasts. This design of S-IPN hydrogels enables to modulate individual cues to design cell specific requirements.
Directing the Protein Interactions of Chondroitin Sulfate Glycopeptides via a Tunable Multivalent Scaffold

Song-Gil Lee, Pei Liu, Liwei Chen, Jerry K. C. Toh, Yi Li Ang, Joo-Eun Jee, Jaehong Lim and Su Seong Lee
Institute of Bioengineering and Nanotechnology, Singapore

Given the challenges inherent in the synthesis of large glycosaminoglycan (GAG) polysaccharides, chemically accessible multivalent glycoligands have been a growing arsenal in the field of GAG mimetics. Nevertheless, the inability of positioning sulfated sugar motifs at desired sites has hindered efforts to precisely tailor their biofunctions. Here, we describe systematic explorations into the importance of scaffold conformation in designing glycopolymers using structurally well-defined chondroitin sulfate-based glycopeptides. In our approach, the precise orientation of sulfated disaccharide motifs has been accomplished by taking advantage of a polyproline scaffold. Our protein binding studies demonstrate that the specific conformational display of pendant sugars is central to direct their multivalent interactions with nerve growth factor (NGF). Computational modeling and cellular studies have further validated that this approach can be used to engineer NGF-mediated signaling by regulating the NGF/TrkA complexation process, leading to enhanced neuronal differentiation and neurite outgrowth of PC12 cells. Taken together, our findings offer a promising strategy for the pinpoint engineering of GAG-mediated biological processes and a novel method of designing new therapeutic agents that are highly specific to GAG-associated disease.
Invited Talk

Designing Polymers with Protein-Like Activity: New Opportunities for T-Cell Biology

Gregory N. Tew
University of Massachusetts Amherst, USA

Our primary research aim is to create new materials using a combination of principles, many of which are inspired by biology. Within this large field, many researchers have explored the ability to recapitulate the essential features of these natural pores using simpler, synthetic scaffolds. These endeavors represent one specific example of a much larger effort to understand how synthetic molecules interact with and manipulate the plasma membrane. Modern biophysical assays highlighted the interplay between the synthetic scaffold and lipid composition leading to negative Gaussian curvature, a requirement for pore formation. The complexity of this interplay between lipids, bilayer components, and the scaffolds remains to be better resolved, but significant new insight has been provided. The combination of unique molecular scaffolds and guanidinium-rich side chains has produced an array of polymers with robust transduction (and delivery) activity. Being a new area, the fundamental interactions between these new scaffolds and the plasma membrane are just beginning to be understood. It has become clear that the combination of molecular design, biophysical models, and biological evaluation provide a robust approach to novel proteinomimetics. Previous work on facially amphiphilic polymers generated antimicrobials inspired by natural host defense peptides, like Magainin and Defensin. We will discuss our newest results in which we have successfully mimicked that biological activity of protein transduction domains like HIV-TAT. Here we report the first detailed structure-activity relationship of a new PTD family of polymers based on a completely abiotic backbone. Cellular uptake studies on three different cell lines (HEK 293T, CHO, and Jurkat T cells) confirm that these synthetic analogs are highly efficient novel protein transduction domain mimics (PTDMs), that are more effective than TAT_{49-57} and nonaarginine (R9) and also highlights the usefulness of polymer chemistry at the chemistry-biology interface.
Invited Talk  

Light/ROS-Responsive Nanovesicles for Anti-Cancer and Anti-Inflammation Therapy  
Sangyong Jon, Yonghyun Lee and Soyoung Lee  
Korea Advanced Institute of Science and Technology, Korea

External or internal stimuli-responsive drug delivery systems have been of great interest in that drug molecules are to be released to the site of a disease in a controlled manner, thereby improving efficacy but minimizing toxicity. Here, we report a dual light/ROS stimuli-responsive bilirubin nanovesicles (BNVs) for potential anti-cancer and anti-inflammation therapy. Bilirubin is a final metabolite of heme and functions as a potent antioxidant by scavenging ROS in our body. More interestingly, bilirubin can undergo photo-mediated isomerization to more water-soluble compounds. To use bilirubin for biomedical applications, it was first converted to pegylated bilirubin, which undergoes self-assembly to yield uniform nanovesicles with size of ~120 nm. In this lecture, the preparation, characterization and stimuli-induced disruption behavior of BNVs will be presented. Furthermore, as proof-of-concept, therapeutic efficacy of BNVs on a mouse colitis model will be also presented.

Oral Presentation  

Green Tea-Sourced Micellar Nanocomplex for Protein Delivery with Synergistic Effects  
Joo Eun Chung1, Susi Tan1, Shu Jun Gao1, Nunnarpas Yongvosootorn1, Soon Hee Kim2, Jeong Heon Lee2, Hak Soo Choi2, Hirohisa Yano1,3, Motoichi Kurisawa1 and Jackie Y. Ying1  
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The carriers of all drug delivery systems so far are just excipients for drug delivery with no relevance for therapeutic effect and can lead to problems associated with their toxicity, metabolism and elimination through their use in unreasonably large quantities. Therefore, the drug-to-carrier ratio has been a considerable limiting factor when designing drug carriers. However, if both the drug and carrier possess therapeutic effects, this issue would not be a restricting factor, and might offer the advantage of combined therapeutic effects. (-)-Epigallocatechin-3-O-gallate (EGCG) is a major ingredient of green tea and has been shown to possess anti-cancer effects, anti-HIV effects, neuroprotective effects and DNA-protective effects, among others. Here we show that sequential self-assembly of the EGCG derivative with anti-cancer proteins form stable micellar nanocomplexes (MNCs) that have greater anti-cancer effects in vitro and in vivo than free protein. The MNC is obtained by complexation of oligomerized EGCG with the anti-cancer protein, Herceptin, to form the core, followed by complexation of poly(ethylene glycol)-EGCG to form the shell. When injected into mice, the Herceptin-loaded MNC showed improved tumor selectivity, longer blood-half-life and reduced tumor growth more efficiently than free Herceptin.
Bio-Inspired Nanogels for Nanomedicine
Kazunari Akiyoshi
Kyoto University, Japan and JST ERATO, Japan

Nanogels can be used as a new biologics DDS by efficiently trapping biomacromolecules, such as DNA, siRNA, peptides and proteins, within the network. We first reported physically cross-linked nanogels by self-assembly of hydrophobized polysaccharides. The proteins are trapped inside the amphiphilic nanogel polymer network without aggregation and are released in the native form (chaperon function). Polysaccharide nanogels act as an ideal antigen delivery system to stably trap antigen protein and to efficiently transport the antigen to the draining lymph node (immuno-transporter). Cationic polysaccharide nanogels were used as a universal protein-based antigen delivery vehicle for adjuvant-free intranasal vaccination. Cationic polysaccharide nanogels were also complexed with nucleic acids, including double-stranded small interfering RNA or plasmid DNA, and acted as multifunctional nucleic acid delivery systems.

The nanogels were also used as building blocks to control the nanostructure of macrogels or particles for advanced biomedical technology including drug delivery system and regenerative medicine. Hydrogels with well-defined nanostructures were obtained by self-assembly and chemical cross-linking of the nanogels as building blocks. Acryloyl or methacryloyl group modified hydrophobized polysaccharides were synthesized as reactive nanogels. By using the nanogels, nanogel-crosslinking nanoparticles, hydrogels, sheet film and nanogel-coating materials were developed.

We describe here our recent progress in the synthesis of functional nanogels by self-assembly of associating polymers for nanomedicine.
Peptide Inks for Bio-Printing 3D Organotypic Biological Constructs

Yihua Loo and Charlotte A. E. Hauser

Institute of Bioengineering and Nanotechnology, Singapore

Self-assembling ultrashort peptides are ideal building blocks to construct ordered three-dimensional (3D) scaffolds for biomedical applications. In particular, stimuli-responsive candidates are of interest as inks for 3D bio-printing, wherein the peptide scaffold is co-deposited with cells in specific and controlled manner to ensure spatial localization. The resulting organotypic biological constructs can be applied towards high-throughput cell screening and regenerative medicine applications.

We discovered that a subclass of unique amphiphilic ultrashort peptides containing lysine or lysine-mimetic residues demonstrate salt and pH-enhanced self-assembly into nanofibrous hydrogels. These peptides are intrinsically biocompatible and non-immunogenic. Consisting of only 3 to 7 aliphatic amino acids, their characteristic motif stimulates self-assembly into helical fibers which further aggregate into three-dimensional nanofibrous networks that entrap water. Tuning the gelation kinetics and mechanical properties, we developed formulations that form gels instantaneously upon exposure to physiologically-relevant salt solutions. Exploiting their stimuli-responsiveness, we encapsulated various human primary and pluripotent stem cells to generate 3D cell-scaffold constructs. These constructs were continuously stable for more than 20 days under standard culture conditions, enabling long term culture of stem cells for differentiation studies. We have also repeatedly passaged the cells in 3D for more than 10 passages by enzymatically dissociating the hydrogel to release the cells for re-encapsulation. To influence cell behaviour, we co-encapsulated bioactive moieties such as genes, molecular compounds, growth factors and cell adhesion motifs. Such biological constructs can be used as organoid models for screening small molecules, studying cell behavior and disease progression, as well as tissue-engineered implants for regenerative medicine.
Micropatterning of Polyvinyl Alcohol for Vascular Tissue Engineering Applications
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Small diameter vascular grafts (< 6 mm internal diameter) are used in bypass or replacement of occluded peripheral arteries. However, there is a lack of commercially available, synthetic small diameter grafts that provide acceptable long-term patency. To improve clinical outcomes, it is necessary to enhance in situ endothelialization of small diameter vascular grafts. Topographical cues may be used to effect the change by influencing the behavior of endothelial cells, such as increasing their migration and proliferation capacities.

Poly(vinyl alcohol) (PVA), a biocompatible and non-thrombogenic hydrogel due to its hydrophilic nature, is an excellent material for vascular tissue engineering, showing short-term patency in a rat abdominal aorta model. Our preliminary data has also demonstrated patency of the 1-mm diameter PVA graft in a rabbit model with multi-level peripheral arterial occlusion at 2 weeks post-implantation.

Although PVA has been proven to be biologically inert, the lack of endothelial cell attachment on the luminal surface of the graft would greatly impact the long-term patency. We have incorporated topographical cues onto planar PVA films, thereby improving endothelial cell viability, proliferation and function in vitro. Platelet adhesion studies on patterned PVA films showed platelets with rounded and less activated morphology. Through a novel casting method, we fabricated PVA small diameter vascular graft with topography on its luminal surface. Implantation of patterned PVA grafts in rat abdominal aorta model exhibited patency and in situ endothelialization after 20 days. Thus, PVA vascular grafts are excellent candidates as a small diameter vascular graft by, preventing thrombosis, stimulating in situ endothelialization and supporting long-term patency.
Nano-Topography Cell Modulation of Corneal Endothelial Behavior

Jodhbir S. Mehta¹, Evelyn Yim² and Gary Peh¹

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The human corneal endothelium plays an important role in maintaining corneal transparency. Human corneal endothelial cells have limited regenerative capability in vivo. Consequently, endothelial dysfunction can occur following corneal endothelial trauma or inherited diseases. To restore endothelial function, corneal transplantation is needed. However, there is a worldwide shortage of donor corneas, motivating the development of a tissue-engineered graft alternative using cultivated endothelial cells. To induce in vitro cell proliferation, much effort has been made to improve culture conditions and to mimic the native extracellular microenvironment. We incorporated topographical and biochemical cues in our in vitro culture of human corneal endothelial cell line B4G12 (HCEC-B4G12) and hypothesized that manipulation of the extracellular environment can modulate cell proliferation, morphometry and phenotype. The topographies tested were nanopillars, microwells and micropillars on polydimethylsiloxane, while the biochemical factors were extracellular matrix protein coatings of fibronectin-collagen I (FC), FNC® coating mix (FNC) and laminin-chondroitin sulfate (LC). Cellular morphometry, Na⁺/K⁺-ATPase and zona occludens 1 (ZO-1) gene and protein expression were analyzed 3 days after cells had formed a confluent monolayer. The cell circularity on all patterns and coatings was above 0.78. On all coatings, cell area was the lowest on micropillars. The coefficient of variation (CV) of the cell area was the lowest on nanopillars with an LC coating. With an FC coating, micropillars induced a better cellular outcome as the cells had the greatest circularity, smallest cell area and highest Na⁺/K⁺-ATPase and ZO-1 gene and protein expression. With the LC coating, HCECs grown on nanopillars resulted in the lowest CV of the cell area and the highest ZO-1 gene expression. Thus, HCEC-B4G12 morphometry and phenotype can be improved using different topographical and biochemical cues.
Surface Chemistry of Carbon Nanotube Scaffolds Modulate Viability, Mitochondrial Superoxides and Enhances Ex Vivo Expansion of Human Umbilical Cord Blood Progenitor Cells

Sudipto Bari1,2, Pat P. Y. Chu3, Sujoy Ghosh4, Andrea Lim4, Xiubo Fan2, Shang Li4, Gigi N. C. Chiu1 and William Y. K. Hwang2,3,4

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Hematopoietic stem cell transplantation using umbilical cord blood (UCB) is limited to pediatrics, as UCB does not have sufficient cell dosage for adults. We identified carboxylic functionalized single-walled carbon nanotubes (f-SWCNT-COOH) as scaffold to support ex vivo expansion of UCB mono-nucleated-cells (UCB-MNC). In this study, we report that surface functional groups of f-SWCNT are critical for UCB-MNC survival, molecular changes and expansion.

UCB-MNC in serum-free medium with cytokines were exposed to f-SWCNTs with varying functional groups namely –COOH, amide (–O-NH2) & polyethylene-glycol (–PEG) at increasing concentrations. Cultures without f-SWCNTs served as control. The treatment effects were characterized by viability, phenotypic and in vitro / in vivo functional assays.

Maximal CD45+ viability was observed with 1.0mg/ml f-SWCNT-COOH followed by –PEG, –O-NH2 and control (p<0.01). Concurrently, f-SWCNT-COOH optimally decreased CD45+ mitochondrial superoxides (mROS) followed by –PEG, –O-NH2 and control (p<0.01) thus depicting negative correlation (R=–0.863) between viability and mROS. f-SWCNT-COOH gave maximal phenotypic and functional expansion of CD34+ hematopoietic progenitors (HPC) followed by –PEG, –O-NH2 and control (p<0.05). Cytokine array illustrated that f-SWCNT-COOH maintained higher proportion of HPC associated cytokines compared to control (p<0.01). Transplanting f-SWCNT-COOH or cytokine only expanded UCB-MNC to immunodeficient mice resulted in lower (p=0.13) bone marrow engraftment of human CD45/71 cells compared to non-expanded grafts. However, f-SWCNT-COOH expanded grafts gave better frequency of human progenitors (p=0.29) compared to control when they were co-transplanted with non-expanded cells from the same UCB. Mice transplanted with expanded cells had higher (p<0.05) survival rate compared to non-expanded grafts due to lower graft-versus-host-disease.

Surface functionalization of SWCNT is critical in mediating viability, mROS and expansion of freeze-thawed, non-enriched UCB-MNC. Moreover, co-transplantation of low dose non-expanded cells along with high dose of f-SWCNT-COOH expanded cells from the same UCB boosted human engraftment kinetics with better survival rates in immunodeficient xenotransplantations.
Microfluidic Platform for Biomimetic Materials and Tissue Engineering Applications

Jianhua Qin
Dalian Institute of Chemical Physics, China

Microfluidic technologies are providing unprecedented opportunities to create biomimetic microsystems and facilitate the advances in understanding and developing new strategies for regenerative medicine and tissue engineering applications. In this talk, I will present a multi-scale microfluidic platform that can be explored for the facile synthesis and assembly of biomimetic materials with different morphologies, such as microsphere, microfiber and droplet-in-fiber. These bioinspired materials were demonstrated not only as functional micro-carriers for cells and biomolecules, but also as building blocks for micro-tissue construction. We believe these bio-hybrid materials would find a wide range of applications in the fields of tissue engineering, stem cell research and regenerative medicine.

A Defined Xeno-Free and Feeder-Free Culture System for the Derivation, Expansion and Direct Differentiation of Transgene-Free Patient-Specific Induced Pluripotent Stem Cells

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A defined xeno-free system for patient-specific iPSC derivation and differentiation is required for translation to clinical applications, conditions which are not met by standard somatic cell reprogramming protocols which rely on the use of MEFs and xenogeneic medium. Here, we describe a well-defined culture system based on xeno-free media and LN521 substrate which supports efficient reprogramming of normal or diseased skin fibroblasts from human of different ages into hiPSCs, long-term self-renewal of hiPSCs, and direct hiPSC lineage-specific differentiation. Using an excisable polycistronic vector and optimized culture conditions, we achieved up to 0.15~0.3% reprogramming efficiencies, which is a 15~30 fold increase over conventional viral vector-based methods. Subsequently, transgene-free hiPSCs were obtained by Cre-mediated excision of the reprogramming factors. The derived iPSCs maintained long-term self-renewal, normal karyotype and pluripotency, as demonstrated by the expression of stem cell markers and ability to form derivatives of three-germ layers both in vitro and in vivo. Additionally, we demonstrated that Parkinson's patient transgene-free iPSCs derived using the same system could be directed toward differentiation into dopaminergic neurons under xeno-free culture conditions. Thus, our approach presents a safe and robust platform for the generation of patient-specific iPSCs and derivatives for clinical and translational applications.
Cost-Effective Differentiation of Hepatocyte-Like Cells from Human Pluripotent Stem Cells Using Small Molecules

Farah Tasnim1 and Hanry Yu1,2
1Institute of Bioengineering and Nanotechnology, Singapore
2Yong Loo Lin School of Medicine, Singapore

Human hepatocytes are valuable tools for hepatotoxicity screening, a crucial step for drug discovery and development. The utility of primary human hepatocyte (PHs), the current cellular system of choice for drug screening and cell therapy, is limited. Therefore, substantial effort has been put into the derivation of hepatocytes from renewable sources such as pluripotent stem cells (hPSCs) using step-wise addition of growth factors and cytokines. However, obtaining and maintaining hepatocyte-like cells that represent adult phenotype still remains a major goal. In addition, a more cost efficient method for differentiation would be highly attractive, owing to the large number of cells required for cell therapy and drug screening. In this regard, small molecules offer an attractive alternative since they would be less expensive, more easily controlled and possibly more efficient than growth factors in directing differentiation. We hypothesized that we could develop a cost-efficient, stable system for differentiating pluripotent stem cells into functional hepatocytes using predominantly small molecules.

Our results showed that, with the exception of Activin A, all growth factors which are currently required for the differentiation of hPSCs could be replaced using specific combinations and concentrations of small molecules. With this combination, the hPSCs could be driven to a definitive endodermal stage, followed by a hepatoblast stage and finally differentiated into hepatocyte like cells (SM-Heps). The gene expression of markers of SM-Heps at these stages were comparable to the expression level of cells differentiated using established growth-factor based differentiation protocol (GF-Heps). In addition, SM-Heps produced albumin and urea at levels similar to that of GF-Heps. The activity of major cytochrome P450 (CYP) enzymes was also comparable. Finally, the response of SM-Heps to paradigm hepatotoxicants was similar to GF-Heps. More importantly, the drug response of SM-Heps was also similar to that of PHs.

We have developed a step-wise method of differentiating hPSCs into functional hepatocytes using predominantly small molecules. Our results suggest that these cells could be a valuable tool for drug screening and development.
Invited Talk  

Microfluidic Nebulization Platform for Pulmonary Drug and Gene Delivery  
Sarah Masoumi¹, Aisha Qi¹, Anushi Rajapaksa², Christina Cortez-Jugo², David Piedrafita², Peggy Chan¹, James Friend¹ and Leslie Yeo¹  
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We demonstrate the use of surface acoustic wave (SAW) microfluidic nebulization platform for non-viral gene delivery. High levels of gene expression are observed in the Western blot analysis of COS-7 cells transfected with post-nebulized naked plasmid DNA encoded with an Influenza A virus surface antigen. This is subsequently verified through systematic and mucosal antibody responses detected via a hemagglutinin inhibition assay in the sera of female Sprague-Dawley rats delivered via intratracheal instillation of the condensed aerosols and in that of female Merino-cross ewe lambs delivered through direct inhalation via a mechanical ventilator. Further, we demonstrate that the technology is a very rapid, efficient and straightforward means for synthesizing 100 nm dimension biodegradable polymeric particles within which therapeutic molecules such as nucleic acids, proteins and peptides can be encapsulated. Finally, the ability to synthesize multiple polyelectrolyte coatings encapsulating these biomolecules is shown using sequential atomization-suspension steps as a fast and efficient alternative to conventional layer-by-layer polyelectrolyte assembly. These multilayer nanocapsules offer the possibility for tuning the drug release profile for controlled delivery over a prolonged period or for targeting the delivery to a specific location within the body. The low costs, control offered over the aerosol/particle size, possibility for flexible and multilayer encapsulation, low power requirement, high delivery efficiency, and the miniaturization of the system altogether suggest that the SAW nebulization platform represents an attractive alternative to current nebulizers and inhalers, which we envisage could constitute the next-generation of devices that will revolutionize pulmonary drug and gene delivery for needle-free vaccination or for the treatment of various diseases in the near future.
Invited Talk

**Magnetotactic Bacteria as Nanorobotic Therapeutic Delivery Agents in Cancer**

*Sylvain Martel*

*École Polytechnique de Montréal, Canada*

A new paradigm known as direct targeting aims at navigating therapeutic agents using the most direct route in order to avoid, or at least minimize, systemic circulation that often affects healthy organs and tissues. To be successful, direct targeting must rely on therapeutic agents that can propel and be directed towards the regions to be treated, such as the hypoxic regions of solid tumors. As such, drug-loaded MC-1 Magnetotactic Bacteria (MTB) have been investigated as delivery vehicles in direct targeting. Preliminary experimental results where the computer-assisted magnetotactic and the fully-autonomous microaerophilic responses of MC-1 bacteria have been exploited, suggest the potential advantages of these cells as sophisticated navigable therapeutic nanorobotic agents over artificial implementations and known therapeutic carriers to target hard-to-treat tumor hypoxic regions.

Oral Presentation

**PARP Inhibitor for Leukemia Treatment: New Uses for an Old Drug**

*Motomi Osato*

*National University of Singapore, Singapore and Institute of Bioengineering and Nanotechnology, Singapore*

Cancer is caused by the accumulation of genetic defects. The RUNX family genes are among the most frequently inactivated genes in human leukemia and other cancers. Yet, it is currently unknown how RUNX-related cancers can be eliminated using targeted therapy. A gene ablation experiment for Runx genes in mice yielded an unanticipated outcome. RUNX-deficient mice died due to two drastic hematopoietic diseases: bone marrow failure (BMF) and leukemia. The former refers to the inability to produce blood cells, whereas the latter means a massive expansion of abnormal hematopoietic cells. These apparently opposing clinical manifestations were reminiscent of a rare human congenital disease called Fanconi anemia (FA). FA is caused by mutations in either of the 15 genes responsible for the repair of a specific type of DNA damage. Subsequent biochemical analyses revealed that RUNX proteins have a critical and central role in the FA pathway by facilitating the recruitment of functionally active FA protein, FANCD2, to sites of DNA damage. This previously unappreciated link between RUNX and FA factors prompted us to test the possibility that PARP inhibitors originally designed for killing cancer cells with DNA repair defects could now be applied for leukemias and cancers harboring RUNX alterations, which were earlier never thought to have DNA repair defects. In fact, PARP inhibitors suppressed the proliferation of leukemic cells with RUNX alteration in cell culture experiments. Further drug efficacy testing is currently underway, employing mouse leukemia models, as a preclinical study.
Antimicrobial Peptide-Mimetic Polymers as a New Line of Defense Against Drug-Resistant Bacteria

Kenichi Kuroda
University of Michigan, USA

The emergence of antibiotic resistance in bacteria is a significant concern to medical practice, rapidly diminishing the number of available treatment options. Our research has been focused on the design and development of antimicrobial polymers, which mimic the functions and structures of naturally occurring antimicrobial peptides (AMPs). AMPs are components of the innate immune system and have proven effective against drug-resistant bacteria. The AMP-mimetic polymers of interest are random methacrylate copolymers with cationic and hydrophobic groups in the side chains, displaying cationic amphiphilic structures. These polymers exert their bactericidal effect by disrupting bacterial cell membranes, which contrast to conventional antibiotics. We have been investigating these polymers for potential therapeutic use because of their broad-spectrum activity, rapid bactericidal effect, and low propensity for resistance development in bacteria, which are the hallmarks of HDPs. The chemical and structural diversity of polymers will expand the possibilities for new antimicrobial applications including coatings, gels, and wound dressings with tailored activities. These synthetic polymers are cost-effective, suitable for large-scale production, and tunable for diverse applications, providing great potential as new therapeutic antimicrobial agents. In this talk, I will discuss the antimicrobial mechanism of polymers, as well as physicochemical parameters for the molecular design and engineering of antimicrobial polymers.
Antimicrobial Polymers and Peptides
Willy Chin, Jeremy Tan, Chuan Yang, Shaoqiong Liu, Ashlynn Lee, Xiyu Ke and Yi Yan Yang
Institute of Bioengineering and Nanotechnology, Singapore

The increased prevalence of antibiotic-resistant infections has led to an urgent need for the development of innovative antimicrobials. Macromolecular antimicrobial agents, such as cationic polymers and peptides, have recently received increasing attention because they can selectively target and disintegrate bacterial membranes via electrostatic interaction and insertion into the membrane lipid domains, avoiding potential bacterial resistance. As a result, a plethora of bio-inspired synthetic polymers and peptides have been proposed, and are achieving considerable success in overcoming many drawbacks found in using peptides. Unfortunately, toxicity has presented a significant problem during in vivo administration.

In this talk, biodegradable antimicrobial polymers and short beta-sheet forming synthetic peptides will be discussed. The antimicrobial polymers are based on biodegradable cationic polycarbonates, which are synthesized via organocatalytic living ring-opening polymerization. This synthetic platform yields polymers with well-defined molecular weight and structure, which are crucial for future clinical applications as individual molecular weight fractions of a polydisperse system are expected to exhibit distinct pharmacological activities in vivo. Polymers with various molecular configurations have been designed and synthesized. The polymers with optimal hydrophilicity/hydrophobicity balance have strong activity against multidrug-resistant Gram-positive and Gram-negative bacteria, as well as fungi without inducing significant toxicity both in vitro and in vivo. The optimized polymers and peptides have been tested in MRSA and P. aeruginosa infectious mouse models, and the results are promising. These antimicrobial polymers and peptides may potentially be used in the prevention and treatment of multidrug-resistant infections.
Molecular Characterization of Circulating Tumor Cells

Say Li Kong¹, Joyce An Yi¹, Igor Cima², Poh Koon Koh²,³, Kenneth J. H. Koh¹, Debarka Sengupta¹, Nur-Afidah Bte Mohamed Suhaimi², Wai Min Phyo², Jess Vo², Daniel Lee², Min Hu², Jackie Y. Ying², Daniel Tan², Iain B. Tan⁴, Min-Han Tan², Shyam Prabhakar¹, Paul Robson¹, Bing Lim¹ and Axel M. Hillmer¹

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Recent advances in cell enrichment, microfluidics and molecular biology technologies allow the detailed characterization of small cell populations down to the single cell level. Circulating tumor cells (CTCs) have the potential to provide a non-invasive means of assessing progressive cancers in real time during therapy, and further, to help direct therapy by monitoring genetic lesions over time. We use two approaches, a microsieve-based method and a spiral fluidics-based method, to enrich CTCs from the blood of patients with colorectal and lung cancer, respectively. Both cancers are among the most common malignancies in humans. I will present data of a gene-targeted sequencing approach and how sequence variants identified in CTCs correlate with mutations of the primary tumor of the same patient. Our study has implications on the use of CTC-derived molecular genetic data for patient management and provides insight into the technical challenges.
A Multigene Assay Identifying Distinct Prognostic Subtypes of Clear-Cell Renal-Cell Carcinoma with Differential Response to Tyrosine-Kinase Inhibition

Yukti Choudhury¹, Xiaona Wei¹, Ying-Hsia Chua², Lay Guat Ng³, Hui Shan Tan⁴, Valerie Koh³, Aye Aye Thike³, Eileen Poon⁴, Ng Quan Sing⁴, Chee Keong Toh⁴, Ravindran Kanesvaran⁴, Puay Hoon Tan³ and Min-Han Tan¹,⁴

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Patients with clear-cell renal-cell carcinoma (ccRCC) have divergent clinical outcomes in terms of survival and therapeutic response. Here, we aim to develop a practical molecular assay that can classify ccRCC patients into clinically relevant subtypes.

Whole genome expression analysis was performed on formalin-fixed paraffin-embedded (FFPE) material from a cohort of 55 ccRCC patients who underwent surgery at the Singapore General Hospital (SGH). An eight-gene expression assay operating on a quantitative PCR (qPCR) platform for FFPE material was developed. Prognostic performance of the predictor was evaluated in an independent cohort of FFPE samples (n=224) from the Singapore General Hospital, in RNA-sequencing data (n=419) from The Cancer Genome Atlas (TCGA) and a public microarray expression dataset from Van Andel Research Institute (n=174). For a subset of 48 patients who received receptor tyrosine-kinase inhibitor (TKI) treatment post-nephrectomy, association of clinical response with the qPCR classification was evaluated.

The eight-gene qPCR predictor separated patients into prognostic groups with differential cancer-specific survival times in three validation cohorts (SGH cohort: hazard ratio (HR) 4·44; 95% CI 2·53-7·81; p=1·48x10⁻⁸; TCGA cohort: (HR) 2·26 CI 1·59-3·21; p=3·06x10⁻⁷; VARI microarray dataset (HR) 2·19 CI 1·22-3·93; p=0·00743). For 48 patients receiving TKI treatment, the prognostic classification was associated with radiological response to treatment ([OR] 0·429 p=5·96x10⁻⁴), as well as increased progression-free survival on TKI treatment ([HR] 3·58 CI 1·16-11·03, p=0·019).

We have developed an eight-gene assay for prognostic classification of ccRCC into meaningful clinical prognostic subtypes. Of note, this classification is correlated with therapeutic response for patients receiving TKI therapy, representing both a prognostic and potentially predictive classification.
Pharmacogenetic Biomarkers for Clinical Use
Ming Ta Michael Lee
RIKEN Center for Integrative Medical Sciences, Japan and Academia Sinica, Taiwan

Genetic markers have been identified to be associated with drug-induced adverse reactions, and this has prompted the US to include genetic information in drug labels. This presentation will focus on the genetic associations identified in Asia, such as HLA-B*1502, which has been shown to associate with carbamazepine induced Stevens-Johnson syndrome. Toxic epidermal necrolysis and polymorphisms in VKORC1 and CYP2C9 are associated with warfarin dosage requirements. The clinical use of these genetic variants as biomarkers for predicting drug-induced adverse events or dosage requirement, and the difficulties of using these will be discussed. Other potential biomarkers for clinical use will also be described.

In addition to pharmacogenetic biomarkers, biomarkers for complex diseases, such as osteoarthritis will be discussed. I will present a strategy we are developing to study these diseases and the potential markers for the progression of diagnosis of these diseases.

Nanoprobe-Based Colorimetric Assay for HLA-B*15:02 Screening
Yanbing Zu, Min-Han Tan, Ee Chee Ren and Jackie Y. Ying
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2Singapore Immunology Network, Singapore

Human leukocyte antigen (HLA) allele, HLA-B*15:02, is a genetic risk marker for carbamazepine-induced Stevens Johnson Syndrome/Toxic Epidermal Necrolysis. It has been strongly recommended to conduct HLA-B*15:02 testing before starting treatment with carbamazepine. The adoption of the pharmacogenomic strategy in clinical settings requires an accurate and cost-effective screening assay.

Here, we report a nanoprobe-based colorimetric method for HLA-B*15:02 screening. Two PCR reactions and four plasmonic nanoprobes have been designed to achieve high specificity. The method has been validated using 40 selected human genomic DNA samples, among which 14 samples were positive and the others bore alleles most similar to HLA-B*15:02 in sequence. The study demonstrated that the PCR primers and the nanoprobes were highly specific in recognizing their targets, allowing the differentiation of HLA-B*15:02 from other similar alleles. The assay also ensured the same phase of the targeted gene sites, ruling out combination ambiguities. The nanoprobe-based assay can be implemented easily in molecular diagnostic laboratories for highly specific and cost-effective HLA-B*15:02 screening.
Digital PCR: Speeding the Use, Easing the Flow and Spurring the Growth

Johnson Ng
JN Medsys, Singapore

Digital polymerase chain reaction (dPCR) is a powerful tool for detecting nucleic acids at very high sensitivity and precision, and is particularly useful for the detection of rare mutations and copy number variations. It works by partitioning a typical PCR reaction into thousands of smaller sub-reactions, such that each has at most a single copy of DNA. As a result, dPCR experiments usually require a long turnaround time, cumbersome workflow, and multiple dedicated instruments and consumables that can be very costly. These limitations make it less compelling for researchers to switch from qPCR to dPCR despite the potentially superior performance of the latter. At JN Medsys, we put ourselves in the users’ shoes and strived to make the dPCR technology better so it can be more widely adopted. The end result is an affordable, high-throughput dPCR system with short turnaround time and easy workflow. Through that, we aim to make performing dPCR as easy as qPCR, while achieving superior sensitivity and precision. This, we hope, would spur the widespread adoption of a very promising technology that would in turn improve the outcomes of life-threatening diseases such as cancer.

Nanosensors with Graphene and Functional Nucleic Acids

Yingfu Li
McMaster University, Canada

My research group is interested in functional nucleic acids, particularly their application as biosensing materials. Functional nucleic acids are single-stranded DNA or RNA sequences that are capable of carrying out ligand binding (aptamers and riboswitches), catalysis (ribozymes and DNAzymes) or both functions (aptazymes). They can be isolated from random-sequence DNA or RNA pools using “in vitro selection”, a Darwinian-like test-tube evolution process. Functional nucleic acids can be combined with nanomaterials for the design of bioanalytical systems with highly advanced functions. In this presentation, I will describe a versatile aptamer-graphene based biosensing platform that is capable of achieving ultrasensitive detection of biological targets. The system features three essential components: reduced graphene oxide (rGO) for its ability to absorb single-stranded DNA molecules non-specifically, DNA aptamers for their ability to bind rGO but undergo target-induced conformational changes that facilitate their release from the rGO surface, and rolling circle amplification (RCA) for its ability to amplify a primer-template recognition event into repetitive sequence units that can be easily detected. The addition of a cognate target for the graphene-absorbed aptamer triggers a bioanalytical relay – the formation of aptamer-target complex, the release of the aptamer probe from rGO, the capture of the probe by a circular DNA template, and round by round copying of the circular template by phi29 DNA polymerase. The broad utility of the platform is illustrated through engineering sensors that are capable of achieving ultra-sensitive detection of protein, DNA and small-molecule analytes.
Non-Invasive Sensitive Detection of KRAS and BRAF Mutation in Circulating Tumor Cells of Colorectal Cancer Patients

Nur-Afidah Mohamed Suhaimi¹, Yu Min Foong¹, Daniel Yoke San Lee¹, Wai Min Phyo¹, Igor Cima¹, Esther Xing Wei Lee¹, Wei Lin Goh², Wei-Yen Lim³, Kee Seng Chia³, Say Li Kong⁴, Gong Min⁴, Lim Bing⁴, Axel M. Hillmer⁴, Poh Koon Koh¹,2, Jackie Y. Ying¹ and Min-Han Tan¹,5

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The characterization of genetic alterations in tumors serves as useful biomarkers in prognosis and treatment management. However, mutation analysis of clinical samples involves the use of invasive tissue biopsies. Circulating tumor cells (CTCs) obtained non-invasively from peripheral blood could provide an alternative source of malignant cells. Using a label-free CTC enrichment strategy that we have established previously, we report the development of two methods for qualitative assessment of tumor genotype in CTCs.

Blood samples from 44 colorectal cancer patients were enriched for CTCs using a size-based microsieve technology. To screen for mutations, we established the high-resolution melt (HRM) and multiplex allele-specific PCR (ASPCR) KRAS-codon 12/13- and BRAF-codon 600- specific assays, and compared the assays’ performance with pyrosequencing and “gold-standard” Sanger sequencing. The resulting CTC genotypes were compared with corresponding tumor from each patient. To ensure specificity in the assays, blood samples from 18 healthy donors were similarly processed.

Both HRM and ASPCR could detect as low as 1.25% KRAS- or BRAF- mutant alleles. HRM detected 14 (31.8%) out of 44 patients with KRAS mutation in CTCs, while 5 (11.3%) patients were found to have BRAF mutation. ASPCR detected CTCs with KRAS and BRAF mutations in 10 (22.7%) and 1 (2.3%) out of 44 patients, respectively. Comparing tumor tissues and CTCs mutation status, we observed 84.1% concordance in KRAS genotype (p=0.000129, Fishers’ exact test; OR=38.7, 95% CI=4.05 to 369) and 90.9% (p=0.114) concordance in BRAF genotype. For patients with discrepant mutation status, the pyrograms from pyrosequencing confirmed the validity of the tumor mutation. The HRM approach demonstrates a sensitivity of 100% and specificity of 91.4% and 95.3% for KRAS and BRAF assays respectively.

We present the economical and sensitive HRM and ASPCR methods to scan for mutations rapidly in CTCs, allowing non-invasive qualitative assessment of tumor genotype.
Orthotopic Caecal Implants for the Creation of Patient-Derived Cancer Xenografts

Hao Yun Yap\textsuperscript{1,2}, Sharon Heng Yee Choy\textsuperscript{3}, Sathivel Ponniah\textsuperscript{3}, Poh Koon Koh\textsuperscript{1,4}, Jackie Y. Ying\textsuperscript{1} and Min-Han Tan\textsuperscript{1}

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Patient-derived xenografts (PDX) are key clinical models for cancer research. The most common method for creating a PDX is usually by surgically implanting the tumor sample subcutaneously in genetically immunocompromised mice. Unfortunately, subcutaneous tumor engraftment rates are less than ideal and the different microenvironment may lead to different PDX tumor growth characteristics as compared to orthotopic implantation. We describe our early experience in creating orthotopic PDXs for colorectal cancer.

We obtained intra-operative tumor samples for 12 patients who underwent curative resection surgery for colorectal cancer. 4x4 mm fragments of samples were surgically implanted into the caecum of SCID mice and subsequently monitored for tumor growth and associated complications. The tumor growth characteristics and survival of these mice were recorded over time.

25 mice received caecal implants of colorectal tumors. The median follow up time was 132 days. 8 mice died over this period, due to non-tumor causes. During the experimental period, 4 mice developed intra-abdominal tumors more than 2 cm and had to be euthanized and their tumors propagated. Histology showed that these tumors were consistent with colorectal carcinoma.

Direct orthotopic tumor implantation is a feasible method of creating PDXs that replicate the original disease with high fidelity. A high level of surgical expertise and intensive animal care is required to ensure maximal success. These PDXs represent crucial tumor models for use in cancer research, particularly therapeutics.
Efficient Identification of Binding Peptides by Ultra-High-Throughput Screening of Bead-Based Peptide Libraries

Jaehong Lim, Joo-Eun Jee, Liqian Gao, Jessica Oon, Yong Siang Ong and Su Seong Lee
Institute of Bioengineering and Nanotechnology, Singapore

The combinatorial one-bead-one-compound (OBOC) peptide libraries are attractive particularly because of their capability to easily generate a vast number of structural diversity based upon a robust chemistry platform. This versatile tool is, however, not utilized as much as other alternatives for affinity screening, such as phage display and aptamers, not to mention the conventional antibody technology. Only a few limited cases have been reported to date, presumably due to scarcely known limitations of the bead-based screening. As a matter of fact, there is no reliable high-throughput approach to expedite the entire screening process. Therefore, we have established an ultra-high-throughput screening platform by automating time-consuming steps, such as library construction, bead sorting and peptide sequencing. With efforts dedicated to identifying the residual problems in the bead-based screening, we have found that one serious drawback lies in various types of non-specific interactions arising from employing polymeric beads as solid support and fluorescence label. Amongst such non-specific/undesired interactions, the fluorescence dyes labelled to target proteins turned out to significantly affect the screening results, causing dominant occurrence of false positives. Recent progress in optimizing each module of the screening campaign will be introduced along with an overview of our current efforts to apply the bead-based screening to several applications, such as protein capture agents for diagnostics and inhibitor development, among others.

Protein Capture Agents for CRP

Jessica Oon, Jaehong Lim, Joo-Eun Jee, Liqian Gao, Yong Siang Ong and Su Seong Lee
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This paper presents the development of binding peptides with high affinity and specificity against C-reactive protein (CRP) using our high-throughput screening platform for bead-based peptide libraries. CRP is a clinically important biomarker in the blood to diagnose inflammation. Via the screening of a 6mer comprehensive bead-based library and subsequent optimization by alanine scanning, suitable 6mer binding candidates were obtained. To further improve binding affinity and specificity, a simple elongation method was carried out on an optimized 6mer ligand. Preliminary tests by SPR, immuno-precipitation, micro-array and bead-based validation were carried out on the hit 9mer peptides. The 9mer ligand with the highest target-binding/non-specific binding ratio was selected for further enhancement of binding affinity and specificity. Using the 9mer candidate as the anchor ligand, several methods were used for the generation of 14mer bi-ligands. These include the screening of a core-shell library and the screening of an encoded, specially designed, dual library. The 14mer bi-ligand candidates were validated by Immuno-precipitation against human serum. The 14mer bi-ligand from the dual-library screening was found to show much improved binding affinity and specificity to CRP, as compared to the original 9mer anchor ligand. Furthermore, the 14mer bi-ligand also showed very similar binding to human serum as a commercial anti-CRP antibody. This result demonstrates that our high-throughput screening method is a very useful tool for the development of stable protein capture agents for biomarkers in a rapid and robust manner.
Simultaneous Determination of Three-Type Bone Turn Over Markers Using an Integrated Automatic Electrochemical Immunosensor

Hyeyoun Kim, Kook Jin Jang, Kooknyung Lee and Min-Ho Lee
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An integrated automatic electrochemical immunosensor has been designed for the simultaneous detection of three bone turn over markers (osteocalcin (OC), parathyroid hormone (PTH), and vitamin D (VitD)). Three different markers were immobilized onto custom-made working electrodes using covalent bonds. The detection is based on current changes caused by the amount of antigen-antibody reaction using a 3-electrode system. The developed prototype is composed of a connecting probe, working stage with three sample holders, and a main control unit. After reaction of each antigen, the current of each electrode was measured simultaneously with three different channels. The dynamic range of detection of the sensors were 0.1~20 ng/mL, 0.5 ~ 200 ng/mL and 0.01 ~ 100 ng/mL for OC, PTH, and Vit D, respectively. The imprecision (CV) of between-run and within run test show less than 3% and 5%. The sensitivity and selectivity of the test were measured to be 3% and 5%, respectively. The developed immunoassay system offers promising results when applied in real serum samples obtained from the patients. In addition, the developed system provides promise for simple, rapid, and cost-effective analysis of multi-analytes.

Rapid Quantification of Bioaerosols Containing Influenza Antigen by Air Sampler and Lateral Flow Immunoassay Reader System

Young Tai Seo, Kook Jin Jang, Kook Nyung Lee and Min-Ho Lee
Korea Electronics Technology Institute, Korea

The recent outbreaks of influenza received much attention for their rapid and worldwide spread, and fatalities. In order for proper measures to be implemented, continuous monitoring of influenza and its early detection are pivotal. An integrated automatic detection platform has been designed for the continuous monitoring of influenza. Rapid detection methods are significantly essential to combine sampling of bioaerosols with analysis for specification and quantification of influenza in air. The cyclone separator was applied for the collection of airborne influenza antigens, a device designed and made for sampling with a high sampling rate (1000 L/min). The aerosol was generated by a nebulizer and a fluidic control system was implemented using a micro diaphragm liquid pump and 3-way solenoid valves. Quantification was achieved by the lateral flow assay sandwich-based immunoassay cartridge and reader system connected to the air sampler. The efficiency of sampling was examined by nebulizing influenza in a chamber and quantifying it with a digital reader system after sampling. The influenza types H1N1 and H3N2 were diluted with a ratio of 1:2, 1:3, 1:4, 1:6 and 1:8, and measured for quantification, which is good for bioaerosol measurements in the environment and interior.
Heat-Shrunken Hierarchical Silica Nanomembrane for High Quality DNA Purification

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Here we describe a novel method to fabricate hierarchical silica nanomembrane by making use of heat-shrinkable polyolefin (PO) films. First, silica was deposited onto the PO film using electron beam vapor deposition. Then the silica-coated PO film was baked in a convection oven to induce shrinking, hence surface wrinkling. The PO film retracts to less than 10% of its original size and the resulting silica membrane exhibits distinctive hierarchical structures ranging from nanometer to micrometer, which are determined by the thicknesses of silica deposition. With 2 nm silica, the membrane displays only micro-scale ridges. At 20 nm, nanowrinkles start to appear on the ridges, forming overlaying hierarchical nanostructures. When the silica layer reaches 100 nm, a large number of silica flakes emerge and interweave with the micro ridges.

To evaluate the efficacy of the silica nanomembrane as a solid substrate for DNA extraction, we compared the quality of DNA isolated using the nanomembrane to those isolated using commercial magnetic silica particles in three aspects: DNA recovery yield, purity and integrity. With 4 μg genomic DNA input, we were able to recover about 80% DNA using 200 nm silica nanomembrane. In comparison, commercial silica magnetic particles only recovered about 20% under the same condition. DNA isolated using silica particles usually suffer from contamination due to the carryover of the lysis/binding buffer and washing buffer into the final eluent, as evidenced by small 260/280 ratios. In comparison, the 260/280 ratio of DNA recovered using nanomembrane was very close to the ideal value 1.8, and the 260/230 ratio was similar to the control DNA, suggesting high purity. Moreover, DNA isolated by magnetic particles were often sheared into small fragments due to mechanical stress. In contrast, DNA isolated using silica nanomembrane were able to retain their integrity when analyzed using gel electrophoresis.
The Application of Conjugated Polymer Nanodots as Long-Term Trackers

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The two remarkable features of mesenchymal stem cells (MSCs), multipotency and self-renewal, as well as ease of isolation and expansion, rapidly positioned them as promising therapeutic agents in regenerative medicine. There has been a rapid surge in clinical trials involving stem cell therapies over the last few years and those trials are establishing the clinical pathways for an emergent new medicine. However, the identity in vivo, heterogeneity, anatomical localization and functional roles in adult tissue homeostasis have remained enigmatic, due to the lack of sufficient technologies to monitor MSC fate in vivo. Herein, we synthesized conjugated polymer (CP) nanodots as the fluorescent trackers with high stability, brightness and low cytotoxicity for tracking of MSCs to reveal their in vivo behaviours. The CP nanodots have shown significantly better long-term tracking ability as compared to a commercial quantum dot tracker without compromising the features of MSCs in terms of proliferation, migration and differentiation. Fluorescence imaging of tissue sections from full-thickness skin wound-bearing mice transplanted with CP nanodot-labelled MSCs suggested that the transplanted MSCs only remained in the regenerated dermis and the paracrine signalling is the predominant contribution to promote skin regeneration.
**A Versatile and Robust Xeno- and Serum-Free Cultivation System for Human Pluripotent Stem Cells**

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Pluripotent stem cells (PSC) have traditionally been cultured on mouse embryonic feeder (MEF) cells which contribute to the maintenance of pluripotency and deposit extracellular matrix components conferring cell attachment. Though constituting a relatively robust cultivation environment when monitored rigorously, mEF-based systems are prone to lot-to-lot variances. Furthermore, the xenogeneic nature of mEF cells and commonly used media components is not compliant with current efforts to establish clinically compatible protocols for the maintenance and differentiation of PSC. Different compositions have been devised in order to maintain pluripotency in feeder-independent conditions. However, most media require extensive adaption periods when cells are transferred from feeder-dependent to feeder-free culture conditions. We have optimized a xeno- and serum-free media formulation that a) allows rapid adaption to feeder-free conditions, i.e. culture on Matrigel or Vitronectin, b) enables robust and efficient expansion of bona fide PSC as single cells, as well as cell-clusters for more than 10 passages while maintaining pluripotency as evidenced by marker expression, in vitro differentiation potential, teratoma formation and karyotyping, c) allows rapid culture initiation after cryopreservation and genetic manipulation, and e) supports episomal reprogramming of human fibroblasts. Furthermore, preliminary observations suggest that the medium supports formation of aggregates from single cell inoculated hPSCs and their expansion in suspension culture. The formulation will allow a rapid translation into a clinical grade medium designed following the recommendations of USP <1043> on ancillary materials and will be suitable for clinical grade expansion of PSC.
Differentiation of Corneal Endothelial-Like Cells from Human Embryonic Stem Cells

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The corneal endothelium, an essential part of the cornea, is composed of a single layer of hexagonal cells that line the innermost layer of the cornea. Corneal endothelial cells (CEC) regulates the corneal hydration, contribute to transparency and optimal visual functions. Damage to CEC leads to loss of transparency and results in severe visual impairment. Corneal transplantation by donor cornea is currently the only method to replace CEC, as these cells exhibit a very limited repairing capacity. Since corneal transplantation is on the rise worldwide, stem cell therapy is one of the promising alternatives to the limited donor cornea. Here we demonstrated the differentiation of human embryonic stem cells (hESC) to CEC-like cells by a 3-step process. First, hESC were differentiated to ectodermal neural crest cells (NCC) using an established protocol. These NCC were further differentiated to periocular mesenchymal precursor cells (POMP) by a defined protocol. Expression of gene markers FOXC1, PITX2 confirmed the generation of POMP. Further, the differentiation of CEC-like cells from POMP cells and their characterization will be discussed.

Patterned Tissue Constructs by Assembly of Polyelectrolyte Hydrogel Fibers

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Micro-patterning technologies are emerging as powerful tools to form three-dimensional (3D) cellular constructs for applications in tissue engineering and regenerative medicine. These technologies, coupled with hydrogel encapsulation, can provide cells with a hydrated 3D microenvironment that mimics the native extracellular matrix. In our lab, we have developed a micro-patterning technique using biodegradable hydrogels formed by the assembly of interfacial polyelectrolyte complexation (IPC) fibers. In IPC, two droplets of oppositely charged polyelectrolytes form an interface, at which the neutralized complexes coalesce to form a hydrogel fiber. The advantage of this cell encapsulation technique is that the fibers are produced under aqueous conditions at room temperature, which makes it amenable to incorporation of biological components inside the fibers. We will describe the use of these fiber-assembled hydrogels to create pre-vascularized tissue constructs and fabricate biomimetic tissue units for in vitro drug screening.
Electrospun Nanofibrous Biocomposite Mats for Post-Menopausal Wound Tissue Engineering

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Post-menopausal wound care management is a substantial burden on health services, since there is an increased risk of developing a number of degenerative pathological conditions, linked by the common theme of excessive inflammation. The controlled estrogen replacement can accelerate healing of acute cutaneous wounds, linked to its potent anti-inflammatory activity. Wound dressing from electrospun nanofibers potentially offers many advantages over conventional processes. Generally, the ultimate goal of the nanofiber design is to provide an ideal structure that can replace the natural extracellular matrix until the host cells can grow and synthesize a new natural cellular matrix. In addition, the unique electrospinning process can be invoked to impregnate the nanofiber membranes with desired therapeutic agents. So here we introduce a new material for wound tissue dressing, in which a composite nanofibrous wound dressing material loaded with β-estradiol was obtained through electrospinning. This study involves the characterization of these nanofibers and analysis of cell growth and proliferation to determine the efficiency of tissue regeneration on these biocomposite polymer nanofibrous scaffolds and to study the possibility of using it as a potential wound dressing material in the in vivo models.

Modification of PLGA Nanofibrous Scaffold by Electron Beam Irradiation for Soft Tissue Engineering

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Biodegradable poly(lactide-co-glycolide) (PLGA) has found widespread use in modern medical practice. However, its degradation rate is relatively slow for soft tissue regeneration, especially for the restoration of skin and mucosa. The purpose of this study is to examine the effect of electron beam irradiation on the degradation behavior of PLGA nanofibers. PLGA (50:50) nanofibrous mats were prepared by electrospinning, and were electron beam irradiated at radiation doses of 50, 100, 150, 200, 250 and 300 kGy. A control PLGA nanofiber (0 kGy) and electron beam irradiated (50, 100, 150, 200, 250 and 300 kGy) PLGA nanofibers were degraded hydrolytically in phosphate-buffered saline solution at 37°C for 7 weeks. The degradation behavior of the PLGA nanofiber was studied by measuring the changes in weight, FTIR spectra, surface morphology and physical properties. The results showed that the morphology of the PLGA nanofibrous mat was not deformed after electron beam irradiation, but an increase of hydroxyl (OH) group peak intensity with increasing radiation dose during degradation experiments indicates that hydrolytic degradation is accelerated by electron beam radiation. From these results, it can be concluded that the biodegradation behavior of PLGA nanofibrous scaffolds can be modulated by electron beam irradiation with varying irradiation dose.
Substrate Stiffness Modulates the Maturation of Embryonic Stem Cell–Derived Cardiomyocytes and Hepatocytes

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The ability to generate mature cells from renewable sources, such as pluripotent stem cells, offers the promise of revolutionizing in vitro toxicity testing and drug screening. However, cells obtained by differentiating embryonic stem cells have an immature fetal/neonatal-like phenotype. We hypothesized that biomechanics, i.e. the afterload for cardiomyocytes and the tissue stiffness for hepatocytes, plays a crucial role in their maturation. Both the above environmental parameters can be mimicked in vitro by culturing cells on substrates of varying stiffnesses.

For mouse embryonic stem cell–derived cardiomyocytes (ES-CMs), we indeed found that culture on compliant substrates that were softer than tissue culture plastic led to enhanced maturation of ES-CMs and enabled up to 15% shortening. The ES-CMs obtained via such dynamic resistance training generated adult CM-like forces, and after 6 weeks of culture on these substrates, we detected cells forming a mature transverse tubule network. This is the first study to demonstrate these properties in ES-CMs and provides proof-of-concept for using this approach to obtain mature human ES-CMs.

The stiffness of the human liver is around 10 kPa. However, for substrates with stiffness less than 50 kPa attachment of human embryonic stem cell–derived hepatocytes (hES-Hs) was not observed. For substrates with a Young’s modulus between 50 and 200 kPa hES-Hs formed compact colonies, whereas on tissue culture polystyrene they formed a diffuse monolayer. Expression of several cytochrome enzymes as well as albumin production was inversely related to the substrate stiffness and was most similar to primary human hepatocytes on substrates with stiffness most similar to human liver tissue i.e. 50 kPa substrates. For ES-Hs cultured on 50 kPa substrates for 12 days, treatment with β-napthoflavone led to a 6-fold induction of Cyp1A2. These results indicate the importance of replicating in vivo biomechanics for maturing cells obtained from embryonic stem cells.
**Formation and Stability of Interpenetrating Polymer Network Hydrogels Consisting of Fibrin and Hyaluronic Acid for Tissue Engineering**

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Fibrin gel is widely used as a tissue engineering scaffold. However, fibrin gel has poor mechanical property which results in rapid contraction and degradation. Several strategies have been developed to improve the mechanical property of fibrin gel, including the optimization of polymerization condition (e.g. pH, ionic strength, calcium, fibrinogen and thrombin concentrations) and the formation of composite materials consisting of a porous synthetic scaffold filled with fibrin gel. In this study, an interpenetrating polymer network (IPN) hydrogel composed of fibrin and hyaluronic acid-tyramine (HA-Tyr) was developed. The fibrin network was formed by cleaving fibrinogen with thrombin, producing fibrin monomers that polymerize rapidly. The HA network was formed through the coupling of tyramine moieties using horseradish peroxidase (HRP) and hydrogen peroxide ($\text{H}_2\text{O}_2$). Fibrin-HA-Tyr IPN hydrogels with different storage modulus ($G'$) were formed by tuning the crosslink density of HA-Tyr network. The crosslink density of HA-Tyr network was controlled by varying the amount of $\text{H}_2\text{O}_2$, as demonstrated in previous studies. While fibrin gels were completely degraded in the presence of plasmin and contracted when embedded with cells, the shape of IPN hydrogels were maintained due to structural support from the HA-Tyr network. Cell proliferation and capillary formation occurred in IPN hydrogels and were found to depend on the $G'$ of the hydrogels. The results suggest that fibrin-HA-Tyr IPN hydrogel is a potential alternative to fibrin gel as a scaffold for tissue engineering applications that require shape stability.

**Enzyme-Mediated Hyaluronic Acid-Tyramine Hydrogels for 3D Propagation of Human Embryonic Stem Cells**

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The propagation of human embryonic stem cells (hESCs) in three-dimensional (3D) scaffolds is highly desirable in regenerative medicine. Herein, we report an enzyme-mediated hyaluronic acid-tyramine (HA-Tyr) hydrogel that encapsulated and propagated hESCs in 3D. HA-Tyr hydrogels were formed by crosslinking tyramine moieties with the use of horseradish peroxidase (HRP) and hydrogen peroxide ($\text{H}_2\text{O}_2$). The stiffness of the hydrogel was finely tuned by varying the concentration of the $\text{H}_2\text{O}_2$. The hydrogel with a storage modulus of ~60 Pa supported the proliferation of hESCs in a chemically-defined medium, and biochemical and histological analysis revealed that hESCs proliferated well and formed spheroid structures in 3D, without undergoing apoptosis. The hESCs released from the hydrogels highly expressed CD44 and pluripotency markers. These cells exhibited the capability to form cell derivatives of all three embryonic germ layers in vitro and in vivo. In addition, the genetic integrity of the hESCs was unaffected in the 3D cultivation system.
Fabrication of an Injectable Hydrogel Made of Modified CMC and Pullulan as Anti-Adhesion Barrier
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In the field of surgical operation, bad post-operative adhesions cause pelvic pain, infertility and intestinal obstruction. Despite much interest in preventing post-operative adhesion, these symptoms persistently appear. That is why we need to insert a physical barrier between the abdominal walls and organs. There are many types of tissue adhesion barriers, such as film, nanofiber and hydrogel. In particular, hydrogel barriers have many advantages such as biodegradability, non-toxicity, non-preform, flexibility, and high permeability of oxygen and nutrients. We have developed an in situ injectable crosslinked hydrogel fabricated by modified carboxymethyl cellulose (CMC) with tyramine and subsequent enzyme reaction using horseradish peroxidase (HRP) and \( \text{H}_2\text{O}_2 \), and subsequently added pullulan into the hydrogel solution for improving adhesive strength and accelerating biodegradation. The modified CMC with tyramine was confirmed by ATR-FTIR. Gelation time ranged from 15 s to 90 s, depending on the concentration of HRP and \( \text{H}_2\text{O}_2 \), and the biodegradation behavior was observed by weight loss and SEM images of the gel. The swelling ratio of the hydrogel containing less pullulan is higher than the one with more pullulan. These results show that pullulan increases the surface area of the hydrogel by diffusing water, leading to accelerated biodegradation. In in vivo animal tests, post-operative tissue adhesion was dramatically decreased by applying in situ injectable hydrogel. From these results, we concluded that the injectable hydrogel made of modified CMC and pullulan has great potential for use as an anti-adhesion barrier.

Crosslinked Ultra-Short Peptide Hydrogel Dressings for Wound Healing
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There are more than 35 million cutaneous wounds that require urgent medical attention annually in America alone. As such, wound healing places a huge strain on healthcare systems worldwide. The premise for using hydrogels in wound repair is that it offers a moist environment conducive for healing. Our group focuses on the development of ultrashort (3-7 residues of naturally-occurring amino acids) peptide hydrogels. Previously, a pioneer cysteine-containing candidate was introduced. After disulfide crosslinking, these gels became stiffer and were better able to maintain their shape after water immersion. Now, a library of crosslinked peptide hydrogel candidates has been further generated. A sequence (LIVAGKC or LK6C) optimal for moist wound healing in terms of gel transparency, ease of handling and cost saving was then evaluated in vivo. Circular dichroism experiments revealed that disulfide bond formation presumably kept the fibers (tens of nm in diameter) together and accounted for the ability of crosslinked gel to survive intact after water immersion. LK6C had limited allergenic potential as it failed to provoke any sensitivity when administered to guinea pigs in the Kligman maximization test. More importantly, when applied topically to mice inflicted with full-thickness excision wounds, the peptide hydrogel significantly (\( p < 0.05 \)) potentiated healing and re-epithelization compared to a commercially available gel product. The peptide hydrogel is thus safe for topical application and promotes a superior rate and quality of wound healing.
De Novo Design and Experimental Characterization of Ultrashort Self-Associating Peptides
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Self-association is a common phenomenon in biology and one that can have positive and negative impacts, from the formation of architectural cytoskeleton of cells to the formation of fibrils in amyloid diseases. Understanding the nature and mechanisms of self-association is important for modulating these systems and in creating biologically-inspired materials. Based on a de novo peptide design framework that can evaluate self-associating peptide systems, the self-association propensities of six tripeptides (Ac-LVE, Ac-YYD, Ac-LLE, Ac-YLD, Ac-MYD, Ac-VIE) were evaluated and compared. Self-association and electron microscopy studies revealed that Ac-LLE formed bead-like microstructures in solution, Ac-LVE and Ac-YYD formed fibrillar aggregates, Ac-VIE and Ac-MYD formed hydrogels, and Ac-YLD crystallized under ambient conditions. These results demonstrate that the intricate interplay between physical properties influence the self-association of the tripeptides. An X-ray crystallographic study was carried out on a single crystal of Ac-YLD, which revealed that each molecule adopts a β-strand conformation that stack together to form parallel β-sheets. A water molecule links three molecules of Ac-YLD via hydrogen bonds. We expect that this peptide design framework will find future application in creating novel self-associating peptides based on unnatural amino acids, and inhibitor peptides of detrimental self-aggregating biological proteins.
Engineering Block Copolymer Self-Assembly with PEGylated Aliphatic Polycarbonates for Biomedical Applications

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The ability to incorporate desired functionalities onto a biodegradable polymer allows for precise engineering of material properties and thereby greatly expands their scope and applicability. Amongst different classes of degradable polymers, due to the relative ease in introducing physico-chemical functionalities, aliphatic cyclic carbonates have emerged as an important synthetic material. With the advent of transition metal-free, organo-catalytic ring opening polymerization (OC-ROP), well-defined polymers can be readily accessed without potential toxicity concerns associated with the trace metals. Concerted developments in the past decade on cyclic carbonate monomer syntheses and controlled ROP polymerization of these monomers have rendered aliphatic polycarbonates well-suited for biomedical applications.

Recently, in our laboratories, we have developed cholesterol- and fluorene-containing novel six-membered cyclic carbonate monomers in a step-efficient manner. By using poly(ethylene glycol) methyl ether of different molecular weights as the macro-initiator and also by varying the degree of polymerization of the functional polycarbonate block, series of amphiphilic diblock copolymers were obtained via OC-ROP process. Aqueous self-assembly of these PEGylated amphiphilic polycarbonates were investigated. Depending upon the actual composition, these block copolymers self-assembled to form nanostructures of different shapes, ranging from conventional (spherical micelles, rod-like elongated micelles) to unconventional (disk-like micelles, stacked-disks and lamellar-sheets) morphologies. Apart from tailoring the aqueous self-assembly, polymer composition can also be tuned for drug delivery applications. With optimal hydrophobic block composition of cholesterol-containing polymers, paclitaxel, a hydrophobic anticancer drug was well encapsulated into nano-size micelles (hydrodynamic diameter = 36 nm; PDI = 0.07; drug loading content =15 wt. %).

In this presentation syntheses and characterization of these functional polycarbonates and their aqueous self-assembly behaviors will be discussed. Application of some these materials as a versatile drug delivery vehicle for hydrophobic drugs and also extension of these materials to ABA-type triblocks to form hydrogels will also be highlighted.
Effects of Surface Functionality on Intracellular Transport of Polymer Particles
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The intracellular transport behavior and cellular uptake mechanism of nanocarriers is essential for the design of efficient and effective drug delivery vectors. In this study, we employed single particle tracking (SPT) and cell function inhibition to investigate the behavior of polystyrene beads with surface functional groups being amine, carboxyl and TAT-peptide, designated as PS-NH₂, PS-COOH, and PS-TAT, respectively. We found from particle tracking that for a short incubation time, more PS-TAT underwent directed motion along the microtubules with higher velocity than both PS-COOH and PS-NH₂. With prolonged incubation, however, the proportion of PS-COOH undergoing directed motion was significantly enhanced from 18.9% to 44.1%. Macropinocytosis has been found to be the major internalization pathway for PS-TAT, and the kinetics of ensuing intracellular transport is faster as reflected by a higher proportion of particles undergoing a directed motion associated with microtubules for the short incubation time. In contrast, caveolae-mediated endocytosis plays a large part for the entry of PS-COOH, thereby leading to a delayed onset of the directed motion. Moreover, our results suggested that positive charge might not be a dominant factor for the efficient transport of TAT-peptide in cells.

Anticarcinogenic Effect of Curcuminoid Loaded Polymethyl Methacrylate Nanoparticles
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Curcuminoids, oleoresins from Curcuma longa L., have known anti-carcinogenic and anti-inflammatory properties but high toxicity, poor aqueous solubility and susceptibility to degradation in body fluid are deterrents to its clinical administration. Poly (methyl methacrylate) nanoparticles (PMMA-NPs) are biocompatible, resilient and can entrap hydrophobic drugs. The present investigation is related to solubilizing curcuminoids by incorporating them in these NPs and a study of the anticarcinogenic effect using lung cancer (A549) cell line. PMMA-NPs were prepared using emulsion polymerization and the freshly extracted oleoresins were post loaded in well dispersed NPs. The presence of the three component oleoresins was confirmed by thin layer chromatography. The size and morphology of void and loaded NPs were determined by DLS, SEM and TEM. The NPs were spherical with size 192.66 ± 5nm (void) and 199.16 ± 5nm (loaded). Drug loading and encapsulation efficiency were 6% and 93%, respectively. From the FTIR spectra the characteristic absorption vibration of PMMA and the bands at 1383 cm⁻¹, 1233 cm⁻¹ and 962 cm⁻¹ for curcuminoid moiety were observed. Drug release up to ten days was estimated in buffer, saline and serum. The highest release of around 50% was noted in serum within 3 days. The in vitro anti-cancer activity of loaded drug was evaluated up to 72 hours by MTT assay using A549 cell line. Cell uptake of dye-loaded NPs was visualized within 30 minutes of incubation. The results revealed that the dose- and time-dependent cell death in case of loaded PMMA-NPs was comparable to that of free curcuminoid. According to the study, the drug-loaded PMMA-NPs appear to be highly suitable for effective, localized and safe chemotherapy.
Sensitization of Cancer Cells via Non-Viral Delivery of Apoptosis Inducing Proteins Using a Cationic Bolaamphiphile

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Non-viral protein delivery systems are extremely important in modulating cell behavior without causing genetic alterations. Here, we investigate the use of a protein delivery system based on a cationic bolaamphiphile to sensitize cancer cells towards apoptosis-inducing drugs as a novel approach for cancer therapy. We demonstrated the efficacy of the system by two strategies. The first strategy involved delivery of a survivin antibody to inhibit survivin activity. As survivin functions to inhibit apoptosis and is specifically expressed in cancer cells, its inhibition by antibodies resulted in sensitization of the cells to the cancer drug, doxorubicin. The IC$_{50}$ of doxorubicin was reduced ~2.5 fold after delivery of survivin antibodies to breast cancer cells and induction of apoptosis was shown by western blotting with apoptosis specific antibodies. Cancer cells could also be sensitized by protein delivery via a second pathway. Delivery of functional wild type p53 into p53-null liver cancer (Hep3B) cells reversed the cancer phenotype, sensitizing the cells towards the p53 pathway drug, Nutlin. Nutlin reduced the viability of Hep3B cells by ~42% at 15µM concentration, demonstrating the effectiveness of p53 delivery. The expression of p21, a downstream target of p53 further confirmed the functional status of the delivered protein.
PD-L1 siRNA Delivery by Folic Acid-Functionalized PEI Enhances Tumour Targeted Killing of CAR T Cells for Ovarian Cancer Immunotherapy

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Epithelial ovarian cancer (EOC) remains the most lethal gynecological cancer, emphasizing the need for better therapies. Adoptive immunotherapy involving the use of T cells expressing chimeric antigen receptors (CARs) is a promising strategy for the treatment of EOC. However, immunosuppressive mechanisms brought about by interactions between signalling molecules, such as programmed death molecule-1 (PD-1) and its ligand PD-L1 expressed on activated T cells and tumour cells respectively, dampen T cell responses. The aim of this study was to block PD-1/PD-L1 interactions by the delivery of PD-L1 siRNA, using various folic acid (FA)-functionalized polyethylenimine (Mn 10 kDa) PEI, to SKOV-3-Luc EOC cells. PEI was successfully modified with various functional groups (FA, polyethylene glycol (PEG), PEG-FA) using either EDC/NHS conjugation or the established methyl-carboxytrimethylene carbonate (MTC) platform to form polymers with well-defined compositions (PEI-FA, PEI-PEG and PEI-PEG-FA). The polymer/siRNA complexes were characterized for particle size, zeta potential, cytotoxicity, PD-L1 knockdown efficiency and specificity of uptake into cancer cells when challenged with the presence of PBMCs. SKOV-3-Luc cells with PD-L1 knockdown were then treated with CAR T cells targeted against the extended ErbB family (T4 cells), which is widely expressed on EOC cells. Changes in T4 functionality were assessed by viability of SKOV-3-Luc cells, INF-γ production by T4 cells and expression of degranulation marker CD107a on T4 cells. Among the polymers synthesized, PEI-FA exerted the lowest cytotoxicity and resulted in the most efficient PD-L1 siRNA knockdown as well as the highest specificity of uptake into tumor cells. In addition, PEI-FA/siRNA complexes had the smallest particle size (104.3 ± 1.72 nm) and maintained a cationic charge density. PD-L1 knockdown in SKOV-3-Luc cells using PEI-FA/PD-L1 siRNA complexes rendered tumor cells more susceptible to T4 cell killing than when SKOV-3-Luc cells were treated with PEI-FA/scrambled siRNA. PD-L1 knockdown in tumor cells enhanced T4 cell responses via increased IFN-γ cytokine release and degranulation of perforin and granzymes. These findings provide insight into targeted delivery of PD-L1 siRNA for improved ovarian cancer therapy.
Surface-Stabilized Calcium Phosphate Nanoparticles for Tumor-Targeted Delivery of siRNA
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Low cytotoxicity and high cellular gene delivery capability are among the most important prerequisites for the selection of a non-viral carrier. Although calcium phosphate (CAP) nanoparticles have long been used for animal cell transfection, its rapid and uncontrollable crystal growth and lack of tissue specificity are among the most challenging problems that limit its use in the clinic. In this study, we report the development of CAP nanoparticles stabilized by a conjugate of the mussel-inspired adhesive molecule, 3,4-dihydroxy-L-phenylalanine (dopa), and a nontoxic hydrophilic natural polymer, hyaluronic acid (HA), for targeted siRNA delivery to tumors. CAP/siRNA/dopa-HA can form compact nanoparticles that effectively protect siRNA from enzymatic degradation despite the structural drawbacks of siRNA, such as low charge density and short and rigid structure. In addition, stabilized CAP nanoparticles were able to maintain their colloidal stability in a physiological salt condition for over a week. The superior ability of CAP/siRNA/dopa-HA to maintain the integrity of encapsulated siRNA and the stability in solution of the nanoparticles allow this formulation to achieve improved intratumoral accumulation of siRNA and a high level of target gene silencing in solid tumors after systemic administration. Considering its biocompatibility, transfection efficacy, and tumor targeting capability, this stabilized calcium phosphate nanoparticle-based gene delivery platform should be considered a promising candidate carrier for systemic siRNA delivery and targeted cancer therapy.

Dendritic Cell-Based Cancer Immunotherapy Through Baculovirus Activation
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Cancer immunotherapy is a powerful therapy in which the patient's own immune cells are used to recognize and target the cancer cells. Dendritic cell (DC)-based immunotherapy employs the patient's own DCs to present cancer antigens to the effector T cells and allow them to recognize the cancer cells and trigger anti-tumor activity. However the need for a strong activator of the immune system and the need for specific cancer antigens pose a challenge in DC-based cancer immunotherapy. In our study, we employ baculovirus (BV) as a mean to activate the dendritic cells. Through the transduction of DCs derived from peripheral blood mononuclear cells (PBMCs), we are able to show a further activation of these DCs as compared to untreated DCs. Co-culture of transduced DCs with cytokine-induced killer cells (CIKs) were then carried out to further activate CIK cells. CIKs are a heterogenous population of natural killer-like cells, and they recognize and kill cancer cells independent of MHC, making them a valuable cell source against cancers that have MHCs downregulated. Characterization of CIK cells that were co-cultured with BV-DCs displayed positive response and thus, hints towards the generation of a more potent and cytotoxic CIKs cells for targeting cancer cells.
**Baculoviral Vectors as an Immunotherapy for Bladder Cancer**

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Bladder cancer is the ninth most common malignant cancer in the world. Bacillus-Calmette-Cuerin (BCG) is often used as an immunotherapy to reduce the recurrence and progression of bladder cancer. However, there exist limitations to BCG immunotherapy – such as refractory, resistance, relapsing and intolerance. Hence, we proposed using insect baculovirus-based vectors as an alternative. We constructed 2 recombinant baculoviral vectors: CD40 ligand (BV-CD40L), interleukin 15 (BV-IL15). Treatments consisted of either BCG or different recombinant baculoviral vectors were instilled into the bladder of C57BL/6 mice that carried mouse urothelial carcinoma MB49 cells. After two treatments, we observed that instilling either BCG or baculovirus alone gave comparable reduction in bladder tumor size. Treatments using BV-CD40L and BV-IL15 gave further reduction of tumor, especially when both vectors were used in combination. These findings indicated that baculovirus is a potential *in vivo* gene therapy vector to deliver therapeutic genes for bladder cancer treatment.

**Targeted Gene Delivery Based on Hyaluronic Acid-Green Tea Catechin Nanogel Complexes**

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Institute of Bioengineering and Nanotechnology, Singapore

A novel nanogel complex comprising hyaluronic acid (HA) – green tea catechin conjugates, branched polyethyleneimine (PEI) and plasmid DNA was developed for targeted gene delivery into cancer cells. HA was exploited for its ability to target CD44 receptors which are overexpressed in many types of cancer cells, while (-)-epigallocatechin-3-gallate (EGCG), the main component of green tea catechins, was chosen for its ability to interact with DNA through hydrogen bonding, hydrophobic and π-π stacking interactions. HA-EGCG conjugates, PEI and DNA self-assembled in aqueous solution to form nanosized complexes. Surface charge of the complexes decreased as a function of increasing HA-EGCG concentration. The strong binding interactions between HA-EGCG and PEI/DNA imparted high stability to the HA-EGCG nanogel complex, contributing to its resistance against heparin challenge. Gene transfection studies revealed that HA-EGCG/PEI/DNA nanogel complexes demonstrated significant enhancement compared to PEI/DNA binary complexes in CD44 overexpressing HCT-116 cancer cells as a result of increased cellular uptake via CD44 receptor-mediated endocytosis. HA-EGCG nanogel complexes also showed superior transfection efficiency over HA complexes because of improved stability. We expect that this nanogel system could potentially be used for cancer gene therapy.
Localization and Immunogenic Properties of DNA Nanotubes In Vivo

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DNA-based nanoconstructs are particularly promising for biological and biomedical applications. DNA structures are biocompatible and survive in cell media, blood serum, and cultured cells for extended periods of time. Furthermore, they can be modified with a plethora of (bio)chemical moieties with nanometer precision and full control over stoichiometry.

Unmethylated CpG-sequences are a hallmark of microbial DNA, and are commonly used as adjuvants for immunostimulation. These CpG-oligonucleotides are recognized by Toll-like receptor 9 (TLR9), present on lymphocytes and antigen-presenting cells, incl. macrophages, and thus initiate an immune response.

In this study, we investigated the use of DNA-based nanotubes as promising carrier systems (CpG delivery) and their effect on immune cells in vivo and in real time.

DNA nanotubes, CpG-decorated DNA nanotubes, and CpG-oligonucleotides (500 nM, 300 nl) were microinjected into the cremaster muscle of anesthetized mice. As assessed by in vivo fluorescence microscopy, all three types of DNA were rapidly internalized by tissue macrophages and colocalized with lysotracker dye in these cells.

Only microinjection of CpG-decorated DNA nanotubes but not of DNA nanotubes or CpG-oligonucleotides induced a significant increase in leukocyte adhesion (30 min after application) and transmigration (60 min after application) in postcapillary venules of the cremaster muscle as observed by in vivo microscopy. Interestingly, CpG-decorated DNA nanotube-elicited leukocyte recruitment was almost completely blocked in animals treated with an inhibitor of mast cell degranulation.

Confocal microscopy of immunostained muscle tissue revealed that only after application of CpG-decorated DNA nanotubes, nuclei of cells surrounding the microinjection site were positive for phosphorylated p65, indicating (TLR-9 mediated) activation of the NF-kB pathway.

Taken together, these in vivo findings suggest that DNA nanotubes are potent delivery vehicles, targeting tissue macrophages and mast cells. The immunogenic potential apparently depends on the decoration of DNA tubes with CpG-oligonucleotides.

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Self-Assembled Micellar Nanocomplexes Comprising Green Tea Catechin Derivatives and Protein Drug for Cancer Therapy

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The drug-to-carrier ratio is an important issue in designing drug carriers because using unreasonably high quantities of carriers can lead to problems associated with carrier toxicity and difficulties in metabolism and elimination. However, if both the drug and carrier possess therapeutic effects, this issue would not be a restricting factor, and might offer the advantage of combined therapeutic effects. (−)-Epigallocatechin-3-O-gallate (EGCG), a major ingredient of green tea, is recognized for its various therapeutic effects, including anticancer effects. Here, we design the core-shell micellar nanocomplex spontaneously constructed by the self-assembly of EGCG derivatives and therapeutic protein. This system is the first to utilize EGCG as a carrier of biological molecules, aiming at the synergistic therapeutic effects between the carrier and the therapeutic protein. We constructed a micellar nanocomplex (MNC), from sequential self-assembly of the EGCG derivatives and anticancer protein, which achieve a greater anticancer effect in vitro and in vivo than the free protein. The MNC is constructed by complexation of oligomerized EGCG with the anticancer protein, Herceptin, to form the core, followed by complexation of poly(ethylene glycol)-EGCG to form the shell. When injected into mice, the Herceptin-loaded MNC showed improved tumour selectivity, longer blood-half-life and reduced tumor growth more efficiently than free Herceptin. This MNC represents a unique and effective drug delivery system which takes advantage of the therapeutic effect of the green tea-based carrier.
pH-Sensitive Polycarbonate Micelles for Enhanced Intracellular Release of Anticancer Drugs: A Strategy to Circumvent Multidrug Resistance

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In this study, we have synthesized two novel amphiphilic diblock copolymers with aldehyde groups via organocatalytic ROP of a functionalized cyclic carbonate monomer (MTC-Bz) using polyethylene oxide (PEG) as the macroinitiator. The polymers were covalently conjugated with an anti-tumor drug doxorubicin (DOX) via a pH-sensitive Schiff-base linkage. The resulting conjugates formed micelles in phosphate-buffered saline (PBS) (pH 7.4) with an average size of about 100 nm and narrow size distribution. The surface charge of the micelles was close to zero. The micelles were stable in both PBS and cell culture media containing 10% FBS up to 5 days. The results obtained from the in vitro release study indicated that DOX release from the micelles was pH-dependent, being faster at pH 5.0 (the endolysosomal environment) than pH 7.4 (the extracellular environment). Human breast cancer MCF-7 cells and DOX-resistant MCF-7/Adr cells were employed to investigate the cellular uptake and cytotoxicity of DOX-loaded micelles. The confocal microscopy and flow cytometry studies showed that the uptake of DOX-loaded micelles by MCF-7 cells was similar to that of free DOX. In sharp contrast, the uptake of DOX-loaded micelles by MCF-7/Adr cells was significantly higher than that of free DOX. The polymers showed no toxicity to MCF-7 and MCF-7/Adr cells. The DOX-loaded micelles killed the cells efficiently. In particular, they were more potent against drug-resistant MCF-7/Adr cells than free DOX due to the higher cellular drug accumulation and pH-triggered intracellular drug release, providing a strategy to navigate around drug resistance. These DOX-conjugated micelles can be a promising carrier for the delivery of anticancer drugs with amine functional groups.
In photodynamic therapy (PDT), the light-induced activation of a photosensitizer (PS) leads to the generation of cytotoxic singlet oxygen that can trigger various mechanisms of cell death. Implementation of controllable singlet oxygen generation within cancer cells would lead to more reliable PDT with minimal phototoxicity to normal cells and enhanced efficacy for photodynamic cancer treatment. With this rationale, we design and synthesize a bioreducible biarmed methoxy poly(ethylene glycol)-(pheophorbide a)$_2$ (mPEG-(ss-PhA)$_2$) conjugate for cancer cell-specific PDT, by which PhA moieties are chemically conjugated at one end of mPEG via disulphide-bond-contained biarmed linkages. The amphiphilic mPEG-(ss-PhA)$_2$ conjugates can form stable self-assembled nanoparticles (NPs) in the aqueous condition. The mPEG-(ss-PhA)$_2$ NPs showed intramolecular and intermolecular self-quenching effects that enabled the NPs to remain photo-inactive in a physiological buffer. However, the cleavage of the disulfide bonds that accelerates the dissociation of the NP structure triggers the rapid release of PhA molecules in a photoactive form in response to intracellular reductive conditions. In cell culture systems, mPEG-(ss-PhA)$_2$ NPs exhibited significant phototoxicity upon light exposure and intracellular uptake. Furthermore, we observed that the dequenching processes of PhA in the mPEG-(ss-PhA)$_2$ NPs highly depended on the expression of intracellular thiols, which supplementary of glutathione monoethylester facilitated more rapid PhA release and enhanced the PhA phototoxicity. These findings indicate that the bioreducible activation mechanism of mPEG-(ss-PhA)$_2$ NPs in cancer cells can maximize the cytosolic dose of active PSs to achieve high cytotoxicity, thereby providing a potential implementation of controllable PDT for cancer treatment.
**Interaction of Plasma Proteins with Ellagic Acid Reduced Gold Nanoparticles and Its Biocompatibility**  
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The importance of nanotherapy in biomedicine has been established and gold nanoparticles (AuNPs) are found to be the most widely employed. As the AuNPs synthesized by chemical route exhibits toxicity to normal cells, biological synthesis has gained immense attention. Recently, reductants with biological properties have been used to synthesize AuNPs. Ellagic acid (EA) is one such medicinal flavanoid with promising characteristics as a reductant. In our study EA has been used as a reducing and stabilizing agent to form spherical and stable AuNPs (eAuNPs) which have been characterized through microscopic and spectroscopic analyses. The mechanism of formation is found to be through temporal evolution. The eAuNPs are also found to be stable in cell culture medium as observed through UV-Visible and zeta-potential analyses. In order to study the interaction of eAuNPs with plasma proteins absorption spectral and fluorescence quenching studies have been carried out. A dose and time dependent increase in adsorption of proteins has been observed, which may indicate the formation of a protein corona on the eAuNPs. The specific proteins interacting with eAuNPs have been revealed through FT-IR and SDS-PAGE analysis. The eAuNPs are found to be cytocompatible towards human blood cells as investigated through hemocompatibility and viability assays conducted in peripheral blood mononuclear cells. These results together indicate the potential of eAuNPs to be applied in diagnostics and nanopharmaceutics.

**Doxorubicin Conjugated CQE-AuNPs and Their Application in Breast Cancer Therapy**  
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Synthesis of gold nanoparticles (AuNPs) using biological sources offers eco-friendly approach compared to traditional chemical methods. In our earlier study, we have reported microwave-assisted single-step green synthesis of AuNPs using aqueous extract of *Cissus quadrangularis* (CQE-AuNPs) and now we show that CQE-AuNPs are stable in different biological buffers and media. They are also found to bind with bovine serum albumin (BSA) in a time and dose dependent manner indicating their stability. We have also formulated a delivery system along with the drug doxorubicin(@Dox).The synthesized conjugates have been characterized using spectroscopic, dynamic light scattering and zeta potential measurements to confirm their binding to AuNPs. Further, cytotoxicity of CQE-AuNPs@Dox to MCF-7 breast cancer cell line has been evaluated and the complex shows significant decrease in cellular viability compared to doxorubicin or CQE-AuNPs alone. CQE-AuNPs@Dox also inhibit VEGF (Vascular Endothelial Growth Factor) induced angiogenesis. These results show the beneficial synergistic action of doxorubicin and bioactive components of CQE present in CQE-AuNPs@Dox. We believe that this conjugate can be a promising candidate for cancer treatment.
Delivery of pGFP Entrapped Calcium Phosphate Nanoparticles to the Vaginal Mucosa
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Vaginal application of nucleic acids holds great potential for the prevention and treatment of various viral infections resulting in genital herpes, AIDS and cervical cancer. An increasing number of nanoparticle-based nucleic acid delivery systems, including calcium phosphate (CaP) nanoparticles (NPs), are currently being evaluated for use as non-viral vector. In our present study monodispersed CaP NPs with polymeric shell encapsulating pGFP have been developed to facilitate delivery to the vaginal mucosa. The pGFP-CaP NPs were prepared in aqueous medium at 4°C and pH 7.2 using CaCl₂·2H₂O, Na₂HPO₄ and C₆H₇Na₃O₇·2H₂O as precursors. The SEM of the pGFP-CaP NPs showed spherical morphology and diameter of <100nm, which correlates to the DLS data. The high negative ς-potential of free DNA was substantially decreased following condensation with Ca²⁺ ions and the formation of the NPs. To obtain bioadhesivity, mucus penetrating property and near neutral surface potential, surface coating of NPs with hydrophilic polymer was carried out. The FTIR spectra of the NPs confirmed the entrapment of the pDNA as well as the absorption of the polymer. In gel retardation assay the movement of pGFP-CaP NPs is less as compared to free pDNA. The dissolution of pGFP-CaP NPs was estimated at pH 5.5, 7.2 and 8.5. The maximum release of pDNA following dissolution, estimated spectrophotometrically and visualized by gel electrophoresis was at pH 7.2. The XRD analysis demonstrated that the void and loaded NPs are highly crystalline with hydroxyapatite and di-calcium phosphate dihydrate as major phases. The NPs were prepared in an aqueous system with low solubility at pH 5.5. Solubility was lowered by coating with bioadhesive polymer, rendered the NPs suitable for delivering the nucleic acid to the vaginal mucosa.
Au-Ni-Pt Striped Nano-Swimmer Motion: Theory and Experiments

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A self-propelling nano-swimmer is proposed with tri-metallic segments: gold non-catalytic portion, nickel central part and platinum catalytic section. This novel type of catalytic nano-swimmer is designed to mimic the function of biomolecular motor protein, which possesses the ability to deliver cargo and naturally exists in biological bodies, and fabricated with a bottom-up approach based on NEMS (Nano-Electro-Mechanical System) technology. The autonomous propulsion of gold-nickel-platinum striped nano-swimmer is induced by oxygen bubble detachment, which is catalytically generated from the decomposition of hydrogen peroxide into oxygen molecule and water. In aqueous hydrogen peroxide solution, platinum can chemically catalyze the decomposition of hydrogen peroxide to produce oxygen bubbles detaching from the surface of platinum, which generate the recoil force reacting on the catalytic nano-swimmer moving forward towards a gold segment. Herein, we investigate the autonomous propulsion mechanism stemming from the momentum change of a catalytic nano-swimmer-oxygen bubble integral system and derive the nano-swimmer velocity formula. From the velocity equation, it is seen that the propulsion velocity is influenced by several parameters, such as fuel hydrogen peroxide concentration, surrounding temperature, interfacial tension, and the size of the nano-swimmer, etc. In addition, the velocity of catalytic nano-swimmer is estimated at different fuel concentrations. Finally, the fabricated Au-Ni-Pt striped nano-swimmer motion is clearly observed under an optical microscope, revealing that the oxygen bubbles are generated at the surface of platinum with the addition of hydrogen peroxide and the motion speed is $10 \mu m$ and $25 \mu m$ in concentrations of $5 mol/m^3$ and $20 mol/m^3$ of aqueous hydrogen peroxide solution, respectively. Finally, the emerging nano-swimmer can also propel autonomous motion either linearly or circularly.
IBN-4 Nanoparticles for Antimicrobial Photodynamic Therapy: 
Characterization and In Vitro Investigation

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Ching-Feng Weng and Chia Hung Lee 
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The rise of antibiotic-resistant bacteria has become a major clinical and public health concern. Moreover, the microorganisms have excogitated effective approaches to counteract the biocidal action of antimicrobials. In recent years photodynamic therapy (PDT) has emerged as an attractive treatment strategy for infectious diseases. Metals were shown to further potentiate the stability and PDT effect of photosensitizers and were therefore used in this study to investigate its ability to potentiate the activity of Indian spice curcumin against bacteria. IBN-4 nanoparticles loaded with photoactivable Vanadium curcumin [VO(cur)(dppz)Cl)] metal complex was prepared and tested for their PDT efficacy on planktonic cells and biofilms of *Staphylococcus aureus*, *Staphylococcus epidermis*, *E.coli* and *B.subtilis*. First, the starting IBN-4 nanoparticles are examined to verify that their synthesis has been successful considering the structural properties, using transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FTIR), nitrogen adsorption/desorption and Brunauer-Emmett-Teller (BET). The Vanadium curcumin [VO(cur)(dppz)Cl)] metal complex was initially functionalized with 3-iodopropyl Trimethoxy silane groups and further loaded in to the IBN-4 nanoparticles. Mild toxicity was observed when planktonic cells and biofilms were treated with the nanoparticles in the dark. However, when the planktonic cells and biofilms showed a significant phototoxicity upon light irradiation. The antimicrobial activity of IBN-4 loaded Vanadium curcumin metal complex was significantly higher than curcumin and Vanadium curcumin metal complex in free form. Further the nanoparticle conjugated metal complex was able to generate ROS up on light exposure that would have contributed to the oxidative stress leading to the enhanced bacterial cell lysis. Fluorescein isothiocyanate (FITC) was loaded in to IBN-4 particles as a model platform to assess its efficacy as a drug delivery tool and the particle uptake mechanisms were studied. These findings suggest that IBN-4 particles hold tremendous promise in delivering photosensitizers demonstrating their potential applicability in medicine.
Effective global control of tuberculosis (TB) is increasingly threatened by the rapid emergence of multidrug-resistant strains and novel efficacious TB therapeutics is urgently needed. To this end, cationic peptides have been identified as a new class of agents with the ability to kill drug-resistant mycobacteria, yet displaying a lower susceptibility for resistance development. However, their inherent poor serum stability and permeability across biological membranes greatly limit their use as oral agents in clinical applications. To address this, we explored the use of surface acoustic wave (SAW) technology for peptide nebulization to provide effective local delivery through the respiratory tract. SAWs are nanometer amplitude electroelastic waves that propagate along the surface of a piezoelectric substrate and prior work has shown that it can act as a powerful microscale actuation mechanism for the successful nebulization of DNA, proteins and cells. Six synthetic anti-mycobacterial peptides previously reported by our group were used as model peptides for SAW nebulization. Using high performance liquid chromatography (HPLC) analysis post-nebulization, we showed that concentration recoveries for the peptides were significantly greater than 90%. Further, together with mass spectrum analysis, we confirmed that the peptides’ integrity was preserved after the nebulization process. The anti-mycobacterial activity of the nebulized peptides was found to be unchanged as compared to control non-nebulized peptides, as shown by effective minimum inhibitory concentrations against M. smegmatis of 62.5-125 mg/L. Further optimization will facilitate the development of this approach as a novel and effective TB treatment modality.
A Rational Same-Centered Design Strategy for the Development of Broad-Spectrum Biodegradable Antimicrobial Polycarbonates

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The prevalence of drug-resistant microbes is an eminent health issue facing society today. A recent report by the World Health Organization on global surveillance of antimicrobial resistance has brought attention to the very real possibility of the twenty-first century moving towards a post-antibiotic era wherein common infections and minor injuries can easily kill. There is thus a dire need for novel classes of antimicrobials to be developed, particularly with alternative modes of action that exhibit reduced propensity towards resistance development.

To this end, biodegradable antimicrobial polymers have recently emerged as a promising solution for combating drug-resistant pathogens while being tolerant towards resistance development due to the physical nature of its antimicrobial mechanism. It has been widely established that the balance between cationic charge and hydrophobicity (i.e. amphiphilic balance) is pertinent in influencing the biological properties of antimicrobial polymers. While considerable design efforts has been directed towards tuning the amphiphilic balance via copolymerization of disparate cationic and hydrophobic monomers (i.e. segregated monomer approach), the same centered design (i.e. hydrophobic moiety directly conjugated to cationic component) has insofar been warranted insufficient attention. We posit that such a design strategy would allow more prudent control of the polymer's global amphiphilicity and effectively delineate pivotal structural determinants that might have a significant bearing on its activity and selectivity. Herein, using a model polycarbonate platform, we will discuss a rational same centered design strategy to develop potent and highly selective macromolecular antimicrobials in both in vitro and in vivo models.
Co-Delivery of Anti-Viral and Anti-Fungal Therapeutics for the Treatment of Sexually Transmitted Infections Using a Moldable, Supramolecular Hydrogel

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According to the World Health Organization (WHO), there were approximately 35.3 million people living with human immunodeficiency virus (HIV) in 2012. While there is currently no cure for HIV infection, effective treatment with antiviral drugs and proper prophylaxis can control and prevent proliferation of the virus, respectively. Furthermore, most HIV infection cases are often complicated by sexually transmitted infections (STIs) such as candidiasis. In this investigation, a therapeutic co-delivery hydrogel system has been developed to provide effective HIV prophylaxis, alongside the prevention and/or treatment of candidiasis. Two components – a HIV reverse transcriptase inhibitor, tenofovir, and a cationic macromolecular antifungal agent derived from a vitamin D-functionalized polycarbonate (VD/BnCl(1:30)) were formulated into a biodegradable vitamin D-functionalized polycarbonate/PEG-based supramolecular hydrogel. The hydrogels exhibit thixotropic properties and can be easily spread across surfaces for efficient drug absorption into biological tissue. A sustained release profile of tenofovir was observed from the hydrogel system, where approximately 85% of the drug was released within 3 h. The VD/BnCl(1:30) polymers did not impede the diffusion of tenofovir from the hydrogel matrix as there was no difference in the drug release profiles in the presence and absence of the polycation.

Antimicrobial efficacy studies indicated that the cationic-formulated hydrogels were able to kill planktonic C. albicans efficiently with a minimum bactericidal concentration (MBC) of 0.25–0.5 g/L. These hydrogels were also able to eradicate biofilm cultures of C. albicans at 4 × MBC used in treating the planktonic organisms. When human dermal fibroblasts (as model mammalian cells) were treated with these hydrogels, the cell viability remained high at above 80%, demonstrating excellent biocompatibility. When applied topically, this dual-functional hydrogel can potentially prevent HIV transmission and eliminate microbes that cause infections in the vulvovagina region.
**Antibacterial/Antifouling Coatings: Role of Block Copolymer Architecture**

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The high prevalence of catheter-associated infections amounts to more than 3 billion dollars annually in hospitals, and functional polymer coatings on catheter surfaces serve as an attractive solution. Most polymer coatings for medical catheters have either antimicrobial or antifouling properties, but rarely both. Recent advances in polymer chemistry have enabled us to develop cationic polycarbonates-based coatings with both antimicrobial and antifouling properties through a polydopamine attachment layer. In this study, triblock polycarbonate polymers consisting of antifouling poly(ethylene glycol) (PEG), antimicrobial cationic polycarbonate and a tethering functional block were synthesized and subsequently grafted onto pre-functionalized catheter surface through covalent bonding under the ambient conditions. The anchoring functional moiety was positioned at either the center or end of the polymer block to investigate its effect on antifouling and antimicrobial properties. The results showed that the surface coated with the polymer containing the end-positioned tethering block was more hydrophilic and the most effective against both S. aureus and E. coli, resisting surface fouling for one week. Conversely, the surface coated with the polymer containing the center-positioned tethering block demonstrated higher bacteria killing efficacy as compared to the controls without polymer coating and the former polymer-coated surface. The surface coated with the more hydrophilic polymer was also able to resist protein fouling and platelet adhesion, and all polymer-coated surfaces did not cause significant hemolysis. Therefore, this series of cationic triblock polycarbonates offer great potential as antimicrobial and antifouling coatings for the prevention of catheter-associated bloodstream infections.
Infections caused by medical devices, especially intravascular and urinary catheters, pose major challenges in hospitals. Various catheter coating methods have been studied to prevent catheter-associated infections. However, it is difficult to obtain an effective and facile coating without elevating the toxicity of the catheter surfaces. In this study, a series of brush-like polycarbonates containing three key components such as pendent dopamine (for adhesion of polymer to inert substrate), PEG (for antifouling property) and antibacterial cations (for electrostatic targeting of bacteria as well as membrane lysis) were synthesized via organocatalytic ring-opening polymerization. The results showed that the presence of dopamine molecules facilitates the anchoring of the polymers onto the inert silicone surface. The polymer coating synthesized with the optimal hydrophobic quaternizing agent was able to kill both Gram-positive and Gram-negative bacteria in bacterial suspension, and prevent bacterial fouling on the catheter surface. In addition, this polymer coating was stable under a simulated blood flow, and its antibacterial and antifouling activities remained even after being in contact with bacterial solution over a long period of time. Importantly, this coating was hemocompatible, and able to prevent protein adsorption and platelet adhesion. Therefore, this brush-like polymer coating has great potential for use in intravascular catheters for prevention of catheter-associated infections.
Synthetic β-Sheet Forming Peptide Amphiphiles for Treatment of Fungal Keratitis

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Fungal keratitis is a leading cause of ocular morbidity. However, diagnosis typically occurs late, as many frequently misdiagnose fungal keratitis as bacterial keratitis. Even if the diagnosis is made correctly, treatment remains a challenge because of the acute lack of safe and effective antifungal agents for clinical use. In recent years, antimicrobial peptides (AMPs) have received considerable attention as potent and broad-spectrum antimicrobial agents with the potential to overcome antibiotics resistance. We previously reported the design of short synthetic β-sheet forming peptides (IKIK)₂-NH₂ and (IRIK)₂-NH₂ with excellent antimicrobial activities and selectivities against various clinically relevant microorganisms including Gram-positive S. epidermidis and S. aureus, Gram-negative E. coli and P. aeruginosa, and yeast C. albicans. In this study, we evaluated the application of the two most promising synthetic β-sheet forming peptide candidates for in vivo fungal keratitis treatment in comparison with a non-β-sheet forming peptide and commercially available amphotericin B. It was found that topical solutions of the designed peptides are safe and as effective as the clinically used amphotericin B. Compared to the costly and unstable amphotericin B, (IKIK)₂-NH₂ and (IRIK)₂-NH₂ are water-soluble, relatively inexpensive and stable. Thus, the synthetic β-sheet forming peptides are presented as promising candidates for the treatment of fungal keratitis.

Engineering Luminescent Silver Nanoclusters for Biomedical Applications

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Ultrasmall silver nanoclusters (Ag NCs), typically consisting of 100 Ag atoms or less, have recently emerged as a new class of functional materials due to their intriguing physical and chemical properties, such as strong luminescence and quantized charging. In particular, the luminescence properties of Ag NCs have been recently used for sensor development and bioimaging application. However, the practical applications of luminescent Ag NCs have been constrained by their relatively low stability in aqueous solutions, especially in the biological systems, largely due to the vulnerability of Ag NCs toward the oxidation and ligand etching. There is therefore a pressing need to develop efficient strategies to synthesize stable Ag NCs that can largely preserve their cluster structures and properties in the practical settings. In this study, we have developed one simple yet efficient method to synthesize highly luminescent Ag NCs in aqueous solution. The as-synthesized Ag NCs also show excellent stability in aqueous solutions and buffers. A number of characterization techniques including electrophoresis, and mass and optical spectroscopy, have been used to determine the molecular formula and the structure of the as-synthesized Ag NCs. The products and engineering principles developed in this study may pave the way of Ag NCs toward biomedical applications, such as biosensors and antimicrobial agents.
Health Effects of Engineered Nanoparticles
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The use of nanomaterials in various products has become increasingly popular in recent years. However, even with the rapid advancement in nanotechnology, the toxicity of nanomaterials is not well known. Silver nanoparticles (AgNPs) and gold nanoparticles (AuNPs) are commonly used nanoparticles today. AgNPs are incorporated in many products from the medical industry; however they were found to be toxic to cells, where mitochondrial functions were disrupted. AuNPs have many potential uses in the biomedical sector, especially in cancer therapy and as drug carriers, but cellular toxicity of AuNPs is poorly understood. This study investigates the effects of AuNPs on human neuronal mitochondria with the use of the ADP/ATP assay protocol, and to compare the results obtained to that of experiments performed with AgNPs, which is the positive control. Results of the ADP/ATP ratio assay revealed that acute exposure of AgNPs to SH-SY5Y cells resulted in mitochondrial dysfunction, corresponding to previous research. However, acute exposure of AuNPs to SH-SY5Y cells showed an opposite trend, where ATP production was increased. While the mechanism for the enhancement of ATP production is unknown, a possible reason may be that the size of the AuNPs (20 nm in diameter) made it less toxic to cells. It has been previously shown that the cytotoxicity of AuNPs is size-dependent. The reason why ADP production dropped is unknown too, and we speculate it to be a result of an increase in ATP synthesis in the cells, which explains why ATP levels were significantly higher than the negative control.
A Human Mesoendoderm Pattern to Detect Developmental Toxicity of Compounds

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Developmental toxicology is the study of effects of toxic chemicals and physical agents on the developing offspring. In the presence of xenobiotics such as certain pharmaceutical drugs and pesticides, deviant embryo development may happen due to their developmental toxicity such as death, malformation, growth retardation, and functional deficiency. Currently, animal-based in vivo models are still the only generally accepted methods for developmental toxicity testing. However, these models are very expensive, animal-consuming, and most importantly, suffer from high inter-species variations in the developmental toxicity responses of test compounds. Here, we developed a new human pluripotent stem cell (hPSC)-based in vitro platform to detect developmental toxicity of xenobiotics. We applied micropatterning technique to spatially induce the mesoendoderm differentiation of hPSCs within circular hPSC colonies. These spatially induced cells would later underwent self-organized directed collective cell migration, forming a distinct mesoendoderm pattern on day 3. This mesoendoderm pattern was tested to be morphologically consistent across different control hPSC colonies and cell lines, and very sensitive to developmental toxicity risk. In the presence of different known developmental toxic drugs such as Thalidomide and Retinoid acid, the spatial distribution of this mesoendoderm pattern was significantly disrupted in a dose-dependent manner. In contrast, non-developmentally toxic drug, such as Penicillin G, would not affect both the morphology and the location of the mesoendoderm pattern, showing no significant differences compared with untreated control colonies. Further validation of this model with more pharmaceutical compounds could make it a powerful human-specific platform for preliminary drug screening in pharmaceutical companies.
An In Vitro 3D Hair Follicle Model for the Testing of Hair Actives

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Hair loss is an important issue that relates to personal care, as it affects men, women and children of all ages. Over their lifetime, 60% of men and 10% of women will have to face hair loss. Hence, there is a steadily growing worldwide demand for hair loss treatment products. A hair follicle model, which recapitulates the biological response of the native hair follicle towards hair actives would be useful to screen for potential drug candidates or actives that either promote or inhibit hair growth. We have developed a hair follicle model by encapsulating the two main hair follicle cell types, dermal papilla (DP) cells and keratinocytes within a polyelectrolyte fiber matrix. The cells can be encapsulated in adjacent layers at resolutions of ~50 µm, allowing them to self-assemble into aggregates of dermal papilla cells that associate closely with the keratinocytes. Gene expression of these constructs showed up-regulation of molecules involved in epithelial-mesenchymal interactions of the hair follicle. Implantation of the follicular structures in SCID mice led to the formation of hair follicle-like structures, thus demonstrating their hair inductive ability. Our studies show that DP cell proliferation in the model provides a measure of the promoting or inhibitory effects of known hair growth regulating agents. Collectively, our results underline the promise of our 3D hair follicle model for in vitro screening and testing of hair actives.
Predictive Renal Models for Safety Screening

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Risk assessment of nanomaterials requires properly validated in vitro models. The human kidney receives 25% of the cardiac output and is a major target organ for nanoparticles. The kidney is also important for the clearance of nanomaterials from the human body. However, there is currently a lack of reliable pre-clinical models for predicting nephrotoxicity. Therefore, nephrotoxicity of novel compounds is typically only detected during late stages of development. Validation of current renal in vitro models was either not performed, or the models were not predictive.

Here we developed an in vitro model based on human primary renal cells. The endpoint was mRNA expression of the pro-inflammatory interleukins (IL)6 and IL8. The in vitro model was validated with 41 well-characterized compounds and all major performance metrics were determined. The results showed that 76% - 85% of predictions made with this model would be correct. This work established the first in vitro model that predicts nephrotoxicity in humans with high accuracy. Comparable predicitivity was achieved when stem cell-derived HPTC-like cells were employed in the in vitro model, demonstrating the first successful application of stem cell-derived human renal cells. For comparison, standard renal cell lines and widely used endpoints were tested, and poor predictivity was obtained.

Further, we addressed the underlying mechanisms of compound-induced IL6 and IL8 up-regulation in HPTCs. The results showed that this was dependent on the nuclear translocation of NF-kB. These results give further insights into the activation of pro-inflammatory pathways in nephrotoxicity, which also play an important role in the pathophysiology of acute kidney injury. Our well-characterized in vitro models that predict nephrotoxicity in humans with high accuracy will now be applied for the screening of nanomaterials.
**Predictive In Vitro Models for Nanotoxicology**

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*In vitro models play an increasingly important role in drug safety screening and nanotoxicology. This is due to altered legislation and the increasing number of compounds and nanoparticles that need to be tested. So far, results obtained with *in vitro* models are often difficult to interpret. Problems are related to the use of not well-characterized materials and a lack of standardization in the assays used. While there is an increasing awareness and improvement to these issues, the lack of predictive cell-based *in vitro* models for human internal organs remains a major problem. Predictive liver- and kidney-specific *in vitro* models would be of particular interest, as these organs are not only major targets of nanoparticles, but are also important for their clearance. We have recently developed the first high content screening (HCS) model that predicts nephrotoxicity in humans with high accuracy (~90%). This model has been validated with well-characterized drugs. This HCS model is suitable for efficient screening of nanomaterials and prediction of their toxicity. Currently it is applied for the characterization of nanosystem for drug delivery.*

**Purification of Biomass-Derived 5-Hydroxymethylfurfural and Its Catalytic Reaction to 2,5-Furandicarboxylic Acid**

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*A simple and effective water extraction method to purify 5-hydroxymethylfurfural (HMF) from biomass dehydration system was reported. Up to 99% of the HMF could be recovered and the HMF in aqueous solution was directly converted to 2,5-furandicarboxylic acid (FDCA) as the sole product. With this purification technique, an integrated process from fructose to FDCA via HMF prepared in isopropanol monophasic system, we got an overall FDCA yield of 83%. From Jerusalem artichoke raw biomass to FDCA via HMF prepared in water/MIBK biphasic system, an overall FDCA yield of 55% was obtained.*
Ligand’s Role on the Properties of Gold Nanoclusters

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Recently, the physicochemical properties of noble metal nanoclusters (NCs) have drawn significant interest in the fields of chemistry, materials, and biology. The rapid advancement of the field has established the crystal structure of several metal NCs. The properties of these metal NCs largely depend on their structure which consists of a metal core and a ligand shell. In this poster, we demonstrate how the surface ligand plays key roles on the structure and properties of metal NCs. In particular, the variation of optical properties of the metal NCs in solution upon functionalization with different ligands on the surface will be addressed. Furthermore, we demonstrate a novel strategy to improve the stability of the metal NCs in solution, which still remains as a major problem toward various practical applications. We believe that this work would stimulate more experimental and theoretical research focusing on the ligand shell engineering which could further pave their ways toward biomedical applications.

Poly-Benzyl Ammonium Chloride Resins as Solid Catalysts for Fructose Dehydration

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5-hydroxymethylfurfural (HMF) is one of the most promising platform molecules, and can be converted into a variety of interesting chemicals. The production of HMF is essentially targeted at the bulk chemicals downstream, such as chemicals for the fuels and plastics industries. One critical challenge in HMF production processes is the bridge to further value-adding reactions in a simple and efficient way (e.g. fewer isolation and purification steps). Herein, a novel poly-benzyl ammonium chloride (PBrNH\textsubscript{3}Cl) resin is developed as a highly efficient and stable catalyst for the dehydration of carbohydrates into HMF. In the isopropanol system, PBrNH\textsubscript{3}Cl produces high purity HMF that is suitable as feedstock for the oxidation to 2,5-furandicarboxylic acid (FDCA). The excellent catalytic properties together with its easy synthesis, low cost, and non-toxic nature make this poly-ammonium resin a promising catalyst for the development of new and efficient processes for biomass-based chemicals.
Calcium Carbide Utilization in Organic Synthesis

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Calcium carbide is traditionally synthesized from coal. Recently, it has been demonstrated that calcium carbide can also be synthesized from lignocellulosic biomass. The low production cost has put calcium carbide in a better position to serve as a sustainable resource for the chemical industry. Furthermore, the use of calcium carbide in organic synthesis is safer as compared to handling acetylene gas. However, due to the low solubility of calcium carbide and the difficulty in reaction controlling, the reality of direct using of CaC₂ in organic synthesis remains the most challenging aspect thus far.

Herein, we disclose efficient catalytic protocols for the synthesis of various functional acetylene derivatives and enaminoes from calcium carbide. A novel catalytic system for the production of propargylamines directly from calcium carbide via AAA or AHA three-component-coupling reaction pathways was disclosed. Mono-substituted aminopropyne products with terminal alkyne function could be produced in high yield at mild conditions. The click reaction between aminopropyne and azide, one pot sequential AAA-AHA coupling and AAA-Sonogashira coupling reactions, and the domino Sonagashira-heterocyclization reaction with calcium carbide demonstrated the versatile applications of this novel methodology in organic synthesis. Furthermore, a mild, metal-free system for the synthesis of propargyl alcohols containing a terminal alkyne from calcium carbide has been developed. It is a safe and easy-to-handle protocol that is applicable to both aldehydes and ketones at relatively low temperatures. Recently, a three component reaction system of calcium carbide, aryl aldehyde and amine was studied and the reaction could lead to enamino or propargyl amine by changing reaction conditions or substrate scope. The new findings also demonstrated the versatile property of acetylide ion which can bridge both electrophiles and nucleophiles.

The use of calcium carbide in organic synthesis is more cost-efficient and safer than the use of acetylene gas. This would offer the chemical industry and organic synthesis platform chemicals a sustainable feedstock. The new synthetic methods presented here demonstrated that calcium carbide could play a major role as a sustainable and cost efficient carbon source in modern organic synthesis.
Size-Dependent Patterned Recognition and Extraction of Metal Ions by a Macrocyclic Aromatic Pyridone Pentamer

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A circularly folded pyridone pentamer, which is synthesized by BOP-mediated H-bonding-assisted one-pot macrocyclization reactions, exhibits many interesting functions such as fiber formation and catalysis. With its suitably sized non-collapsible cavity of 1.4 Å in radius formed by five convergently aligned interior-pointing carbonyl O-atoms, this macrocyclic pentamer further exhibits selective recognition and efficient extraction of several larger ions in the decreasing order of Cs⁺ > Ba²⁺ > Tl⁺ > Au⁺ > K⁺ > Rb⁺ in the presence of many other smaller metal ions using biphasic water–CHCl₃ system. The patterned extraction displayed by the pentamer appears to be size-dependent with better efficiencies than 18-crown-6, 21-crown-7 and valinomycin containing six or more oxygen-donor atoms. The first principle computations at the B3LYP/6-31G(d,p) level provide the structural insights into the behavioral origins of the ion binding by this pyridone pentamer being that the “rigid” framework in it discourages or even prohibits concurrent binding of its three or more interior O-atoms toward smaller ions whose binding is additionally disfavored by the substantial repulsions from the vicinity amide H-atom. Our current systematic investigation has laid a solid foundation for us to pursue more challenging issues, focusing on the speedy evolution of circularly folded hybrid pentamers derived from monomeric pyridone, methoxybenzene, fluorobenzene, or pyridine building blocks for highly selective recognition of metal ions.

Porous MnO/Mn₃O₄ Nanocomposite for Electrochemical Energy Storage

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Controlling the morphology of nanostructured Mn oxide materials can be an effective way to improve the capacitance for supercapacitor application. Herein we demonstrate for the first time the synthesis of tetrahedral MnO/Mn₃O₄ nanocomposites with porous structures, and a new method to synthesize porous urchin-shaped MnO/Mn₃O₄ nanocomposites, as evidenced by the detailed analysis of transmission electron microscopy images and power X-ray diffraction patterns. In comparison, the octahedral MnO nanoparticle with lower surface area was synthesized. The porous urchin-shaped MnO/Mn₃O₄ nanocomposites exhibit superior capacitance toward supercapacitor application. The porous tetrahedral MnO/Mn₃O₄ nanocomposites exhibit superior stability. Superior capacitance and stability for supercapacitor application of these porous nanocomposites can be explained in terms of a much higher surface area of porous structure. This porous nanocomposite structure also offers a vivid example to investigate the morphology and surface area effect of nanocomposites, and their influence on the capacitance of the nanocomposites.
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